Gender- and age-related differences in osteoclast formation from circulating precursors

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Abstract

A number of bone diseases characterised by excessive osteolysis (e.g. osteoporosis and Paget's disease) exhibit a marked gender difference in prevalence and are more common in the elderly population. Bone resorption is carried out by osteoclasts, which are formed by fusion of circulating mononuclear precursor cells of haematopoietic origin. In this study, we have determined whether there are gender- and age-related differences in osteoclast formation from circulating precursors. Peripheral blood mononuclear cells (PBMCs) were co-cultured with UMR106 osteoblast-like cells in the presence of macrophage-colony stimulating factor (M-CSF) and 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) or cultured alone in the presence of sRANKL (soluble receptor activator of nuclear factor κB ligand) and M-CSF. As assessed by the formation of tartrate resistant acid phosphatase (TRAP)-positive ($TRAP^+$) and vitronectin receptor-positive (VNR^+) multinucleated cells (MNCs), there was no difference in the number of circulating osteoclast precursors in males and females. Lacunar resorption carried out by osteoclasts formed from these precursors was generally increased in males compared with females ($P=0{\cdot}03$). An increase in the number of $TRAP^+$ and VNR^+ MNCs formed from male PBMCs was noted in response to $1,25(OH)_2D_3$ ($P<0{\cdot}005$). An increase in lacunar resorption in cultures of PBMCs (10^5 per well) from males was also noted in response to 10^{-9} M $1,25(OH)_2D_3$ ($P<0{\cdot}05$) and sRANKL ($P=0{\cdot}05$), but not M-CSF. The addition of dexamethasone resulted in a marked increase in osteoclast formation and lacunar resorption in both males and females. Post-menopausal females and males of comparable age showed similar levels of osteoclastogenesis. Pre-menopausal women showed similar levels of osteoclastogenesis but less resorption ($P=0{\cdot}01$) compared with males of comparable age. These results show that there are specific gender/age-related differences in osteoclast formation and bone resorption and have implications for evaluating osteoclastogenesis in skeletal diseases such as primary osteoporosis and Paget's disease.

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Introduction

Bone is a dynamic tissue in which bone resorption by osteoclasts is tightly coupled to bone formation by osteoblasts. A number of bone diseases which are characterised by excessive osteolysis, such as osteoporosis and Paget's disease, exhibit discordant coupling of bone resorption and bone formation. These conditions are both more common in the elderly population and exhibit a marked gender difference in prevalence, primary involutional osteoporosis occurring more commonly in females and Paget's disease more commonly in males.

The osteoclast is a multinucleated cell (MNC) derived from the pluripotent haematopoietic stem cell (Suda *et al.* 1992). It has been shown that the human osteoclast precursor circulates in the monocyte fraction and exhibits a monocyte/macrophage phenotype (Fujikawa *et al.* 1996). Differentiation of these cells into osteoclasts requires the presence of macrophage-colony stimulating factor (M-CSF) and involves interaction between osteoclast precursors, which express the receptor activator of nuclear factor κB (RANK), and osteoblastic cells, which express RANK ligand (RANKL) (Lacey *et al.* 1998, Nakagawa *et al.* 1998, Yasuda *et al.* 1998). This accords with the finding that human osteoclast formation from circulating mononuclear phagocyte precursors *in vitro* requires the presence of M-CSF and either contact between osteoclast precursors and RANKL-expressing osteoblasts (Fujikawa *et al.* 1996) or the presence of soluble (s)RANKL (Matsuzaki *et al.* 1998, Quinn *et al.* 1998). 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is also required for osteoclast formation when human monocytes are co-cultured with osteoblastic UMR106 cells (Fujikawa *et al.* 1996). $1,25(OH)_2D_3$ has also been shown to

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stimulate osteoclast activity via osteoblasts (McSheehy & Chambers 1987) and to promote osteoclast formation by increasing the ratio of RANKL to its soluble decoy receptor, osteoprotegerin (Tsukii *et al.* 1998, Nagai & Sato 1999).

Differences in skeletal mass are known to be age- and sex-related (Looker *et al.* 1995). Increasing our understanding of the effects of age and gender on bone is important in the context of improved prevention and treatment of metabolic diseases of bone. A number of bone disorders which are characterised by a decrease in bone mass are known to occur more commonly in elderly individuals and to exhibit a marked gender difference. In this study we have focused on investigating whether there are gender- and age-related differences in osteoclast formation and activity.

Materials and Methods

Materials

All cell incubations were performed in alpha minimal essential medium (MEM) (Gibco, Paisley, Strathclyde, UK) supplemented with glutamine (2 mM), benzyl penicillin (100 IU/ml), streptomycin (100 μg/ml) and 10% fetal calf serum (FCS) (Gibco) in a humidified atmosphere of 5% CO_2 at 37 °C. Cloned, hormone-responsive, calcitonin receptor-negative, osteoblast-like UMR106 cells (from a rat osteosarcoma-derived cell line) were obtained from Prof. T J Martin, Melbourne, Australia (Forest *et al.* 1985). $1,25(OH)_2D_3$ (Solvay Duphar, Weesp, NL) and dexamethasone (Sigma Chemical Co., Poole, Dorset, UK) were dissolved in absolute alcohol and stored at −20 °C. Human M-CSF (R&D Systems Europe, Abingdon, Oxon, UK) and sRANKL (Amgen Inc., Thousand Oaks, CA, USA) were dissolved in MEM/FCS and stored at −20 °C.

Preparation of monocyte–UMR106 co-cultures

Monocytes were isolated from the peripheral blood of a total of 22 normal males (age range 25–86 years) and 22 normal females (age range 29–85 years). The menopausal status of the female subjects was ascertained at the time of blood collection by interview and all subjects classed as <50 years and >50 years were confirmed to be pre- and post-menopausal respectively. After collection, blood was diluted 1:1 in MEM, layered over Ficoll-Hypaque (Pharmacia, Milton Keynes, Bucks, UK), centrifuged at 693 ***g*** and washed and resuspended in MEM/FCS. The number of cells in the resulting suspension of peripheral blood mononuclear cells (PBMCs) was counted in a haemocytometer, after lysis of red cells with a 5% (v/v) acetic acid solution.

Dentine slices (4 mm diameter), prepared as previously described (Boyde *et al.* 1984), and glass coverslips (6 mm diameter) were placed in 96-well tissue culture plates. UMR106 osteoblast-like cells (2×10^4) were added to each well and then cultured on the dentine slices and coverslips for 24 h in MEM/FCS. The cell suspension of PBMCs (10^5 cells/well) was then settled on these coverslips and dentine slices for 2 h. The coverslips and dentine slices were then removed from the wells, washed vigorously in MEM/FCS to remove non-adherent cells and cultured in 24-well tissue culture plates containing 1 ml MEM/FCS, with $1,25(OH)_2D_3$ (10^{-7} M), M-CSF (25 ng/ml) and dexamethasone (10^{-8} M) for up to 21 days. Media and added factors were entirely replaced every 3–4 days.

Preparation of monocyte cultures in the presence of sRANKL

Monocytes were isolated from the peripheral blood of a total of 16 normal males (age range 25–83 years) and six normal females (age range 31–78 years) as detailed above then seeded (alone) at 5×10^5 cells/well onto dentine slices/coverslips. After 2 h incubation, non-adherent cells were removed by washing in MEM/FCS and cell cultures on dentine slices and coverslips were placed in 24-well tissue culture plates containing 1 ml MEM/FCS, in the presence of sRANKL (30 ng/ml), M-CSF (25 ng/ml) and dexamethasone (10^{-8} M) and cultured for up to 21 days.

Assessment of the number of circulating osteoclast precursors in the monocyte fraction and relative sensitivity to osteoclastogenic factors

To compare the number of circulating osteoclast precursors present in male and female subjects, isolated PBMCs were serially diluted (10^2–10^5 cells/well) and co-cultured for 14–21 days with UMR106 cells on coverslips and dentine slices. Their relative sensitivity to $1,25(OH)_2D_3$ (10^{-10}–10^{-7} M) was assessed in this PBMC–UMR106 co-culture system. The relative sensitivity of male and female circulating osteoclast precursors to sRANKL and M-CSF was assessed in the absence of UMR106 cells. PBMCs were seeded onto dentine slices and coverslips and cultured in the presence of sRANKL, M-CSF and dexamethasone as described above. The concentration of either sRANKL (1–30 ng/ml) or M-CSF (5–25 ng/ml) added to the PBMC cultures was altered.

Histochemical and immunohistochemical characterisation of cultured cells

Histochemical staining for tartrate resistant acid phosphatase (TRAP) was carried out using a commercially available kit (Sigma). Cell preparations were fixed in citrate/acetone solution and stained for acid phosphatase, using naphthol AS-BI phosphate as a substrate, in the presence of 1·0 M tartrate; the product was reacted with Fast Garnet GBC salt (Minkin 1982).

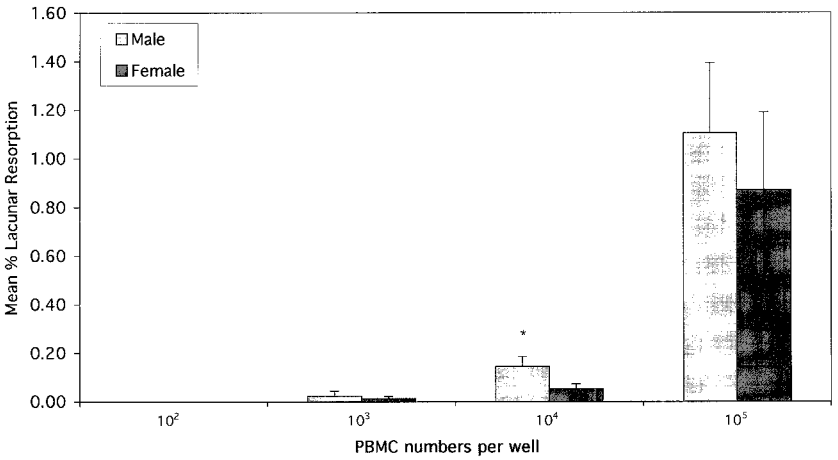

Figure 1 The effect of PBMC concentration on lacunar resorption in co-cultures of PBMCs from 20 age-matched male and female subjects incubated with UMR106 (2×10^4) cells in the presence of $1,25(OH)_2D_3$ (10^{-7} M), M-CSF (25 ng/ml) and dexamethasone (10^{-8} M). The results are expressed as the mean percentage area resorbed per dentine slice ± S.E.M. *$P<0{\cdot}05$, compared with females.

Cell preparations on coverslips were also stained immunohistochemically, by an indirect immunoperoxidase technique (Gatter *et al.* 1984), with the monoclonal antibody 23C6 (Serotec, Kidlington, Oxon, UK); this is directed against CD51, the vitronectin receptor (VNR), a highly osteoclast-associated antigen (Horton *et al.* 1985). Cell preparations were similarly stained with the monoclonal antibody GRS1, directed against CD14, a macrophage-associated antigen not known to be expressed by osteoclasts (Athanasou & Quinn 1990). Coverslips were examined under light microscopy. The number of $TRAP^+$ and VNR^+ MNCs was counted in four fields of view (× 10 objective) per coverslip and the mean calculated. Cells containing three or more nuclei were considered multinucleated.

Functional evidence of osteoclast differentiation: detection of lacunar resorption

At the end of the co-culture period, dentine slices were placed in NH_4OH (1 M) for 2 h and adherent cells removed by ultrasonication. The slices were then washed with distilled water and stained with 0·5% (v/v) toluidine blue, then washed again. The surface was examined for evidence of lacunar resorption under light microscopy. This was quantified by calculating the percentage area of resorption on each slice, using image analysis software (Photoshop 5·5; Adobe, Adobe Systems UK, Oxbridge, Middlesex, UK).

Statistical analysis

Data are presented as means ± S.E.M. Significant differences were determined using a two-tailed *t*-test. For the purposes of analysis, percentage data were normalised by arcsine transformation. $P<0{\cdot}05$ was taken as significant.

Results

Osteoclast formation and bone resorption in UMR106–PBMC co-cultures: estimation of the relative proportion of circulating osteoclast precursors in males and females

Isolated PBMCs from both males and females cultured on coverslips showed the phenotypic profile of monocytes and not osteoclasts (Athanasou & Quinn 1990). These cells expressed CD14 and were negative for the osteoclast markers TRAP and VNR after 24 h incubation. No evidence of lacunar resorption was noted following 24 h culture of PBMCs on dentine slices. Both male and female PBMCs co-cultured with UMR106 cells differentiated into osteoclasts as evidenced by the formation of numerous $TRAP^+$ and VNR^+ MNCs in 14 day co-cultures on coverslips and the production of lacunar resorption pits in 21 day co-cultures on dentine slices. In cultures of serial dilutions of PBMCs and UMR106 cells, incubated in the presence of 10^{-7} M $1,25(OH)_2D_3$, 25 ng/ml M-CSF and 10^{-8} M dexamethasone, osteoclast formation (as evidenced by TRAP and VNR expression and lacunar resorption) was first observed in both males and females when 10^3 PBMCs were added to dentine slices and coverslips (Fig. 1).

Gender and age-related differences in osteoclast formation and bone resorption

No difference was noted in the number of $TRAP^+$ and VNR^+ MNCs formed in co-cultures of UMR106 cells

Table 1 Mean ± S.E.M. of $TRAP^+$ and VNR^+ MNCs (per high-power field) present in 14 day cultures on glass coverslips, in the presence of 10^{-7} M $1,25(OH)_2D_3$, 25 ng/ml M-CSF and 10^{-8} M dexamethasone

	$TRAP^+$ MNCs	VNR^+ MNCs
Male (29–86 years)	3·90 ± 0·54	10·22 ± 2·96
Female (29–85 years)	3·88 ± 0·50	6·32 ± 1·37
Male <50 years	5·31 ± 1·21	8·67 ± 2·23
Female <50 years	3·88 ± 0·83	5·80 ± 1·48
Male >50 years	3·28 ± 0·54	11·00 ± 4·33
Female >50 years	3·88 ± 0·62	6·61 ± 2·86

with male and female PBMCs (10^5 cells/well) (Table 1). When the data were split in terms of age, i.e. <50 years and >50 years, there was also no significant difference in the number of $TRAP^+$ or VNR^+ MNCs formed in culture. When 10^4 PBMCs were added to dentine slices, significantly more resorption was seen in males compared with females (Fig. 1, $P=0·03$). This increase in resorption was not associated with a corresponding increase in the number of $TRAP^+$ or VNR^+ MNCs formed in culture. When 10^5 PBMCs were added to dentine slices there was no overall difference in the extent of lacunar resorption in males and females. However, when the data were split in terms of age, a significant increase in lacunar resorption was noted in males <50 years compared with females <50 years. The extent of lacunar resorption was similar in males and females >50 years (Fig. 2). An increase in lacunar resorption in females >50 years compared with females <50 years was also noted but this did not reach statistical significance.

Sensitivity of circulating osteoclast precursors to osteoclastogenic factors in males and females

$1,25(OH)_2D_3$ When serial dilutions of $1,25(OH)_2D_3$ were added to PBMC–UMR106 co-cultures, formation of $TRAP^+$ and VNR^+ MNCs and lacunar resorption was first noted in the presence of 10^{-9} M $1,25(OH)_2D_3$ in both males and females (Fig. 3). No osteoclast formation was noted in co-cultures to which 10^{-10} M $1,25(OH)_2D_3$ was added. An increase in the number of $TRAP^+$ and VNR^+ MNCs was noted in males when 10^{-9} M and 10^{-8} M $1,25(OH)_2D_3$ was added to co-cultures ($P<0·005$ and $P<0·05$ respectively). The mean number ± S.E.M. of $TRAP^+$ and VNR^+ MNCs formed when 10^{-9} M $1,25(OH)_2D_3$ was added to co-cultures was 4·20 ± 1·13 and 9·13 ± 3·73 respectively in males and 0·11 ± 0·08 and 1·75 ± 0·68 respectively in females ($P<0·005$ and $P<0·05$ respectively as compared with male subjects). The mean number of $TRAP^+$ and VNR^+ MNCs formed when 10^{-8} M $1,25(OH)_2D_3$ was added to co-cultures was 7·20 ± 1·29 and 8·08 ± 3·31 respectively in males and 3·00 ± 0·57 and 2·00 ± 1·07 respectively in females ($P<0·05$ respectively as compared with male subjects). The mean number of $TRAP^+$ and VNR^+ MNCs formed when 10^{-7} M $1,25(OH)_2D_3$ was added to co-cultures was 6·20 ± 0·85 and 7·18 ± 2·92 respectively in males and 3·24 ± 1·67 and 3·89 ± 2·17 respectively in females. There was no significant difference in the mean number of $TRAP^+$ and VNR^+ MNCs formed in cultures containing PBMCs isolated from male or female subjects. Although all concentrations of $1,25(OH)_2D_3$ resulted in an increase in the extent of lacunar resorption in male UMR106–PBMC co-cultures, compared with female subjects, the most significant difference was observed only when 10^{-9} M $1,25(OH)_2D_3$ was added to these co-cultures (Fig. 3).

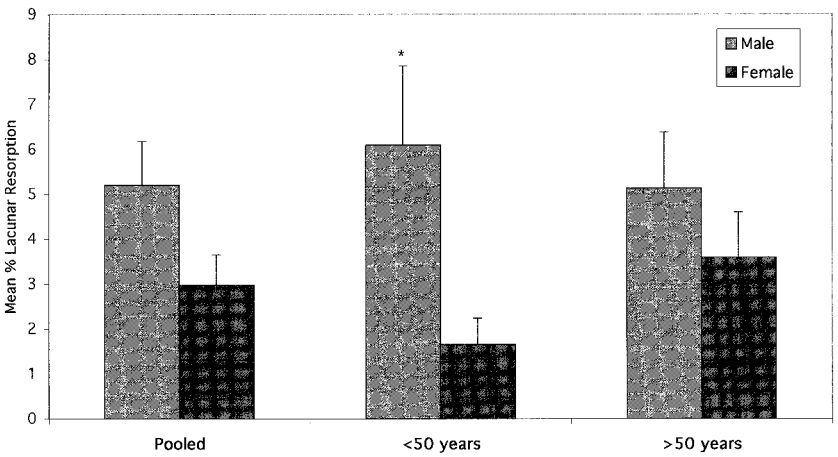

Figure 2 The effect of age on the extent of lacunar resorption in co-cultures of PBMCs (10^5) from 22 age-matched male and female subjects incubated with UMR106 cells in the presence of $1,25(OH)_2D_3$ (10^{-7} M), M-CSF (25 ng/ml) and dexamethasone (10^{-8} M). The results are expressed as the mean percentage area resorbed per dentine slice ± S.E.M. *$P<0·05$, compared with females.

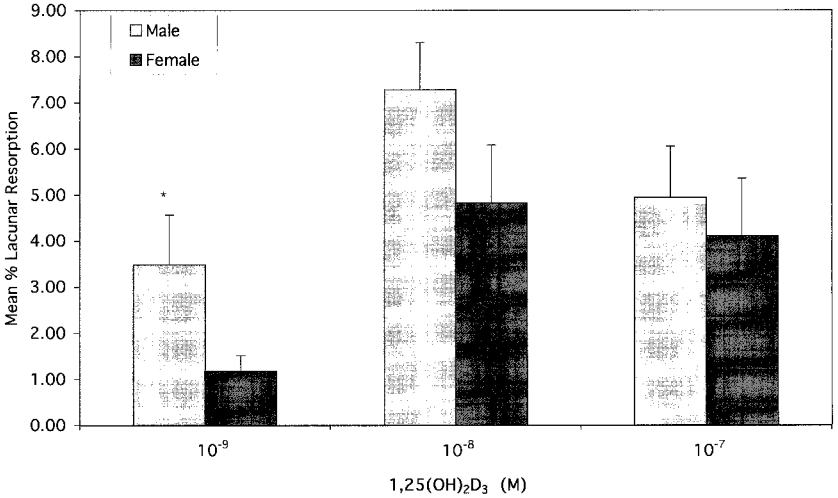

Figure 3 The effect of $1,25(OH)_2D_3$ on lacunar resorption in co-cultures of PBMCs (10^5) from 18 age-matched males and females incubated with UMR106 cells (2×10^4) in the presence of M-CSF (25 ng/ml) and dexamethasone (10^{-8} M). The results are expressed as the mean percentage area resorbed per dentine slice ± S.E.M. *P<0·05, compared with females.

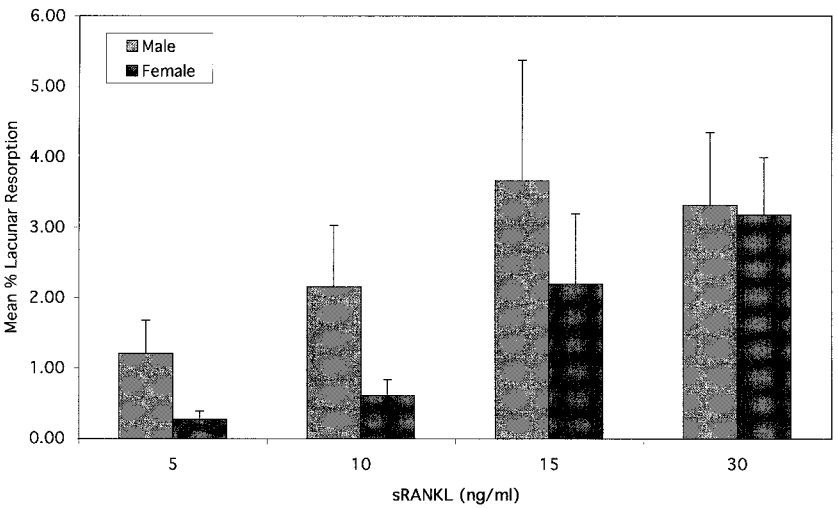

Figure 4 The effect of sRANKL on lacunar resorption in cultures of PBMCs (10^5) from 12 males and 6 females (all age-matched) incubated in the presence of M-CSF (25 ng/ml) and dexamethasone (10^{-8} M). The results are expressed as the mean percentage area resorbed per dentine slice ± S.E.M.

RANKL PBMCs incubated in the presence of sRANKL, M-CSF and dexamethasone formed $TRAP^+$ and VNR^+ MNCs in 14 day cultures on coverslips and lacunar resorption pits in 21 day cultures on dentine slices. When 30 ng/ml sRANKL was added to PBMC cultures, no difference in the extent of lacunar resorption between males and females was noted (Fig. 4). However, at lower concentrations of sRANKL, there was a trend for increased lacunar resorption in males compared with females. This reached statistical significance in PBMC cultures to which 5 ng/ml sRANKL was added (Fig. 4, P=0·05). There was no evidence of osteoclast formation following the addition of 1 ng/ml sRANKL. No significant difference in the mean number of $TRAP^+$ and VNR^+ MNCs in male and female cultures was noted in response to the addition of varying concentrations of sRANKL. The mean number of $TRAP^+$ and VNR^+ MNCs formed in PBMC cultures to which 30 ng/ml sRANKL was added is shown in Table 2.

M-CSF Sensitivity of osteoclast precursors from males and females to the osteoclastogenic effects of M-CSF was

Table 2 Mean ± S.E.M. of $TRAP^+$ and VNR^+ MNCs (per high-power field) present in 14 day cultures on glass coverslips, in the presence of 30 ng/ml sRANKL and 25 ng/ml M-CSF with or without 10^{-8} M dexamethasone (Dex)

	$TRAP^+$ MNCs	VNR^+ MNCs
Male		
+Dex	1·94 ± 0·52[a]	6·17 ± 2·53[a]
− Dex	0·71 ± 0·32	0·17 ± 0·11
Female		
+Dex	3·01 ± 0·57[b]	4·75 ± 1·56[b]
− Dex	0·98 ± 0·18	1·25 ± 0·70

[a]$P<0·05$ compared with males − Dex; [b]$P<0·05$ compared with females − Dex.

assessed in cultures of PBMCs incubated in the absence of UMR106 cells but in the presence of sRANKL (30 ng/ml) and M-CSF (5–25 ng/ml). The number of $TRAP^+$ and VNR^+ MNCs formed in culture was similar in males and females at all of the concentrations studied; the mean number of $TRAP^+$ and VNR^+ MNCs seen when 25 ng/ml M-CSF was added is shown in Table 2. No difference was noted in the extent of lacunar resorption in cultures of PBMCs from males or females at any of the concentrations studied (Fig. 5).

Corticosteroids Corticosteroids are known to influence bone remodelling with long-term use being associated with an increased risk of osteoporosis. The addition of dexamethasone to PBMC cultures, in the absence of UMR106 cells but in the presence of sRANKL and M-CSF, resulted in a significant increase in the formation of $TRAP^+$ and VNR^+ MNCs in both males and females (Table 2; $P<0·05$ and $P<0·05$ respectively). There was also an increase in the extent of lacunar resorption seen in cultures of both male and female PBMCs incubated in the presence of dexamethasone (Fig. 6; $P<0·0001$ and $P<0·01$ respectively). No significant difference in the extent of osteoclast formation (Table 2) or lacunar resorption (Fig. 6) was noted between male and female PBMCs cultured in the presence of dexamethasone.

Discussion

Differences in the formation and/or activity of osteoclasts in males and females could account for the marked gender difference in prevalence of some osteolytic bone disorders such as osteoporosis and Paget's disease. Mononuclear osteoclast precursors, which are known to circulate in the monocyte fraction of peripheral blood, form MNCs that express all the phenotypic characteristics of osteoclasts when cultured in contact with osteoblast-like UMR106 cells in the presence of M-CSF and $1,25(OH)_2D_3$ (Fujikawa *et al.* 1996), or when cultured alone in the presence of M-CSF and sRANKL (Matsuzaki *et al.* 1998, Quinn *et al.* 1998).

By observing the extent of osteoclast formation in cultures of serial dilutions of these circulating precursors in age-matched males and females, we found no marked difference in the relative proportion of mononuclear osteoclast precursors in the peripheral blood of males and females. There was no difference in the number of $TRAP^+/VNR^+$ MNCs formed from circulating precursors in age-matched males and females; these osteoclast-associated markers are expressed to a variable extent at different stages of osteoclast formation (Takahashi *et al.* 1994, Faust *et al.* 1999). The extent of lacunar resorption by osteoclasts formed in these PBMC cultures was similar in post-menopausal females and age-matched males but this was not the case for cultures of PBMCs from pre-menopausal females in which significantly less lacunar resorption was noted than in PBMC cultures from males of corresponding age. This observation accords with those of a recent study on age-related changes in bone turnover which found that there is an increase in markers of bone turnover, including bone resorption in young males (Fatayerji & Eastell 1999). As we noted there was no significant increase in osteoclast formation in males less than 50 years, it would appear that this increase in resorption is due to increased functional activity or survival of osteoclasts formed from circulating precursors derived from males in this age group.

Although not directly comparable, it is interesting to consider our findings in the light of bone histomorphometry studies which have looked at the number of osteoclasts and the lacunar resorption surface area in males and females in different age groups. Most histomorphometry studies which have analysed the number of osteoclasts in bone have shown that this does not exhibit a marked gender difference and that osteoclast numbers in fact varies quite widely within a particular age group (Sedlin *et al.* 1963, Schenk *et al.* 1969, Meunier *et al.* 1973, Melsen *et al.* 1979, Rehman *et al.* 1994). Schenk *et al.* (1969) noted that the extent of Howship's lacunae covered by osteoclasts was similar in all age groups from 20–80 years, indicating a remarkably constant state of osteoclast formation and activity. However, in other studies, no significant change with age or sex was noted in resorption area or resorption cavity depth, although these measurements are more likely to reflect osteoclast activity than osteoclast formation (Meunier *et al.* 1973, Croucher *et al.* 1991). Some studies have reported an increase in the extent of resorption in females in their sixth decade (Sedlin *et al.* 1963, Melsen *et al.* 1979), but this has been refuted by others (Meunier *et al.* 1973). Although our findings on osteoclast formation and bone resorption cannot strictly be equated to those of bone histomorphometry studies, it should be noted that post-menopausal females and age-matched males exhibited a similar level of lacunar resorption in our study.

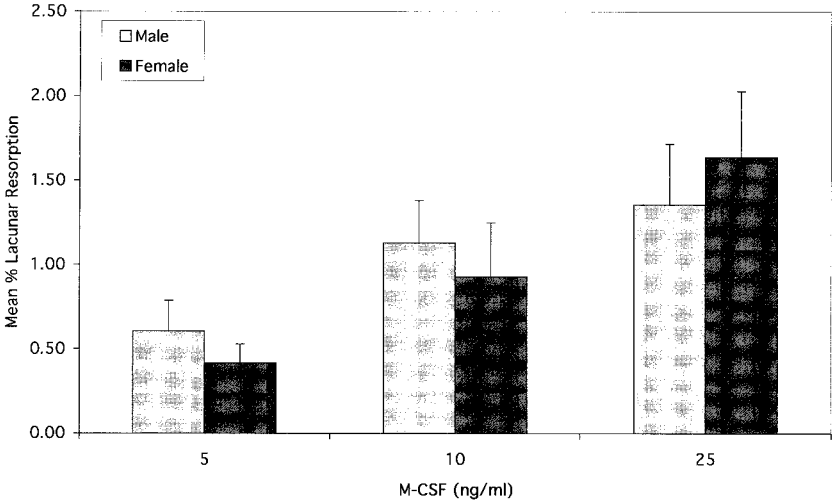

Figure 5 The effect of M-CSF on lacunar resorption in cultures of PBMCs from 16 males and 6 females (all age-matched) incubated in the absence of UMR106 cells but in the presence of sRANKL (30 ng/ml) and dexamethasone (10^{-8} M). The results are expressed as the mean percentage area resorbed per dentine slice ± S.E.M.

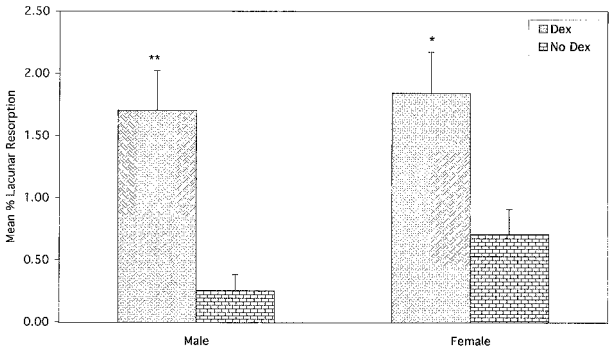

Figure 6 The effect of dexamethasone (Dex) on lacunar resorption in cultures of PBMCs from 16 males and 6 females (all age-matched) incubated in the absence of UMR106 cells but in the presence of sRANKL (30 ng/ml) and M-CSF (25 ng/ml). The results are expressed as the mean percentage area resorbed per dentine slice ± S.E.M. *P<0·05, **P<0·001, compared with females.

Of the osteoclastogenic factors examined, only $1,25(OH)_2D_3$ was found to show a marked gender difference in osteoclast formation. The number of $TRAP^+$ and VNR^+ MNCs formed in co-cultures of UMR106 cells and PBMCs was increased in males compared with females; this increase in the number of $TRAP^+$ and VNR^+ MNCs in males was associated with a corresponding increase in lacunar resorption. This increased resorption in males could in part be due to $1,25(OH)_2D_3$ stimulation of osteoclast formation; however, it should be noted that $1,25(OH)_2D_3$ is also known to promote osteoclastic bone-resorbing activity via stimulation of osteoblastic cells (which were present in the co-cultures used in this study) (McSheehy & Chambers 1987). Although no differences in serum levels of $25(OH)_2D_3$ and $1,25(OH)_2D_3$ have been noted in males and females (Haddad 1992), our finding of increased resorption in males is of interest with regard to the observation that PBMCs derived from patients suffering from Paget's disease, a condition which occurs predominantly in males, were also found to be hypersensitive to $1,25(OH)_2D_3$ (Neale *et al.* 2000).

RANKL, a recently identified membrane-bound protein of the tumour necrosis factor (TNF) receptor family, promotes preosteoclast fusion and osteoclast survival and activity (Hofbauer *et al.* 2000). PBMCs from males and females were cultured in the presence of varying concentrations of sRANKL in order to assess their relative sensitivity. No difference in terms of the formation of $TRAP^+/VNR^+$ MNCs was noted in cultures of PBMCs from males and females at any of the concentrations studied. However, a trend towards increased lacunar resorption by osteoclasts formed from male PBMCs at low concentrations of sRANKL was noted. This increased resorption may have been due to RANKL stimulation of osteoclast activity and survival rather than an effect on osteoclast formation (Burgess *et al.* 1999, Jimi *et al.* 1999). No significant difference was noted between males and females in response to varying concentrations of M-CSF, in terms of either TRAP and VNR expression or the extent of lacunar resorption. Although it has been shown that the membrane form of M-CSF is differentially regulated in rat bone marrow by oestrogens and androgens (Lea *et al.* 1999), our findings suggest that there is no M-CSF-induced difference in osteoclast formation in males and females.

Corticosteroid treatment is a major risk factor for osteoporosis (Nishimura & Ikuyama 2000). Dexamethasone is often used to promote osteoclastogenesis *in vitro* (Udagawa *et al.* 1990, Fujikawa *et al.* 1996, Itonaga *et al.* 1999). A significant reduction in bone resorption was seen when dexamethasone was excluded from cultures of both male and female PBMCs, although between the sexes no difference in the extent of osteoclast formation or lacunar resorption was noted following the addition of dexamethasone. Thus, it would appear that corticosteroids stimulate osteoclast formation and bone resorption and that this effect is not gender-related. Matsuzaki *et al.* (1998) showed that the increase in $TRAP^+$ cell numbers after dexamethasone administration was not significant but they did not study the effect of this treatment on the RANKL-induced lacunar resorption. Dexamethasone has been shown to increase osteoclast formation and lacunar resorption in human bone marrow cultures (Sarma *et al.* 1998). Dexamethasone is known to promote expression of membrane-bound M-CSF in murine osteoblasts but the mechanism whereby it stimulates osteoclast formation from circulating precursors is unknown (Rubin *et al.* 1998). Our own and previous studies suggest that this is due primarily to an effect on osteoclast proliferation and differentiation rather than osteoclast activity (Tobias & Chambers 1989, Hirayama *et al.* 2000).

Specific gender and age differences in activity and hormonal response have been noted in osteoblastic bone-forming cells obtained from human bone (Katzburg *et al.* 1999). The data presented in this study show that there are also differences in the formation and activity of bone-resorbing cells in males and females. PBMCs from males were found to be more sensitive to $1,25(OH)_2D_3$ than those of females, and osteoclasts formed in cultures from male PBMCs were more active in response to sRANKL. Our finding of a difference in the bone-resorbing activity of osteoclasts formed from PBMCs derived from pre-menopausal females and males of comparable age, and our observation that this difference is not evident in post-menopausal years, would also be in keeping with a role for female sex hormones in exerting an anti-resorptive effect in pre-menopausal years. Levels of oestrogen are significantly reduced in females following menopause, leading to changes in bone metabolism. Oestrogen is thought to exert its bone-protective effects by reducing production of cytokines and growth factors (e.g. interleukin-1 (IL-1), TNF-α, IL-6 and M-CSF) which promote osteoclast formation and bone resorption (Manolagas & Jilka 1995, Kimble *et al.* 1996, Pacifici 1996, Lin *et al.* 1997, Jilka 1998, Lea *et al.* 1999). Oestrogen also suppresses RANKL-induced osteoclast differentiation (Nakagawa *et al.* 1998, Shevde *et al.* 2000). Whether our findings reflect differences in the sensitivity of male and female osteoclast precursors or osteoclasts themselves to the effect of oestrogen and other humoral/hormonal factors requires further investigation. Certainly, these gender differences in osteoclast formation and activity should be taken into account when assessing studies of bone resorption in disorders that exhibit a marked gender difference in prevalence.

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