The Journal of Bone and Joint Surgery

EDITORIALS AND ANNOTATIONS

STUDIES IN THE TRANSPLANTATION OF BONE: A NEW APPROACH

Cardinal Polignac, trying hard to impress Madame de Deffaud with the miracles of St Denis, is said to have told how the saint carried his severed head from the headsman's block in Montmartre to the site of the abbey which bears his name. This, declared the priest, "was a distance of no less than two leagues." "Ah Monseigneur!" replied the Marquise, "I believe in such circumstances that it is only the *first* step that counts."

The first step in unleashing the present intense activity in immunological investigations, which have suddenly replaced an approach to the subject almost exclusively concerned with the study of infectious disease, was the posing of the question by Burnet and Fenner (1949): What is the mechanism whereby the cells of the body can recognise "self" from "non-self"?

It is an obvious fact that a man or an animal lives in harmony with his own cellular components, but if cellular material from almost any other human individual or from an animal is introduced into his tissues, antibody production or some other immunological reaction is evoked. "It is axiomatic," insists Sir Macfarlane Burnet (1961) "that any modern concept of the nature of immunity must start with an interpretation of this capacity of the organism to differentiate foreign chemical configurations from those native to its own structure."

The problem of how foreignness is recognised has been tackled in recent years by Jerne (1960), who believes that the mature individual must possess an elaborate store of "information" to allow it to react immunologically with any foreign configuration that enters the body. He compares this store of information to a "purged xenotypic dictionary" from which all words corresponding to the organism's own chemical configurations have been removed.

The information is stored, according to Burnet's (1959) "Clonal Selection Theory," in the mesenchymal cells such as lymphocytes and plasma cells. The role of the foreign antigen is to select cells which carry the complementary reactive pattern. When the foreign antigens take the form of a skin homograft the cellular infiltration which accompanies the tissue breakdown at the time of rejection presumably represents such selectively mobilised cells, and, according to some, the destruction of the graft cells is directly achieved by the activity of these cellular "messengers of destruction."

Orthopaedic surgeons are well acquainted with the use of stored bone for grafting. The cells of such grafts cannot be expected to survive permanently after transplantation, but the inert substances which form a relatively large proportion of this tissue act to provide a supporting framework for the invading cells of the host.

Even if this so-called *homostatic* graft is replaced by a *homovital* homograft, the living cells will usually be prevented from contributing to the growth of the graft because they provoke an immunological reaction. In both man (Longmire, Cannon and Weber 1954; Phemister 1951; Carnesale and Spankus 1959) and in animals (Enneking 1957) it is widely accepted that autografts are more successful than homografts, and are incorporated into the host skeleton earlier and more completely.

It is of great importance to understand the reasons why homologous bone is less readily accepted than autologous bone. By analogy with the skin homograft, it is tempting to attribute

the inferior results with homografts to an immunological incompatibility between the cells of the host and the graft. In support of this, Nisbet, Heslop and Zeiss (1960) have shown that a transfusion of spleen cells to newborn recipient rats will induce a state of specific immunological tolerance, so that when the same animals were grafted, as adults, with cortical bone from the same donors, the homograft reaction and osteogenesis were modified so as to resemble that seen around autografts.

Most of the fundamental work on transplantation immunity has been concerned with skin grafts, and there is a great need for basic studies of the antigenic components of bone. Medawar (1945), studying skin homografts in rabbits, gave seven as the minimum number of antigens likely to differentiate two unrelated rabbits. On this basis it would be very seldom that a compatible donor for all of this number of antigens could be found, except in the case of an identical twin. In practice, however, not all antigens may be of similar potency in provoking homograft rejection. Thus, working with a mouse sarcoma, Gorer (1938) showed that if mice were compatible for a major antigen, a graft might persist for three months or more, while if they were not so, it regressed in about three weeks. It is therefore possible that we might greatly prolong the life of human homografts if we could match them for a relatively small number of antigens.

Using the erythrocytes of the mouse suspended in a dextran-containing medium and mouse leucocytes, Amos, Gorer, Mikulska, Billingham and Sparrow (1954) were able to detect the appearance, after skin homograft rejection, of serum antibodies which agglutinated both red and white blood cells of the donor. Haemagglutinating antibody has also been found in dogs after massive bone transplantation by Curtiss and Herndon (1955). In the November 1961 issue of this Journal Burwell and Gowland (1961*a*) described their efforts to uncover by various serological tests the antigens involved in a bone-homograft reaction in the rabbit. They were unable to detect the appearance of iso-agglutinins in the serum.

During the past decade much effort has been directed towards assessing the relative importance of free antibody and of lymphoid cells in the rejection of tissue grafts. Even today a definite answer cannot be given. As a rule, serum is unable to transfer skin homograft immunity into a normal animal, whereas this can be achieved with lymphoid cells derived from the spleen or lymph nodes. But it is now clear that there is more than one type of homograft immunity, and the role of the humoral antibodies depends very much on the nature of the target tissue. Passive transfer of immunity by means of serum was shown possible against leukaemia cells by Gorer and Amos (1956). This is not irrelevant to the question of bone grafts, which, at least for cancellous bone, must include marrow tissue. Gorer and Boyse (1959) showed that they could abolish the protective action of bone marrow in fatally irradiated mice by administration of pertinent anti-sera, and there can be little doubt that humoral antibodies play a considerable role in the destruction of incompatible myeloid cells, whether normal or malignant.

What of the osteocytes? Algire (1957) has shown that cells maintained within a porous capsule, which transmits antibody globulin but not cells, are capable of survival in the peritoneal cavity of immunised homologous hosts. It has been suggested that the osteocytes within a bone homograft are in an analogous situation since the bone canaliculi are too small to allow the host inflammatory cells to gain access to them. Heslop, Zeiss and Nisbet (1960) found that osteocytes could survive up to 250 days near the outer surface of a bone homograft, thus showing the ineffectiveness of the immune response in this situation.

But if the osteocytes can survive the homograft reaction, why can a homograft form so little new bone? Heslop, Zeiss and Nisbet (1960) stress that no correlation could be found in their material between osteogenesis and osteocyte survival, which was little more in autografts than in homografts. Indeed, the survival of the osteocytes in a homograft, by maintaining the supply of histocompatibility antigens may exacerbate the inflammatory response around the graft and so interfere with the reparative osteogenic activity of the host.

Evidence has gradually accumulated that the main agents of skin homograft rejection are the host cells, notably the lymphocytes, and in particular a strong analogy has been drawn with the cellular changes in the delayed-type allergic response as exemplified by the tuberculin reaction. In their second paper, Burwell and Gowland (1961*b*) studied the cellular events which follow the insertion of pieces of fresh cancellous bone into the subcutaneous tissue of the rabbit ear. As had been shown in many studies using bacterial and other foreign antigens, the main cellular response occurs in the first regional lymph node, which increases considerably in size and after four days comes to contain an increased number of large and medium pyroninophilic lymphoid cells. These occur in the diffuse cortex and reach maximum at five days after the insertion of the bone homografts. In the case of subcutaneously deposited "classical" antigens such as ovalbumin or diphtheria toxoid the main cellular responses in the regional node have been clearly shown to be linked with the production of antibody. Thus, Leduc, Coons and Connolly (1955) and White, Coons and Connolly (1955) showed by the fluorescent antibody technique that the pyroninophilic cells, which formed the three stages (haemocytoblast, immature and mature plasma cell) in a differentiating family of plasma cells, all contained antibody specific for the injected antigen. The relationship of these events to the cellular changes described by Burwell and Gowland (1961*b*) remains obscure, for they omit any account of cells corresponding with mature plasma cells. Also they find the main site of the pyroninophilic cells to be the cortex of the lymph node rather than the medulla, which is the usual site of antibody-producing plasma cells.

Descriptions of the cellular reactions within reticular tissues are notoriously bedevilled by terminology. Burwell and Gowland give no less than fourteen possible synonyms for their "large and medium lymphoid cells." In the last analysis it matters little what a cell is called so long as the name is accompanied by an accurate description. It is disquieting therefore to witness the birth of a completely new term "the medium lymphoid cell" without any formal description or dimensions. Without such accurate morphology the newer cellular immunology is destined for chaos.

Whatever the theoretical interpretation of their observed cellular responses to bone homografts, Burwell and Gowland, in this number of the Journal, have used these to assess the antigenicity of variously treated rabbit cancellous bone homografts. This method appears valid, although the reader is left in some doubt whether a graft-versus-host reaction (rather than *vice versa*) might not better account for some of the cellular responses in the regional lymph node. Thus, Gowans (1961) has shown that a transfusion of homologous parental thoracic duct lymphocytes into F₁ hybrid recipients results in the appearance of cortically located pyroninophilic cells, and in this case the host-versus-graft response is precluded. Marrow-free cancellous bone is found not to produce cytological evidence of an immune response. This could mean that the antigens responsible for homograft reaction reside principally in the nucleated marrow cells, or that the latter themselves act as precursors of the observed cortical pyroninophilic cells.

In another paper in the present Journal Chalmers and Ray have examined the claim that immature bone homografts can produce extensive new bone. Homografts of eighteen-day foetal femora in pure strains of mice showed no fundamental difference in behaviour from grafts of more mature mouse bone. From these results it seems that there is no reason to suppose that bone from an immature source is likely to behave more favourably than more mature bone homografts in current use.

These recent investigations, while providing no easy means for circumventing the bone homograft reaction, have explored many questions fundamental to orthopaedic immunology. It is apparent that the characterisation of the number and potency of histocompatibility antigens in bone deserves urgent future consideration, and that we must continue to explore ways for modifying the immunological responses by which the individuality of the body is so strictly preserved.

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