REACTION OF THE RECIPIENT'S TISSUE BED TO TRANSPLANTATION OF ALLOGENEIC BONE TISSUE STORED FOR DIFFERENT TIMES

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Antigenic activity of allogeneic bone tissue was reduced with an increase in the duration of its storage at temperatures of between −25 and −30°C.

The principal carriers of antigenic specificity in bone tissue are the osteocytes and red marrow cells [9, 10, 15]. These cells are destroyed during prolonged conservation by freezing [2, 3]. The possibility is thus not ruled out that an increase in the period of storage is one possible method of reducing the antigenic activity of allogeneic bone tissue. To determine this activity, the method most widely used is that of transplantation into muscle tissue [7, 13, 14, 18]. In this case, against the background of the usual inflammatory reaction, cells performing definite functions in the immunological process appear around the grafted bone [4-6, 8, 16, 17]. By determining the number of these cells and the duration of the period when they can be found, the antigenic activity of the graft can be estimated.

In the investigation described below histological methods were used to study the effect of the period of storage of bone tissue on its antigenic activity.

EXPERIMENTAL METHOD

Experiments were carried out on 150 guinea pigs (24 donors and 126 recipients). Grafts weighing 100 mg were cut from the metaphysis and epiphysis of the donor's femur and tibia, containing cortical and cancellous tissue with bone marrow cells. Fresh grafts and also grafts stored for 1, 3, 6, 9, and 12 months at −25°C were used for the operation. The grafting was carried out as follows: an incision, 0.5 cm long, was made in the skin of the posterior surface of the middle third of the thigh. The graft was introduced between separated layers of the muscles. The skin was sutured with silk.

In series I fresh grafts were transplanted, in series II grafts stored for 1 month, in series III grafts stored for 3 months, in IV for 6 months, in V for 9 months, and in series VI grafts stored for 12 months were transplanted. The number of animals used in each series was 21. The guinea pigs were sacrificed 4, 8, 12, 20, 30, 60, and 90 days after transplantation and the graft was removed from the recipient's soft tissues surrounding it. The material was fixed in 12% neutral formalin, and decalcified in 5% nitric acid. Pieces of tissue were embedded in paraffin wax or celloidin. Sections were stained with hematoxylin and eosin and by Van Gieson's method.

EXPERIMENTAL RESULTS

In the experiments of series I, 4 days after the operation well-marked exudative inflammatory changes (edema of the fibrous connective tissue, diffuse infiltration of that tissue by lymphocytes, neutrophils, and

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eosinophils) were found in the tissues surrounding the fresh graft. On the 8th day these phenomena were reduced in intensity. Meanwhile, localized collections of lymphocytes and solitary plasma cells appeared near the graft. Starting with the 4th day after the operation, a vigorous proliferative reaction was observed around the graft, with the formation of a well-developed layer of fibrous connective tissues. Later this tissue invaded the vascular canals of the cortical layer and the intertrabecular spaces of the cancellous zone and almost completely filled them by the 30th day. On the 8th day, islands of osteogenic cells and bone trabeculae were observed beside the graft. Later, degenerative changes began to develop in this tissue, accompanied by lysis of the osteocytes, and by the 30th-60th day it was undergoing resorption. After the 8th day lacunar resorption appeared on the surface of the graft, with numerous osteoclasts inside the lacunae. By the 30th day, the grafts were much reduced in size. By the 60th-90th day, they were found only as microfragments, varying in size and basophilic in their staining properties. In the newly formed fibrous connective tissue surrounding the bone graft, considerable diffuse infiltration with lymphocytes and polymorphs appeared from the 8th to the 60th day. After the 20th day, collections of large, undifferentiated connective-tissue cells, with a pale, oval nucleus, appeared in the same zone. At all periods of observation in this series of experiments, capillaries distended with blood were seen in the tissues surrounding the graft, and recent diapedetic hemorrhages were present.

In the experiments of series II the reaction of the tissues to a graft stored for one month was identical with the tissue reaction in the experiments of series I. Hence, around grafts of cortical and cancellous bone, including osteocytes and red marrow cells, either obtained freshly or stored for 1 month, differentiation of the cells was clearly defined. On the 4th-8th day after transplantation, these grafts were surrounded by numerous neutrophils and cells characteristic of the reaction of hypersensitivity of delayedtype (lymphocytes, lymphocyte-like cells), as well as histiocytes, macrophages, and solitary plasma cells. The majority of the lymphocytes and lymphocyte-like cells which were observed surrounded the graft for a long time (60-90 days). The possibility is not ruled out that, as well as the osteoclasts, which were found in large numbers, and which play the principal role in bone tissue destruction, the lymphocytes also played some part in this process. Increased resorption of the graft not only led to the appearance of lymphocytes, macrophages, and other cells, but was also accompanied by the appearance of large, undifferentiated, connective-tissue cells with a palely stained, oval nucleus 20 days after transplantation. This type of infiltration is characteristic of antigenic stimulation and is observed in tissues bordering allogeneic grafts [11, 12]. Another characteristic feature of this series of experiments was the appearance of young, newly formed bone trabeculae close to the bone graft, but after 30-60 days these underwent resorption. It is doubtful whether the osteogenesis around the grafted tissue arose on account of the donor's cells. Evidently the fresh osteocytes and red marrow cells, with their ability to induce osteogenesis, stimulated the formation of new bone cells. However, resorption of the newly formed tissue under the influence of antigenic stimulation was evidence of its allogeneic nature. Consequently, further investigations are necessary in order to shed light on this problem.

The characteristics of the cellular reaction as described above were also observed around grafts stored for 3, 6, 9, and 12 months, but to a much lesser degree and at much later periods after transplantation. Polymorphocellular infiltration of the tissues surrounding the graft was less marked, there were fewer of the large, undifferentiated connective-tissue cells with a palely-stained, oval nucleus, and they appeared at later periods, on the 30th-60th day after transplantation. By this time the fibrous connective tissue near the graft was mainly normal in structure. Loose fibrous connective tissue had grown into only about half of the total number of vascular canals and intertrabecular spaces 60-90 days after transplantation of the tissue. Resorption of the grafts was only slight in degree. The formation of new bone tissue near the graft as a rule was not found in these series of experiments (except in two animals).

A study of the tissue reaction of the bed to an allogeneic bone graft containing red marrow cells thus leads to the conclusion that its antigenic activity is reduced in the course of conservation.

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