Twenty-Five Years of Clinical Experience with Bone Banking in Israel*

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Abstract

Background: Bone banking and the clinical use of banked tissue are the most common forms of allopreservation and transplantation in modern medicine.

Objectives: This article reviews 25 years (1973–98) of experience in bone banking in Israel.

Methods: A nationwide survey on the clinical application of the banked musculoskeletal tissues during 1996 was conducted by means of a written questionnaire sent to all orthopedic departments in Israel.

Results: The response rate to the questionnaire was 84%. A total of 257 cases were allocated bone allografts: the majority comprised 225 spongy bones, 26 were massive bone allografts and 6 were soft tissue allografts.

Conclusion: Improvement of quality control and quality assurance of the banked tissues, together with development of skills in the use of osteoinductive and osteoconductive materials, cast the future of musculoskeletal tissue banking.

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The clinical practice of bone banking began during the 1973 Yom Kippur (Day of Atonement) war. Many wounded soldiers suffered from traumatic bone loss, mainly due to high velocity missile injuries. The urgent need for human bones to replace these defects called for the establishment of a "bone bank" based on bones from amputated limbs. These "heroic" procedures were the result of the initiative and drive of the late Professors I. Farine and H. Horoszowski. The successful results in these severely injured limbs later led to heuristic efforts in the research of musculoskeletal tissue banking and its clinical application [1]. The National Bone Bank was formally established In 1987 and initiated the delivery of bones and other musculoskeletal tissues to most of the orthopedic departments in Israel. This could not have been achieved without the active support and participation of the late Prof. H. Horoszowski.

We report here the results of the first nationwide survey on the use of musculoskeletal tissues. The results of this survey, a review of our experience in the clinical use of banked bones, and future expectations are presented and discussed.

Materials and Methods

The first Israeli nationwide survey on the use of allogenic musculoskeletal tissue was undertaken at the beginning of 1997. A questionnaire was distributed among all the orthopedic departments in Israel. The survey addressed the following: a) the number of cases in which allogenic bone was used during 1996; b) the type of banked tissue used, i.e., massive allografts, osteoarticular allografts, femoral head used either as crushed to chips or as structural grafts, and tendons; and c) procedures at local institutions regarding the use of these tissues. Consequently, records of all the cases at our institution (level I trauma center) in which musculoskeletal allografts were used between 1987 and 1996 were retrieved and analyzed. We received an 84% response to the questionnaire, and the reported parameters were recorded.

Results

According to a 1996 nationwide survey that received a response of 84%, allogenic musculoskeletal tissues were used in 257 cases. Massive bone allografts were used in 26 cases (11 segmental, 13 structural, and 2 osteoarticular); crushed spongy bone chips were used in 225 cases, and soft tissue allografts in 6 cases (2 to reconstruct the anterior cruciate ligament, 2 to reconstruct the Achilles tendon, and one each to repair the rotator cuff in the shoulder and elbow ligaments). A follow-up chart attached to every bone was returned in 95% of the cases. No adverse reaction was reported by the surgeons who carried out the transplants, except for two cases of *Streptococcus epidermidis* infections (nosocomial?), which resolved after antibiotic therapy. Of the overall national use, 42%, 41% and 16% of the massive allografts, femoral heads and tendon allografts, respectively, were used at our institution.

Since 1996, in 23% of the institutions that responded, a local bone bank for femoral heads has been established and is managed in accordance with the Ministry of Health regulations for bone banking. The remaining institutions responded that they mostly rely on the National Bone Bank for their allogenic needs.

Allogenic musculoskeletal tissues used at the Sheba Medical Center, 1987–96

Between November 1987 and December 1996, allogenic musculoskeletal tissue from the bone bank was supplied to 622 patients. These consisted of 122 massive bone allografts (19%), 6 tendon allografts (2%) and 495 femoral heads (79%).

The clinical indications for use according to the type of bone are summarized in Table 1. A relatively even distri-

* A Tribute to Professor Henry Horoszowski

bution to tumor, trauma and revision total joint in the massive allograft cases is seen, compared to femoral heads, which were used mostly to augment comminuted fractures.

The reported complications associated with the use of these tissues comprised infection in 56 cases (9%), non-union of massive allograft necessitating bone graft in 9 (7%), and fracture of the allograft in 5 cases (4%). Of our 1996 cases, two developed infection — namely two *S. epidermidis,* and one methicillin-resistant *Staphylococcus aureus*. The first two cases resolved after antibiotic therapy, and the methicillin-resistant *S. aureus* case underwent debridement. In one case, non-union of massive allograft arthrodesis of the knee necessitated autogenous iliac bone grafting. During 1996, there were no fractures among the allografts. However, we retrieved five cases with fractures from our records: three proximal tibia, one distal femur and one humerus. The fractures were treated by exchange of the allograft in three cases and internal fixation in the other two. From these fractures it was concluded that in the proximal tibia the metaphyseal cortical form of allograft should be avoided, and that preferably cortical only, or "composite" cortical allografts and prostheses should be used.

Apart from these patients who were operated on in the Orthopedic Wing, there were an additional 53 neurosurgical patients in whom specially shaped tricortical iliac crest grafts were used as structural grafts in cervical spine fusion. No adverse reactions were reported following these operations, and full union was achieved in the grafts. However, these patients, who are followed by the neurosurgical ward, are beyond the scope of this report.

Discussion

The clinical use of banked musculoskeletal tissue has gained widespread acceptance in modern orthopedic surgery [2–4]. Estimated at over a million a year throughout the world, such allotransplantations are thus the most common form of allotransplantation performed [5–8]. The results of our survey reflect the world trend towards the use of these grafts — namely, the use of crushed femoral heads to augment comminuted fractures, and massive allografts for structural defects. In the former form the allograft serves as a scaffold for new host bone growth and replacement, and in the latter as a permanent structural solution [9–12].

Accordingly, orthopedic scientists are seeking osteoinductive and osteoconductive materials that will promote spongy bone incorporation and replacement, such as the bone morphogenic protein group of peptides. Alternatively, in the massive grafts, incorporation is expected only at the graft/host junction, otherwise whole graft incorporation may cause weakening and breakage. Thus, it is our aim to preserve selective osteoinductive and osteoconductive properties of these grafts. However, the increasing occurrence of transmissible diseases, such as AIDS or hepatitis, urges strict regulations concerning musculoskeletal graft control and assurance [13,14].

Often, the mandatory procedures for graft preparation inevitably have some deleterious effects on the osteoinductive and osteoconductive properties of the grafts [15–17]. It is clear, therefore, that a major aspect of the future of musculoskeletal tissue banking and its clinical applications lies in the following processes:

- Developing capabilities to harvest and restore embryonal musculoskeletal tissue
- The ability to modulate stored musculoskeletal stem cells into a preplanned scaffold of allografts
- Improving screening and sterilization methods of the grafts with minimal jeopardy to its biomechanical properties.

In conclusion, strict adherence to national and professional regulations of tissue banking is necessary to ensure the high quality of precious human tissue earmarked for use in transplantation.

Table 1. Indication of bone allografts at Sheba Medical Center, 1987–96

	Massive allograft (n = 122)		Femoral head (n = 495)	
Tumor	43	35%	173	35%
Trauma	42	34%	229	47%
Revision total joint	37	31%	93	18%

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Capsule

Gene may provide new route to potent vaccines

For protection against an infectious disease, few things can beat a vaccine made of the living organism. But every live vaccine is a balancing act: the pathogen has to be vigorous enough to trigger an immune defense by the host, yet too weak to lead to serious illness. A team from the University of California, Santa Barbara, has come up with a possible solution. They have found a gene that seems to orchestrate the activity of dozens of other genes needed for a full-blown infection by *Salmonella typhimurium*. When they knocked out the gene, the bacteria became powerless to cause disease but still elicited a fiery immune response in mice; in other words, the bacteria had apparently become the ideal vaccine.

Because the gene that produces the protein DNA adenine methylase (Dam) is shared by many other pathogens, the researchers believe that easy-to-produce vaccines for a range of diseases — from meningitis to the plague — may lie within reach. For some of these, no vaccine is currently available. Pathogens like *Salmonella* have many so-called virulence genes, which are switched off when the bacteria are living on a petri dish or a chicken in the refrigerator, but spring into action once they enter the gut of a mammal where they help the bacterium to penetrate the gut lining, travel throughout the body, and use the host's resources to grow and divide. Mahan's team had already discovered some 250 of these genes in *Salmonella.* Little was known about how they are regulated, although earlier studies had shown that a protein called PhoP controls the expression of some of them.

In their search for other regulators, the team tried many candidates. One of them was Dam, which was known to be involved in DNA repair. In *Escherichla coli* strains that cause urinary tract infections, Dam also controls the formation of pili. To see whether the protein might have a wider role, the team created a S. *typhimunium* strain that lacked the *dam* gene and found that it was utterly innocuous to mice, even in huge doses; when administered orally, the bacteria entered the mucosal tissue lining the gut but did not colonize organs such as the liver and the spleen. Measurements of gene activity showed that the absence of Dam altered the expression of at least 20 virulence genes. According to Mahan, further experiments have shown the real number to be at least 40. Dam apparently acts as a master switch for these genes, since it can glue methyl groups to DNA strands at specific sites. By doing so, the enzyme decreases the ability of some regulatory proteins to bind to DNA, while increasing that of others. Each of these proteins can, in turn, up or slow down the transcription of one or more genes.

The authors claim that this finding may give scientists several new weapons in the relentless race against bacterial infections. For one, drugs that block Dam could slow down bacterial growth and possibly result in a whole new generation of antibiotics. When the team immunized 17 mice with Dam-negative *S. typhimurium,* the strain proved to be an effective vaccine. Five weeks later the immunized mice all withstood terrifying doses of the normal bacterium — up to 10,000 times the amount that killed nonimmunized mice. To explain how such enfeebled bacteria could provoke such a strong immune response, Mahan theorizes that knocking out *dam* actually renders the bacteria easier to detect by the immune system. Normally, bacteria turn every gene on as briefly as possible to prevent the host's immune system from detecting and attacking foreign proteins. But with the Dam switch shut off; some genes may be expressed for a very long time, within easy sight of the host.

Because different *Salmonella* strains probably share quite a few proteins, one Dam-negative strain may even elicit an immune response that also covers others. In a work not yet pubished, says Mahan, the team shows that mice immunized with *S. typhimurium* were also immune to a related *Salmonella* strain that infects chickens and eggs. The reverse was also true, and Mahan is testing whether the vaccine covers more of the 2,500 *Salmonella* strains currently known. If it does, inoculating cattle and chickens with a vaccine based on the technique may help banish *Salmonella* from the food chain, although it will have to compete with other vaccines in various stages of development.

Genetic studies have shown that other gut-colonizing bacteria like *Vibrio cholera, Haemophilus influenzae, Yersinia pestis, Shigella,* and *Treponema pallidum* all have Dam; perhaps they too can be crippled by knocking out the *dam* gene.

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