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Expression of human decay accelerating factor or membrane cofactor protein genes on mouse cells inhibits lysis by human complement

D. J. G. White, T. Oglesby, M. K. Liszewski, I. Tedja, D. Hourcade, M.-W. Wang, L. Wright, J. Wallwork, and J. P. Atkinson

From the Clinical School of Medicine, University of Cambridge Addenbrooke's Hospital, Hills Rd., Cambridge CB2 2QQ, and The Howard Hughes Medical Institute at Washington University, St. Louis, MO, USA

Abstract. Mouse cells expressing the human complement regulatory proteins decay accelerating factor (DAF) or membrane cofactor protein (MCP) were produced both by hybridoma technology and by transfection with the appropriate cDNAs. The expression of either or both of these products protected the mouse cell from lysis by human (though not rabbit) complement in the presence of naturally occurring human anti-mouse antibody. This effect could be abrogated by the addition of monoclonal antibody against DAF or MCP. These data suggested that the production of animals transgenic for human complement regulatory proteins should in principle be similarly protected from hyperacute xenograft rejection.

Key words: Decay accelerating factor – Membrane cofactor – Mouse cells

Vascular grafts transplanted between distantly related species are hyperacutely rejected minutes after revascularisation. Numerous investigations have demonstrated that complement is critically involved in this process [13, 14] although the precise mechanisms by which this occurs are poorly understood. Platt et al. [15] have demonstrated that pig endothelium can be activated as assessed by the release of hipuran sulphate by the fixation of C3b via the classical pathway. Forty and coworkers [5] have demonstrated that platelet thrombus formation induced by classical pathway activation of complement causes rapid failure of rabbit hearts perfused with human blood. However both he [4] and others [8, 11, 18] have also demonstrated a role for the alternate pathway of complement in hyperacute xenograft rejection. The enzymatic cleavage of C3 by the C3 convertases is a key step in complement-mediated destruction via both pathways and is regulated b a family of proteins termed the regulators of complement activation (RCA) [16]. This family includes serum proteins (factor H and C4 binding protein), receptors (CR1 and CR2), and membrane bound proteins [decay accelerating factor (DAF) and membrane cofactor protein (MCP)]. Since DAF and MCP are believed to protect autologous tissue from endogenous complement activation [12], we sought to determine whether these human proteins could protect discordant mammalian cells from human complement-mediated destruction.

Offprint requests to: D. J. G. White

Materials and methods

Human/mouse cell hybrids generated by fusion of human EBV transformed B cells with the non-secreting mouse myeloma X63-AG8.653 [9] were screened for the presence of human chromosome 1. This was performed by hybridization with oligonucleotide primers specific for human chromosome 1 following PCR amplification of DNA extracted from cell lines. Both upstream (5′-CCACAGGT-GTAACATTCTGT-3′) and downstream (5′-GAGATAGTGT-GATCTGAGGC-3′) primers were from the sequence of human antithrombin II (AT3) gene [22]. A human/mouse hybridoma, B10, (which secretes human anti-tetanus antibody, N. Hughes-Jones personal communication) retained human chromosome 1. An EBV transformed human tonsilar B cell line, T5 and a mouse/mouse hybridoma, DB3 (also produced by fusion with X63-Ag8.653; [21]) were used as negative and positive controls respectively.

Chromium release assay was performed as previously described [3]. Complement was absorbed at +4°C with mouse spleen cells (2 mls/spleen) to remove lytic naturally occurring anti-mouse antibodies. Human or pig serum was used as a source of naturally occurring antibodies and was heat inactivated at 56°C for 30 min. Monoclonal antibodies were added to labelled cells immediately prior to the start of the assay at 10 μg/ml.

Mouse fibroblasts were transfected with DAF and MCP cDNAs subcloned in the forward orientation into the EcoR1 site of the expression vector pHBapr-1-neo [6]. An additional construct, in which the MCP sequences were cloned into the expression vector in the reverse orientation, was isolated and used as a control. Cloned cDNA for DAF and MCP were obtained and characterized as previously described [1, 10]. Plasmid DNA was prepared using the pZ523 kit (5′-3′ Inc West Chester, Pa. USA). NIH 3T3 cells were transfected utilizing lipofectin (Bethesda Research Laboratories, Gaithersburg, Md. USA) and then cultured for 2 days in DME supplemented with 10% horse serum following which geneticin (Gibco Grand Island NY USA) was added to the medium at an active concentration of 0.5 mg/ml. Cells were subsequently maintained in this medium. DAF transfectants were further selected by fluorescence activated cell sorting (EPIC 75, Coulter Corp, Hialea, Fla. USA).

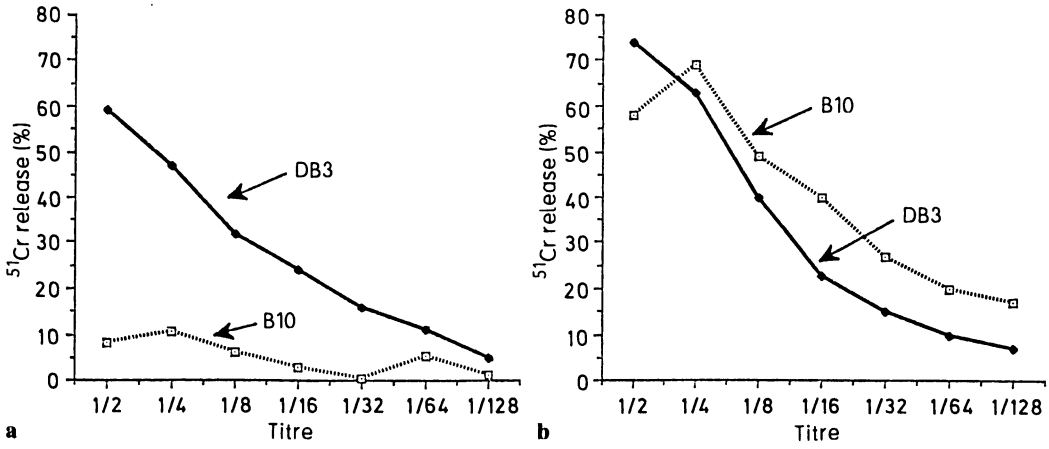

Fig. 1. Shows a comparison between the ability of **a** human and **b** rabbit complement, in the presence of "naturally occurring" human anti-mouse antibody, to lyse either a mouse/human hybrid, B10 possessing human chromosome 1 and expressing human DAF or a mouse/mouse hybrid control, DB3

Results

FACS analysis using the monoclonal antibodies 1A10 [17] and E4.3 [20] showed that the selected human/mouse hybrid, B10, expressed both human DAF and MCP as did the human tonsilar cell line T5. The mouse/mouse hybrid did not express these RCA products. Figure 1 a shows that naturally occurring human anti-mouse antibodies and human complement were able to lyse DB3, the mouse/mouse hybridoma, but not B10, the human/mouse hybridoma. Both cell types were killed by rabbit complement (Fig. 1 b). T5 exposed to pig anti-human antibodies was lysed in the presence of rabbit complement but not human complement (data not shown).

Cell lines transfected with the DAF or MCP constructs were isolated and shown by FACS analysis to be expressing DAF or MCP. The MCP transfectant (unsorted) expressed approximately 1×106 copies per cell (in the range of a malignant epithelial cell line [19]) while the DAF transfectant (sorted twice) expressed approximately 30000 copies per cell, similar to a normal peripheral nucleated cell. The cytoprotective effect of transfecting the individual genes for DAF or MCP was tested by treating all three cell lines (the two expressing cell lines and the control transfected in the reverse orientation) with human complement and naturally occurring human anti-mouse antibodies. The mouse fibroblasts expressing human DAF (Fig. 2 a) or MCP (Fig. 2 b) were almost completely protected from lysis by human complement while the reverse MCP control line was lysed. Appropriate controls demonstrated that this cell lysis was complement mediated. The protective effect was abrogated by the addition of monoclonal antibodies against DAF (IC6 [2]) or MCP (GB24 [7]) to the appropriate assay. A nonspecific monoclonal served as a control in these assays.

Discussion

A precise understanding of the steps of the complement reaction that participate in hyperacute xenograft rejection will be needed to design appropriate strategies for therapeutic intervention. This paper describes experiments demonstrating the complement regulatory activity of human DAF and MCP on the surface of hybrid and transfected mouse cells. These data also provide a demonstra-

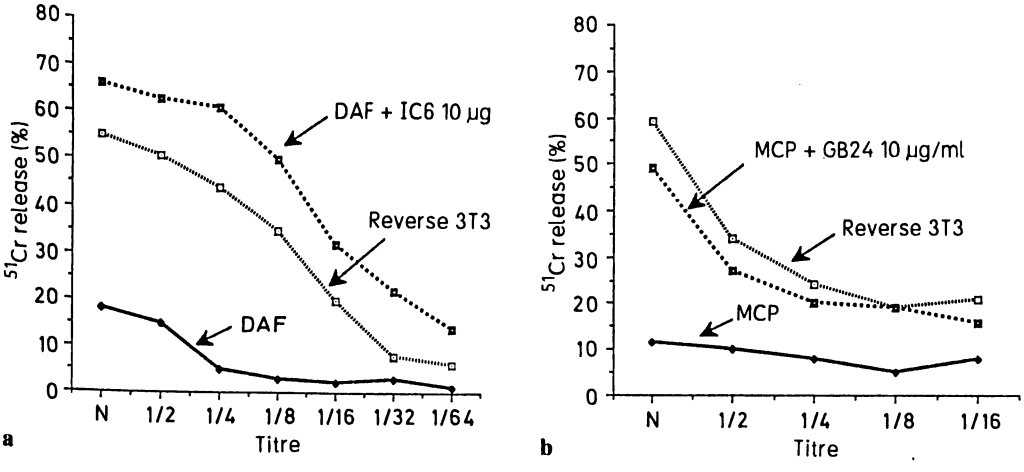

Fig. 2 a, b. The cytoprotective effect of transfecting a mouse fibroblast cell line (3T3 NIH) with **a** MCP or **b** DAF. Controls are the transfected cell line blocked with monoclonal antibody against the expressed product and 3T3 cell line transfected with the MCP gene in the reverse orientation *(Reverse)*

tion of cytoprotection from complement by genetic manipulation of the target cell. The assay used is in many respects analogous to the mechanisms involved in hyperacute xenograft rejection. Recently Yannoutsos and co-workers produced mice transgenic for human DAF (manuscript in preparation). The production of such transgenic animals expressing human complement regulatory products such as DAF and MCP at appropriate levels, should, in principle, permit organs derived from such animals to be transplanted into man without hyperacute rejection. What subsequent immune damage such an organ xenograft might suffer is not known.

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