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Bone formation following intrarenal transplantation of isolated murine chondrocytes: chondrocyte–bone cell transdifferentiation?

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Summary

Isolated syngeneic epiphyseal chondrocytes transplanted into a muscle formed cartilage in which matrix resorption and endochondral ossification began at the end of the second week after transplantation. After 56 days cartilage was converted into an ossicle. In 7-day-old intrarenal transplants, epiphyseal chondrocytes formed nodules of cartilage. In 10-day-old transplants, islands of bone appeared. Slight resorption of cartilage was first noted in 14-day-old transplants of chondrocytes. After eight weeks, transplants contained mainly bone. Intramuscularly transplanted rib chondrocytes formed cartilage which did not ossify. Nevertheless, bone islands appeared in intrarenal transplants of rib chondrocytes. Bone was not formed in allogeneic intrarenal transplants of epiphyseal or rib chondrocytes, but appeared in such transplants in animals immunosuppressed by anti-thymocyte serum and procarbazine. When spleen cells from animals immunized with allogeneic chondrocytes were transferred to immunosuppressed chondrocyte recipients two weeks after intrarenal chondrocyte transplantation, the majority of osteocytes in bone islands was dead. On the other hand, endochondral bone formed in intramuscular transplants of allogenic epiphyseal chondrocytes in immunosuppressed recipients was not damaged by sensitized spleen cells. This suggested that bone in 10- to 14-day-old intrarenal transplants of chondrocytes arose from injected cells and not by induction. To see whether bone was formed by chondrocytes or by some cells contaminating the chondrocyte suspension, the superficial layer of rib cartilage was removed by collagenase digestion and only more central chondrocytes were used for transplantation. Intrarenal transplants of these chondrocytes also yielded bone. Furthermore, in some experiments intrarenal transplants of syngeneic rib chondrocytes were stained *in toto* by Alcian blue and Alizarin red S 7 and 14 days after transplantation and examined under a dissecting microscope. In 7-day-old transplants only cartilage nodules could be found while in the older ones cartilage and bone or only bone islands were present. These results strongly suggest that in intrarenal transplants epiphyseal and rib chondrocytes transdifferentiated into bone cells. It also seems that chondrocytes first formed cartilage matrix which, after their transdifferentiation, was substituted by bone matrix.

Key words: chondrocytes, bone cells, intrarenal transplantation, cartilage, bone, transdifferentiation, mouse.

Introduction

Induction of bone by intramuscular transplants of transitional epithelium (Huggins, 1931), gall bladder epithelium (Huggins and Sammet, 1933), transformed cell lines (Anderson *et al.* 1964; Włodarski *et al.* 1970) or decalcified bone matrix (van de Putte and Urist, 1966; Reddi and Anderson, 1976) is a well-recognized phenomenon. It has also been demonstrated, however, that these inductors fail to produce bone in kidney, liver or spleen (Huggins *et al.* 1936; Urist *et al.* 1969; Chalmers *et al.* 1975; Wlodarski, 1978).

In recent years, strong bone-inductive properties of cartilage produced by transplanted isolated epiphyseal chondrocytes has also been demonstrated (Thyberg and Moskalewski, 1979; Ksiazek and Moskalewski, 1983; Wright *et al.* 1985). Such chondrocytes transplanted intramuscularly form cartilage in which central chondrocytes hypertrophy, the matrix calcifies and 2 weeks after transplantation endochondral ossification begins. Within 6 weeks, cartilage is almost completely replaced by bone tissue forming an ossicle. Endochondral bone seems to be induced by calcified cartilage matrix (Thyberg and Moskalewski, 1979; Ksiazek, 1983).

In this work, we wanted to establish whether bone formation, after transplantation of epiphyseal chondrocytes, occurs only in muscles or also in a parenchymatous organ. For this purpose, isolated epiphyseal chondrocytes were injected into a kidney. Unexpectedly, as well as cartilage nodules produced in kidney parenchyma, islands of bone tissue appeared during the second weeks after injection of cells, without prior resorption of cartilage. Thus, in the kidney, reputed to be an unsuitable place for induction (Huggins *et al.*

1936), after transplantation of chondrocytes, bone appeared earlier than in muscle and by a mechanism different from endochondral ossification. This raised the question whether the bone was formed by the recipient's cells under the influence of cartilage or by chondrocytes or cells contaminating the chondrocyte suspension, under the influence of local environment. Therefore, the aim of this paper was to describe bone produced in kidney parenchyma after transplantation of chondrocytes and to study its origin.

Materials and methods

Animals

Cartilage for transplantation or for isolation of chondrocytes was taken from 4-day-old inbred CFW/Ll (CFW) or C57Bl/10 (B10) mice of both sexes. Skin for isolation of fibroblasts was taken from 19-day-old CFW fetuses. Adult CFW males served as recipients of transplants.

Isolation and transplantation of chondrocytes and fibroblasts

Chondrocytes were isolated either from cartilaginous epiphyses of long bones or from rib cartilage. The cartilages were cleared of the surrounding tissues under a dissecting microscope. Rib cartilage was cut off at a distance from the sternum and costochondral junction to avoid contamination of chondrocytes from rib cartilage with those from the rib growth plate. After preliminary cleaning, rib cartilage was additionally treated with 0.25 % trypsin in phosphate-buffered saline (PBS), at 37°C for 50 min to remove remnants of the perichondrium.

Chondrocytes were isolated as described earlier (Malejczyk *et al.* 1985) by 4–5 h digestion in collagenase. In some experiments, digestion of rib cartilage was interrupted after 1 h, the liberated cells were discarded and digestion was continued with a fresh solution of the enzyme. The yield was $2.5–3.0\times10^6$ of epiphyseal and $1.5–1.8\times10^6$ of rib chondrocytes from one donor. Isolated cells were injected into the kidney parenchyma after standard surgical exposure of this organ or into the posterior tibial muscle. The number of chondrocytes per transplant was 2.5×10^6, suspended in 0.05 ml of Eagle's minimal essential medium (MEM). Special care was taken to disperse chondrocytes throughout the kidney parenchyma in transplants that were to be stained *in toto*. Some animals also received intrarenal transplants of the femoral head or rib cartilage.

Fibroblasts were isolated by digestion of fetal skin fragments with 0.25 % trypsin, rinsed by centrifugation in MEM with 10 % calf serum, filtered through a 60 μm mesh mylon cloth and injected into a kidney in the same number as chondrocytes. Fibroblast suspension also contained some epidermal cells.

Immunosuppressive treatment

CFW recipients of B10 epiphyseal or rib chondrocytes injected into a kidney, and epiphyseal chondrocytes injected intramuscularly, were immunosuppressed by treatment with procarbazine hydrochloride (PCH; Hoffman La Roche, Basel, Switzerland) and rabbit anti-mouse anti-thymocyte serum (ATS) (Malejczyk and Moskalewski, 1988). The animals were treated with four consecutive doses of PCH ($100\,mg\,kg^{-1}$ of body weight) and ATS (0.5 ml per mouse) given simultaneously on every second day, beginning on the day of transplantation. The ATS preparation had a similar immunosuppressive potency as described previously (Malejczyk and Moskalewski, 1988). Two weeks after intrarenal and six weeks after intramuscular grafting of chondrocytes, some of the immunosuppressed recipients of allogeneic B10 chondrocytes received syngeneic, specifically sensitized spleen cells.

Sensitization was accomplished by intramuscular transplantation of B10 chondrocytes. Sensitized spleen cells were isolated two weeks after immunization using a glass homogenizer with a loosely fitted pestle. They were suspended in MEM containing 50 units ml^{-1} of heparin and 5×10^7 cells per animal were administered through a tail vein.

Histological examination

Recipients of transplants were killed at various times, up to 8 weeks after transplantation. The number of transplants in various experimental groups is given in Table 1. Fragments of muscle or kidney containing cartilage were fixed in 10 % buffered formalin, decalcified when necessary in 14 % edetic acid, pH 7.6, dehydrated and embedded in paraffin. Sections cut at 10 μm were stained with hematoxylin–eosin, with Alcian blue, pH 0.4, prepared as described previously (Moskalewski *et al.* 1983) or by von Kossa method for calcium.

In toto *staining of cartilage and bone in intrarenal transplants*

Mice bearing intrarenal transplants of rib chondrocytes were killed 7 and 14 days after transplantation and the kidneys were *in toto* stained with Alcian blue and Alizarin red S according to De Gennaro *et al.* (1980). The numbers of cartilage and bone islands were counted under the dissecting microscope. Then the fragments of kidney containing transplants were decalcified when necessary and subjected to routine histological examination.

Table 1. *Occurrence of bone in intramuscular and intrarenal transplants of isolated epiphyseal chondrocytes*

Chondrocytes	Duration of transplant (days)	Number of transplants and frequency of bone formation: Kidney	Muscle
Syngeneic	7	0/7	0/6
	10	$7/7^n$	nd
	14	$13/13^n$	$2/14^e$
	28	$6/6^{n?}$	$7/7^e$
	56	$5/5^{n?}$	$6/6^e$
Allogeneic	14	0/5	0/5
Allogeneic (immunosuppression)	28	$5/5^n$	nd*
Allogeneic (immunosuppression and sensitized spleen cells)	28	$7/7^n$	nd
	56	nd	4/4

nd – not done
e – endochondral
n – non-endochondral
n? – non-endochondral, possibly also some endochondral
* – described previously (Malejczyk and Moskalewski, 1988)

Results

With the exception of two intrarenal grafts of syngeneic rib chondrocytes in which only bone was present, all chondrocyte transplants resulted in cartilage formation. The frequency of bone formation after transplantation of epiphyseal chondrocytes is given in Table 1 and rib chondrocytes in Table 2. The detailed morphological description of cartilage produced in syngeneic and allogenic intramuscular transplants of epiphyseal chondrocytes was presented previously (Ksiazek and Moskalewski, 1983). Here, only a general description, as a background to other types of transplants, is given.

Intramuscular syngeneic transplants of epiphyseal chondrocytes

Cartilage nodules formed in 7-day-old transplants were surrounded by granulation tissue. In some nodules, the central cells were enlarged as compared with the peripheral ones, but the matrix was not calcified. In 14-day-old transplants, the central chondrocytes were hypertrophied and the matrix surrounding them was calcified. In some areas, granulation tissue invaded cartilage and formed deep erosion pits or channels. A few bone trabeculae deposited within the calcified matrix were present in only 2 out of 14 transplants. In neither case was bone present at the periphery or in the vicinity of cartilage (Fig. 1). In 28-day-old transplants, the resorption cavities were enlarged and the majority contained bone trabeculae. Bone was also present in the peripheral layer of cartilage which underwent resorption. In 56-day-old transplants resorption of cartilage and bone deposition were far advanced and the transplants were organized into ossicles (Fig. 2).

Table 2. *Occurrence of bone in intramuscular and intrarenal transplants of isolated rib chondrocytes*

Chondrocytes	Duration of transplant (days)	Number of transplants and frequency of bone formation	
		Kidney	Muscle
Syngeneic	7	0/5	nd
	14	8/8[n]	0/6
	28	5/5[n]	0/5
	56	nd	0/6
Syngeneic (central cells)	14	3/3[n]	nd
Allogeneic	28	0/3	nd
Allogeneic (immunosuppression)	28	4/4	nd
Allogeneic (immunosuppression and sensitized spleen cells	28	4/3	nd
Syngeneic (stained *in toto*)	7	5/5	nd
	14	5/5*	nd

* – In two transplants cartilage was absent and only bone was found.

Intrarenal syngeneic transplants of epiphyseal chondrocytes

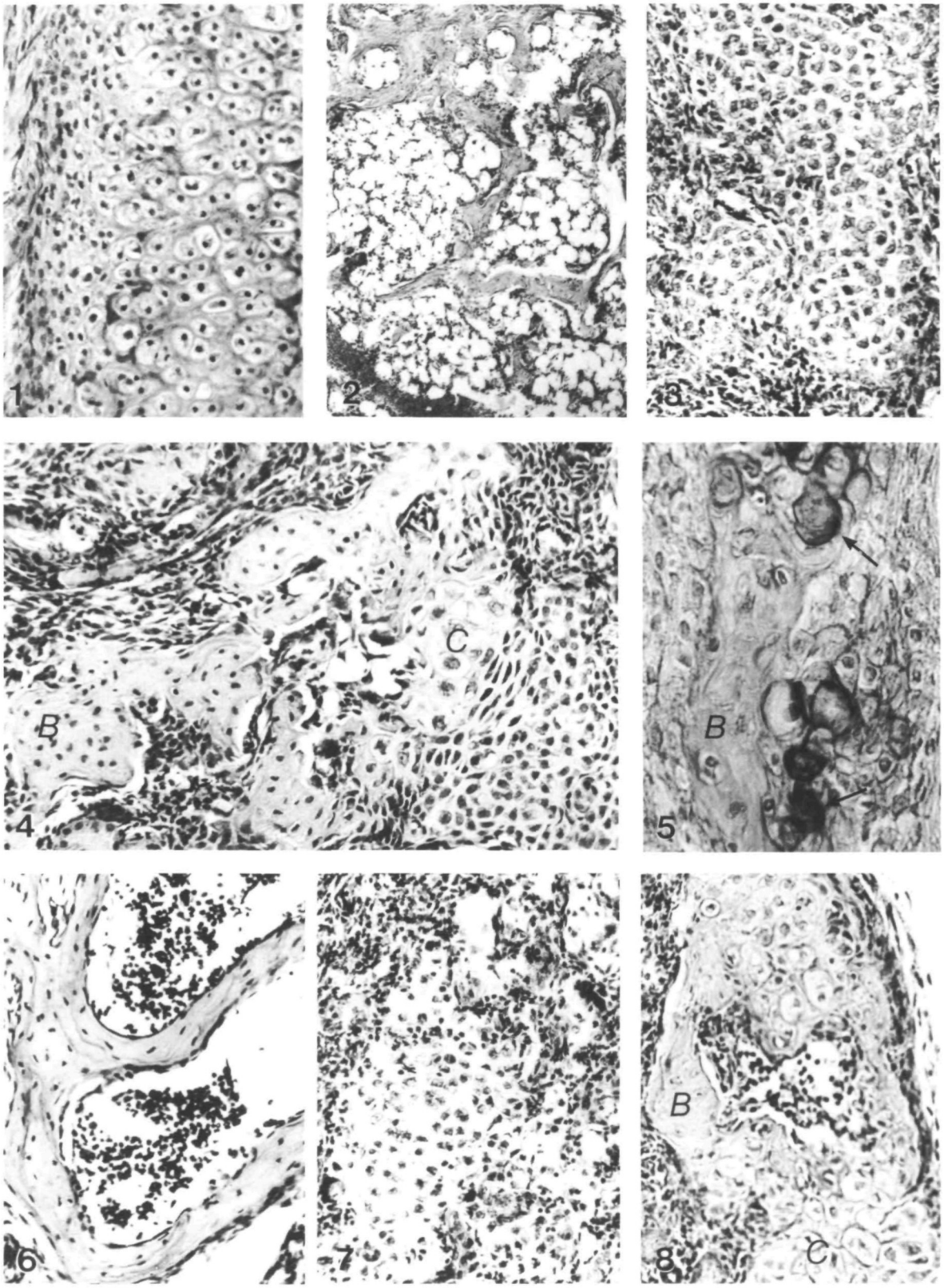
Cartilage nodules were present mainly within the kidney parenchyma and were distributed along the channel formed by the needle during injection. Kidney parenchyma in this region was damaged and partially substituted by granulation tissue. The nodules in the kidney were rounded, oval or irregularly shaped. They also differed considerably in size. In some cases cartilage was also found under the kidney capsule. It was usually narrow and followed the curvature of the kidney. In 7-day-old transplants several cartilage nodules were present, but bone was not observed (Fig. 3). In all 10- and 14-day-old transplants both cartilage and bone occurred (Fig. 4). Bone tissue adhered to the cartilage or was separated from it by a band of the granulation tissue. Some bone islands were found at a distance from the cartilage. The numerical ratio of bone islands to cartilage nodules varied considerably in various transplants. In some of them several bone islands and only a few or no cartilage nodules were found. The latter were, however, usually larger than the former.

Cartilage in 7-day-old transplants contained only a small amount of intracellular substance as compared with analogous intramuscular transplants and was devoid of hypertrophied chondrocytes. In 14-day-old cartilage nodules, a few hypertrophied cells appeared and in their region calcification of matrix occurred, but the amount of matrix remained still low. Occasionally some resorption of matrix by granulation tissue at the periphery of cartilage could be observed. Bone matrix was strongly calcified. In decalcified material both tissues could be easily distinguished after hematoxylin–eosin staining since the matrix of cartilage was homogeneous and pale, while the matrix of bone was fibrous and strongly acidophilic. In some cartilage nodules, however, acidophilic areas with slightly fibrous texture were present. The stainability of bone matrix with eosin was usually similar within the whole cross-section of the bone island. The difference between cartilage and bone matrix was even more conspicuous after Alcian blue staining since the former was strongly positive while in the latter only a narrow rim of osteoid surrounding bone lacunae was stained (Fig. 5).

In 28-day-old transplants intracartilage channels containing blood vessels could be distinguished. A small amount of bone could be occasionally observed at the periphery of such channels. Resorption was more conspicuous in cartilage formed under the kidney capsule than within the parenchyma. In 56-day-old transplants, bone with well-developed bone marrow predominated (Fig. 6). Cartilage was absent or occurred in small foci usually connected with bone.

Intramuscular, two-week-old, allogeneic transplants of epiphyseal chondrocytes

Cartilage produced in these transplants was similar to that in syngeneic ones, but was surrounded and partially resorbed by infiltrating cells, mainly lymphocytes and macrophages. Bone tissue was absent.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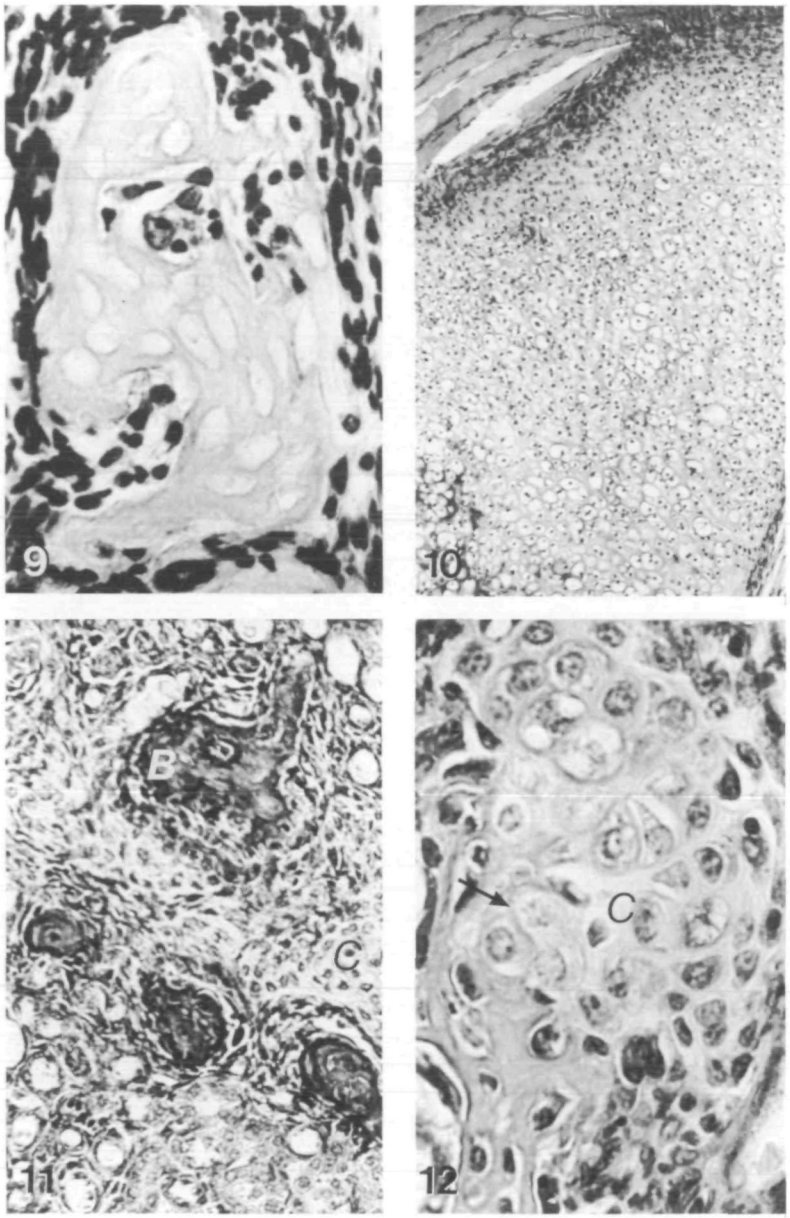

Figs 1, 2. Intramuscular transplants of isolated syngeneic chondrocytes. Hematoxylin–eosin.
Fig. 1. Cartilage produced by epiphyseal chondrocytes 14 days after transplantation. ×160.
Fig. 2. Ossicle formed within cartilage produced by epiphyseal chondrocytes, 56 days after transplantation. ×63.
Figs 3–6. Intrarenal transplants of syngeneic epiphyseal chondrocytes.
Fig. 3. Cartilage produced 7 days after transplantation. H. E. ×160.
Fig. 4. Cartilage (C) and bone (B) in 14-day-old transplant. H. E. ×160.
Fig. 5. Cartilage (arrow) closely adhering to bone (B) in 14-day-old transplant. Alcian blue – Kernechtrot. ×160.
Fig. 6. Bone with bone marrow in 56-day-old transplant. H. E. ×160.
Figs 7–9. Intrarenal transplants of allogeneic epiphyseal chondrocytes. H. E.
Fig. 7. Cartilage produced in 14-day-old transplant. Note infiltrating cells surrounding and invading cartilage. ×160.
Fig. 8. Cartilage (C) and bone (B) in immunosuppressed animal, 28 days after transplantation of chondrocytes. ×160.
Fig. 9. Bone surrounded by infiltrating cells with bone lacunae devoid of osteocytes in immunosuppressed animal which 14 days after transplantation of epiphyseal chondrocytes received sensitized spleen cells. ×400.
Fig. 10. Intramuscular transplant of syngeneic rib chondrocytes lasting 56 days. Only cartilage is present. ×63.
Figs 11, 12. Intrarenal transplants of syngeneic rib chondrocytes. H. E.
Fig. 11. 14-day-old transplant with cartilage (C) and bone (b) in non-decalcified material. ×160.
Fig. 12. 14-day-old transplant of chondrocytes from central part of rib cartilage with cartilage (C) and bone (B). Cartilage matrix in some areas (arrow) appears slightly acidophilic and fibrous. ×400.

Intrarenal allogeneic transplants of epiphyseal chondrocytes

Transplants, lasting 14 days, contained cartilage nodules surrounded by infiltrating cells, some of which penetrated into its periphery. The border of cartilage was rough, suggesting that some matrix was resorbed (Fig. 7).

Intrarenal allogeneic transplants of epiphyseal chondrocytes in immunosuppressed animals

These transplants were taken for examination after 28 days. Both cartilage nodules and islands of bone were present. They were surrounded by infiltrating cells but their matrix was not invaded and no signs of resorption were noted (Fig. 8). Occasionally, a few bone lacunae without osteocytes were encountered but the majority of osteocytes displayed a normal appearance and stainability. In animals that received sensitized lymphoid cells two weeks after transplantation of chondrocytes, both cartilage nodules and bone islands were surrounded by infiltrations. In bone islands, from 50 to 100% of osteocytes were dead as judged by the presence of empty lacunae or lacunae with shrunken cells which lost stainability with hematoxylin (Fig. 9). Some peripheral lacunae were open and contained a few infiltrating cells.

Intramuscular transplants of allogeneic epiphyseal chondrocytes in immunosuppressed animals

In such 6-week-old transplants endochondral ossification took place but the amount of produced bone was

small compared with that in syngeneic transplants (Moskalewski and Malejczyk, 1988). Therefore, in the present work, immunosuppressed animals with chondrocyte transplants were given sensitized splenocytes six weeks after transplantation of chondrocytes and the transplants were taken for examination after two weeks. The amount of produced bone was low, but no signs of necrosis of osteocytes could be observed.

Intramuscular transplants of syngeneic rib chondrocytes

These transplants, lasting 14, 28 or 56 days contained cartilage nodules surrounded by granulation tissues. The central chondrocytes were hypertrophied and some calcification of matrix was evident, particularly in older transplants, as in rib cartilage *in situ* (Ksiazek, 1983). Cartilage was not resorbed and bone tissue was absent both within the cartilage and its surroundings (Fig. 10).

Intrarenal transplants of syngeneic rib chondrocytes

In 7-day-old transplants, only cartilage nodules were present. Transplants, lasting 14 and 28 days, contained both cartilage nodules and well-calcified islands of bone (Fig. 11). Cartilage matrix was acidophilic and fibrous here and there. Signs of resorption of cartilage nodules could not be observed in any of the transplants whatever its age.

Transplants of chondrocytes from cartilages with the peripheral layer of cells removed by 1 h digestion with collagenase yielded cartilage and bone as in the transplants described above. In these transplants, cartilage matrix in some areas also appeared slightly acidophilic and fibrous (Fig. 12).

Intrarenal transplants of allogeneic rib chondrocytes in normal and immunosuppressed animals

Cartilage nodules formed in transplants from animals not subjected to immunosuppression were surrounded by infiltrating cells which often penetrated into the matrix. Bone tissue was absent. In immunosuppressed animals islands of bone were formed. They were surrounded by infiltrating cells, but the majority of osteocytes remained viable as judged by normal nuclear stainability. On the other hand, in bone islands from animals that received sensitized splenocytes, many osteocytes were dead.

In toto *stained intrarenal transplants of syngeneic rib chondrocytes*

In a group of five mice killed 7 days after intrarenal transplantation of rib chondrocytes, the kidneys contained from two to seven cartilage nodules. The size of nodules varied considerably. 'Large nodules' were those with their largest dimension above 0.5 mm. Bone tissue did not occur. All five kidneys taken for examination 14 days after transplantation of chondrocytes contained bone islands. In two kidneys, cartilage nodules could not be found and in the remaining ones only large cartilage nodules were present. Histological examination confirmed that islands of tissue stained by Alizarin red S represented bone and not some unspecific calcification of kidney parenchyma.

Intrarenal syngeneic transplants of epiphyseal or rib cartilage fragments

In these 14-day-lasting transplants, cartilage of both types was surrounded by granulation tissue. Both tissue could not be found either in the surroundings of transplanted cartilage or within the cartilage itself. Only within the femoral head were there some bone trabeculae that were not removed during preparation of the transplant.

Syngeneic intrarenal transplants of skin fibroblasts

Within the kidneys taken for examination two weeks after injection of the cells, areas of fibrous tissue with nests of keratinizing epidermis were seen. Bone tissue was absent.

Discussion

Injection of epiphyseal chondrocytes into a kidney was followed by cartilage formation during the first week after transplantation and by appearance of bone during the second week in all transplants. Formation of bone in these early transplants was not preceded by cartilage resorption, thus it could not arise endochondrally. In analogous intramuscular transplants, a small amount of bone was formed endochondrally in cartilage produced by epiphyseal chondrocytes only in 2 out of 14 transplants. It is particularly noteworthy that bone formation regularly also occurred in intrarenal transplants of rib chondrocytes although it never appeared in intramuscular transplants of these cells. The region of cartilage from which the latter originated calcifies but remains non-ossified *in vivo* (Ksiazek, 1983).

Bone in kidney could be formed either by host cells stimulated by some inductor released from cartilage or by injected cells under the influence of the kidney environment. To distinguish between these possibilities, allogeneic transplants of chondrocytes were used. It was previously observed (Ksiazek and Moskalewski, 1983) that, in intramuscular transplants of allogeneic epiphyseal chondrocytes, bone formation, which regularly occurred in syngeneic transplants, was inhibited. This inhibition could be prevented by immunosuppression (Malejczyk and Moskalewski, 1988). Similarly, in transplants of allogeneic chondrocytes into a kidney, bone formation was absent, but occurred in immunosuppressed animals. In the latter case, bone islands were surrounded by infiltrating cells, but no clear signs of rejection were present. If, however, two weeks after transplantation of epiphyseal or rib chondrocytes, the immunosuppressed recipients received an injection of spleen cells from syngeneic donors previously sensitized with chondrocytes, the infiltrating cells eroded the bone matrix and many osteocytes were dead. Thus, rejection of bone was evident, strongly suggesting that this bone was formed by the injected cells and not by induction.

Isolated chondrocytes may contain a small admixture of other cell types (Bryan, 1968). Bone in intrarenal transplants could, therefore, be produced either by chondrocytes or by contaminating cells. However, transplants of chondrocytes prepared from rib cartilages, subjected to two-step digestion with collagenase to reduce possible contamination by perichondrial cells, contained bone as in standard transplants. It appears, therefore, that bone in transplants was formed by the chondrocytes themselves.

Since 7-day-old transplants contained only cartilage, it seems that chondrocytes first deposited cartilage matrix and only afterwards transdifferentiated into bone cells and began to produce bone matrix which substituted cartilage matrix. As a matter of fact in some areas cartilage matrix appeared slightly acidophilic and fibrous suggesting transition between cartilage and bone. This surmise is supported by observations of intrarenal transplants of syngeneic rib chondrocytes stained *in toto*. In such 7-day-old transplants only cartilage was present. In kidneys bearing 14-day-old transplants either both cartilage and bone occurred or only bone was present. Thus, cartilage that was present in 7-day-old transplants but which disappeared in the older ones, must have transdifferentiated into bone, since no signs of cartilage resorption were noted.

It may be questioned whether the evidence presented here is sufficient to accept transdifferentiated of chondrocytes into bone cells. Therefore, we suggest this explanation as a hypothesis that requires confirmation by other techniques, but which seems to be compatible with all observations reported in this work.

In older intrarenal transplants, most of the cartilage became substituted by bone. In transplants of rib chondrocytes, no signs of endochondral ossification were noted, but in transplants of epiphyseal cells it was difficult to decide whether this substitution represented continuation of transdifferentiation or whether some endochondral ossification similar to that occurring in the intramuscular transplants took place.

In accord with a previous report (Lacroix, 1951), no transdifferentiation of epiphyseal or rib cartilage into bone was observed after intrarenal transplantation of cartilage fragments. It seems, therefore, that this process occurs only in freshly formed cartilage which might, for example, be more susceptible to the influence of the kidney environment. Transplants of fibroblasts and epidermal cells did not evoked bone formation. This indicates that bone formation in chondrocyte transplants was not caused by unspecific irritation of the kidney.

As far as the relationship between chondrocytes and osteocytes is concerned, there is general agreement that the bone marrow stroma contains precursors of both these cell types (Friedenstein, 1976; Jotereau and Le Douarin, 1978; Owen, 1980; Hirano and Urist, 1981). It has also been demonstrated that cells of the perichondrium and periosteum may give rise either to osteoblasts or chondroblasts, depending on the environment (Tonna and Pentel, 1971; Silberman *et al.* 1983; Nijweide *et al.* 1986). It is, however, uncertain whether the population of stem cells forms both cartilage and bone, because the individual stem cells are bipotential or because the population is a mixture of separate chondrogenic and osteogenic stem cells (Hall, 1981, 1982; Stutzmann and Petrovic, 1982).

It appears that some mature chondrocytes may transdifferentiate into osteoblasts in birds (Lufti, 1971; Kahn and Simmonds, 1977). Transdifferentiation of hypertrophic chondrocytes into osteoblasts in the mammalian epiphyseal growth plate has also been postulated on the basis of autoradiographic and ultrastructural observations (Knese and Knoop, 1961; Holtrop, 1966, 1972; Crelin and Koch, 1967), but has not been confirmed in other studies (Hanaoka, 1976; Miki and Yamamuro, 1987). In intramuscular transplants of isolated epiphyseal chondrocytes in which endochondral ossification took place, bone cells originated from host tissues and no evidence of transdifferentiation of chondrocytes into bone cells was noted (Thyberg and Moskalewski, 1979; Wright *et al.* 1985). Such origin of bone cells was particularly evident when devitalized cartilage with areas of calcified matrix was used as an intramuscular transplant. Host tissues invaded the transplant and bone was formed within the calcified matrix, which suggested that such matrix acts as inductor (Thyberg and Moskalewski, 1979; Ksiazek, 1983). This conclusion is also confirmed by observations reported in this paper. Allogeneic intramuscular transplants of epiphyseal chondrocytes in immunosuppressed animals contained endochondral bone which was not rejected by sensitized splenocytes. This suggests that the bone was of host origin.

Recently, transdifferentiation of hypertrophic chondrocytes into bone cells was described in organ cultures of embryonic mouse metatarsalia cocultured with fragments of embryonic kidney or with young embryos (Thesingh and Scherft, 1986). In our system, however, transdifferentiation seemed to concern mainly chondrocytes which were not hypertrophied. It is, therefore, uncertain whether observations of these investigators and ourselves deal with the same phenomenon.

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