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Effect of Age and Ovariectomy on Fibroblastic Colony-Forming Unit Numbers in Rat Bone Marrow

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SUMMARY. It is now thought that osteoblasts (OB) stem from mesenchymal precursor cells (fibroblastic colony forming units or CFU-f) in the bone marrow. As the availability of these cells may have a profound effect on bone formation, the effect of age and ovariectomy (OVX) on CFU-f numbers was studied. It was found that with increasing age the numbers of CFU-f in the marrow were drastically reduced. OVX had no effect on CFU-f levels in 4 week and 4 month old rats but raised CFU-f levels in 1 year old rats back to levels seen in younger rats. Sham operation alone had an effect on CFU-f levels in older rats and the OVX effects were indistiguishable from the sham effect until 20 d post operation when the sham levels returned to baseline. The results show that CFU-f levels follow a similar trend to the rates of bone formation seen in aged and OVX rats.

INTRODUCTION. Although it is well known that OVX in rats results in osteopaenia, it is not always appreciated that both bone formation and resorption are increased and that the net bone loss seen is due to the inability of the increased bone formation to compensate for the increase in resorption. This increase in bone formation can be seen *in vivo* as an increase in OB and mineralising surface, mineral apposition rate and serum osteocalcin levels (1, 2, 3). As these are reversed by treatment with oestradiol, it is likely that the increase in formation and resorption are regulated by common mechanisms. In contrast, the osteopaenia seen in senile osteoperosis is associated with a decrease in bone formation and resorption (4).

The osteogenic potential of bone marrow stromal cells (BMSC) has been demonstrated *in vivo* by the formation of bone-like tissue by BMSC transplantated in diffusion chambers (5) and the differentiation of BMSC into OB-like cells *in vitro* (6, 7). The role of BMSC in the regulation of bone formation, however, has not been clearly established. Recently, Manolagas and co-workers showed that a possible mechanism for the increased osteoclastogenesis seen after OVX may be an increase in the synthesis of cytokines in the bone marrow resulting in increased numbers of osteoclast (OC) precursors (8). As the OB and OC precursors in the bone marrow share a common environment and that both resorption and formation are decreased by administration of oestrogen, it seems logical that a similar mechanism may be responsible for the increase in OB seen after OVX. For this reason, we investigated the influence of age and OVX on levels of osteoblast precursor cells in bone marrow in rats.

METHODS. Bone marrow cells (BMC) were obtained from the tibias of female Wistar rats and assayed for CFU-f as described previously (9). Colonies were stained for:- a) APase with naphthol phosphate (0.05 mg/ml) in Tris (0.08 M, pH 8.5) containing fast red bb (1 mg/ml), b) calcium with 0.5% alizarin red pH 6.2, collagen with 1% sirius red F3BA in saturated picric acid and total colonies with 1% methylene blue in borate buffer (10 mM, pH 8.8). Total fibroblastic colonies were termed Col-f and considered to be derived from CFU-f. Colonies that also stained positive for APase, calcium and/or collagen were termed Col-AP, Col-Ca and Col-co respectively.

RESULTS. Rats aged 4, 14, 52 weeks were either OVX or sham operated (6 rats per group) and then killed after a further 30 days. OB precursors in the bone marrow were then assessed using the CFU-f assay as described above. OVX had no significant effect on Col-f number or on the relative proportions of Col-AP, -Ca or -co in 4 or 14 week old rats. However, the numbers colonies of all types were drastically reduced in 52 week old rats to very low levels and in these animals OVX increased Col-f and -Ca numbers to levels above (but not statistically significantly) those of the younger animals (fig. 1).

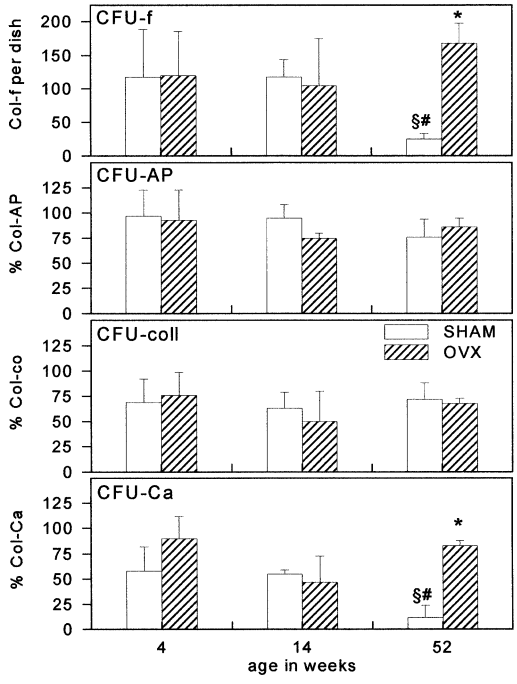

Fig. 1. Effect of OVX on bone marrow CFU-f numbers in 4, 14 and 52 week old rats. Results shown are means ± S.D. n = 6. $p<0.05$, * relative to sham, # relative to 4 week old rats, § relative to 14 week old rats.

To study the time course of this effect, groups of 6, 52 week old rats were either sham or OVX operated, killed after 0, 10, 15 and 20 days and then CFU-f levels analysed as described. It was noted that the sham operation alone increased CFU-f levels reaching a maximum after 15 days and then returning to baseline levels by 20 days. OVX also increased Col-f, -AP, -Ca and -coll levels to above those of the controls, however, after 20 days, when the sham group had returned to baseline levels, levels from the OVX group remained high (fig.2).

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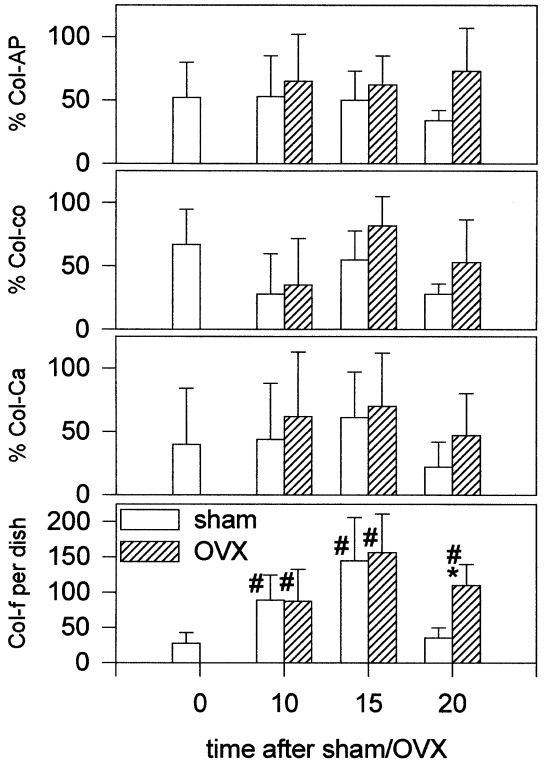

Fig. 2. Time course of sham/OVX effect on CFU-f numbers in rat bone marrow. Results shown are means ± S.D. n=6, $p<0.05$ * relative to sham, # relative to time 0.

We have previously shown that, in the presence of dexamethasone, OB precursors in the bone marrow exist as non-adherent and adherent forms and that the addition of PGE_2 could induce the transition from the former to the latter (9). To see if OVX had an effect on these non-adherent precursors, BMC from 20 d post sham or OVX groups were also treated with 10^{-7}M PGE_2. Cells from both groups behaved similarly with PGE_2 increasing numbers of all types of colonies in both groups (fig. 3).

DISCUSSION. With increasing age, the number of CFU-f in the bone marrow is drastically reduced. This confirms the findings of other groups (10, 11), however, as these cultures were also allowed to calcify, we can confirm that this reflects a decrease in the number of available OB precursor cells in the bone marrow. This may be one reason for the decrease in bone formation seen in old age. OVX had no effect on CFU-f numbers in younger animals. As these animals are still actively growing, this may be due to an already high basal level of bone turnover which would be reflected by higher basal CFU-f levels. On the other hand, OVX in aged rats, which have low basal levels of CFU-f, gave rise to a large increase in the number of OB precursors in bone marrow. In contrast to these results, Egrise et al found an increase in CFU-f levels in 4 month old rats where as we found no effect (12). The results from the aged rats show that the response obtained is highly dependent on the time of sampling. In the Egrise study the animals were killed 42 days post OVX whereas in this study they were left for only 30 d. This difference in sampling could account for the differences in response seen and would suggest that a more thorough investigation including a time course should be carried out. The fact that BMC from sham and OVX animals responded to PGE_2 with a similar increases in colony numbers of all types suggests that these differences are due to changes in absolute numbers of CFU-f in the bone marrow and not colony forming efficiency.

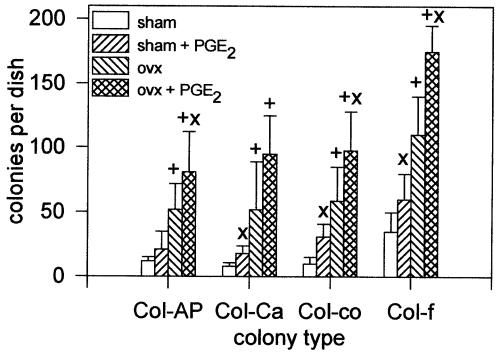

Fig. 3. Effect of PGE_2 on colony formation by BMC from OVX and sham operated rats. Results shown are means ± S.D. n = 6. $p<0.05$ + relative to sham, x relative to PGE_2 free controls.

These data outline 2 important points. Sham alone has a positive but short lived effect on CFU-f numbers in aged rats. In order to measure the OVX effect in isolation, the marrow has to be analysed at least 20d after OVX. Younger animals have high basal levels of CFU-f and because of this older animals are much better suited for studies designed to show increases in CFU-f levels.

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