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Activation of the alternative pathway of complement is an important component of hyperacute rejection of rabbit hearts by human blood

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Hyperacute discordant xenograft rejection can be simulated by blood perfused working isolated heart [1]. 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 results in immediate graft destruction by a thrombotic process. Prevention of this process results in rapid rejection at about 20 min by the alternative pathway of complement.

Key words: Heart graft thrombosis – Hyperacute rejection – Alternative pathway of complement

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 inactivated by the addition of 10 μg purified cobra venom factor (CoF) [3] to 240 ml plasma. Alternative pathway inactivation alone was produced by heating plasma at 50°C for 20 min to destroy factor B. Anti-rabbit antibody (ARA) was absorbed from human plasma by incubation with rabbit blood cells for 80 min at 4°C.

Hearts were perfused as working preparations [1] until their functional failure. Lytic human ARA titres were measured before and after perfusion [1]. Complement classical pathway activity was measured by 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 of 300 min with little functional change. Hearts perfused with human blood underwent immediate graft thrombosis (IGT) ($P < 0.001$). This is initiated by IgM anti-rabbit antibody and the classical pathway of complement, is mediated by platelet activating factor and is effected by platelets [2]. Hearts perfused with human blood modified as above did not undergo this immediate process.

In systems where IGT was prevented, rejection occurred at a median time of 20 min ($P < 0.001$) [2]. Examination of rejected hearts showed neutrophil and lymphoid infiltrates with interstitial IgG and endothelial IgM deposits. C9 deposition was in excess of C4.

Perfusion with blood, complement-inactivated with CoF, delayed organ failure to a median time of 207 min ($P < 0.001$). No C3, 4 or 9 was seen. Perfusion with heat-inactivated blood resulted in survival to 300 min with little functional change ($P < 0.001$). IgM, IgG and C3 were deposited. Classical pathway CH50 remained normal. Rejection at a median time of 33 min still occurred with blood from which the ARA had been absorbed. No IgM was seen in these hearts though the interstitial IgG remained. C3 was present as was C9 in excess of C4. Organ survival is summarized in Figure 1.

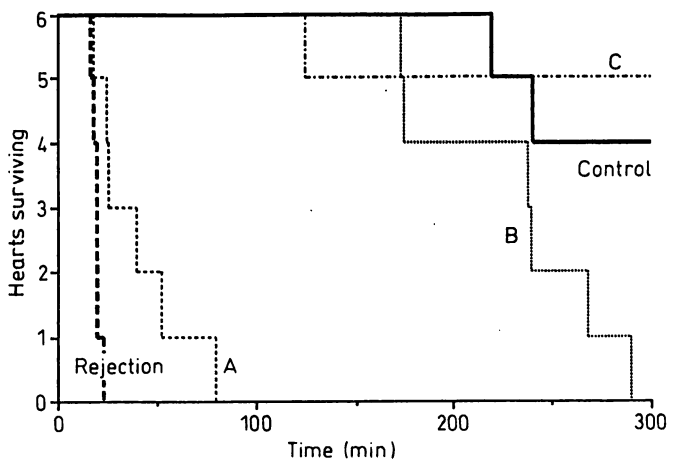

Fig. 1. Organ survival. *A*, antibody absorbed; *B*, complement inactivated; *C*, alternative pathway inactivated

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Discussion

Hearts which had been rejected at about 20 min in the absence of IGT had C9 deposition in excess of C4 suggesting that activation of the alternative pathway of complement had occurred. The part played by complement, by whichever pathway, is confirmed by the prevention of rejection by complement inactivation with CoF. The important role of the alternative pathway of complement is demonstrated by the prevention of rejection by heart inactivation, to destroy factor B and inactivate this pathway, despite the presence of normal plasma titres and deposition of anti-rabbit antibody and a normal classical pathway CH50. Failure of absorption of heterophile antibody to prevent rejection confirms the unimportance of this antibody in this second component of rejection.

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

The assumption that hyperacute discordant xenograft rejection is the consequence of a heterophile antibody [6] has been challenged [4, 5]. These results suggest that rejection is multifactorial and that an important component is mediated by the alternative pathway of complement.

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