Lipid extraction decreases the specific immunologic response to bone allografts in rabbits

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The immunologic response against frozen bank bone is generally considered to have no clinical importance. In a previous study, the bone formation rate in frozen allografts was measured at standardized conditions in the Bone Harvest Chamber. By defatting frozen allografts, the bone formation rate increased. In the present study, the effect of defatting was further investigated, as the specific immunologic influence was excluded by using autografts. Pairs of grafts were frozen and one graft in each pair was defatted with chloroform/methanol. With these autografts there was no difference in bone formation rate between defatted and non-defatted implants, measured with ^{99m}Tc–MDP. Combined with the previous experiments, the results indicate that the increased bone formation rate in defatted allografts is caused by the removal of specific cell surface antigens. Thus, the immunologic reaction to bank bone can be diminished by defatting.

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In frozen bank bone, the marrow and bone cells are dead and the specific immunologic reaction is reduced, compared with fresh allografts. In clinical use, frozen allografts usually become sufficiently incorporated, but in some cases they fail (Burchardt 1987). Defatting with chloroform/methanol facilitates the incorporation of new bone in animal models (Prolo et al. 1982, Aspenberg and Thorén 1990). In our previous rabbit experiment, we could not determine whether this facilitation was due to decreased specific immunologic reactions or to a decreased general inflammatory response to the marrow debris. The aim of the present study was to evaluate the role of the specific immunologic reaction to cell surface antigens by using autografts in a similar experimental model.

Material and methods

We used the Bone Harvest Chamber (Albrektsson et al. 1984, Aspenberg and Thorén 1990), a threaded titanium cylinder, which is screwed into a 7 mm hole in the tibia, just anterior to the medial collateral ligament. The chamber is pierced by a 1-mm wide canal, which is placed so that the openings are covered by cortical bone. The skin wound is closed subcutaneously. 5 weeks after implantation, the canal is spontaneously filled with a cancellous bone rod (approximately 1×1×5 mm). Such cancellous bone rods can be harvested repeatedly from the chamber.

We implanted the Bone Harvest Chamber bilaterally in the tibia in 9 adult lop-eared rabbits (>9 months old, weighing 4.6–6.2 kg). 2 rabbits were excluded because of wound infections which were obvious before opening the chambers. In the remaining 7 rabbits, altogether 11 grafting procedures were performed. The bone rods from the first harvests were not used. Rods harvested after 5 more weeks were kept as autograft bones (primary rods) for implantation later. The pair (one from each tibia) was kept sterile and frozen at –70 °C for at least 4 weeks. The day before implantation one bone rod in each pair was deposited in a test tube with 1/1 chloroform/methanol overnight, then rinsed 3 times with methanol, 3 times with water and finally deposited in saline. The non-defatted rod was thawed in saline before implantation. The pair was implanted in the Bone Harvest Chambers as autografts. The defatted rod was alternately inserted on the right and left sides. The grafts were harvested after 3 weeks, at which time we believe the mineralization process to be at its maximum level, according to previous experience. 3 hours before harvest, the rabbits received an injection of 45 MBq ^{99m}Tc-labeled methyldiphosphonate (MDP).

The bone-forming activity was measured by ^{99m}Tc-MDP scintimetry immediately after harvest. The bone rods were decalcified, sectioned longitudinally and stained with hematoxylin-eosin.

For each pair of grafted rods, the scintimetric values were analyzed by forming a ratio between defatted and non-defatted. These ratios were tested for a difference from 1 by the Wilcoxon test.

Results

No difference in scintimetric activity was found between defatted and non-defatted autografts. There was no difference between left and right sides or between defatted and non-defatted grafts in the primary bone rods, when they were harvested to become grafts.

On histological examination, most of the bone appeared to be newly formed. The total bone mass appeared larger than in the nongrafted chambers used in other studies. Most of the bone had cells in the lacunae and osteoblast linings, but areas of dead bone could be defined. There was a difference between the defatted and non-defatted autograft samples. On blinded examination, defatted samples contained more new living bone and less remaining dead implanted bone in all pairs. The bone structure was more mature, with thin organized laminas in contrast to the non-defatted samples, where there was more woven bone. Some frozen primary rods were examined directly after thawing. They contained small fat droplets in the former marrow tissue. In the recovered non-defatted grafts some of these fat droplets had not vanished. In the recovered defatted grafts there was only loose connective tissue and new marrow between the bone laminas.

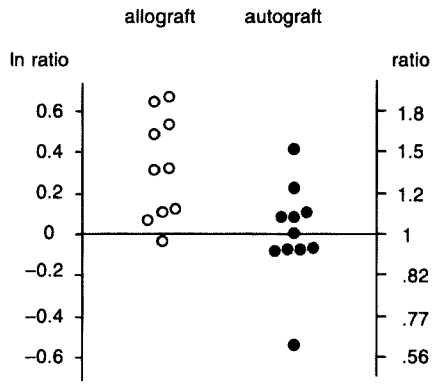

Figure 1. Scintimetric activity 3 weeks after grafting. Each value represents a ratio between defatted and non-defatted grafts.

Discussion

According to our previous studies with allografts in rabbits, the effect of lipid extraction could be due to a reduction in specific immune reactions and/or a reduction in a general inflammation that disturbs bone formation (Aspenberg et al. 1988b). In the bone ingrowth canal of our titanium chamber, the inflammation caused by small amounts of demineralized bone matrix is enough to reduce bone formation (Aspenberg et al. 1988a). The time needed by the recipient animal to remove all the debris from the bank bone may also further delay bone formation.

The experimental model in the present study was equal to the previous allograft experiment (Aspenberg and Thorén 1990). In that allograft experiment, defatted grafts had an increased scintimetric activity compared to non-defatted (Wilcoxon $P < 0.005$). This is different from the present autograft study (Mann-Whitney $P < 0.01$), in which defatting did not have this effect (Figure 1). Thus, the greater effect of defatting allografts must be due to removal of allogenic antigens. The ^{99m}Tc-MDP uptake 3 weeks after implantation demonstrates the bone formation activity at that time. In our chamber model, ^{99m}Tc-MDP uptake correlates with ^{45}Ca uptake (r 0.8; $P < 0.01$). Both allo- and autogeneic grafts, whether defatted or not, will probably become fully remodeled with time in this model. Thus, ^{99m}Tc-MDP incorporation will decrease and equalize between different kinds of grafts. In humans, where full incorporation of allografts cannot be expected (Enneking and Mindell 1991), a similar difference between defatted and non-defatted grafts may persist and improve the end result.

Our study suggests that removal of cell-surface antigens by destruction of the cell membranes plays an important role during early phases of incorporation. By freezing or lyophilization, the marrow and bone cells die and the specific immunologic reaction against frozen or lyophilized bone is reduced compared with fresh allograft implantation (Heiple et al. 1963, Burchardt 1983). It is difficult to measure the specific immunologic reaction. In some studies, using a microcytotoxicity assay, sensitization towards frozen allografts did not occur (Bos et al. 1983). In others, there was sensitization against some of the samples (Friedlaender et al. 1976, 1978, 1984). Another method for measuring the immunologic influence is to use immunosuppression. In cases with a strong transplantation barrier, immunosuppression leads to an increased incorporation (Goldberg et al. 1984). In the present study we avoided the problems of measuring a specific immunologic response by comparing standardized allo- and autografts. We hypothesized that the increased bone formation after defatting was due to a decrease of both an immunologic response and a general inflammatory reaction. The decreased immunologic response seems to be the main cause of the effects of lipid extraction, because there was a clear difference between the allograft and the autograft groups. However, as we found histological differences within pairs in the autograft group, defatting also seems to diminish the non-specific response.

It has been considered advantageous, but not practically feasible, to HLA-match bone grafts (Stevenson et al. 1992). Lipid extraction destroys cell surface antigens. This may be an easier way to optimize graft acceptance than HLA tissue-matching.

This research has practical importance. We have shown that immunological barriers reduce bank bone incorporation and that this effect can be diminished by lipid extraction.

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