Human bone bank allografts stimulate bone resorption and inhibit proliferation in cultures of human osteoblast-like cells

Steen Mejdahl[1], Carl Å Hansen[1], Henrik Skjødt[2] and Inge Reimann[1]

Incorporation of a frozen human bone allograft requires osteoclast activity and ingrowth of recipient osteoblast precursors. We examined the effects of allografts on human osteoblasts. Allografts stimulated a release of factors from normal human osteoblast-like cells, capable of inducing osteoclastic bone resorption in vivo. Further allografts inhibited osteoblast proliferation in cultures. The response was detectable within 4 days of culture and was still present after 3 weeks. Devitalized bone autografts had a similar effect. This suggests that bone bank grafts may induce a resorptive reaction at the recipient site by stimulating release of factors from osteoblasts capable of inducing osteoclastic resorption. The storage temperature was crucial for preservation of the response, since the activity was lower in allografts stored for 6 months at –20°C than in those stored at –80°C.

[1]Department of Orthopaedic Surgery, Herlev Hospital, Herlev Ringvej, DK-2730 Herlev, Denmark. Tel +45 4453-5300. Fax -5332; [2]Laboratory of Medical Immunology 7544, Rigshospitalet, Copenhagen, Denmark
Submitted 97-01-17. Accepted 97-09-11

The effect of bone bank allografts on recipient bone cells is of interest because incorporation of the devitalized graft depends on tissue remodeling in the graft area. In particular, graft bone matrix may contain constituents which regulate the recruitment, numbers and activity of osteoblasts and osteoclasts, including growth factors (Sampath and Reddi 1984, Hauschka et al. 1986, Urist 1989, Guo et al. 1991). The matrix-producing osteoblasts also appear to play a central role in bone resorption. Thus, osteoblasts may mediate the osteoclast-activating effect of many hormonal factors, such as PTH, $1,25(OH)_2D_3$, thyroid hormone, IL-1, TNFα and TNFß (McSheehy and Chambers 1986, Thomson et al. 1986, 1987, McSheehy and Chambers 1987, Britto et al. 1994).

Knowledge of direct effects of frozen allografts on recipient bone cells is limited and the level of storage temperature is based on empirical knowledge rather than experiments (Tomford et al. 1986, Association of American Tissue Banks 1987, Eastlund et al. 1992). We examined the effect of bone bank allografts on recipient osteoblasts, in terms of bone resorbing activity and cell proliferation, to see whether the storage temperature had any effect on these responses.

Material and methods

Bone bank allografts. Femoral heads were obtained from 5 women and 1 man aged 72 (64 –78) years, operated on because of coxarthrosis. Each specimen was divided into vertical sections 15 mm thick. The sections were then placed in freezers within 30 minutes and stored for either 2 weeks or 6 months at –80 °C or –20 °C. Prior to the experiments, the sections were thawed and washed in phosphate-buffered saline (PBS) with a syringe to remove any remains of marrow and soft tissue. Trabecular bone samples, 3 × 3 × 3 mm, were excised and placed in tissue culture multiwell plates, with human osteoblast-like cell monolayers, for the stated length of culture. In some experiments, the grafts were separated from the bone cell monolayers in a two-chamber system by a cell-impermeable membrane (Transwell, Costar, supplied by Meda, Copenhagen, Denmark).

Human osteoblast-like cells. Discarded bone was obtained from patients with arthrosis undergoing hip replacement. Trabecular bone specimens were isolated and minced, according to Skjødt et al. (1985). The bone specimens were washed in PBS with a syringe to remove blood and bone marrow, seeded in flasks and cultured in Eagle's Modified Essential Medium (EMEM), supplemented with 25 mM Hepes buffer, 100 U/mL penicillin and 100 µg/mL streptomycin, 2

Table 1. Effect of bone bank allografts on the release of factors by human osteoblast-like cells inducing osteoclastic resorption expressed as ^{45}Ca release treated/control ratio, mean (SEM) for 4 calvaria from 1 of 2 experiments [a]

Bone bank allograft tp (°C)	Duration of coculture (days)					
	2	3	4	6	14	21
1 (–80)	1.06 (0.03)	1.46 (0.09) [b]	1.58 (0.11) [b]	1.62 (0.13) [b]	1.46 (0.15) [b]	1.35 (0.06) [b]
1 (–20)	1.12 (0.09)	1.35 (0.07) [b]	1.41 (0.10) [b]	1.40 (0.09) [b]	1.36 (0.27)	1.33 (0.09) [b]
2 (–80)	1.20 (0.12)	1.42 (0.10) [b]	1.63 (0.14) [b]	1.68 (0.09) [b]	nt	1.29 (0.05) [b]
2 (–20)	1.09 (0.06)	1.30 (0.06) [b]	1.54 (0.17) [b]	1.50 (0.16) [b]	nt	1.36 (0.13) [b]
3 (–80)	0.97 (0.11)	1.28 (0.06) [b]	1.62 (0.24) [b]	1.60 (0.21) [b]	1.40 (0.13) [b]	1.43 (0.23) [b]
3 (–20)	1.04 (0.07)	1.36 (0.04) [b]	1.70 (0.19) [b]	1.65 (0.14) [b]	1.52 (0.19) [b]	1.36 (0.15) [b]
4 (–80)	1.25 (0.14)	1.69 (0.19) [b]	1.87 (0.18) [b]	1.81 (0.28) [b]	1.42 (0.11) [b]	1.17 (0.09)
4 (–20)	1.11 (0.07)	1.55 (0.14) [b]	1.72 (0.25) [b]	1.74 (0.13) [b]	1.59 (0.20) [b]	1.29 (0.05) [b]
5 (–80)	1.37 (0.16) [b]	1.40 (0.08) [b]	1.69 (0.21) [b]	1.70 (0.54)	nt	1.31 (0.10) [b]
5 (–20)	1.25 (0.08) [b]	1.52 (0.17) [b]	1.60 (0.09) [b]	1.52 (0.13) [b]	nt	nt
6 (–80)	1.05 (0.03)	nt	1.79 (0.26) [b]	1.62 (0.14) [b]	1.50 (0.09) [b]	1.25 (0.07) [b]
6 (–20)	1.13 (0.08)	nt	1.86 (0.15) [b]	1.48 (0.22) [b]	nt	nt

[a] Kinetics of the release of factors by human osteoblast-like cells inducing bone resorption in response to grafts stored for 2 weeks at –80 °C and –20 °C, respectively. Cells were cocultured with grafts at 37 °C in 96-multiwell plates and bone resorptive activity assessed in supernatants pooled from multiplicates using the mouse calvaria bioassay (see Material and methods). Calvaria were incubated with 1:2 dilutions of test supernatants (treated) or control supernatants from human osteoblast-like cell-cultures without grafts (control). Grafts cultured for 2, 3, 4, 6, 14 and 21 days without human osteoblast-like cells released no factors inducing bone resorption (data not shown). The grouped supernatants (graft donors 1–6) were tested in separate calvaria assays. nt not tested.

[b] significantly different from control ($p < 0.05$; Student's t-test).

mM L-glutamine, 50 µg/mL ascorbate and 10% fetal calf serum (FCS; heat-inactivated). The cultures were kept at 37 °C in a humid atmosphere of 95% air, 5% CO_2, with change of medium once weekly. The bone chips were removed as soon as a confluent monolayer was reached (usually within 3–6 weeks) and the cells were detached by incubation with 0.5 g/L trypsin, 0.2 g/L EDTA at 37 °C. The cells were subsequently replated in multiwell plates and cultured in complete EMEM. More than 85% of $1,25(OH)_2D_3$-stimulated human osteoblast-like cells (10^{-8}M; 48 h at 37 °C) stained for the osteoblast-specific protein, bone gla protein (BGP; osteocalcin), using a specific rabbit anti-human-BGP antibody. This phenotypic test was repeated on monolayers after exposure to bone bank grafts. No difference between cultures before and after coculture with grafts was detected. The experiments were carried out on confluent monolayers at first passage, unless otherwise stated.

Bone resorption assay. Newborn (< 2 days old) NMRI outbred white mice were injected subcutaneously with 2 µCi $^{45}CaCl_2$. 4 days after injection, the mice were killed, the calvarias were removed and divided into 2 halves. The calvarias were then preincubated individually for 24 h in 24-well plates in 1 mL BGJ-B medium (Fitton-Jackson modification), supplemented with 5% heat-inactivated rabbit serum (Wellcome, MEDA, Copenhagen, Denmark; a single lot used), 2 mM L-glutamine and antibiotics. Pairs of half-calvaria were transferred for an additional 48 h period, one half-calvaria being cultured with medium containing 1:2 dilutions (unless otherwise stated) of test supernatant, the other with appropriate control medium. The release of ^{45}Ca into the medium and the amount of remaining ^{45}Ca in the half-calvaria (dried, 90% formic acid-digested) were measured by liquid scintillation counting and the bone resorption was calculated as the percentage of total ^{45}Ca released from each half-calvaria. Treated/control (T/C) bone resorption ratios above 1.0 then reflected stimulation of bone resorption by test supernatants. Results were expressed as means (SD) for multiple (≥ 4) pairs of calvaria. The percentage of release of ^{45}Ca into the medium of the amount of ^{45}Ca incorporated into the control half-calvaria varied in experiments from (% (SEM)) 8.2 (1.09) to 11.4 (0.96). Salmon calcitonin (20 nM) was used to find out whether osteoclast inhibition reversed the ^{45}Ca-release induced by supernatants.

Assay for bone-cell growth. The cells were seeded in multiwell plates at a density of 5×10^3 cells/cm^2. They were grown to a confluent monolayer in complete EMEM. Bone allo- and autografts were then added to the cells for the stated duration of coculture (Table 4). The incorporation of thymidine in DNA was measured after an 8 h pulse with 1 µCi per well [^{3}H]TdR in human osteoblast-like cells cocultured with bone graft samples. Human osteoblast-like cells cultured in medium alone were used as controls. Cultures were collected, using a semi-automated cell har-

Table 2. 6-day 2-chamber cocultures of bank bone allografts and human osteoblast-like cells' effect on release of factors by human osteoblast-like cells inducing bone resorption expressed as ^{45}Ca release treated/control ratio, mean (SEM) of 4 calvaria [a]

Bone bank allograft	Medium alone (control) [b]		+ Allograft (contact) [c]		+ Allograft (no contact, 2-chamber) [c]	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
1	1.04 (0.07)	0.95 (0.10)	1.84 (0.16) [d]	1.91 (0.24) [d]	1.41 (0.07) [d, e]	1.38 (0.11) [d, e]
2	0.93 (0.11)	1.01 (0.05)	1.70 (0.19) [d]	1.63 (0.09) [d]	1.53 (0.15) [d]	1.31 (0.06) [d, e]

[a] Human osteoblast-like cells were cultured at 37 °C in medium alone and with bone bank allografts stored for 2 weeks at –80 °C either in one-chamber (contact) or two-chamber (no contact; divided by a cell-impermeable membrane) 24-well Transwell plates (see Material and methods). Measurements were done by day 6, since release of factors inducing bone resorption was maximal at this time (Table 1). Bone resorption was assessed as described in Table 1.
[b] Medium from human osteoblast-like cell cultures was tested against control medium incubated at 37 °C for 6 days without cells.
[c] Medium from human osteoblast-like cell-graft cocultures was tested against control medium from human osteoblast-like cell cultures without grafts.
[d] Significantly different from control ($p < 0.05$; Student's t-test).
[e] Significantly different from contact ($p < 0.05$; analysis of variance).

Table 3. The role of prostaglandins in the release of factors inducing bone resorption by graft-stimulated human osteoblast-like cells expressed as ^{45}Ca release treated/control ratio, mean (SEM) for 4 calvaria[a]

Addition	Day 4		Day 6	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Medium alone [b]	0.97 (0.04)	1.05 (0.07)	1.01 (0.03)	1.08 (0.10)
Graft [c]	1.77 (0.20) [d]	1.65 (0.14) [d]	1.83 (0.20) [d]	1.62 (0.14) [d]
Graft + indomethacin (10^{-6}M) [c]	1.25 (0.06) [d, e]	1.37 (0.09) [d, e]	1.50 (0.16) [d]	1.29 (0.08) [d, e]

[a] Release of factors inducing bone resorption in human osteoblast-like cell cultures exposed to bone bank allografts (–80 °C, 2-week storage) for 4 and 6 days—the maximal response of bone resorption (Table 1). Indomethacin was added at culture start (osteoblast and bone allograft) at a concentration level known to block prostaglandin E_2 release by human osteoblast-like cells[13]. The dilution of the test supernatant in this experiment was 1:10. Indomethacin was added to the controls at the same concentration as in the 1:10 dilution. Experiments shown are representative of a total of 4, using different donor allografts. Thymidine incorporation rates measured concomitantly were not altered by indomethacin (10^{-6}M); % of control ± SEM were on day 4: 103.5 ± 6.4 and day 6: 98.9 ± 4.8.
[b] Medium from human osteoblast-like cell cultures was tested against control medium incubated at 37 °C for 4 or 6 days, respectively, without cells.
[c] Medium from human osteoblast-like cell-graft cocultures was tested against control medium from human osteoblast-like cell cultures without grafts.
[d] Significantly different from medium alone ($p < 0.05$; Student's t-test).
[e] Significantly different from graft-human osteoblast-like cell cultures without indomethacin ($p < 0.05$; analysis of variance).

vester (Skatron, Lier, Norway) and [^{3}H]TdR incorporation in DNA measured by liquid scintillation. A hemocytometer was used to count 100 µL aliquots of disaggregated single cell suspensions, which were released from wells at intervals by trypsin/EDTA incubation, as described, and gently agitated. The number of cells in 10 fields was counted.

Reagents. Methyl-[^{3}H]thymidine (5 Ci/mmol) and ^{45}Ca (10–40 mCi/mg calcium (Amersham, Birkerød, Denmark); culture media, PBS, FCS (heat-inactivated; a single batch used), ascorbate, L-glutamine, antibiotics, trypsin-EDTA (Gibco; Life Technologies, Roskilde, Denmark) and culture plates (Greiner Co., Austria; supplied by InVitro, Hillerød, Denmark) were purchased. Indomethacin and salmon calcitonin came from Sigma Co. (Bie & Berntsen, Copenhagen, Denmark).

Statistics. Significance of differences was calculated by analysis of variance or the Student's t-test.

Results

Allografts stimulated within 72 h a release of factors from human osteoblast-like cells capable of inducing osteoclastic bone resorption (Table 1). The release of these factors reached maximum after 6 days of culture and were still detectable after 3 weeks. Autologous

Table 4. Effect of bone bank allografts on the proliferation of normal human osteoblasts (human osteoblast-like cells) assessed by incorporation of tritiated thymidine expressed as mean [^{3}H]-TdR incorporation (% of control (SEM)) of triplicate wells from 1 of 2 experiments[a]

Bone bank allograft tp (°C)	Duration of coculture (days)					
	2	3	4	6	14	21
1 (–80)	96 (7)	98 (5)	83 (3)[b]	60 (6)[b]	70 (5)[b]	79 (6)[b]
1 (–20)	99 (3)	91 (10)	77 (4)[b]	56 (7)[b]	59 (8)[b]	73 (5)[b]
2 (–80)	101 (2)	99 (3)	82 (3)[b]	65 (5)[b]	70 (6)[b]	81 (4)[b]
2 (–20)	97 (4)	102 (7)	78 (4)[b]	59 (3)[b]	64 (4)[b]	77 (6)[b]
3 (–80)	97 (4)	94 (8)	86 (2)[b]	70 (4)[b]	74 (6)[b]	94 (6)
3 (–20)	107 (10)	97 (2)	90 (6)	73 (3)[b]	80 (4)[b]	88 (3)[b]
4 (–80)	96 (6)	93 (6)	82 (6)[b]	59 (6)[b]	65 (7)[b]	70 (4)[b]
4 (–20)	94 (9)	88 (1)[b]	78 (5)[b]	56 (4)[b]	59 (5)[b]	74 (5)[b]
5 (–80)	101 (4)	96 (4)	81 (4)[b]	56 (4)[b]	60 (3)[b]	63 (6)[b]
5 (–20)	95 (9)	97 (2)	84 (2)[b]	60 (5)[b]	nt	nt
6 (–80)	104 (6)	nt	80 (3)[b]	66 (3)[b]	68 (5)[b]	68 (4)[b]
6 (–20)	101 (3)	nt	75 (7)[b]	60 (7)[b]	nt	nt

[a] Kinetics of the proliferative response of human osteoblast-like cells to grafts stored for 2 weeks at –80 °C and –20 °C, respectively. Cells were cocultured with grafts at 37 °C in 96-multiwell plates. Values measured after 6-h and 24-h coculture were not significantly different from control values (data not shown). In 2 experiments, values were also measured after 5-, 7- and 10-day coculture: in both cases, values were lowest on day 6 (data not shown). No difference in effect was found between temperature groups.
nt not tested.
After 2, 3, 4, 6, 14 and 21 days cells were counted, using a hemocytometer, as described. Results of 1 representative experiment in a total of 6 were (% of control ± SEM): –80 °C: 92.9 ± 3 .8 (day 2), 95.0 ± 7.1 (day 3), 78.8 ± 1.5 (day 4), 63.9 ± 2.6 (day 14), 76.4 ± 6.1 (day 21). There was no significant difference between temperature groups.
[b] Significantly different from control ($p < 0.05$; analysis of variance).

Table 5. 6-day 2-chamber cocultures of bank bone allografts and human osteoblast-like cells' effect on human osteoblast-like cells ^{3}H-thymidine incorporation expressed as mean cpm (SEM) of triplicates [a]

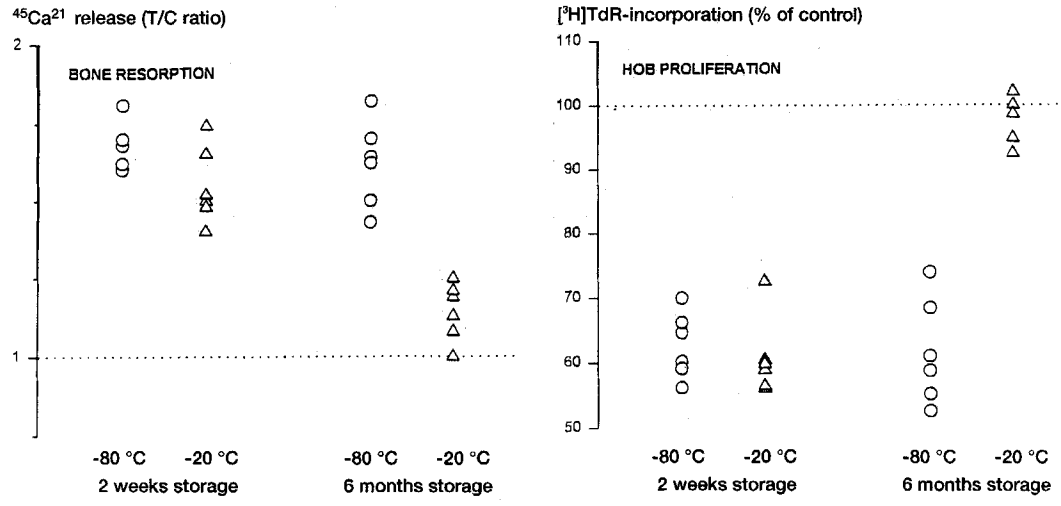
Bone bank allograft	Medium alone (control)		+ Allograft (contact)		+ Allograft (no contact, 2-chamber)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
1	4630 (306)	3981 (266)	2700 (162)[a]	2051 (82)[a]	2591 (208)[a]	1944 (72)[a]
2	2879 (102)	5030 (319)	1377 (59)[a]	2805 (114)[a]	1427 (90)	2920 (175)[a]

[a] Human osteoblast-like cells were cultured in medium alone and with bone bank allografts, as described in Table 2. 6-day cocultures were chosen, since the response peaked by day 6 (Table 4). Proliferation was assessed by the incorporation of thymidine. Values are mean cpm ± the standard error of the mean of triplicates.
There was no difference between the contact and no contact groups. Experiments are representative of a total of 4 bone bank allografts tested. Grafts stored for 2 weeks at –20 °C induced a similar response (data not shown).
[b] Significantly different from medium alone ($p < 0.05$; analysis of variance).

bone induced a similar response (data not shown). The reaction partially depended on allograft-human osteoblast-like cell contact, since two-chamber cultures induced less osteoclastic bone resorption (Table 2). The release of factors involved prostaglandin production, since indomethacin (10^{-6} M; a dose known to inhibit human osteoblast-like cell PGE_2 production (Gowen et al. 1985)) partially inhibited the response (Table 3). Calcitonin (20 nM) blocked the bone resorptive action, indicating an osteoclast-mediated effect (data not shown).

Allografts inhibited human osteoblast-like cell replication, as detected by decreased thymidine incorporation (Table 4). An autologous set-up induced a similar response (data not shown). The antiproliferative response was still detectable, but less pronounced after 3 weeks. The reduction of human osteoblast-like cell replication appeared independent of any direct contact between graft material and the cells (Table 5). No signs of cell toxicity were detected (eosin exclusion test).

The grafts induced similar responses from human osteoblast-like cells after 2 weeks' storage at –80 °C and –20 °C (Tables 1 and 4). After 6 months' storage, the



Comparison of the effect of bone bank allografts stored at –80 °C (○) and –20 °C (△) for 2 weeks and 6 months, respectively, on release of bone resorptive activity (left) and [^{3}H]TdR-incorporation (right) in human osteoblast-like cells after 6 days' culture. Control = human osteoblast-like cells with medium alone. Values are means. 6 different graft specimens were tested. Results for the 20 °C storage group were significantly different after 6 months, as compared to 2 weeks (ANOVA, $p < 0.05$)

activity was retained in –80 °C grafts, but was markedly reduced in allografts stored at –20 °C (Figure).

Discussion

Early phases of bone graft incorporation may involve resorption, which allows invasion of vascular buds and osteoblasts along the periphery (Friedlaender 1987, Kirkeby 1991). Indeed, bone implants induce osteoclast recruitment, depending on recipient osteoblasts in vivo (Akamine et al. 1994). These data are consistent with our findings that human osteoblast-like cells release factors capable of inducing osteoclast activity on exposure to devitalized bone material. The reason why the bone resorption declines after 6 days is unknown, but it could be due to destruction of these factors, or to production of an inhibitor. Results were similar in allogeneic and autologous graft-human osteoblast-like cell cocultures, suggesting that no immunological reactions were involved. The response was particularly pronounced in cultures with direct graft-cell contact, which suggested that human osteoblast-like cell-bone matrix interaction might be involved. For example, adhesion molecules have been demonstrated on bone cells, including the human osteoblast-like cell (Clover et al. 1992), which might mediate signals from bone graft matrix (Juliano and Haskill 1993). In speculative terms, the marked antiproliferative human osteoblast-like cell-response during release of factors inducing bone resorption, in the first week of allograft-human osteoblast-like cell culture, might also reflect an initially facilitated osteoclast access at the recipient site, due to reduced osteoblast numbers in vivo.The possibility that the human osteoblast-like cell [^{3}H]-TdR-incorporation measurements might appear reduced due to a misinterpreted cell migration to grafts during cocultures was excluded by the similar responses in the two-chamber assays.

Prostaglandins, particularly prostaglandin E_2 (PGE_2), are produced by the human osteoblast-like cell (MacDonald et al. 1984) and stimulate bone resorption (Raisz and Martin 1983). Our data indicate that prostaglandins contributed to release of bone resorptive activity. However, prostaglandin inhibition did not alter the antiproliferative response, suggesting that although bone grafts stimulate prostaglandins in human osteoblast-like cells, as revealed by the effect of indomethacin on bone resorption, prostaglandins do not mediate the growth inhibition.

The human osteoblast-like cell response to allograft material was completely retained after 6 months' storage at –80 °C. Grafts stored for 6 months at –20 °C lost the human osteoblast-like cell modulating effects, which were present after 2 weeks' storage. Temperatures below –70 °C are generally recommended for storage up to 5 years in bone banks, a view largely based on empirical knowledge of success (American Association of Tissue Banks 1987, Eastlund et al. 1992). This freezing level may be required to retard autolysis sufficiently by proteolytic enzymes (Tomford et al. 1986). However, bone frozen to –20 °C has been used successfully (Burwell

1969) and has been suggested for storage up to 6 months (Standards of The American Red Cross Tissue Services 1992). Our data indicate that temperature-dependent alterations after 6 months' graft storage at –20 °C completely change the human osteoblast-like cell response, which may alter graft incorporation in vivo. Graft temperature-sensitive molecules may primarily include growth modulatory substances since the regulatory effect on human osteoblast-like cell proliferation was lost, whereas the release of factors inducing bone resorption was merely reduced. Mechanisms including proteolytic enzyme activity (Tomford et al. 1986) and ice crystal formation (Skinner 1990) at –20 °C might contribute. Further studies are indicated to examine the role of graft-induced responses of recipient osteoblasts in vivo. Our studies suggest that caution should be taken in utilizing bank bone stored for a long time at –20 °C.

Acknowledgments

This study was supported in part by the Danish Rheumatism Association. We thank Helen Nielsen for technical assistance. The rabbit antihuman BGP was supplied by Dr. I. Clemmensen

References

Akamine A, Kimura R, Maeda K. Implanted bone particles induce osteoclast development in the presence of osteoblast lineage cells. Bone 1994; 15: 343-9.

American Association of Tissue Banks. Standards for tissue banking. Va, McLean, AATB, 1987.

Britto J M, Fenton A J, Holloway W R, Nicholson G C. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology 1994; 134: 169-76.

Burwell R G. The fate of bone grafts. In: Recent advances in orthopaedics (Ed. Apley A G). The Williams & Wilkins Co., Baltimore 1969: 115-29.

Clover J, Dodds R A, Gowen M. Integrin subunit expression by human osteoblasts and osteoclasts in situ and in Culture. J Cell Sci 1992; 103: 267-72.

Eastlund D T, Campagnari K D. Standards of The American Red Cross Tissue Services. The American Red Cross, 1992.

Friedlaender G E. Current concepts review: Bone grafts. The basic science rationale for clinical applications. J Bone Joint Surg (Am) 1987; 69: 786-90.

Gowen M, Wood D D, Russell R G G. Stimulation of the proliferation of human bone cells by human monocyte products with interleukin-1 activity. J Clin Invest 1985; 75: 1223-9.

Guo M Z, Xia Z, Lin L B. The mechanical and biological properties of demineralized cortical bone allografts in animals. J Bone Jt Surg (Br) 1991; 73: 791-4.

Hauschka P V, Mavrakos A E, Iafrati M D, Doleman S E, Klagsbrun M. Growth factors in bone matrix. Isolation of multiple types by affinity chromatography on heparin-sepharose. J Biol Chem 1986; 261:12665-72.

Juliano R L, Haskill S. Signal transduction from the extracellular matrix. J Cell Biol 1993; 120: 577-85.

Kirkeby O J. Revascularization of bone grafts in rats. J Bone Joint Surg (Br) 1991; 73: 501-5.

MacDonald B R, Gallagher J A, Ahnfelt-Ronne I, Beresford J N, Gowen M, Russell R G G. Effects of bovine parathyroid hormone and 1,25-dihydroxyvitamin D3 on the production of prostaglandins by cells derived from human bone. FEBS Lett 1984; 169: 49-52.

McSheehy P M J, Chambers T J. Osteoblastic cells mediate osteoclastic responsiveness to parathyroid hormone. Endocrinology 1986; 118: 824-28

McSheehy P J M, Chambers T J. 1,25-dihydroxyvitamin D3 stimulates rat osteoblastic cells to release a soluble factor that increases osteoclastic bone resorption. J Clin Invest 1987; 80: 425-9.

Raisz L G, Martin T J. Prostaglandins in bone and mineral metabolism. Bone Miner Res Ann 1983; 2: 286-310.

Sampath T K, Reddi A H. Distribution of bone inductive proteins in mineralized and demineralized extracellular matrix. Biochem Biophys Res Commun 1984; 119: 949-54.

Skinner H B. Alternatives in the selection of allograft bone. Orthopaedics 1990; 13: 843-6.

Skjødt H, Gallagher J A, Beresford J N, Couch M, Poser J W, Russell R G G. Vitamin D metabolites regulate osteocalcin synthesis and proliferation of human bone cells in vitro. J Endocrinol 1985; 105: 391-6.

Thomson B M, Saklatvala J, Chambers T J. Osteoblasts mediate interleukin 1 stimulation of bone resorption by rat-osteoclasts. J Exp Med 1986; 164: 104-12.

Thomson B M, Mundy G R, Chambers T J. Tumor necrosis factors alpha and beta induce osteoblastic cells to stimulate osteoclastic bone resorption. J Immunol 1987; 138: 775-9.

Tomford W W, Ploetz R N, Manknin H J. Bone allografts of femoral heads: Procurement and storage. J Bone Joint Surg (Am) 1986; 68: 534-7.

Urist M R. Bone morphogenetic protein, bone regeneration, heterotopic ossification and the bone—bone marrow consortium. Bone Miner Res Ann 1989; 6: 57-112.