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Perfusion of rabbit hearts with human blood results in immediate graft thrombosis, a temporally distinct component of hyperacute rejection

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Hyperacute discordant xenograft rejection can be simulated by a blood-perfused working isolated heart [2]. The survival of the heart is dependent on its functional integrity, and the preparation is thus sensitive to early myocardial damage.

Perfusion of rabbit hearts with human blood produces immediate graft destruction by a thrombotic process which is a distinct component of hyperacute rejection.

Key words: Heart graft thrombosis – Hyperacute rejection

Methods

Hearts of 1.7 kg New Zealand White rabbits were perfused with rabbit or human group AB blood. Blood of either species was collected into heparin (6500 units/l) and was reduced to a haematocrit of 25%. Human blood was unmodified, or had previously perfused another heart, or had been treated as detailed below. A log-rank analysis of survival of the six hearts in each group was performed.

Complement was depleted by the additon of 10 μg purified cobra venom factor (CoF) [3] to 240 ml plasma. Platelets were removed (99.5%) with a Pall RC50 filter. Platelet activating factor was inactivated by addition of WEB 2170, a specific antagonist. Anti-rabbit antibody (ARA) was absorbed from plasma by incubation with rabbit blood cells for 80 min at 4 °C.

Hearts were perfused as working preparations [2] until functional failure. Lytic human ARA titres were measured before and after perfusion [2]. Complement classical pathway activity was measured using the CH50 technique [3]. Hearts were examined after perfusion by conventional and immunohistological methods (IgG, IgM, C3, C4, C9).

Results

Homologous perfusion resulted in organ survival for 300 min with no functional change. Hearts perfused with human blood failed at 1 min ($P < 0.001$). This failure was characterized by cessation of coronary flow and an ischaemic appearance and ECG. Examination of hearts revealed platelet occlusion of small vessels and immediate deposition of interstitial IgG, endothelial IgM and C3, 4 and 9. This event has been termed immediate graft thrombosis (IGT). Heart perfused with modified human blood did not thrombose.

Blood which had previously perfused another heart produced rejection at a median time of 20 min ($P < 0.001$). Perfusion with platelet-free blood resulted in rejection at a median time of 22 min ($P < 0.001$). Platelet activating factor block with WEB 2170 produced rejection at a median time of 20 min ($P < 0.001$). Perfusion with blood from which the ARA had been absorbed resulted in rejection at a median time of 33 min ($P < 0.001$).

Examination of rejected hearts revealed neutrophil and lymphoid infiltrates. Interstitial IgG and endothelial IgM were again seen. C9 was deposited in excess or absence of C4. Treatment with CoF delayed organ failure to a median time of 207 min ($P < 0.001$). No C3, 4 or 9 was seen. Organ survival is summarized in Fig. 1.

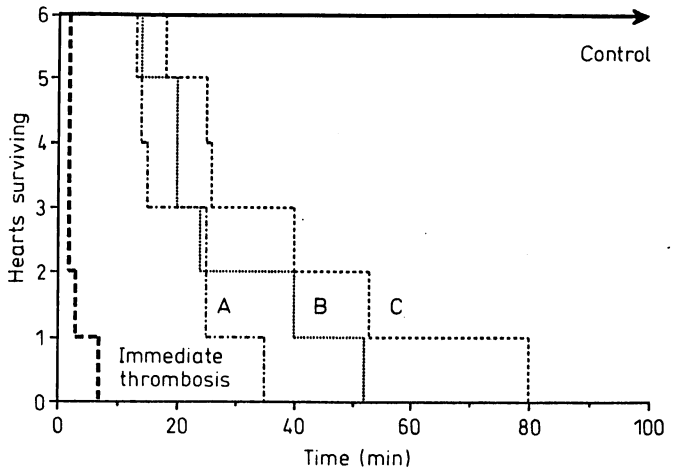

Fig. 1. Organ survival. *A*, WEB 2170; *B*, platelet free; *C*, antibody absorbed

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Discussion

Rejection in this species combination has two components. The first is immediate and thrombotic, and can be prevented by removal of platelets or inhibition of platelet activating factor. Removal of IgM anti-rabbit antibody also presents the thrombosis but has no effect on subsequent rejection. The central part played by complement, by whichever pathway, is confirmed by the prevention of both IGT and subsequent events by treatment with CoF. Immediate graft thrombosis, then, is a process initiated by IgM anti-rabbit antibody and mediated by complement by its classical pathway. It is then effected by platelet activating factor presumably of endothelial origin and the formation of platelet plugs. Heart which are rejected in the absence of IGT have C9 deposition in excess C4 suggesting that the alternative pathway of complement is responsible for this second process. This hypothesis has been confirmed in other studies [1].

The assumption that hyperacute discordant xenograft rejection is the consequence of heterophile antibody [6, 7] is in part confirmed. However, a distinction has been made between immediate thrombosis and another rapid event mediated by the alternative pathway of complement. The potential importance of this pathway has been commented upon by other investigators [4, 5].

The distinction that has been made between immediate graft thrombosis and alternative pathway mediated rejection in discordant organ perfusion has been made only with the use of a new, sensitive technique.

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