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Failure of Thymic Grafts to Stimulate Resorption of Bone in the Fatty/Orl-*op* Rat

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SUMMARY

An infusion of compatible normal bone marrow stimulated bone resorption in both unthymectomized and thymectomized Fatty/Orl-op osteopetrotic rats. Bone resorption was not stimulated by compatible normal thymic grafts in either unthymectomized or thymectomized mutants. It is concluded that the thymus is not fundamental in the cure of osteopetrosis in this strain of osteopetrotic rat and that the defect lies in myeloid rather than thymic regulation of osteoclastic function.

INTRODUCTION

Considerable advances have been made in elucidating the origin of the osteoclast, the cell responsible for the resorption of bone. For instance it is now known that the osteoclast is derived from a different source from the bone forming osteoblast, the former arising from the myeloid tissue of bone marrow (1,2,3) and the latter from the mesenchymal tissues (4), although the two types of cell are thought to collaborate closely in bone resorption (5,6). A failure of osteoclastic function in mammals including man causes a disease called osteopetrosis, in which the medullary cavities of long bones are restricted by calcified material giving a characteristic opaque radiological appearance. Indeed the discovery that a graft of compatible normal bone marrow was capable of stimulating bone resorption in osteopetrotic rodents (7) gave the impetus to the advances in our knowledge of the resorption of bone at the cellular level and to its clinical application (8,9).

A report (10), apparently based on two animals, that a thymus graft alone was able to stimulate bone resorption in the osteopetrotic Fatty/Orl-op rat required confirmation. We repeated the experiments with the same mutant strain and found no evidence that the thymus alone could stimulate the resorption of petrotic bone, nor that the curative effect of bone marrow grafts was thymic dependent.

MATERIALS AND METHODS

Animals. The Fatty/Orl-op rat strain was obtained by the generosity of Dr. René Moutier S.N.R.S. Orleans-la-Source, France (11) and is maintained by brother and sister matings. Intra-strain skin grafts have proved immunological uniformity. The mutants (op/op) are fed on crushed food pellets because the teeth are unerupted and they received tap water ad libitum. The mutant op/op can be distinguished from the unaffected heterozygote (op/+) and normal homozygote (+/+) by radiography four days after birth.

Thymectomy. Just before or soon after weaning op/op mutants were anaesthetised with Avertin (Winthrop) intraperitoneally. Through a vertical skin incision the manubrium sterni was split to expose the gland which was removed by blunt dissection. The skin incision was closed with metal skin clips. Treatment by thymic or bone marrow grafts was given a few days later.

Bone marrow grafts. The femurs and tibias of young op/+ and +/+ donors were cleared of soft tissues and the ends cut off. The marrow was flushed out with TC 199 medium

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(Wellcome) with a needle and syringe into sterile centrifuge tubes and washed once with medium. Unthymectomized and thymectomized op/op mutants received 80-120 x 10^6 bone marrow cells intraperitoneally or intravenously into a tail vein.

Thymus grafts. Thymuses were removed from young op/+ and +/+ donors, matched for sex and immediately grafted into the recipients. Thymectomized op/op mutants received one lobe subcutaneously and one lobe intraperitoneally. Unthymectomized op/op mutants received a whole gland subcutaneously.

Assessment of bone resorption. Progress of bone resorption was followed by diagnostic radiography every two weeks with a conventional portable X-ray apparatus.

Post mortems. At kill a careful search was made of the superior mediastinum and any thymectomized op/op mutant with retained thymic tissue was excluded from the results (6 out of 23). The state of the thymus grafts was noted. The long bones were split and examined under a binocular dissecting microscope and several were examined in standard histological preparations.

RESULTS AND DISCUSSION

In 8 thymectomized op/op mutants, subcutaneous and intraperitoneal thymic grafts (Fig.1) did not stimulate bone resorption 50 days after grafting (Fig.2 & 3).

Five unthymectomized op/op mutants similarly grafted and followed for a longer period were also unaffected. On the other hand in 9 thymectomized op/op mutants that received a graft of compatible normal bone marrow, intraperitoneally or intravenously,

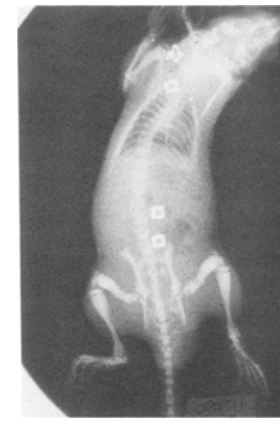

Fig.2. Radiograph 50 days after treatment of a thymectomized op/op mutant that received subcutaneous and intraperitoneal thymic grafts at the age of 21 days. The skeleton remained osteopetrotic.

resorption was complete in the long bones by 4 weeks (Fig.4,5).

In 40 unthymectomized op/op mutants given bone marrow, advanced or complete bone resorption occurred by 4 weeks after treatment.

These observations do not support the claim made by Milhaud et al (10), that a thymic graft can cure the Fatty/Orl-op mutant. Further, in contrast to Milhaud et al we found that thymectomized op/op mutants responded just as well and as quickly to compatible normal bone marrow grafts as unthymectomized mutants. This result is supported by Moutier et al (12), who bred an osteopetrotic-athymic double mutant rat in which allogeneic bone marrow grafts stimulated bone resorption. In addition we have not

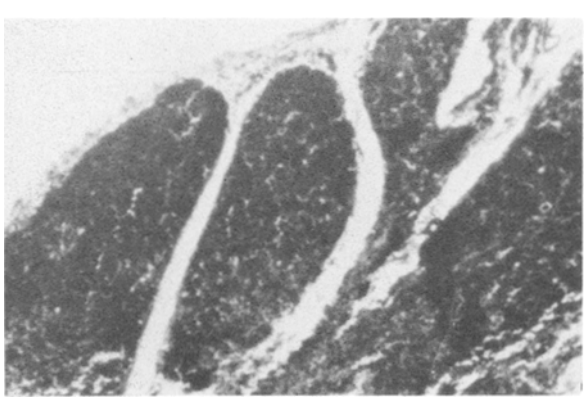

Fig.1. Histological section of an intraperitoneal thymic graft showing normal cellularity and structure (x 100).

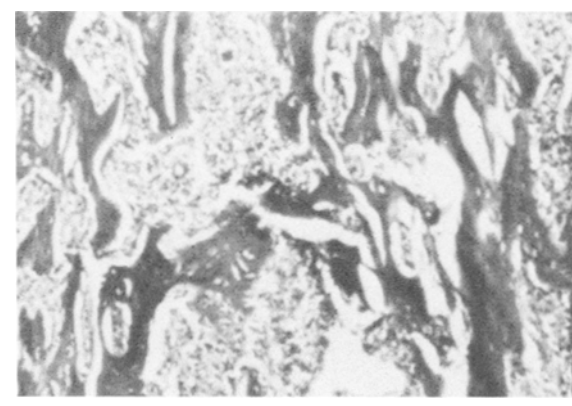

Fig.3. Histological section of part of the femur shown in fig.2. The medullary cavity is obliterated by unresorbed bone. The bone marrow is fibrotic (x 150).

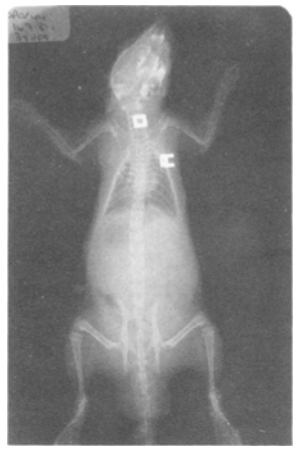

Fig.4. Radiograph 50 days after treatment of a thymectomized op/op mutant that received 120 x 10^6 bone marrow cells intraperitoneally at the age of 21 days. Bone resorption is complete.

found osteopetrotic changes in the rnu/rnu athymic rat, as might be expected if bone resorption was thymic dependent (unpublished observations). Dissociated thymic cells or thymic grafts do not stimulate bone resorption in either thymectomized or unthymectomized osteopetrotic microphthalmic mice (13,14). Further, in unthymectomized microphthalmic mutants successfully treated with compatible normal bone marrow, the thymus varied greatly in size (14). The thymic atrophy in the op/op (10) is also found in cachectic microphthalmic mice and is probably a result of the debilitating nature of the disease. The ia osteopetrotic rat appears to be the only one that can be cured by dissociated thymic cells (15), although this statement must be treated with caution. Marks (15) used a lethal dose of radiation to suppress the recipient's reaction to the graft because the ia gene was not on inbred stock. He claimed to rescue from the effects of radiation and cure the osteopetrosis by injection of thymic cells. As Marks himself pointed out in discussion hemopoietic stem cells are necessary for the restoration of hemopoiesis and the survival of the animals to the stage when cure of osteopetrosis becomes apparent. Thus, it may be concluded that the cure of osteopetrosis was brought about by the transfer of hemopoietic stem cells rather than any specifically thymic cell.

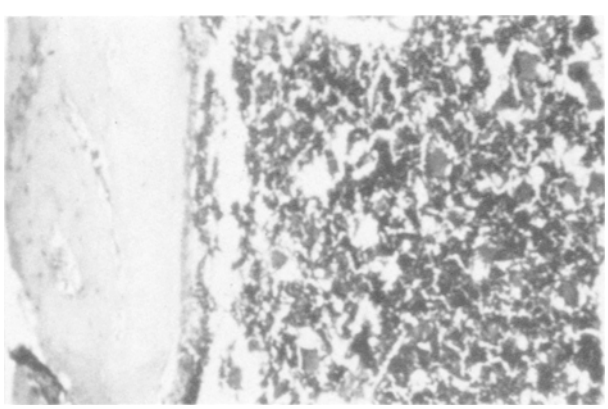

Fig.5. Histological section of part of the femur shown in Fig.4. Note that the petrotic bone has been resorbed and the medullary cavity formed contains hemopoietic bone marrow (x 100).

We postulate that the defect in the op/op mutant causing osteopetrosis is similar to that in the microphthalmic mouse and results in an incompetent osteoclast population. This incompetence is due primarily to an inadequacy in the development of the osteoclast from the myeloid tissue of bone marrow (16) rather than to any impairment of thymic influence, although we do not exclude the possibility of thymic effects on bone resorption.

REFERENCES

1. Göthlin, G., Ericsson, J.L.E. The osteoclast. Clin.Orthop.Rel.Res.120:201-231, 1976.

2. Jotereau, F.V., Le Douarin, N.M. The developmental relationship between osteocytes and osteoclasts, a study using quail-chick nuclear marker in endochondral ossification. Develop.Biol. 63(2): 253-265, 1978.

3. Ash, P., Loutit, J.F., Townsend, K.M.S. Osteoclasts derive from hematopoietic stem cells according to marker, giant lysosomes of beige mice. Clin.Orthop.Rel.Res.155: 249-258, 1981.

4. Owen, M. The origin of bone cells in the postnatal organism. Arth.Rheum.23:1073-1080, 1980.

5. Rodan, G.A., Martin, T.J. Role of osteoblasts in hormonal control of bone resorption - A hypothesis. Calcif.Tiss.Int. 33:349-351, 1981.

6. Chambers, T.J. Phagocytic recognition of bone by macrophages. J.Pathol.135:1-7, 1981.

7. Walker, D.G. Control of bone resorption by hematopoietic tissue. The induction and reversal of congenital osteopetrosis in mice through use of bone marrow and splenic transplants. J.Exp.Med.142:651-663, 1975.

8. Coccia, P.F., Cervenka, J., Teitelbaum, S.L. Kahn, A., Clawson, C.C., Brown, D.M. Reversal of human osteopetrosis by bone marrow transplantation: etiologic implications. Clin.Res.27:483, 1979.

9. Sorell, M., Kapoor, N., Kirkpatrick, D. et al. Marrow transplantation for juvenile osteopetrosis. Am.J.Med. 70:1280-1287, 1981.

10. Milhaud, G., Labat, M-L., Graf, B., Thillard, M-J. Guérison de l'ostéopétrose congénitale due Rat "op" par greffe de thymus. C.R. Acad.Sc.Paris, 283:531-533, 1976.

11. Moutier, R., Toyama, K., Charrier, M.F. Genetic study of osteopetrosis in the Norway rat. J.Hered.65:373-375, 1974.

12. Moutier, R., Toyama, K., Lamendin, H., Ballet, J-J. Guérison de l'ostéopétrose par injection de moélle osseuse allogénique chez le Rat double-mutant "ostéopétrotique-athymique' C.R. Acad.Sc.Paris, 289:919-921, 1979.

13. Loutit, J.F., Sansom, J.M. Osteopetrosis of microphthalmic mice - a defect of the hematopoietic stem cell? Calcif.Tiss.Res. 20:251-259, 1976.

14. Nisbet, N.W. Bone absorption and the immune system. Scand.J.Immunol.14: 599-605, 1981.

15. Marks, S.C. Studies of the cellular cure for osteopetrosis by transplanted cells: specificity of the cell type in ia rats (1). Am.J.Anat.151:131-137, 1978.

16. Loutit, J.F., Nisbet, N.W. The origin of osteoclast. Immunobiol.161: 1982, 193-203.