SEPARATION OF SELF FROM NON-SELF IN THE COMPLEMENT SYSTEM: A ROLE FOR MEMBRANE COFACTOR PROTEIN AND DECAY ACCELERATING FACTOR

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INTRODUCTION

The complement (C) system provides a potent means of recognizing and eliminating foreign elements. It is critical, however, to focus these actions on foreign particles and to prevent inadvertent attack against host tissue. Integral to this function are regulatory proteins of the complement system. Such components provide a means of separating "self" from "nonself" during complement attack (1). Prior to 1980, several plasma proteins were known to fulfill this role. However, in the early 1980's two additional regulatory proteins of the C system, decay accelerating factor (DAF) and membrane cofactor protein (MCP), were identified (reviewed in 2-6). Their biologic activities, "decay accelerating activity" for both C3 convertases in the case of DAF and "factor I dependent cofactor activity" for both C4b and C3b in the case of MCP, overlapped completely with that of two plasma regulatory proteins C4bp and factor H. The deficiency of DAF in paroxysmal nocturnal hemoglobinuria was instrumental in illustrating the critical role that membrane proteins play in protection against C deposition on self tissue. Also, the ability of DAF, because of its glycolipid anchor, to reinsert in a biologic membrane provided a means to assess its regulatory activity. These same approaches were not possible to demonstrate a protective role in the case of MCP. In this report selected structural and molecular aspects of DAF and MCP are summarized and their role in protecting tissue from C attack illustrated.

DECAY-ACCELERATING FACTOR (DAF OR CD55)

In 1969, Hoffmann reported that a substance remaining in the aqueous phase from an extraction of human erythrocyte stroma with n-butanol could inhibit C-mediated hemolysis thorugh an acceleration in the decay of the classical pathway C3 convertase (7,8). More than a decade later Nicholson-Weller and colleagues (9) purified an intrinsic membrane glycoprotein that expressed decay accelerating activity from guinea pig and later human erythrocyte stroma by butanol extraction.

A. Protein

DAF is present on virtually all peripheral blood cells, epithelial surfaces and endothelial cells. Human intracellular pro-DAF and mature DAF have M_rs of 45,000 and 70,000 respectively on SDS-PAGE under reducing conditions. DAF has one N-linked complex-type oligosaccharide and multiple, highly sialylated O-linked oligosaccharides. DAF is anchored by glycophospholipid.

B. CDNA

The deduced amino acid sequence codes for a protein of 347 amino acids. Starting at the amino-terminus, there are four contiguous short consensus repeat (SCR) units of approximately 60 amino acids each. These SCRs are homologous to modules found in over 30 proteins including 11 proteins of the C system (reviewed in reference 6). The SCRs are followed by a 70 amino acid region that is rich in ser/thr/pro residues, a site of clustered o-glycosylation. At the beginning of the second SCR there is one site for an N-linked sugar. The protein ends in a 24 amino acid segment of hydrophobic character but lacks both an anchor sequence and a cytoplasmic tail. This carboxy-terminal peptide is removed posttranslationally and a glycophospholipid anchor is attached.

C. Gene

DAF is a single-copy gene composed of 10 exons spread over 50 kb. These exons encode (sequentially in a 5' to 3' direction) the 5' untranslated region/signal peptide, SCR-1, SCR-2, SCR-3 (split between two equally sized exons), SCR-4, ser/thr/pro-rich region (three exons), and the carboxy-terminal hydrophobic extension peptide/3'-untranslated region together on the final exon. The DAF gene is located at 1q32 in the regulators of complement activation cluster. Within an 800-kb segment, the order of the genes are MCP-CR1-CR2-DAF-C4bp.

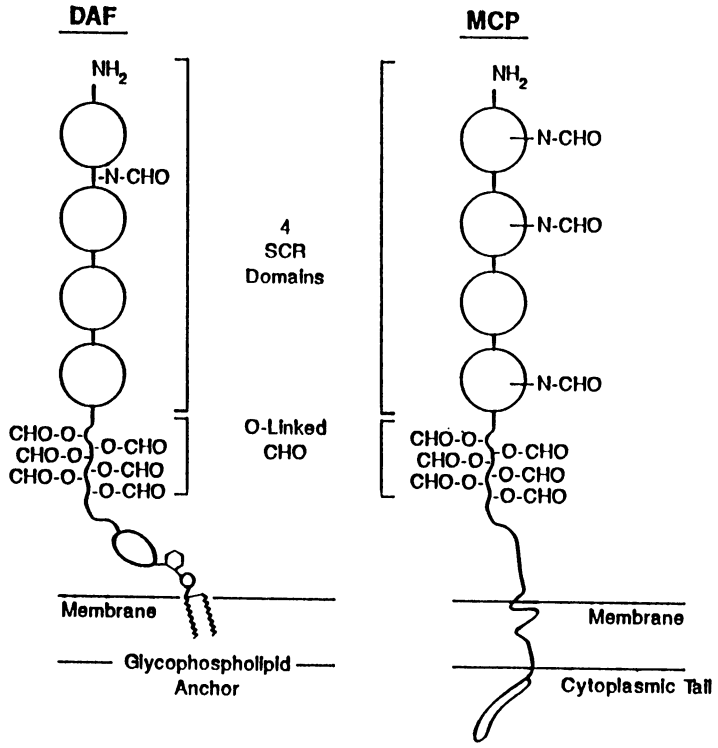

Figure 1. Structure of membrane glycoproteins decay-accelerating factor(DAF) and membrane cofactor protein (MCP) deduced from cDNA sequence and biochemical studies. The short consensus repeats (SCR) are depicted as globular structures and sites of N- and O-linked carbohoydrates (CHO) are indicated.

MEMBRANE COFACTOR PROTEIN (MCP OR CD46)

Cole et al. (9) identified MCP, then termed gp 40-70, as a third class, in addition to CR1 and CR2, of electrophorectically distinct C3-binding membrane proteins of human leukocytes. Soon thereafter Seya et al. (10) purified the protein and demonstrated its cofactor activity.

A. Protein

MCP is present on all peripheral blood cells (except erythrocytes), epithelial surfaces and endothelial cells. A distinguishing strcutural characteristic of MCP on SDS-PAGE is the presence of two broad protein species with M_rs of 59,000 to 68,000 and 50,000 to 58,000. The larger mol wt species contains more sialic acid. Also, much of the M_r heterogeneity of the two forms is secondary to variability in glycosylation. The two precursors of MCP with M_rs of 41,000 and 43,000 possess three N-linked high mannose units. In the Golgi, these sugars are converted to a complex type and multiple (5 to 10) O-linked units are added. Three SDS-PAGE patterns are observed (upper band predominant, lower band predominant, or equal band pattern). These quantitative differences represent a codominantly inherited expression polymorphism of the protein (11).

B. cDNA

The mature MCP protein exists as a family of similar isoforms which vary from 335 to 357 amino acids. Beginning at its amino-terminus, MCP is composed of four SCRs, a ser/thr/pro-enriched area of variable length, a short segment (12 amino acids) of unkown significance, a hydrophobic membrane-spanning domain with a basic anchor, and one of two cytoplasmic tails. Alternative splicing of the ser/thr/pro domains produces three cDNA patterns relative to this area. In addition, two cytoplasmic tails, one of 16 amino acids and one of 23 amino acids, are regularly utilized. Peripheral blood cells and cell lines express variable quantities of four of these isoforms (12). Several lines of evidence including transfection experiments, indicate that the higher mol wt form of MCP corresponds to isoforms containing a 15 amino acid ser/thr/pro-rich exon (with either tail) (12). The lower mol wt form correlates with isoforms lacking this exon (with either tail). Although this ser/thr/pro segment is only 15 amino acids in length, the addition of sugars to the OH-groups on serines and threonines would account for the increase in mol wt.

C. Gene

The MCP gene consists of 14 exons and is a minimum length of 43 kb. A "split" exon encodes SCR-2 while the three other SCRs are encoded by single exons. The split occurs at the second nucleotide of glycine 34. At least one such split exon is present at the same nucleotide position in the other RCA proteins incuding SCR-3 of DAF. Three closely spaced but variably utilized exons encode the ser/thr/pro region. Exon 13 encodes for one cytoplasmic tail and, if utilized, converts the other cytoplasmic tail sequence (which is within the last exon) into part of the 3' untranslated region.

PROTECTION OF TISSUE FROM C MEDIATED LYSIS

To more directly demonstate the regulatory activity of MCP and DAF, we asked if their expression on foreign cells could protect them from human C-mediated lysis (13).

In a classical pathway dependent system, the control MCP transfectant was lysed while there was no lysis of the transfectant expressing MCP. The mAb, GB24, which blocks MCP's function, abrogated this cytoprotective effect. This result indicates that expression of MCP alone is protective. The MCP transfectant expressed an MCP copy number similar to that of certain tumor cell lines. In the same system, the expression of DAF, in a copy number similar to that present on leukocyes afforded similar protection.

In a second model, lysis of the control CHO cells was observed with human or rabbit C in the presence of rat anti-hamster Ab. With the CHO transfectant expressing MCP, there was lysis by rabbit C but not by human C. A similar amount of protection was afforded by DAF-transfection of CHO cells.

These data indicate that MCP protects autologous tissue from damage mediated by the C system. Recently, Seya et al (14) demonstrated that human tumor cells expressing MCP as the only membrane C regulator became "activators" of the alternative pathway if treated with a mAb which blocks MCP's function.

These cell protection experiments are also an example of DAF's ability to inhibit C activation if it is the only membrane regulator expressed. Using cultured human glomerular epithelial cells, Quigg et al (15) have shown DAF to protect these cells from human C-mediated lysis. Cheung et al (16) demonstrated differences in the degree of C sensitivity of certain human melanoma cell lines based on whether or not DAF is expressed. The earlier work of Medof et al (3) clearly established that DAF could protect cells from C mediated lysis if the purified DAF protein was reincorporated into erythrocyte membranes.

We are currently investigating the quantitative and possible cooperative nature of the inhibitory activities of DAF and MCP on C activation. Since MCP, DAF and the membrane regulatory proteins of the membrane attack complex are usually expressed concomitantly, we are interested in their combined effects. Also, DAF was previously shown to act intrinsically, by inhibiting C activtion only on the cell on which it is expressed (3). Preliminary results utilizing the cell lines and methodology outlined above suggest that MCP also works primarily in an intrinsic fashion (T.J. Oglesby and J.P. Atkinson, unpublished).

CONCLUSION

Regulatory proteins account for the manner in which the C system has handled the "tricky" question of separting self from non-self (1). The alternative pathway is an independent immune system. It is not only ancient on the phylogenetic scale but also almost certainly preceded the appearance of antibody (17,18). The alternative pathway can amplify on foreign but not host tissue. Microbes, for the most part, do not possess C regulatory proteins. As a result, C activation occurs on many types of organisms. No such activation occurs on self-tissue. Thus, self/non-self discrimination in the C system is made possible by its regulatory proteins. It is a remarkably simple yet highly effective solution to a difficult problem. The fact that the vaccinia and the herpes simplex viruses (HSV) express C4b- and C3b-binding proteins (virulence factors) that mimic the function of C regulatory human proteins strongly supports this concept (19).

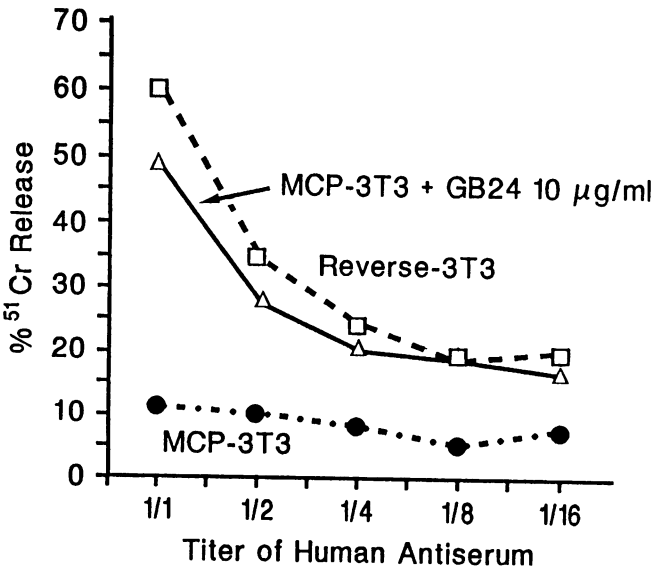

Figure 2. Cytoprotection from human C by expression of human MCP on the 3TR murine cell line. Cell lysis (% ^{51}Cr release) of 3T3 cells transfected with MCP cDNA in reverse (Reverse-3T3, control) or forward orientation (MCP-3T3) are compared. GB24, a mAb known to block MCP function, was added to a final concentration of 10 μg/ml. A control mAb of the same isotype as GB24 had no effect. In this experiment, the C source was normal human serum (NHS, 1/9 final dilution) absorbed with mouse spleen cells at 4°C and the Ab source was 56°C-heated NHS.

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