THE SITE DEPENDENCE OF THE ARTICULAR CARTILAGE TRANSPLANT REACTION

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Allografts of immature joint cartilage from the knees of lambs were transferred heterotopically into an intramuscular site in animals which had been presensitised by two sets of skin grafts from the same donors. All of these grafts were found to be largely destroyed by the immune response as early as four weeks after transfer. Similar grafts transferred orthotopically into the knees of the recipients, on the other hand, were found to be thriving even after twelve weeks and evoked a minimal response. Heterotopic autografts also provoked a mild though non-specific inflammatory reaction which the orthotopic grafts did not.

It is concluded that cartilage matrix is capable of protecting grafts to a remarkable degree even from a severe immunological assault but only when the nutrition is adequate. It is suggested that the conflicting results of similar previous experiments may be explained by variations in the nutritional state of the graft which may be affected by the technique of transplantation used.

Reports of the prolonged survival of allografts of cartilage have led to its recognition as a “privileged” tissue in relation to the homograft reaction. However, this privilege is clearly not absolute, and while some grafts have indeed survived for many months and even years without significant evidence of rejection (Young 1945; Gibson, Davis and Curran 1958) other similar transfers have shown varying degrees of round-cell infiltration and cellular death (Loeb 1926; Dupertuis 1941; Craigmyle 1955). Even within the same experiment examples of remarkable survival may be found side by side with others showing strong rejection (Loeb 1926; Gibson et al. 1958; McKibbin 1971; Lloyd et al. 1973) and these inconsistencies have not been satisfactorily explained.

It has been established that chondrocytes possess transplantation antigens common to other cells in the same animal (Moskalewski, Kawiak and Rymaszewska 1966; Heyner 1973; Elves 1974) but these can be displayed only after enzymatic dissolution of the surrounding matrix, thus supporting the view put forward by Bacsich and Wyburn in 1947 that it is this substance which is responsible for the immunological privilege enjoyed by hyaline cartilage which is at best but weakly antigenic. It is less clear, however, whether the cartilage matrix is able to confer additional protection by acting as a barrier to the immune response in an already sensitised animal.

Heyner (1969) found that while a series of allografts carried out with cartilaginous limb rudiments in rats survived intact in a non-presensitised animal, a previous skin graft caused them to be rapidly rejected. In contrast, Craigmyle (1960) reported that in rabbits presensitisation did not appear to impair the survival of grafts of costal cartilage, and McKibbin (1971) also found that grafts of joint cartilage could survive as long as fourteen months in the presence of a well-developed cell-mediated response. Elves and Zervas (1974) reported that even when highly immunogenic osteocartilaginous heterografts were used, viable chondrocytes remained as long as seven weeks after grafting, suggesting that there was interference with the normal process of rejection.

These conflicting results are characteristic of this field of study and in attempting to reconcile them it is important to ensure that we are comparing like with like. In many of the experiments already cited the grafts were heterotopic and usually consisted of free transfers to a subcutaneous or intramuscular site. It has been suggested elsewhere (McKibbin 1971; Răliş and McKibbin 1974) that the resistance of cartilage to rejection is dependent on an adequate source of nutrients, and it may be doubted therefore whether such free grafts are adequately provided for in this respect—certainly their circumstances do introduce a degree of unpredictable variability which may be responsible for some of the inconsistencies referred to earlier.

In order to test this hypothesis a comparison has been made between the fate of such a series of free allografts of joint cartilage from lambs with a series of comparable grafts transferred orthotopically into the knee of the host by a technique previously described.
(McKibbin 1971). In these latter circumstances the normal mechanism of nutrition from both the synovial fluid and subchondral bone becomes rapidly re-established, and if nutrition is an important factor in survival the latter may be expected to fare better than the free transfers.

In order to ensure a strong and uniform immunological response the host animals were first presensitised with two sets of skin allografts from the same donor who supplied the cartilage, and in order to identify any differences arising from nutrition alone a series of autografts in the two anatomical locations were also studied.

**MATERIAL AND METHOD**

Altogether forty-five sheep were used. Donor cartilage which can only be taken from very young animals was obtained from twelve lambs which were obtained from a distant farm to avoid any possible consanguinity with the hosts. These were of unspecified breed. The remaining thirty-three sheep were of the Kerry Hill/Beulah Cross strain and were bred on the experimental station.

<table>
<thead>
<tr>
<th>Table I. Experimental material</th>
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<tr>
<td>Orthotopic transplants (and duration)</td>
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<tr>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Autografts</td>
</tr>
<tr>
<td>4 (12 weeks)</td>
</tr>
<tr>
<td>Allografts</td>
</tr>
<tr>
<td>11 (12 weeks)</td>
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<tr>
<td>Total</td>
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Twenty-four of these, aged six to ten months, were used as hosts for the allografts and the remaining nine, which were young animals aged six to nine weeks, were used for autografts.

**EXPERIMENTAL PLAN**

**Autografts.** The donor cartilage was taken from the knees of nine young sheep and, under the same anaesthetic, it was transferred either to an identical site in the opposite knee (orthotopic autografts, five animals) or, alternatively, to the gluteal muscle (heterotopic autografts, four animals). All nine sheep were killed at twelve weeks and the specimens were examined in the same way as the allografts (Table I).

**Allografts.** Each of the twelve donor lambs was assigned to two adult sheep. Under general anaesthesia, skin grafts were transferred from the former to the latter and placed alongside similar skin autografts for comparison. Approximately eight days later, when the skin allografts were showing signs of rejection, a further series of skin grafts from the same donor were then performed. At the same time articular cartilage grafts were taken from the knee of the donor and implanted either into the articular surface of the knee of the host (orthotopic) or into the gluteal muscle (heterotopic). Initially, therefore, there were twelve animals in each group but one died after operation, leaving eleven animals with orthotopic grafts and twelve with heterotopic grafts for study. The animals bearing heterotopic grafts were killed in groups of four at four, seven and nine weeks after operation while the orthotopic group were left for twelve weeks. The grafts were then removed together with the surrounding tissues and examined by morphological techniques to be described.

**Technique for skin grafts.** In each animal three discs of full thickness skin approximately 1.5 centimetres in diameter were transferred from the flank of the donor to a corresponding defect in the flank of the recipient. These were placed alongside three similar skin autografts which had been removed completely and then returned to their original site (Fig. 1). All the grafts were held in place by an occlusive dressing which was removed after five to seven days. A second set of skin grafts were transplanted eight days later in the same way together with the cartilage allografts.

**Technique for cartilage grafts.** All grafts in each group consisted of a rectangular plaque of cartilage approximately 1 centimetre square raised from the lower femoral condyles of the donor animal by a technique previously described (McKibbin 1971). Since the donors were immature, this consisted of articular cartilage of reasonable

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**Fig. 1** Sheep skin autografts (AU) and first set allografts (ALL) eighth day after transplantation. The rejected allografts are in obvious contrast with the healed autografts.

**Fig. 2** Section through the articular cartilage graft done routinely before transplantation in order to assess its appearance and to ensure that no bone was transplanted with it. (Haematoxylin and eosin, ×60 approx.)
thickness but without any underlying bone. A portion of each specimen was removed for microscopic study to confirm this and also to establish the thickness of the graft (Fig. 2).

For the orthotopic grafts a bed was prepared very accurately in a corresponding position in the knee of the host so that the graft could be retained by a push fit assisted by the pressure of the overlying patella. Retaining sutures were not used in these experiments.

Table II. Results of orthotopic and heterotopic articular cartilage transplantation

<table>
<thead>
<tr>
<th></th>
<th>Orthotopic grafts</th>
<th>Heterotopic grafts</th>
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<tbody>
<tr>
<td></td>
<td>Autografts</td>
<td>Allografts</td>
</tr>
<tr>
<td><strong>Graft:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in size</td>
<td>27%</td>
<td>40%</td>
</tr>
<tr>
<td>Active ossification</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Amount of destruction by host</td>
<td>1%</td>
<td>6%</td>
</tr>
<tr>
<td>Amount of necrosis</td>
<td>4%</td>
<td>9%</td>
</tr>
<tr>
<td><strong>Host's reaction:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity of destruction</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Small round cells</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Fibrous tissue</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Granulation tissue</td>
<td>±</td>
<td>+</td>
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The heterotopic grafts were buried about 2 centimetres deep in the middle portion of the gluteus maximus muscle just below the iliac crest. A cut-gut stitch through the overlying aponeurosis was used to prevent displacement.

In the autograft experiments the graft was taken from one knee and transferred by a similar technique to a corresponding position in the contralateral joint or into the gluteal muscle.

Morphological assessment. The gross appearance of the grafts was noted and their thickness measured. By comparing this measurement with the height of the slice taken from the graft at the time of implantation, any change in its thickness could therefore be calculated. In some instances this was increased due to the continuing growth of the cartilage, while in the others it was reduced by a destructive process of the tissues from the host.

Specimens containing bone were first decalcified in formic acid and histological preparations were made of all the excised material. Several sections were taken from each graft and stained with haematoxylin and eosin, Masson's trichrome, Lendrum's MSB and by the Unna-Pappenheim method.

During the subsequent microscopic examination quantitative estimates of the amount of destruction were made by comparison with the original thickness of the graft. All areas of dead cartilage were recorded, directly measured on histographs (Ráliš and Blake 1976) and the amount expressed as a proportion of the whole grafted area on that particular section. The type of cellular response to the graft was recorded and an attempt was made to quantify its intensity by the award of a number of arbitrary "plus" gradings. Finally, using a similar grading system, an estimate was made of the extent to which endochondral ossification of the graft was proceeding.

RESULTS

Skin grafts (Fig. 1). These behaved predictably. All the autografts survived and appeared healthy at three weeks while the allografts showed uniform rejection. A sudden development of paleness and swelling of the affected skin was the earliest sign of rejection. This was evident within six to eight days in the first set of grafts and within two to four days in the second set, most of which showed the characteristic "white graft" reaction. Satisfactory presensitisation therefore appeared to have been achieved in all the allografts.

Cartilage grafts (Table II, Fig. 3)

**Autografts.** As in previous experiments (McKibbin and Holdsworth 1967; McKibbin 1971) the orthotopic autografts were well incorporated at three months. normal endochondral ossification had been resumed, and although there were small areas of dead cartilage at the margin of the specimen (1 per cent) this appeared to have excited no abnormal cellular response.

The intramuscular heterotopic autografts were also substantially intact but the proportion of dead chondrocytes was greater than in the corresponding orthotopic grafts (6 per cent) and ossification seemed to be somewhat suppressed. There was a mild surrounding cellular reaction of a non-specific, predominantly fibroblastic type (Fig. 4).

**Allografts. Orthotopic.** These transplants survived well and the external appearance was often indistinguishable from that of the autografts even at three months (Fig. 5). In all cases ossification continued and the average increase in thickness was 40 per cent. When compared with the autografts the amount of dead graft was slightly increased (9 per cent), and there were moderate accumulations of small round cells and small collections of plasma cells in the surrounding tissues (Fig. 6).

**Heterotopic.** The appearance of all the intramuscular grafts was quite different from that of their orthotopic
counterparts. As early as four weeks massive rejection was already evident (Fig. 7) and the major proportion of the grafts had been totally destroyed by nine weeks. The quantitative comparisons are illustrated in Table II and Figure 3, but it should be borne in mind that these figures tend to underestimate the true degree of contrast since the heterotopic grafts were studied as early as four weeks while all the orthotopic grafts were left for twelve weeks.

DISCUSSION
That portion of these experiments which deals with the fate of heterotopic allografts confirms the findings of Heyner (1969), that when cartilage is transplanted as a free graft to the tissues of a presensitised animal it is soon destroyed by the small lymphocytes of the host.

However, the importance of the present results lies in the constraining fate of the orthotopic grafts in the knee. Even at three months the gross appearance of the transplants was normal and the joints were fully functional. Microscopic examination did reveal a slight increase in the amount of cartilage death and graft destruction compared with the corresponding autografts (Fig. 3) but most of the transferred material appeared to be healthy, and significant growth and ossification of the graft had occurred.

The survival of an allograft in the face of presensitisation is a very severe test of immunological privilege, and the fact that the accompanying second set of skin grafts were rejected within a few days confirms the unique degree to which cartilage is protected from the cellular and presumably also from the humoral elements of the immune response. Even the cornea cannot survive unscathed in these circumstances (Maumenee 1951) nor can any other tissue even with the aid of potent immunosuppressive techniques.

It remains to consider, therefore, why the heterotopic grafts were not similarly protected. There has been no previous reason to think that the knee is an immunologically privileged site, and although there is some evidence that synovial fluid contains a specific agent which resists the degradation of cartilage matrix (Ogston and Phelps 1961; Sweet and Solomon 1971), Sengupta (1974) found that free grafts of allogenic cartilage within the knee were offered no special protection.

It is fundamental to the theory of Bacsich and Wyburn (1955) that it is the quality rather than the quantity of the matrix which is important. They found that young cartilage was more resistant to rejection than old, even though the proportion of matrix to cells is less, and they also found that damage to the matrix by infection increased the chance of rejection.

In the present experiments it may be suspected that the vitality of the matrix in the heterotopic grafts has been compromised by interference with the normal mode of nutrition of the cartilage both from the synovial fluid, assisted by the mechanical action of the joint, and

Figure 4—Heterotopic articular cartilage autograft nine weeks after transplant into muscle. Note the mild ‘encapsulating’ tissue reaction. (Haematoxylin and eosin, ×60 approx.) Figure 5—Orthotopic articular cartilage allograft. This allograft was transplanted twelve weeks previously. It is well established and ossifying in a normal way. There is only a minute zone of diminished viability on the surface and the tissue response of the host is minimal, often just represented by scattered accumulations of small round cells at the base of the graft, as shown in Figure 6. (Toluidine blue, ×60 approx.)
from the subchondral blood vessels. This is confirmed by the difference found in the autografts in the two locations when no immunological considerations were involved. Thus the orthotopic grafts in the knee were virtually indistinguishable from normal while the free grafts failed to grow, showed a reduction in the amount of endochondral ossification and a greater amount of damage to cartilage. In the case of heterotopic allografts, therefore, it is perhaps not surprising that a strong immunological reaction met with little resistance from such enfeebled cartilage.

The orthotopic grafts, on the other hand, enjoy their normal mechanism of nutrition, and vascular connections with the underlying bone are soon re-established. However, previous experiments have shown that even with orthotopic grafts any technical imperfections leading to displacement of the cartilage are penalised by rapid rejection (Pap and Krompecher 1961; McKibbin 1971) and that those grafts which do not remain level with the surrounding cartilage, or which become covered by a pannus after operation, also suffer more immunological damage, again suggesting that the protective effect of the matrix has been lost due to interference with its normal nutrition.

If this explanation is accepted, it becomes possible to explain some of the contradictory findings of previous
workers on the basis of variations in the nutritional status of the graft used.

It would appear that the common practice of studying the fate of free transfers of cartilage, convenient though it is, cannot be relied on to provide an adequate level of nutrition of the graft, thus imposing serious limitations on the conclusions which can be drawn from such a model.

In conclusion, therefore, it may be stated that cartilage is indeed a tissue which enjoys a degree of immunological privilege which is virtually unique, and that this is the result not only of its lower antigenicity, but also of the ability of its matrix to resist the onslaught of an established immunological reaction. However, this privilege is dependent on the preservation of an adequate degree of nutrition of the graft, which must be taken into account in future experimental studies. At the same time, it should be recognised that if this privilege is to be exploited clinically by the repair of defects in joint cartilage, success can only be expected if the position of the graft is so accurate and secure that the normal mechanism of synovial nutrition is unimpaired.

The authors wish to thank Mr J. Wilson and his staff from the Experimental Research Unit at Sully Hospital for their invaluable help. The work was supported by a grant from the Medical Research Council, No. G 970/612/C.

REFERENCES


THE JOURNAL OF BONE AND JOINT SURGERY