IMMUNOLOGY

Graft-versus-host Specificity of Lymphoid Cells transformed from Bone Marrow Allograft in Supralethally X-irradiated Dogs

IT has been shown that bone marrow allografts in supralethally X-irradiated dogs transform into lymphoid cells[1]. Soon after transplantation, so-called 'hyperbasophilic' cells may be observed in bone marrow punctures, and within 6–7 days they and transitional to small lymphocyte forms appear in great numbers in the peripheral blood. We have supposed that these lymphoid cells transformed from bone marrow stem cells are immunologically competent cells which are able to induce the 'graft-versus-host' reaction. This communication presents some evidence for this hypothesis. The participation of these cells in transplantation immunity was tested by their ability to induce a hypersensitivity reaction of the delayed type, called a 'transfer reaction' by Brent *et al.*[2].

Within 7 days of allogeneic bone marrow transplantation in dogs X-irradiated with a dose of 1,000 r., their own blood lymphocytes were injected intradermally into each dog. Leucocytes were isolated from 100–200 ml. of heparinized arterial blood, washed twice with medium 199 and resuspended in this medium to make up a count of nearly 5×10^7 per ml. 0·1 ml. volumes of cell suspension were injected into the lateral skin of the chest; two injections were normally made. In evaluating the reaction erythema, induration and swelling were taken into consideration, but the diameter of the skin lesion was considered the most important sign when scoring. The approximate scores given for the various diameters of the skin lesions were: +, (weak, 6–10 mm); 2+, (moderate, 11–15 mm); 3+, (strong, 16–20 mm); 4+, (21–25 mm); 5+, (very strong, 26–30 mm); and > 5+, (strongest, more than 30 mm). Reactions less than 2+ were considered as unreliable.

Five out of six animals demonstrated strong to strongest skin lesions within 18 h (see the upper part of Table 1). Surprisingly, in one dog the reaction was unreliable; other workers[2,3] have also noticed that not all experimental animals respond to immune lymphocytes. In one animal, the skin lesion presented a huge haemorrhage in the form of a circle with diameter 30 mm.

The immunological specificity of the reaction described is high. Lymphocytes tested in other dogs, intact or irradiated, either provoked moderate reactions (in about half the animals) or did not provoke any reliable reaction (see the lower part of Table 1). These strong skin lesions are therefore the result of a specific interaction between transplantation antigens and the immune lymphocytes committed against them. Some positive non-specific reactions may perhaps be explained by the presence of common transplantation antigens in pairs of animals selected at random. Nor can the possibility be excluded that immuno lymphocytes quickly degenerate in the sites of injection, releasing some active substances which damage tissues non-specifically. It should be mentioned that, according to our unpublished data, these cells have a sharply decreased viability in tissue culture.

Table 1. SKIN REACTION TO TRANSFORMED LYMPHOID CELLS

Lymphocyte donors No.	Lymphocyte recipients No.	Leucocyte No. in injection ($\times 10^6$)	Percentage of lymphoid cells in injection	Reaction in 18 h
116	116	5·0	100	3+
118	118	4·8	97·5	+
139	139	5·0	100	> 5+, 2+
143	143	4·1	94	> 5+, 3+
154	154	3·5	100	4+, 3+
159	159	4·0	74	3+, 3+
143	142*	4·1	94	2+, 2+
156	154	6·4	81	2+, +
139	Intact 1	5·0	100	±
154	,, 3	3·5	100	+, +
154	,, 4	3·5	100	2+, +
154	,, 5	3·5	100	2+, 2+
156	,, 3	6·4	81	+, ±
159	,, 6	4·0	74	±, ±
159	,, 7†	2·4; 3·2	74	±, ±

* Thymectomized dog, 1,000 r. + bone marrow allograft.
† Dog survived 1·5 years after 1,000 r. + two bone marrow allografts.

Non-immune lymphocytes in amounts up to 4×10^6 provoked no reliable reaction either in autologous or in allogeneic injections both in intact and irradiated dogs. The latter fact again confirms the belief that the 'graft-versus-host' reaction is the only immunological component of the skin reaction provoked by tested lymphocytes in X-irradiated allografted dogs.

These experimental results support the hypothesis that the stem cells of bone marrow allografts in supralethally X-irradiated dogs transform into immunologically competent lymphoid cells which are directed against the transplantation antigens of the recipient. Such a transformation leads to interruption of the normal haemopoiesis of bone marrow allograft and removes its protective effect; the aggressive trend of the transformed cells against the recipient can only aggravate the recipient's radiation disease. The picture that we have observed in concentrated form in skin lesions can be expected to hold throughout the body, and it is a matter for speculation whether this reaction has a total diffuse character or is directed against certain 'targets'.

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HISTOLOGY

Mitochondria in Odontoblastic Processes

THE odontoblastic processes of human dentin are projections of the odontoblast cytoplasm into spaces in the dense collagenous, highly mineralized dentin matrix[1]. These projections can be up to several mm in length resulting in the cytoplasm being a long distance (2,000–3,000μ) from the odontoblast nucleus. The contents of odontoblastic processes in published electron micrographs have shown a varied appearance. In general they display a granular texture but do not contain recognizable cytoplasmic organelles[2–4]. Most of these specimens were prepared with standard osmium fixatives which have a very poor penetrating ability. We have investigated the ultrastructure of dentin fixed with glutaraldehyde which is particularly favourable for the preservation of membrane systems and has good penetrating ability. A freshly extracted tooth (severed from its blood supply no longer than 8 min) was broken into small fragments with at least one dimension 0·5–1 mm thick. These were immersed in cold fixative containing 1·25 per cent glutaraldehyde in *s*-collidine buffered to *p*H 7·4–7·55 and adjusted to a final osmolality of 360 milliosmols. Samples were post-fixed in 4 per cent osmium tetroxide for 48 h and then decalcified in a nitric acid–formalin mixture[5] for 12 h. They were then dehydrated in an alcohol series and embedded in 'Epon' *B* (ref. 6). Sections were cut with a diamond knife on an *LKB* 'Ultratome' and examined in an RCA *EMU-3-G* electron microscope.

Figs. 1 and 2 show sections of odontoblast processes from the root of an impacted lower left 3rd molar of a 26-year-old woman. The sections of processes were taken from a point midway between the cementum–dentin and the dentin–predentin junctions. The odontoblast cytoplasm contains (Figs. 1 and 2) mitochondria, membranous elements of endoplasmic reticulum and free and associated ribosome-like granules.

The mitochondria are spheroid, with a very distinct double membrane, and contain a variable number of inter-