HISTOLOGICAL FEATURES OF LARGE BONE ALLOGRAFTS

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We performed biopsies during reoperation for minor complications in two active young patients 9 and 19 months after massive bone allograft implantation for bone tumour. The grafts were dead and resorption-apposition activity, when present, was predominantly in subperiosteal areas. Inflammatory infiltration was very seldom found.

Features considered as 'microfractures' or 'microcracks' were noted in the cortical ring together with the formation of woven bone, in areas with remodelling. Such cracks are likely to be of mechanical origin and do not inevitably lead to complications.

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When limb salvage is indicated for a malignant bone tumour, skeletal reconstruction can be performed using deep-frozen allografts (Parrish 1973; Merle D'Aubigné 1981; Mankin et al 1982; Hernigou, Delepine and Goutalier 1986; Loty et al 1990). The biological outcome of these allografts has been documented in animal experiments (Burwell 1969; Burchardt et al 1978; Heiple et al 1983; Goldberg and Stevenson 1987; Delloye et al 1992), and the results seemed promising for clinical practice. There is, however, a high rate of clinical failure, particularly as a result of infection (Lord et al 1988; Tomford et al 1990) or fracture (Mankin et al 1982; Berrey et al 1990; Thompson, Pickvance and Garry 1993).

These failures involve a variety of problems, including immunological rejection, inadequate revascularisation and

lack of bone remodelling (Deleu and Trueta 1965; Bos et al 1983a,b; Goldberg and Stevenson 1987; Enneking and Mindell 1991). The wider clinical use of allografts requires a better understanding of their biological response in clinical practice if complications are to be reduced. Histological studies of retrieved specimens have been reported for many cases (Parrish 1973; Kandel et al 1984; Brostrom, Nilsson and Nilson 1988; Delloye et al 1988; Oakeshott et al 1988; Enneking and Mindell 1991; Picci et al 1993), but biopsies from living patients without serious complications are rare (Salenius et al 1982; Hernigou et al 1986; Langlais and Vielpeau 1989).

We therefore report our findings in two series of biopsies performed during reoperation for minor complications.

PATIENTS AND METHODS

From 1987 to 1992, we implanted 12 large-segment allografts for skeletal reconstruction during tumour surgery in the Orthopaedic Department of the University Hospital of Nantes.

Two biopsies were performed 9 and 19 months after skeletal reconstruction during reoperation for minor complications. The allografts used had been irradiated (25 000 Gy), and were deep-frozen at -80°C .

Case 1. A 30-year-old woman was referred to us with a recurrent giant-cell tumour in the distal part of the femur. Because of its aggressiveness and subchondral spread we performed a wide resection and reconstruction using a total knee prosthesis, two screwed plates and a 10 cm allograft to replace the distal part of the femur. There were no local or general complications and adjuvant treatment was not required. Nine months later, a limitation of knee movement required further surgery. The opportunity was used to take three biopsies at 2, 4 and 7 cm from the apparently healed host-graft bone junction.

Case 2. A 33-year-old woman presented with a solitary metastasis in the midshaft of the femur from a clear-cell carcinoma of the kidney. We performed resection of part of the femoral shaft after removal of the primary tumour, with reconstruction with an 18 cm allograft, and a locked intramedullary nail. There was painful nonunion of the proximal junction and reoperation for autografting was performed 19 months later. Again, three biopsies were taken, one at 1 cm below the proximal nonunion and the others at 2 and 5 cm above the apparently healed distal junction.

Methods. The specimens were fixed in neutral formaldehyde and either decalcified by electrophoresis (case 1) or

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not decalcified (cases 1 and 2). Sections of decalcified samples (5 μ m) embedded in pycolitis (PSF 115) were stained with haemalum-phloxine-saffron and studied by light microscopy. Non-decalcified specimens were embedded in polymethylmethacrylate (PMMA) after dehydration in alcohol and cut by a low-speed diamond saw into sections approximately 100 μ m thick. Microradiographs of these sections were obtained (Phillips PW IOI2, 20 min, 20 kV, 25 mA) and studied by light microscopy. Some sections were stained with monochrome cyanine. The remaining PMMA-embedded bone from the second case was polished and carbon-coated for backscattered electron microscopy (Jeol JSM 6300). Finally, dispersive X-ray microanalysis was performed on this specimen (Link Pentafel, Oxford, UK).

RESULTS

Neither case showed any evidence of deep infection or local tumour recurrence.

Case 1. Both distal biopsies showed the same pattern. There was a thin layer of new bone surrounding the cortical ring, with predominantly osteoclastic activity in subperiosteal areas. This had enlarged the Haversian canals, which were incompletely filled by osteoblastic cells. The newly deposited bone was not fully mineralised, and the bone marrow was completely replaced by fibrous tissue.

There was very little remodelling in the proximal biopsy, but there were two longitudinal cracks, 0.1 to 0.5 mm in width, which were partially filled with non-lamellar fibrous tissue. Microradiography showed this to be less mineralised than the allograft (Fig. 1). With monochrome cyanine staining, their red-orange colour contrasted markedly with the colour of the remaining graft. These features were suggestive of the formation of woven bone in the cracks.

Case 2. The two proximal biopsies showed intense superficial osteoclastic activity, with wide Haversian canals,

formation of new bone within these canals and increasing peripheral porosity. There were no lamellar bone layers around these areas. Both distal biopsies showed the pattern of 'microcracks' seen in case 1. No areas of remodelling were found in the cortical graft, which appeared necrotic on microradiography and on SEM (Figs 2 and 3). Cracks, 0.25 to 0.4 mm wide, were completely filled with non-lamellar tissue clearly visible in polarised light (Fig. 3b). Microradiography, SEM, monochrome cyanine staining and X-ray microanalysis showed some mineralisation of this non-lamellar tissue, which was suggestive of woven bone. Microradiography, however, showed that this woven bone was less mineralised than the surrounding cortical graft. This was confirmed by microanalysis at 36 points on non-lamellar bone and 48 points on lamellar bone graft which showed $28.28 \pm 3.41\%$ and $36.21 \pm 1.52\%$ respectively of the total element weight for Ca and P. The immature, less mineralised bone had a Ca/P ratio (3.14 ± 0.34) which was higher than that of the cortical allograft (2.36 ± 0.8).

DISCUSSION

A larger number of biopsies would be required to assess the behaviour of deep-frozen allografts, but our two series of biopsies of 'functioning grafts' does provide some indication of their biological outcome. With the exception of the reports of Salenius et al (1982) and of Langlais and Vielpeau (1989) most authors either give few details about histological results or review only allografts retrieved after clinical failure (Parrish 1973; Kandel et al 1984; Broström et al 1988; Delloye et al 1988; Oakeshott et al 1988; Enneking and Mindell 1991; Picci et al 1993).

Our two cases both showed the main features reported in the literature after the use of allografts. The grafts remained dead and anucleate, even after follow-up of 9 and 19 months, and necrotic cortical bone predominated (Merle D'Aubigné 1981; Kandel et al 1984; Hernigou et al 1986;

Fig. 1a

Fig. 1b

Case 1. Figure 1a – Polarisation microscopy shows two microfractures (m), the left completely filled and the right incompletely filled with non-lamellar tissue ($\times 20$). Figure 1b – Microradiography shows that the non-lamellar tissue is less mineralised than the remaining graft ($\times 20$).

Fig. 2a

Fig. 2b

Case 2. Figure 2a – SEM of the full cortical thickness with the periosteal surface on the left. Two strips (m), 0.25 to 0.4 mm wide, are filled with less mineralised tissue than in the rest of the graft (g). Figure 2b – Higher magnification shows a microcrack (m) completely filled with the less mineralised tissue in sharp contrast to the cortical graft (g).

Fig. 3a

Fig. 3b

Case 2. Adjacent sections of the same biopsy on a microradiograph (a) and under polarised light (b) show the strips of less mineralised tissue embedded in the remaining strongly mineralised part of the graft ($\times 20$). This was confirmed by monochrome cyanine staining (not shown).

Broström et al 1988; Oakeshott et al 1988; Langlais and Vielpeau 1989). This was in very close relation to living bone (Salenius et al 1982; Delloye et al 1988) and a thin new layer surrounded the cortical ring (Kandel et al 1984; Delloye et al 1988; Enneking and Mindell 1991), with some bone remodelling in subperiosteal areas (Delloye et al 1988; Enneking and Mindell 1991).

We found that large longitudinal cracks, which we presume to be microfractures, had spread into the diaphyseal cortex. The presence of mineralised tissue in these cracks excluded any possibility that they were artefacts. Such microcracks have been described in freeze-dried allografts (Pelker et al 1984), but our grafts had undergone no specific treatment other than freezing to -80°C and irradiation by gamma rays to 25 000 Gy, which are not known to cause such complications. In patients with an allograft in a weight-bearing segment and no oncological or orthopaedic

complications, such cracks are likely to be of mechanical origin.

Although there is no demonstrable change in the initial mechanical properties of dead bone (Enneking et al 1975; Pelker et al 1984), outer and inner graft resorption must increase the porosity and impair its mechanical properties (Enneking et al 1975; Burchardt et al 1978; Delloye et al 1992). Fractures may be related to more rapid revascularisation in subchondral bone with resultant resorption leading to weakness (Berrey et al 1990; Gebhardt, Roth and Mankin 1990). Our study suggests, however, that microfractures occur in graft areas in which no bony apposition is found and in areas of bone without subchondral resorption. This also applies to macrofractures in allografts (Zehr et al 1991; Thompson et al 1993).

Microcracks do not inevitably cause harm; there is no evidence that they are the first step in the formation of

macroscopic fractures. As reported by Zehr et al (1991) and Thompson et al (1993) both chemotherapy and plate fixation are significantly correlated with allograft fractures. Neither of our patients had received preoperative chemotherapy, a plate had been used only in the first case and the screws were over 3 cm from the biopsy site. The defects created by these microcracks were filled by mineralised non-lamellar woven bone, and may thus be a possible route for new bone apposition, especially in necrotic areas without subperiosteal remodelling.

Such behaviour has been reported at the macroscopic level: fatigue fractures in allografts sometimes heal without further surgery (Hernigou et al 1986; Berrey et al 1990; Thompson et al 1993). It can be presumed that this microscopic and macroscopic behaviour may be due to the presence of an osteoinductive factor, such as the bone morphogenetic protein contained in cortical bone matrix (Urist et al 1967), which could have been released by the microfractures. When the allograft is supported by an implant for fixation, it is likely that microfractures allow revascularisation and bony apposition, especially in necrotic areas.

All the illustrations showed some compression of the Haversian systems in the grafted bone. There are two possible explanations for this appearance: either a large amount of new bone had filled the Haversian canals or the allograft had the same pattern at the time of implantation and there was no bone turnover. Allografts, in intracortical areas, show very little resorption and new bone apposition. It is likely that the appearance is caused by the type of bone used for allografts: this is generally taken from young, previously active donors with thick heavy femoral cortices.

More work is required on the incidence and possible effects of such microfractures in allografts; this will need further biopsies from living patients with no major complications from their allografts.

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