IMMATURE JOINT CARTILAGE AND THE HOMOGRAFT REACTION

B. McKibbin, Sheffield, England

From the Department of Orthopaedics, University of Sheffield

It is apparent from studies of the homograft reaction that cartilage is exceptional in that when it is transferred to another animal of the same species it does not provoke the same vascular and cellular responses that occur with most other tissues. In consequence, such cartilage transfers may survive for a long time in a viable state: instances of human grafts which remained healthy for as long as twenty-two years have been recorded (Gibson, Davis and Curran 1958).

This special behaviour is usually ascribed to the avascular nature of cartilage, the cells of which are separated from those of the host by matrix which acts as a physical barrier and which may, in addition, have a specific protective capacity (Bacsich and Wyburn 1947).

Much of the experimental work on this subject has been done on costal and ear cartilage transferred into heterotopic sites, but it is generally believed that this relative freedom from immunological rejection is shared by articular cartilage. Nevertheless, there appears to be no record of the successful repair of a defect of articular cartilage by a homograft of pure articular cartilage.

Technical factors are in part responsible (Gibson 1965) because adult articular cartilage is very thin and friable, difficult to detach from the underlying bone, and with obvious difficulties in its permanent incorporation in the host without the intervention of blood vessels.

Attempts to avoid these difficulties by transferring the cartilage with a few millimetres of underlying bone to facilitate attachment have been made: Pap and Kompecher (1961) claimed long survival of the cartilage with this method but others have been unable to confirm these findings (De Palma, Tsaltas and Mauler 1963; Campbell, Ishida, Takahashi and Kelly 1963). Although the clinical usefulness of such transfers awaits further study, from the point of view of an immunological study, the procedure is of limited value because the picture is confused by the effects of simultaneous transfer of antigenic bone.

During a study of the properties of joint cartilage in immature animals (McKibbin and Holdsworth 1967) it became apparent that immature cartilage was much more suitable for transfer than its adult counterpart; when used as an autograft it became incorporated rapidly and permanently. This is because such cartilage is in reality two tissues, a superficial layer of true articular cartilage and a deeper layer of proliferating cartilage which is involved in endochondral ossification; the latter is easily separated from the bone (Fig. 1) and readily becomes reattached through the agency of the many blood vessels which invade its deeper layers. Furthermore, since it enjoys a dual source of nutrition (McKibbin and Holdsworth 1966) it can survive, sustained by the synovial fluid, until vascular connections are re-established.

The behaviour of such cartilage as a homograft is of interest, not only because its transfer is technically feasible, but also because of this composite nature. If the resistance of cartilage to immunological rejection is related to its avascularity it might be expected that the two elements of this tissue, the avascular articular layer and the well vascularised proliferating layer, might be differently treated by the host.

For these reasons it was decided to use immature joint cartilage from lambs to repair articular defects in other unrelated lambs and in adult sheep, and to study the survival of such grafts together with any reaction which might be produced.
MATERIAL AND METHOD

Sheep were chosen as the experimental animals because of their large joints. Derbyshire moorland sheep were used, and although a detailed genealogy of these animals is not obtainable efforts were made to ensure that the transfers would be true allografts.

Donor cartilage was obtained from lambs two to three months old. A knee was opened under aseptic conditions and a plaque of cartilage approximately 10 millimetres square was raised from the underlying bone by blunt dissection. This separation is facilitated by a natural plane of cleavage which exists between the bone and the most distal expanded cells in the ossifying cartilage, exactly as in traumatic separation of the growth plate (Fig. 1), and once the plane is entered the cartilage is easily removed in quite large sheets of easily workable material.

The recipient animals were anaesthetised and the right knee was opened. A defect was created in the femoral condyle of the same size and shape as those of the graft. Both cartilage and bone were removed, so that the bed consisted of bleeding bone. The graft was secured with a silk suture which was passed through the bone of the condyle and tied (Fig. 2). The joint was then closed. In some of the animals two defects were made but only one of them was grafted.

Altogether twenty-four sheep received allografts. Fifteen of them were mature, but the other nine were immature and of similar age to the donor lambs.

Recovery from the operation was rapid and all the animals were walking normally within ten days.

The adult sheep were killed in sequence at intervals between five days and fourteen months after operation and the femoral condyles removed. After fixation in formalin and decalcification in EDTA the specimens were embedded in paraffin and sections cut to include the grafted cartilage. These were stained with haematoxylin and eosin or toluidine blue.
Similar preparations were made from the lambs, which were killed at intervals between ten days and one year.

Control autografts were performed in twelve lambs. After removal of the cartilage plaque the opposite knee was opened and the graft inserted there in the usual way. These animals were killed at regular intervals between ten days and six weeks after operation and sections prepared as before.

Figure 3 - Autograft at two weeks. The grafted cartilage is on the left. Normal ossification is occurring and the appearances are almost indistinguishable from those of the undisturbed cartilage on the right. (Haematoxylin and eosin. · 100.) Figure 4 - Homograft at one year. The graft cartilage appears to be healthy although the surface is slightly irregular.

RESULTS

Autografts—Nine of the twelve grafts were found to have survived in a healthy condition. Vascular reattachment was well developed by ten days and endochondral ossification resumed soon afterwards so that by three weeks parts of the graft were indistinguishable from the undisturbed surrounding cartilage (Fig. 3). There was no sign of an abnormal vascular or cellular reaction. At the edges of the graft local abnormalities in the cartilage were sometimes seen; these were ascribed to local damage with some distortion of architecture and the occasional formation of chondrocyte clones. In regions where the plaque fitted tightly against the surrounding cartilage physical union between the two appeared to have occurred. In three cases infection occurred, with an intense polymorph reaction in the bone. The cartilage in these circumstances showed normal vascular reattachment but there was loss of matrix basophilia with some localised areas of chondrocyte death.

Allografts in adult sheep—All but one of the fifteen experiments were technically satisfactory. In the gross specimens the grafted cartilage appeared to be healthy with an intact shiny surface which did, however, show slight irregularities in the surface contour (Fig. 4). In a few of the later specimens there was a thin covering of pannus at the margins of the graft. The
Deep surface of a homograft in a sheep at twelve weeks. Endochondral ossification has now begun and some accumulations of lymphocytes are evident (the vertical fissure is an artefact). (Haematoxylin and eosin, √180.)

THE JOURNAL OF BONE AND JOINT SURGERY
Figure 7 - Homograft in a sheep at seventeen weeks, showing the junctional region between the immature cartilage graft on the left and the mature cartilage of the host on the right. Lymphocyte accumulations are present. (Haematoxylin and eosin, ×100.)

Figure 8 - Detail of the cellular accumulation in seventeen-week specimen. The cells are predominantly small lymphocytes and plasma cells. (Haematoxylin and eosin, ×1000.)

Figure 9 - Deep surface of the seventeen-week homograft. Normal endochondral ossification is in progress. Collections of lymphocytes can be seen whose areas of maximum concentration are at some distance from the site of vascular invasion. (Haematoxylin and eosin, ×180.)
histological appearances in this group showed a progressive series of changes depending on the time the graft had been in place.

The early specimens, obtained at five and twelve days, resembled the autografts at a similar stage. There was slight loss of basophil staining of the matrix and early vascularisation. At six weeks the cartilage had regained its normal staining properties and was considerably thicker than when first transplanted. Vascular connections had been re-established on the deep surface but there was no sign of endochondral ossification (Fig. 5). The surrounding tissues had a normal appearance and there was no evidence of a homograft reaction.

By twelve weeks the cartilage looked very healthy; it was less thickened than in the previous specimen but there was by now normal chondrocyte maturation with column formation and endochondral ossification (Fig. 6). Deep to the graft there were unmistakable signs of round cell infiltration but no sign of cartilage destruction.

The appearances at seventeen weeks were similar. Endochondral ossification was well established (Fig. 7), but accompanied as before by ominous collections of round cells, most of which were lymphocytes (Fig. 8); these were aggregated in places into what almost amounted to lymph follicles. It was noted that these cellular collections always remained at some distance from the actual site of vascular invasion (Fig. 9).

By six months there was much less proliferative activity in the cartilage, and although endochondral ossification was still proceeding it was at a noticeably slower rate. The general appearance suggested that the cartilage was becoming walled off by a developing plate of subchondral bone (Fig. 10). The usual lymphocyte collections were present, but these were less densely populated than formerly and in places appeared to consist of little more than a residual collagen framework (Fig. 11).
FIG. 14
Homograft in a sheep at three weeks. The graft has become displaced below surface level, its normal staining properties are lost and active rejection is in progress. (Haematoxylin and eosin, ×180.)
By one year these tendencies were confirmed and there was little evidence of round cell activity. A well marked subchondral bone plate was present in some areas and endochondral ossification had practically ceased (Fig. 12). The cartilage stained normally but areas of chondrocyte clumping were noticeable.

Similar appearances were seen at thirteen months, but by fourteen months the isolation of the cartilage from the bone was even more marked. There were now very few vascular connections between the two tissues, and in addition to a well marked subchondral bone plate there was seen for the first time a "tide mark" indicating a zone of calcification in the deeper layers of the cartilage (Fig. 13). Very little round cell activity was seen in this specimen, the general appearance of which closely resembled that of normal adult cartilage.

There was one notable exception to this regular sequence of changes which occurred in the specimen from an animal killed at three weeks. In this case the cartilage showed considerable loss of basophilia, and although there were some zones of apparently normal cartilage there were extensive areas in which the chondrocytes were dead. The graft was surrounded by vascular tissue in which small round cells predominated. Erosion and destruction of the cartilage was occurring, and proliferation and endochondral ossification were not seen (Fig. 14).

**Allografts in immature lambs**—These transfers were less successful. In five the graft was technically unsatisfactory in that the subchondral bone appeared to have given way and the graft had sunk below surface level. In these circumstances the cartilage showed poor staining with many areas of chondrocyte death (Fig. 15). This appearance was always accompanied by an intense round cell reaction which appeared as early as three weeks after operation (Fig. 16) and was associated with varying degrees of dissolution of the graft. Once again the cellular accumulations were of the small round cell type (Fig. 17). In the four technically successful experiments, in which the seating of the graft was intact, the changes at ten days, six
IMMATURE JOINT CARTILAGE AND THE HOMOGRAFT REACTION

Figure 16—Homograft to a lamb at three weeks. The matrix has lost its basophilia and an early homograft reaction with destruction is present. (Haematoxylin and eosin, ×100.) Figure 17—Detail of cellular reaction from the same specimen as Figure 15. The cells are predominantly small lymphocytes and plasma cells.

Figure 18—Homograft to a lamb at six weeks. The cartilage is healthy and there is intense proliferation. Endochondral ossification is just beginning. (Haematoxylin and eosin, ×180.) Figure 19—Homograft to lamb at twelve weeks. The cartilage is healthy, endochondral ossification is well established and although lymphocyte accumulations are present there is no cartilage destruction. (Haematoxylin and eosin, ×450.)
weeks, seven weeks and five months were very similar to those seen in the adult specimens. Thus at six weeks there was healthy cartilage showing intense proliferation and the beginning of endochondral ossification (Fig. 18), and by five months ossification was well developed and the usual accompanying lymphocytes were present (Fig. 19).

**Ungrafted defects**—These were found to be filled with fibrous tissue with no evidence of new cartilage formation.

**DISCUSSION**

These results confirm that immature joint cartilage may be readily transferred as an orthotopic autograft because of the ease with which vascular connections can be re-established with the ossifying zone; also allografts of such cartilage can survive and function for periods of at least fourteen months, and although there were some instances of graft failure, which will be discussed separately, in most of those that survived a consistent pattern of change could be discerned in the successive specimens.

In the transfers to adult sheep the cartilage behaved essentially as it would had it remained in the donor animal. After an initial delay there was a resumption of proliferation and ossification in the deep zone which eventually stopped to leave only the articular layer, which gradually lost its vascular connections with the bone and finally became demarcated from it by the development of a basal zone of calcified cartilage. This is the normal pattern of development of such cartilage over the same time intervals, and it is interesting that the immature cartilage should have adhered to this pattern even in the environment of an adult animal. Such behaviour illustrates the importance of intrinsic mechanisms in growth and development.

The series of successful grafts in lambs was incomplete, but the appearances in the survivors suggested that this cartilage was also developing in the normal way.

A homograft reaction was evident in many of these grafts but it did not appear to modify their behaviour. It remains to consider the implications of these findings.

The analogy has been drawn between a cartilage graft and the experimental situation in which a graft is enclosed in a diffusion chamber in which cellular contact with the host is prevented, so that the latter cannot be sensitised or the former destroyed (Gibson *et al.* 1958). In the case of cartilage the matrix performs the function of the diffusion chamber membrane. If this is accepted then the problem is to explain, not why cartilage homografts have a prolonged survival since this is to be expected from such a mechanism, but rather why they ever become involved in the homograft reaction at all.

Gibson (1965) has indeed denied that there is any such involvement, and although in some of the human material which he subsequently studied (Gibson *et al.* 1958) there was a limited reaction, he attributed this to the simultaneous transfer of perichondrium which was normally antigenic. Young (1945) also reported survival of grafts in dogs for up to eighteen months without any trace of a reaction.

Others have found lymphocytic accumulations in a variety of experimental grafts together with limited areas of cartilage destruction (Loeb 1926a and b, Dupertuis 1941, Bacsich and Wyburn 1947, Craigmyle 1955), and Craigmyle (1958), by studying the response of the local lymph node and using second set grafts, was able to show that cartilage was undoubtedly antigenic.

Reasons for these differences will be suggested later. For the moment it is proposed to concentrate on the findings in the present experiments in an attempt to explain the undoubted participation in the homograft reaction which was evident on this occasion.

A simple explanation of the findings may be made on a quantitative basis. If it is assumed that the matrix barrier is not perfect then a slow leakage of antigen could be postulated which would give rise eventually to an attenuated and delayed response. If this were so, it would be expected that there would be a gradual increase in the response with time whereas in fact, although the onset was delayed, a peak of activity was reached at about fourteen weeks, after
which there was a gradual decline. All the evidence pointed to the fact that the activity of the response was related to the amount of vascular invasion and endochondral ossification which was occurring at the time; when proliferation and ossification ceased the response died out. The process of endochondral ossification therefore appears to be associated with the liberation of antigenic material.

This conclusion creates further problems, however, for the generally accepted concept of endochondral ossification implies that as the chondrocyte proceeds along its column it is undergoing a process of gradual degeneration, so that when the ossifying capillary finally breaks into the lacuna at the end of the column the chondrocyte itself is dead. Thus even in these circumstances there should be no effective contact between viable chondrocytes in the graft and the vessels of the host.

The presence of the homograft response therefore suggests either that the death of the chondrocyte has not resulted in a loss of its antigenicity or, more likely, that the chondrocyte is not in fact dead. There is an increasing body of evidence that the changes in the chondrocyte column during ossification do not represent a process of degeneration but rather a process of active maturation (McKibbin and Holdsworth 1966).

Some aspects of metabolic activity actually increase as the process continues (Greer, Janice and Mankin 1968), and labelling techniques have shown that a living chondrocyte stripped of its matrix may emerge at the end of the process (Urist and Adams 1968, McKibbin 1970), a situation which would readily explain a limited homograft response and its relationship to the ossification process.

While it is possible to account in this way for the sensitisation of the host which occurred, it still remains to explain why, in spite of such sensitisation and the presence of considerable accumulations of lymphocytes, no destruction of the cartilage appeared to result, even when the reaction was at its zenith.

The suggestion has already been mentioned that cartilage owes its privileged position to its avascularity, in that destructive elements are prevented from penetrating it. Woodruff (1960) pointed out that avascularity in itself is an insufficient explanation because allografts of pure epidermis, which are likewise avascular, are rejected in the same way as full-thickness skin (Billingham and Sparrow 1954). He suggested that the sheer bulk of a cartilage graft served to isolate most of its contained tissue from the invading host cells. However, Bacsich and Wyburn (1955) showed that the degree of protection afforded in this way was not related to the amount of matrix present but rather to the vitality of the chondrocyte itself, which they took as providing further support for their earlier suggestion (Bacsich and Wyburn 1947) that the matrix had a specific protective function which they thought was provided by certain mucoproteins which the matrix contains in common with the cornea.

The present findings strongly support this theory. Figure 9 shows a graft in which undoubted sensitisation has been produced, yet in spite of this, vessels can be seen ramifying freely in the deeper layers of the cartilage in the very region where the antigen is being liberated. But far from this resulting in destruction of the cartilage, the process of endochondral ossification is proceeding normally. It is especially noteworthy that in this, as in all of the successful grafts, the lymphocytic accumulations always remained at a respectful distance from the site of vascular invasion as if inhibited from a nearer approach. This suggests a specific action on the part of the matrix which acts, not by preventing the access of blood vessels, but by direct inhibition of the destructive cells themselves.

A still more difficult problem is provided by those grafts which failed totally. These were remarkable for the speed with which the changes developed; significant lymphocytic accumulations were present as early as three weeks, soon to be followed by dissolution of the graft.

These findings contrast with the generally reported experience with cartilage grafts in which, if there was any response at all, it was usually very late and limited in extent. There is
also a marked difference from the behaviour of most of the successful grafts in this series; so it is important to identify the factor which is responsible for such a dramatic alteration in the treatment of the transferred tissue.

The feature which appeared to link the unsuccessful grafts was that in all of them some displacement of the plaque had occurred. Usually this consisted of a sinking of the graft below the level of the surrounding cartilage, and this may explain why five out of six failures occurred in immature lambs who lacked a subchondral bone plate to provide support. Such a collapse is likely to impair the nutrition of the graft, and in fact in all the six the cartilage showed signs of diminished vitality with loss of basophilic staining and lack of proliferative activity. This raises the possibility that it was this impairment which was responsible for precipitating the homograft reaction in these instances.

It is of course difficult to separate cause and effect in this situation, for it could equally well be argued that the cartilage impairment was the result of the reaction rather than its cause. Nevertheless the regularity with which graft displacement was associated with a severe reaction was striking. Pap and Krompecher (1961) found similar changes in their osteochondral grafts when the cartilage lay below the level of the surrounding joint surface, but they attributed this to a loss of functional capacity rather than specifically to a failure of nutrition. Similarly Bacsich and Wyburn (1947) found that if a cartilage graft is subsequently damaged by infection this may precipitate a rejection process.

If it was in fact this loss of vitality which was responsible for the vulnerability of these failed grafts then the situation must be contrasted with that in which the cartilage is dead. There seems no doubt from clinical experience with preserved homologous cartilage that prolonged stability of such grafts can occur, and this has been confirmed experimentally (Dupuytren 1941); so it seems that cartilage has a better chance of remaining stable if its cells are dead than if they are alive but with their vitality impaired. This may explain some of the variations which have been reported in the behaviour of live grafts, whose vitality will be dependent on the quality of the host bed provided.

It is to be expected that the success of healthy cartilage grafts and the paradoxical prolonged stability of dead cartilage will have differing explanations.

In the case of dead cartilage it is likely that there are no transplantation antigens present (Woodruff 1960), and any subsequent destruction of the cartilage will be due to a non-specific foreign body reaction. Living cartilage on the other hand does appear to be antigenic and relies on the special properties of its matrix to hold the invading cells at bay, so that any reduction in chondrocyte activity may impair the quality of the matrix with a loss of this property without a corresponding reduction of antigenicity, leaving the way open to the invasion of destructive elements.

This conclusion may have significance in relation to the possible clinical use of cartilage homografts. If the cartilage remains well nourished there seems to be no theoretical reason why it should not survive indefinitely provided its nutrition is maintained. Nevertheless the Damoclesian sword of rejection will forever remain poised, awaiting even a temporary faltering in the vitality of the cartilage to begin a vicious circle of invasion and destruction. It follows that such cartilage will be vulnerable to a slight injury which would not harm normal cartilage, and it may be wise to help the cartilage over any such crisis by the temporary use of immunosuppressive agents.

**SUMMARY**

1. Grafts of joint cartilage from immature lambs were used to repair articular cartilage defects in other lambs and in adult sheep.
2. Stability of these grafts in a functional state was found in most for periods up to fourteen months. Although a limited homograft reaction occurred this did not lead to destruction of the cartilage, even though parts of it were well vascularised.
3. The results suggest that the process of endochondral ossification is associated with the liberation of antigenic material leading to sensitisation of the host. Destruction of the cartilage is prevented by an inhibitory action which the matrix appears to exert on the destructive elements themselves and which is itself dependent on the vitality of the chondrocytes.

4. The avascularity of cartilage is not a sufficient explanation for its privileged position in relation to the homograft reaction.

REFERENCES


