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How endothelial cells protect themselves from complement by expressing decay-accelerating factor (DAF)

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Keywords

Complement, cytokine, decay-accelerating factor, endothelial cell, inflammation

Context

How do endothelial cells (ECs) protect themselves against damage by complement, particularly when they are present at the interface between blood and inflamed tissue? Cell surface proteins have evolved which prevent EC damage by the cytotoxic defence system, decay-accelerating factor (DAF), protectin (CD59) and membrane cofactor protein (MCP). DAF prevents the formation and accelerates the decay of complement 3 (C3) convertases, MCP binds to C3b and C4b promoting their degradation and CD59 inhibits the membrane-attack complex (MAC). How expression of these factors is controlled is not clear. The Hammersmith group has investigated control mechanisms since they are pertinent to the understanding of chronic inflammatory conditions such as rheumatoid arthritis and systemic lupus erythematosus. To test the hypothesis that inflammatory cytokines and complement(C) MACs play a role in the expression of DAF, CD59 and MCP, and to investigate the intracellular signalling pathway involved in DAF expression.

Significant findings

HUVECs and DMECs were shown to constitutively express DAF, MCP and CD59. DAF and CD59 were removed by PIPLC showing them to be GPI-anchored, while MCP was not. The possibility of deleterious effects by trypsin/EDTA was excluded by demonstrating the same cell surface protein expression on ECs which had been harvested by non-enzymatic means.

Of the three surface proteins, DAF was the only one to show a significant increase after a 48 h incubation with TNF-? or IFN-?, and increased further if the two were added together. Time course experiments showed DAF to be detectable at 24 h and maximal by 72 h. Cytokine-induced DAF expression was not inhibited by the PKC antagonist, unlike the PKC agonist (PBu)-induced DAF expression. Cycloheximide completely inhibited both cytokine- and PBu-induced DAF expression. These results show that cytokines cause DAF expression via a PKC-independent pathway and requires

de novo protein synthesis. Northern blot analysis found low levels of DAF mRNA in unstimulated cells. Following cytokine stimulation for 6 h, levels rose seven fold in DMECs and four fold in HUVECs.

HMECs exposed to MAC had a three fold increase in DAF (response maximal by 24 h and maintained at 48 h). The absence of response with heat-inactivated human serum or C7-deficient serum confirmed dependence on C activation. Additionally, C8-deficient serum also abrogated the response, suggesting generation of C5b-7 alone is insufficient to induce DAF. PKC antagonism did not inhibit DAF expression.

The combined stimulation of cytokines and the MAC revealed a dose-dependent increase in DAF with a maximal increase greater than with either stimulus alone. Results of flow cytometry for C3 bound to HMECs showed DAF to be reduced in the cytokine-stimulated ECs. Furthermore, inhibitory anti-DAF monoclonal antibodies reversed this response, while the inhibitory anti-CD59 did not, showing DAF inhibits C3 binding to HMECs.

Comments

Furthering our understanding of EC control mechanisms is integral to understanding how damage is limited in chronic inflammatory diseases. It allows us to speculate on the causes of diseases affecting ECs, such as the vasculitides, and provides potential avenues of intervention.

Methods

Human umbilical vein ECs (HUVECs), dermal microvascular ECs (DMECs) and cell line human dermal microvascular ECs (HMECs) were used. Flow cytometry determined expression of the three factors, by resting HUVECs and DMECs. Incubation with Phosphatidylinositol-specific phospholipase C (PIPLC) prior to flow cytometry allowed investigators to find whether any of the three surface proteins were GPI-anchored. Results obtained using trypsin/EDTA as a detaching agent were compared with a non-enzymatic cell-dissociation solution.

The effect of pro-inflammatory cytokines, TNF-a, IL-1? and IFN-?, on HUVECs and DMECs expression of DAF, CD59 and MCP was examined. An antagonist of protein kinase C (PKC) was added to see whether the PKC pathway was mediating DAF expression. The PKC agonist phorbol 12,13-butyrate (PBu) was also used and compared with cytokine stimulation.

Cycloheximide was used to determine if protein synthesis was needed for DAF expression, and Northern blots were used to investigate DAF mRNA levels following cytokine stimulation. To study the effect of MAC on HMEC expression of DAF, EC monolayers were opsonised with IgG2a anti-endoglin monoclonal antibody, thus optimising complement fixation ability.

ECs were incubated with 2.5% normal human serum (NHS) to generate the MAC. Control experiments used heat-inactivated NHS, C7 and C8-deficient sera, and the PKC antagonist.

Pro-inflammatory cytokines and the MAC were then examined together, ECs were incubated overnight with TNFa, or IFN-?, washed and opsonised, then incubated with NHS to generate the MAC. The effect of DAF on C3 binding to ECs was investigated. Stimulated HMECs were opsonised, incubated with NHS, then C3 binding quantified using flow cytometry. Blocking experiments used non-complement-fixing anti-DAF or anti-CD59 monoclonal antibodies.

References

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