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To Go or Not to Go: Migration of Human Mesenchymal Progenitor Cells Stimulated by Isoforms of PDGF

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Abstract The recruitment of mesenchymal progenitor cells (MPCs) and their subsequent differentiation to osteoblasts is mandatory for bone development, remodeling, and repair. To study the possible involvement of platelet-derived growth factor (PDGF) isoforms, primary human MPCs and osteogenic differentiated progenitor cells (dOB) were examined for chemotaxic response to homodimeric human platelet-derived growth factor AA, -BB, and heterodimeric PDGF-AB. The role of PDGF receptors was addressed by preincubation with PDGF receptor alpha and beta chain specific antibodies. Migration of MPCs, dOB, and primary osteoblasts (OB) was stimulated by the addition of rhPDGF-AA, rhPDGF-BB, and rhPDGF-AB. The effect was highest in MPCs and for rhPDGF-BB, and declining with osteogenic differentiation. Preincubation with the receptor alpha specific antibody decreased the CI to borderline values while pretreatment with the receptor beta specific antibody led to a complete loss of chemotactic response to PDGF isoforms. In control experiments, basal migration values and rhBMP-2 as well as rxBMP-4 induced chemotaxis of MPC were not influenced by the addition of receptor alpha or beta antibodies. Interestingly, without preincubation the parallel exposure of MPC to rhTGF-β1 instantaneously leads to a selective loss of migratory stimulation by rhPDGF-AA. The chemotactic effect of PDGF isoforms for primary human MPCs and the influence of osteogenic differentiation suggest a functional role for recruitment of MPCs during bone development and remodeling. Moreover, these observations may be useful for novel approaches towards guided tissue regeneration or tissue engineering of bone. J. Cell. Biochem. 93: 990–998, 2004.

Key words: PDGF; chemotaxis; mesenchymal progenitor cells; PDGF receptor; osteoblast

A continuous source of osteoblast recruitment for bone growth, remodeling, and fracture repair is ensured by mesenchymal progenitor cells (MPCs) which have been identified in bone marrow and other tissues [Caplan, 1991; Young et al., 1995]. Therefore, migration of bone forming cells is an important event during various physiological and pathological situations. The chemotactic response of osteoblasts to growth factors such as transforming growth factor beta 1 (TGF-β1), bone morphogenetic proteins (BMP), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor A (VEGF-A) has been shown [Lind, 1996; Mayr-Wohlfart et al., 2001]. More recently, the ability for chemotactic response to various growth factors including PDGF-BB has been reported for human MPCs [Fiedler et al., 2002].

PDGF, a polypeptide formed by two amino acid chains of two related genes is one of the most potent mitogens for skeletal cells. The products of the *PDGF A* and *PDGF B* genes can form homodimers or heterodimers, so PDGF could act in three isoforms: PDGF-AA, PDGF-BB, or PDGF-AB. The PDGF is a potent regulator of bone cells either alone or in combination with other factors. PDGF increases the number of preosteoblastic cells, induces chemotaxis, and matrix apposition [Cochran et al., 1993; Horner et al., 1996]. Nash et al. [1994] demonstrated that PDGF-BB also increases the density and volume of callus formed in a rat tibial osteotomy model.

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Osteoblastic cells express two distinct PDGF receptors, the alpha receptor that binds both PDGF chains and the beta receptor, which binds only the B chain of PDGF [Centrella et al., 1992]. Structurally and functionally both receptors are related and binding of PDGF results into a receptor dimerization and activation of intrinsic tyrosine kinase activity [Yarden et al., 1986; Claesson-Welsh et al., 1988; Heldin et al., 1989]. While human osteoblasts contain a similar number of alpha and beta receptors with similar dissociation constants [Centrella et al., 1992], nothing is known about MPCs. The activated receptors could stimulate various signal-transduction pathways, via the PKC, protein kinase A, or by changing the amount of intracellular calcium depending on the cell type [Claesson-Welsh, 1994].

Since human platelets contain all three PDGF isoforms which may be released from fracture hematoma with PDGF-AB representing about 70% [Hammacher et al., 1988; Hart et al., 1990] and osteonectin (SPARC) a non-collagenous protein of bone matrix inhibits PDGF B-chain binding to its receptors [Lane and Sage, 1994] the biological relevance of PDGF-AB and PDGF-AA for recruitment of MPCs is an important question.

Therefore, the aim of this study was to assess the chemotactic effects of, rhPDGF-AA, -BB, and -AB on primary human MPCs in comparison to in vitro differentiated MPCs, and primary human osteoblasts and to elucidate the involvement of PDGF receptors alpha and beta by preincubation with specific antibodies blocking their biological activity. Since a selective inhibition of PDGF-AA-induced chemotactic stimulation by TGF-β1 has been reported for other cell types [Soma et al., 2002] the possible interference of this growth factor with PDGF-induced chemotaxis was additionally tested.

MATERIALS AND METHODS

Cell Culture

MPCs were harvested from human bone marrow derived from routine surgical procedures (pelvic osteotomies) with informed consent from six female patients (age 19–51 years) and in accordance with the terms of the ethics committee of the University of Ulm.

MPCs were isolated as described earlier [Fiedler et al., 2002]. This isolation is specific for adherent MPCs and maintains the progenitor phenotype [Bruder et al., 1998; Pittenger et al., 1999]. The MPC phenotype was proved by FACS analysis with a subset of MPC positive surface markers (CD9, CD44, CD54, CD90, and CD166), as well as CD45 (negative), and by the potential to differentiate in osteoblasts, chondrocytes, or adipocytes [Deans and Moseley, 2000; Gronthos et al., 2001]. Usually over 95% of the cells were positive for MPC typical combinations and 100% negative for hemopoietic surface markers [Fickert et al., 2003]. Only MPC preparations lacking mature osteoblast specific gene expression were used for chemotaxis experiments.

After isolation, the cells were cultured in a basal medium consisting of DMEM with 10% FCS, 1% glutamine, and 1% penicillin/streptomycin (all Biochrom Seromed, Berlin, Germany) at 37°C, 5% CO_2 in 95% humidity. In order to differentiate the MPCs into osteoblasts, the basal medium was supplemented with 10^{-7} M dexamethasone, 50 μg/ml ascorbic acid, and 2,16 μg/ml β-glycerophosphate (Sigma, Germany) for 14 days. Medium was changed twice a week. The osteogenic differentiation was proved by RT-PCR of osteoblast specific genes.

Osteoblasts were harvested from cancellous human bone fragments derived from routine hip and knee replacements within surgical procedures. Donor material obtained from six different patients (age 50–70 years) was plated after collagenase digestion for 2 h and cultured in DMEM with 10% FCS as described previously [Mayr-Wohlfart et al., 2001]. Experiments were performed only in the first three cell passages as well as in the stage of cell maturation.

Growth Factors and Receptor Antibodies

Three different growth factors were used: recombinant human platelet-derived growth factor AA (rhPDGF-AA), recombinant human platelet-derived growth factor BB (rhPDGF-BB), and recombinant human platelet-derived growth factor AB (rhPDGF-AB), all were purchased from DPC Biermann (Bad Nauheim, Germany). As we could describe previously for various growth factors including rhPDGF-BB tested from 0.001 to 10 ng/ml, there was no significant difference in the chemotactic response between 1 and 10 ng/ml [Fiedler et al., 2002]. Thus, for all growth factors concentrations of 1 ng/ml were tested in the chemotaxis assay. Single control experiments with 0.1 and

10 ng/ml confirmed the results obtained with 1 ng/ml. Anti-human PDGF receptor alpha (PDGFRα) and receptor beta (PDGFRβ) antibody were purchased from R&D Systems (Wiesbaden, Germany) and were used in finals concentrations of 10 μg/ml in order to reach a minimum of 75% neutralization of PDGF-mediated activity according to the supplier's information.

Recombinant human transforming growth factor beta 1 (rhTGF-β1) was purchased from TEBU GmbH (Frankfurt, Germany), recombinant human bone morphogenetic protein-2 (rhBMP-2) was provided by the Theodor-Boveri-Institut of the University of Würzburg, Germany [Kübler et al., 1998], and recombinant *Xenopus* bone morphogenetic protein-4 (rxBMP-4) was produced as described earlier [Mayr-Wohlfart et al., 2001].

Chemotaxis Assay

Chemotactic responses were measured by a modified Boyden chamber assay [Falk et al., 1980] using a 48-well micro chemotaxis chamber (NeuroProbe, Inc., Baltimore, MD) with polycarbonate filters with 5-μm pores (Whatman Biometra, Göttingen, Germany) as described previously [Fiedler et al., 2002; Mayr-Wohlfart et al., 2002]. The cells were trypsinized, counted, and partly used for RT-PCR as well as the chemotaxis assay. In PDGF receptor inhibition experiments, cells were preincubated for 30 min with 1 μg/ml PDGFRα or -β antibodies in DMEM, while the controls were incubated in DMEM only. Afterwards all samples were washed once by centrifugation and suspending in DMEM. Growth factor dilutions in DMEM were filled into the lower wells and covered by the chemotaxis filter. Cells (1×10^4) in 50 μl DMEM were filled in the upper wells. After a 4-h incubation, the filter was carefully removed, non-migrated cells on the upper side eliminated by rinsing with cold PBS and scraping over a rubber wiper. The remaining migrated cells on the lower side of the filter were fixed with 4% formaldehyde and stained with toluidine blue. In control experiments, the migrated MPCs on the filter were stained for CD9, CD54, CD 90, and CD166 (BD Biosciences Pharmingen, Heidelberg, Germany) in order to prove whether MPCs or other subpopulations respond to the tested factors. All growth factors were tested in triplicate for each concentration. Control wells with DMEM only in the bottom well were applied for each experiment. Conditioned medium of human fetal osteoblast-cultures was used as positive control in analogy to the use of conditioned medium from fetal cultures for fibroblast chemotaxis [Brenner et al., 2001]. While in the control wells containing only DMEM, the number of cells migrated was about 20–40 cells, the amount of migrated cells in the wells with conditioned medium was always greater than 1,000 cells.

The number of migrated cells in control and stimulated wells was counted for 12 random fields per well at 100× magnification. Results were expressed as a chemotactic index (CI). This index was determined as the average number of migrated cells in stimulated wells divided by average number of migrated cells in control wells. A Zigmond–Hirsch checkerboard analysis was performed in triplicate to distinguish between concentration-dependent cell migration (chemotaxis) and random migration (chemokinesis). The analysis was performed after eliminating the concentration gradient by adding the chemoattractants to the upper chamber with the cell suspension [Zigmond and Hirsch, 1973].

RT-PCR Analysis

The differentiation status of the MPCs, the differentiated progenitor cells, and primary osteoblasts was affirmed by RT-PCR for the expression of osteoblastic markers: alkaline phosphatase (AP), osteocalcin (OCN), and osteonectin (ON; SPARC). Collagen type I alpha 1 (COL1), and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used to verify the PCR results as internal standards. Therefore, total RNA was isolated from 10^5 cells with the RNeasy® System and reverse transcription was done with Omniscript™ RT Kit (all QIAGEN®, Hilden, Germany) following the manufacturer's instructions. PCR-primers for GAPDH, COL1, AP, OCN, and ON were described elsewhere [Fiedler et al., 2002]. Amplicon size was set from 180 to 250 bp for GAPDH, COL1, AP, OC, and to 400 bp for ON. Temperature optimum was set to 60°C. In all cases, the best primer pair was used. Primer and conditions for PDGF-Rα, and PDGF-Rβ were described elsewhere [Lokker et al., 2002].

PCR-reactions were performed with a Robocycler® (Stratagene, Amsterdam, The Netherlands) using HotStarTaq™ Master Mix Kit (QIAGEN, Hilden, Germany). PCR was

performed under linear conditions using the cycle profile: initial incubation 15 min. at 95°C, followed by 30 cycles annealing 45 s at 60°C, extension 45 s at 72°C, denaturation 60 s at 94°C, and terminating with 15 min at 72°C. PCR products were separated on a 1.5% agarose gel and stained with ethidium bromide, visualized and digitalized with an ImageMaster VDS system (Amersham Biosciences, Freiburg, Germany).

Statistics

Experiments were performed from six donors in independent experiments for the PDGF isoforms and the receptor inactivation testing. Results are presented as mean ± SD. The significance of differences between control and stimulation groups was determined using ANOVA and one-paired Wilcoxon tests. Values less than $P = 0.05$ were considered significant.

RESULTS

Chemotaxis of MPCs

In all experiments, the markers of the differentiated bone cell phenotype (high expression of AP and osteocalcin) was absent in MPCs and present in osteogenic differentiated cells (dOB) and primary osteoblasts (OB) as presented in Figure 1A. All tested cell populations expressed both PDGF receptor chains as shown in Figure 1D.

The chemotactic response of MPCs depended on the PDGF isoform applied. While rhPDGF-AA and rhPDGF-AB induced a chemotactic index (CI) of 2.9, the average index of rhPDGF-BB was 6.6 (±1.84) (Fig. 2).

Blocking PDGFRα with specific antibodies, the CI decreased significantly in most cases, but not to a complete inhibition of migration. In OB for PDGF-AB and -BB, the reduction did not reach significance. When using rhPDGF-AA and rhPDGF-AB, a CI of 1.6 (±0.3) or 1.6 (±0.4) respectively was detectable in the presence of the blocking antibody. In the case of rhPDGF-BB, the CI dropped down to 2.5 (±0.4) as shown in Figure 2.

The preincubation with PDGFRβ specific antibodies leads to a complete inhibition of migration in all PDGF experiments. The CI was reduced to 1.2 (±0.2) for rhPDGF-AA, 1.1 (±0.37) for rhPDGF-BB, and 1.0 (±0.25) for rhPDGF-AB.

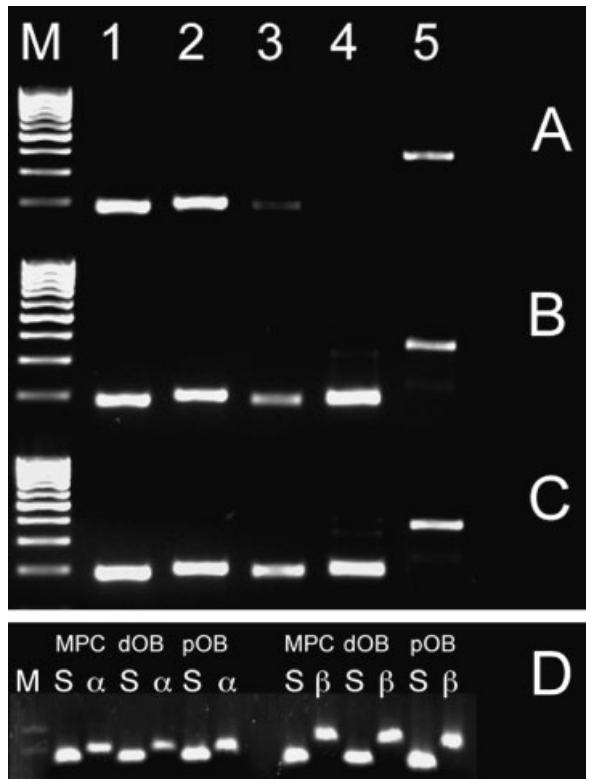

Fig. 1. Differences in the expression of osteoblastic marker genes by mesenchymal progenitor cells (**A**), osteogenic differentiated progenitor cells (**B**), and primary osteoblasts (**C**) detected by RT-PCR analysis. M 100-bp size marker, 1 glyceraldehyde-3-phosphate dehydrogenase, 2 collagen type I, 3 alkaline phosphatase, 4 osteocalcin, 5 osteonectin. **D**: The expression of PDGFRα and -β was detectable in all cells in equal amounts. M 100-bp size marker, S housekeeping gene, α PDGF receptor alpha, β PDGF receptor beta.

The PDGF receptor antibodies used did not influence the chemotaxis of MPC, dOB, and OB in response to recombinant human BMP2 and recombinant *Xenopus* BMP4. This showed that the decrease of migration induced by these receptor antibodies was specific to PDGF (data not shown). Furthermore, blocking of the PDGFRα or -β did not markedly inhibit cell migration by itself (Fig. 4).

The chemotactic responses of MPCs and osteogenic differentiated cells were tested in a checkerboard analysis to determine whether cell migration was due to chemotaxis or chemokinesis. Checkerboard analysis in the tested concentration of 1 ng/ml showed that the stimulation of migration by all PDGF isoforms was present at a positive concentration gradient. This indicates that the cell migration seen in response to these growth factors was due to true chemotaxis, not chemokinesis (Table I).

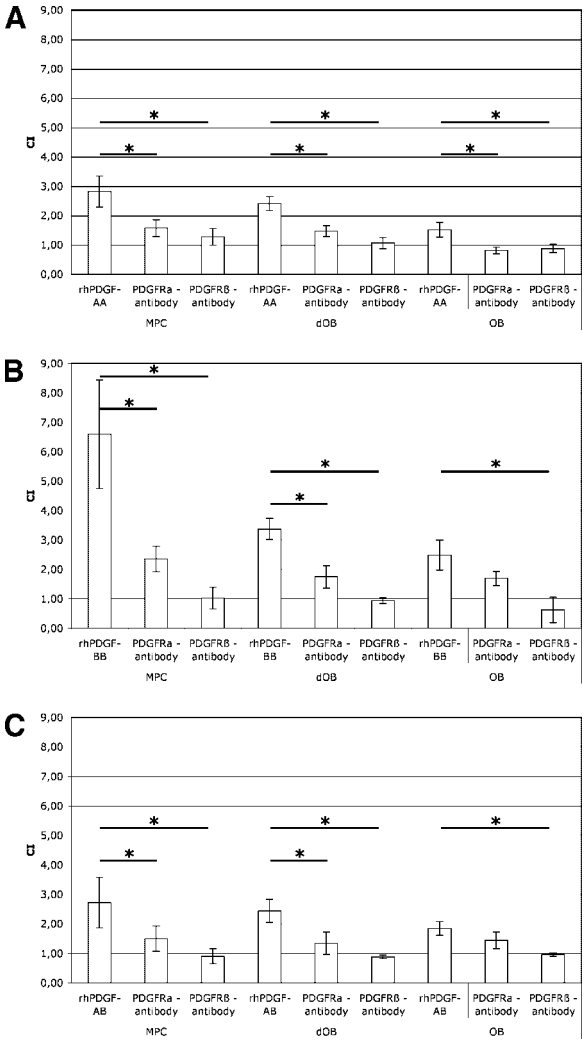

Fig. 2. Chemotaxis of human mesenchymal progenitor cells, osteogenic differentiated progenitor cells, and primary human osteoblasts induced by isoforms of PDGF in the absence or presence of receptor alpha or beta specific antibodies. All experiments were performed in at least six independent experiments in triplicate each. Shown is the CI in the presence of the PDGF isoforms (rhPDGF-AA, -BB, -AB) and PDGF receptor antibodies (PDGFRα antibody, PDGFRβ antibody). Mean ± SD, *P* values from ANOVA and one-paired Wilcoxon's tests less than $P = 0.05$ were considered significant and marked by an asterix (*).

Because of using primary human cells, we had to prove that only MPC chemotaxis was measured by the migration assay. Migrated cells were therefore stained after the chemotaxis experiment on the membrane with typical MPC surface marker antibodies [Gronthos et al., 2001; Fickert et al., 2003]. As shown in Figure 3, all cells were positively stained by CD9, CD54, CD90, and CD166 either stimulated by rhPDGF-AA (Fig. 3A,D,G,J), rhPDGF-BB (Fig. 3B,E,H,K), or rhPDGF-AB (Fig. 3C,F,I,L). These data suggest that no other subpopulation like endothelial progenitors falsifies our data.

The chemotactic response to the conditioned medium of human fetal osteoblast cultures was used as a positive control, but the number of migrated cells could not be counted, because of the high response (>1,000 migrated cells per well). In general, without addition of growth factors, 20–40 cells were counted because of chemokinesis. The addition of growth factors that induce chemotaxis raised the number of cells up to 300 to 400 for 1 ng rhPDGF-BB.

Chemotaxis of Differentiated MPCs and Primary Osteoblasts

Osteogenic differentiated MPCs (dOB) responded in a similar way to primary human osteoblasts (OB) but markedly less than MPCs to rhPDGF-BB as shown in Figure 2. In contrast, their response to rhPDGF-AA and -AB was similar to the CI from MPCs. The same behavior was detectable when the specific PDGF receptor antibodies were added. Thus, in vitro differentiation of MPCs changed the responsiveness to the growth factors towards that of primary osteoblasts in case of rhPDGF-BB but remained at a similar level in the presence of rhPDGF-AA and rhPDGF-AB.

Inhibition of PDGF-AA Mediated Cell Migration by TGF-β1

Parallel application of 10 ng/ml of rhTGF-β1 in the upper and lower well of the chemotaxis chamber led to a complete loss of migration activity induced by rhPDGF-AA as shown in Figure 4 ($CI = 0.9 \pm 0.2$). Surprisingly, chemotaxis induced by rhPDGF-BB and PDGF-AB was slightly but not significantly reduced by about 20%. This is in contrast to Soma and coworkers [Soma et al., 2002] because no long time incubation was necessary prior the chemotaxis experiment to inhibit chemotaxis and that in addition also PDGF-BB and -AB seemed to be affected.

DISCUSSION

The coordinate recruitment of MPCs and more differentiated cells of the osteogenic lineage have to be well regulated and a better understanding of these processes may provide important information for novel therapeutic strategies.

TABLE I. Checkerboard Analysis

Growth factor	Growth factor in upper well	Chemotactic index at 1 ng/ml in lower well			
		MPC		dOB	
		Mean	±SD	Mean	±SD
rhPDGF-AA	−	4.09	0.29	2.82	0.19
	+	0.59	0.17	0.71	0.13
rhPDGF-BB	−	9.45	1.01	4.17	0.35
	+	0.88	0.19	0.70	0.17
rhPDGF-AB	−	5.41	0.61	3.26	0.24
	+	0.68	0.22	0.80	0.15

The table shows data from the checkerboard analysis. The concentration gradient between the wells (−) was eliminated by adding growth factors to the cell suspension in the top well (+). All growth factors were tested in the concentration of 1 ng/ml to induce stimulation of migration. In the table, the migration is expressed as chemotactic index. Data are means of at least triplicate wells with ±SD.

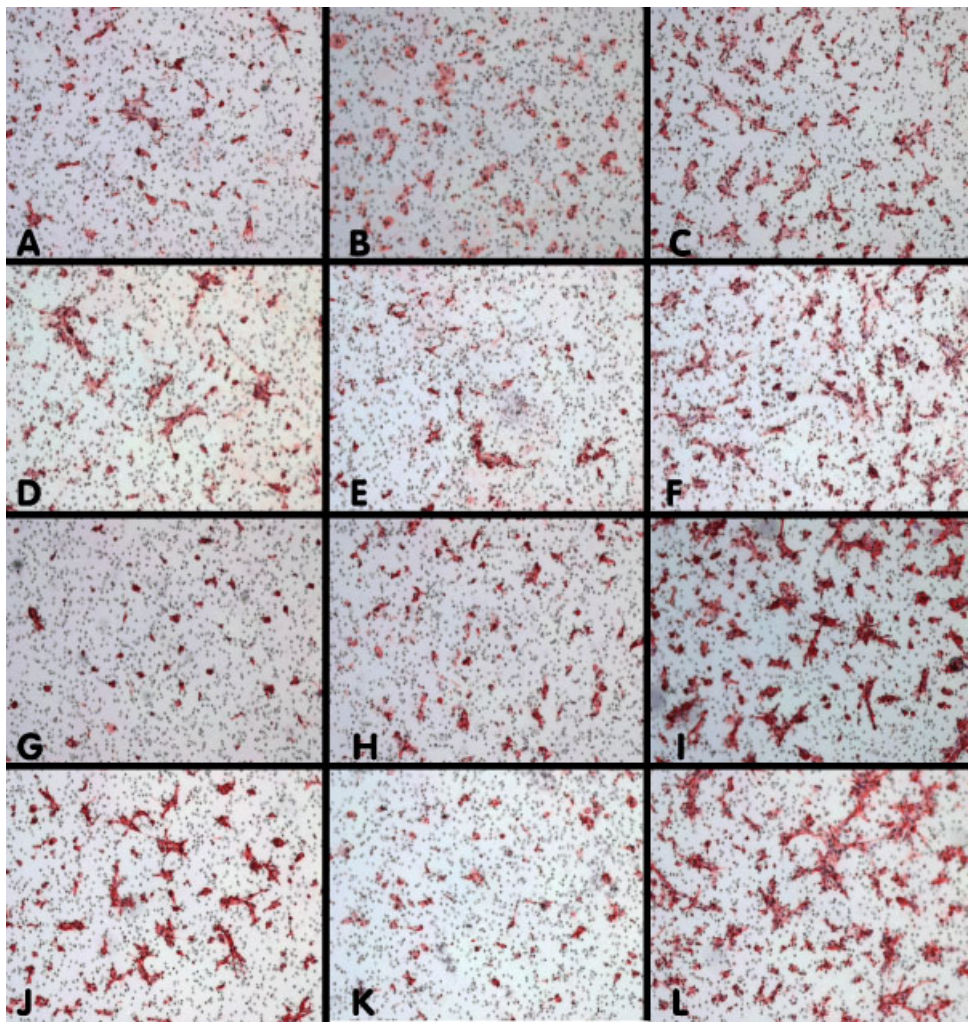

Fig. 3. Staining of human mesenchymal progenitor cells after PDGF induced migration on chemotaxis membranes. To exclude that contaminating subpopulations of endothelial cells for example falsify the CI from MPC, an immunohistological staining with a subset of known surface marker proteins was done [Deans and Moseley, 2000; Fickert et al., 2003]. Staining for CD9 (**A**) rhPDGF-AA, (**B**) rhPDGF-BB, (**C**) rhPDGF-AB, for CD54 (**D**) rhPDGF-AA, (**E**) rhPDGF-BB, (**F**) rhPDGF-AB, for CD90 (**G**) rhPDGF-AA, (**H**) rhPDGF-BB, (**I**) rhPDGF-AB, and for CD166 (**J**) rhPDGF-AA, (**K**) rhPDGF-BB, (**L**) rhPDGF-AB. Magnification 200×. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

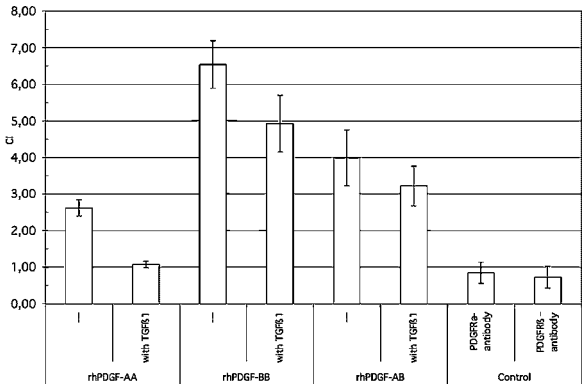

Fig. 4. Addition of 10 ng/ml rhTGF-β1 in the upper and lower well of the chemotactic chamber inhibited the cell response in the case of rhPDGF-AA completely. While the chemotactic migration in case of rhPDGF-AB was reduced about 20%, rhPDGF-BB induced chemotaxis was reduced to 75% of migration. Mean ± SD of two independent experiments in triplicate determination.

Our study focused on the behavior of primary human MPCs, which can differentiate into chondrocytes, adipocytes, osteoblasts, and other cells of mesenchymal origin. The MPC phenotype and the osteogenic differentiation were proved by RT-PCR. The expression of ON coincides with previous reports that MPCs do express ON [Aubin, 1998a,b]. Maybe the expression of ON was increased as part of a shock response upon separation [Sauk et al., 1991; Kudo et al., 1994]. The expression of osteogenic markers like high levels of alkaline phosphatase (AP) and osteocalcin (OCN) in differentiated osteoblasts and primary osteoblasts was sufficient to distinguish between progenitor cells and differentiated cells. Moreover, in experiments with MPCs immunohistochemical staining of migrated cells for typical cell surface markers [Fickert et al., 2003] revealed that indeed progenitor cells and not other subpopulations, like endothelial cells, had migrated. PDGF was discovered in serum as the major mitogenic activity responsible for growth of cultured mesenchymal cells [Sporn and Roberts, 1990]. The mitogenic effect of PDGF-BB has also been found in human osteoblasts and various osteogenic cell-lines [Canalis et al., 1992]. In addition, PDGF-BB was found as well to be a powerful chemotactic factor for mesenchymal cells, including osteoblasts from human and rat tissue [Hughes et al., 1992; Lind et al., 1995; Godwin and Soltoff, 1997; Fiedler et al., 2002]. The PDGF-BB-induced chemotactic index of osteoblasts was described to be in a range of two to three. While our data suggest that the chemotactic effect of PDGF-AB is in the range of PDGF-BB-induced chemotaxis in case of OB, it seems possible that PDGF-AB as the major isoform in platelets is the most relevant chemoattractant for OB in fracture hematoma, while PDGF-BB may be more specific for MPC and their recruitment from long distances, being chemoattractive for the progenitor cells even in lowest concentrations. Since ON expression was similar in MPC and osteogenic differentiated cells, an inhibition of PDGF-B chain binding by this non-collagenous protein described by Lane and co-workers could not account for the different response [Lane and Sage, 1994]. The chemotactic activity of PDGF-AB and -AA for MPCs has not been reported so far and indicates a possible role of all three PDGF variants for cell recruitment in bone tissue. Furthermore, checkerboard analysis revealed that the effect of all isoforms can be attributed to directed cell migration and not to enhanced chemokinesis.

Besides migration of human MPC, MPCs after osteogenic differentiation, and primary human osteoblasts, in response to recombinant human PDGF-AA, recombinant human PDGF-BB, and recombinant human PDGF-AB, we investigated the influence of PDGF receptor specific antibodies on migration behavior. In all cell populations, the expression of receptor alpha and receptor beta chains was detectable by RT-PCR. Thus we could show for the first time that the expression of PDGF-receptor chains in human MPC is similar to the situation in osteoblasts published previously [Centrella et al., 1992]. Interestingly, the addition of PDGF receptor specific antibodies (alpha or beta chain) significantly inhibited cell migration for all PDGF isoforms in MPCs and OB. While the PDGFRβ specific antibody inhibited stimulation of migration in all cases completely, the preincubation with PDGFRα specific antibody only the rhPDGF-AA induced migration was suppressed. In terms of rhPDGF-BB and -AB, a reduction of about 20% was observed. In part these are astonishing results. First it is unclear why PDGFRβ antibodies inhibit PDGF-AA induced migration while PDGF-AA should only interact with the PDGFRα chains [Heldin and Westermark, 1999]. Due to the supplier' information, the monoclonal PDGFRβ antibody should show no crossreaction with the receptor

alpha chains. So further experiments are necessary to explain this behavior. The complete suppression of PDGF-AB effect could be explained if this heterodimer only stimulates migration via the PDGFRα/β dimers but until today there is no information available, if the alpha/beta heterodimer could induce cell migration. The negative effect of the PDGFRα antibodies on all PDGF isoforms induced chemotaxis could be due to the fact, that it is a polyclonal antibody with slight crossreactivity to PDGFRβ. More likely PDGF-BB and PDGF-AB induce migration either via PDGFRβ/β or PDGFRα/β and PDGFRα/α pathways. This would explain the observation that migration was not completely inhibited. Nevertheless, these new results have to undergo further investigation.

A major effect of the receptor antibodies on basal migration and the response to other chemotactic factors like rhBMP-2 and rxBMP-4 [Fiedler et al., 2002] could be excluded. However, a minor negative effect of an inhibition by the PDGF-β receptor antibody itself cannot be excluded and may contribute to the results discussed above.

In accordance to previous studies on other cell types like skin fibroblasts [Soma et al., 2002], we found a selective loss of chemotactic activity of rhPDGF-AA under the influence of rhTGF-β1 in human MPCs. Since TGF-β1 is known to influence the differentiation of MPCs [Tuli et al., 2003; Wang et al., 2003], we tested the effect of a short-term application. In the other cell types, the effect was attributed mainly to a down-regulation of PDGF receptor alpha, which was rather unlikely in our experimental setting because of the short time span of only 4 h exposure. Therefore, additional mechanisms induced by rhTGF-β1 seem to interfere with the chemotactic stimulation by rhPDGF-AA. Although this point clearly deserves further studies, our results unravel complex interactions that may be highly important in the in vivo situation.

In conclusion, we could show that MPCs are targets for all PDGF isoforms—especially PDGF-BB—and that the developmental stage as well as the presence of additional factors has important influence on the migratory response. Thus PDGF isoforms may contribute to the recruitment of MPCs in the context of bone formation, remodeling, and fracture healing and could possibly be useful for novel approaches towards guided tissue regeneration or tissue engineering.

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REFERENCES

Aubin JE. 1998a. Advances in the osteoblast lineage. Biochem Cell Biol 76:899–910.

Aubin JE. 1998b. Bone stem cells. J Cell Biochem Suppl 31:73–82.

Brenner RE, Felger D, Winter C, Christiansen A, Hofmann D, Bartmann P. 2001. Effects of dexamethasone on proliferation, chemotaxis, collagen I, and fibronectin-metabolism of human fetal lung fibroblasts. Pediatr Pulmonol 32:1–7.

Bruder SP, Ricalton NS, Boynton RE, Connolly TJ, Jaiswal N, Zaia J, Barry FP. 1998. Mesenchymal stem cell surface antigen SB-10 corresponds to activated leukocyte cell adhesion molecule and is involved in osteogenic differentiation. J Bone Miner Res 13:655–663.

Canalis E, Varghese S, McCarthy TL, Centrella M. 1992. Role of platelet derived growth factor in bone cell function. Growth Regul 2 :151–155.

Caplan AI. 1991. Mesenchymal stem cells. J Orthop Res 9:641–650.

Centrella M, McCarthy TL, Kusmik WF, Canalis E. 1992. Isoform-specific regulation of platelet-derived growth factor activity and binding in osteoblast-enriched cultures from fetal rat bone. J Clin Invest 89:1076–1084.

Claesson-Welsh L. 1994. Platelet-derived growth factor receptor signals. J Biol Chem 269:32023–32026.

Claesson-Welsh L, Eriksson A, Moren A, Severinsson L, Ek B, Ostman A, Betsholtz C, Heldin CH. 1988. cDNA cloning and expression of a human platelet-derived growth factor (PDGF) receptor specific for B-chain-containing PDGF molecules. Mol Cell Biol 8:3476–3486.

Cochran DL, Rouse CA, Lynch SE, Graves DT. 1993. Effects of platelet-derived growth factor isoforms on calcium release from neonatal mouse calvariae. Bone 14:53–58.

Deans RJ, Moseley AB. 2000. Mesenchymal stem cells: Biology and potential clinical uses. Exp Hematol 28:875–884.

Falk W, Goodwin RH, Jr., Leonard EJ. 1980. A 48-well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration. J Immunol Methods 33:239–247.

Fickert S, Fiedler J, Brenner RE. 2003. Identification, quantification and isolation of mesenchymal progenitor cells from osteoarthritic synovium by fluorescence automated cell sorting. Osteoarthritis Cartilage 11: 790–800.

Fiedler J, Roderer G, Gunther KP, Brenner RE. 2002. BMP-2, BMP-4, and PDGF-bb stimulate chemotactic migration of primary human mesenchymal progenitor cells. J Cell Biochem 87:305–312.

Godwin SL, Soltoff SP. 1997. Extracellular calcium and platelet-derived growth factor promote receptor-mediated chemotaxis in osteoblasts through different signaling pathways. J Biol Chem 272:11307–11312.

Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. 2001. Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 189:54–63.

Hammacher A, Hellman U, Johnsson A, Ostman A, Gunnarsson K, Westermark B, Wasteson A, Heldin CH. 1988. A major part of platelet-derived growth factor purified from human platelets is a heterodimer of one A and one B chain. J Biol Chem 263:16493–16498.

Hart CE, Bailey M, Curtis DA, Osborn S, Raines E, Ross R, Forstrom JW. 1990. Purification of PDGF-AB and PDGF-BB from human platelet extracts and identification of all three PDGF dimers in human platelets. Biochemistry 29: 166–172.

Heldin CH, Westermark B. 1999. Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 79:1283–1316.

Heldin CH, Ernlund A, Rorsman C, Ronnstrand L. 1989. Dimerization of B-type platelet-derived growth factor receptors occurs after ligand binding and is closely associated with receptor kinase activation. J Biol Chem 264:8905–8912.

Horner A, Bord S, Kemp P, Grainger D, Compston JE. 1996. Distribution of platelet-derived growth factor (PDGF) A chain mRNA, protein, and PDGF-alpha receptor in rapidly forming human bone. Bone 19:353–362.

Hughes FJ, Aubin JE, Heersche JN. 1992. Differential chemotactic responses of different populations of fetal rat calvaria cells to platelet-derived growth factor and transforming growth factor beta. Bone Miner 19:63–74.

Kudo H, Hirayoshi K, Kitagawa Y, Imamura S, Nagata K. 1994. Two collagen-binding proteins, osteonectin and HSP47, are coordinately induced in transformed keratinocytes by heat and other stresses. Exp Cell Res 212: 219–224.

Kübler NR, Reuther JF, Faller G, Kirchner T, Ruppert R, Sebald W. 1998. Inductive properties of recombinant human BMP-2 produced in a bacterial expression system. Int J Oral Maxillofac Surg 27:305–309.

Lane TF, Sage EH. 1994. The biology of SPARC, a protein that modulates cell-matrix interactions. FASEB J 8:163–173.

Lind M. 1996. Growth factors: Possible new clinical tools. A review. Acta Orthop Scand 67:407–417.

Lind M, Deleuran B, Thestrup-Pedersen K, Soballe K, Eriksen EF, Bunger C. 1995. Chemotaxis of human osteoblasts. Effects of osteotropic growth factors. Apmis 103:140–146.

Lokker NA, Sullivan CM, Hollenbach SJ, Israel MA, Giese NA. 2002. Platelet-derived growth factor (PDGF) autocrine signaling regulates survival and mitogenic pathways in glioblastoma cells: Evidence that the novel PDGF-C and PDGF-D ligands may play a role in the development of brain tumors. Cancer Res 62:3729–3735.

Mayr-Wohlfart U, Kessler S, Knochel W, Puhl W, Gunther KP. 2001. BMP-4 of *Xenopus laevis* stimulates differentiation of human primary osteoblast-like cells. J Bone Joint Surg Br 83:144–147.

Mayr-Wohlfart U, Waltenberger J, Hausser H, Kessler S, Gunther KP, Dehio C, Puhl W, Brenner RE. 2002. Vascular endothelial growth factor stimulates chemotactic migration of primary human osteoblasts. Bone 30: 472–477.

Nash TJ, Howlett CR, Martin C, Steele J, Johnson KA, Hicklin DJ. 1994. Effect of platelet-derived growth factor on tibial osteotomies in rabbits. Bone 15:203–208.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. 1999. Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147.

Sauk JJ, Norris K, Kerr JM, Somerman MJ, Young MF. 1991. Diverse forms of stress result in changes in cellular levels of osteonectin/SPARC without altering mRNA levels in osteoligament cells. Calcif Tissue Int 49: 58–62.

Soma Y, Mizoguchi M, Yamane K, Yazawa N, Kubo M, Ihn H, Kikuchi K, Tamaki K. 2002. Specific inhibition of human skin fibroblast chemotaxis to platelet-derived growth factor A-chain homodimer by transforming growth factor-beta1. Arch Dermatol Res 293:609–613.

Sporn MB, Roberts AB. 1990. Handbook of experimental pharmacology. Growth factors and their receptors. New York: Springer Verlag.

Tuli R, Tuli S, Nandi S, Huang X, Manner PA, Hozack WJ, Danielson KG, Hall DJ, Tuan RS. 2003. TGF-beta 1-mediated chondrogenesis of human mesenchymal progenitor cells involves N-cadherin and MAP kinase and Wnt signaling crosstalk. J Biol Chem 278:41227–41236.

Wang WG, Lou SQ, Ju XD, Xia K, Xia JH. 2003. In vitro chondrogenesis of human bone marrow-derived mesenchymal progenitor cells in monolayer culture: Activation by transfection with TGF-beta2. Tissue Cell 35:69–77.

Yarden Y, Escobedo JA, Kuang WJ, Yang-Feng TL, Daniel TO, Tremble PM, Chen EY, Ando ME, Harkins RN, Francke U, Fried VA, Ullrich A, Williams LT. 1986. Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. Nature 323:226–232.

Young HE, Mancini ML, Wright RP, Smith JC, Black AC, Jr., Reagan CR, Lucas PA. 1995. Mesenchymal stem cells reside within the connective tissues of many organs. Dev Dyn 202:137–144.

Zigmond SH, Hirsch JG. 1973. Leukocyte locomotion and chemotaxis. New methods for evaluation, and demonstration of a cell-derived chemotactic factor. J Exp Med 137:387–410.