Cooperation Between Decay-Accelerating Factor and Membrane Cofactor Protein in Protecting Cells from Autologous Complement Attack

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Cooperation Between Decay-Accelerating Factor and Membrane Cofactor Protein in Protecting Cells from Autologous Complement Attack

William G. Brodbeck,* Carolyn Mold,† John P. Atkinson,‡ and M. Edward Medof*‡

Decay-accelerating factor (DAF or CD55) and membrane cofactor protein (MCP or CD46) function intrinsically in the membranes of self cells to prevent activation of autologous complement on their surfaces. How these two regulatory proteins cooperate on self-cell surfaces to inhibit autologous complement attack is unknown. In this study, a GPI-anchored form of MCP was generated. The ability of this recombinant protein and that of naturally GPI-anchored DAF to incorporate into cell membranes then was exploited to examine the combined functions of DAF and MCP in regulating complement intermediates assembled from purified alternative pathway components on rabbit erythrocytes. Quantitative studies with complement-coated rabbit erythrocyte intermediates constituted with each protein individually or the two proteins together demonstrated that DAF and MCP synergize the actions of each other in preventing C3b deposition on the cell surface. Further analyses showed that MCP’s ability to catalyze the factor I-mediated cleavage of cell-bound C3b is inhibited in the presence of factors B and D and is restored when DAF is incorporated into the cells. Thus, the activities of DAF and MCP, when present together, are greater than the sum of the two proteins individually, and DAF is required for MCP to catalyze the cleavage of cell-bound C3b in the presence of excess factors B and D. These data are relevant to xenotransplantation, pharmacological inhibition of complement in inflammatory diseases, and evasion of tumor cells from humoral immune responses. The Journal of Immunology, 2000, 165: 3999–4006.

A structural feature that is unique to DAF among human regulators of complement activation proteins is that it contains a posttranslationally added GPI anchor that confers on the cell the ability to sequester cell-bound C3b, which is a key step in the amplification of the alternative pathway (1). The GPI-anchored form of DAF also is known to enhance the ability of MCP to catalyze the cleavage of C3b (2, 3), and this cooperativity is enhanced when the two proteins are present together on the cell surface (4). The cooperative action of DAF and MCP is consistent with their common structural motif of complement control protein repeats (CCPs) (2, 3). Members of this family are encoded within the gene cluster shared by the alternative pathway C3 convertases (8). In the present study, we constructed a recombinant MCP variant that contains a GPI anchor and employed this technology for the purpose of studying how DAF and MCP interrelate functionally on the cell surface.

Our aims were to study whether DAF and MCP work in a cooperative fashion in preventing C3b deposition, and, if so, how they influence each other’s function. For this purpose, we used rabbit erythrocytes (E rab) and studied alternative pathway activation using purified components. E rab are well-suited for such studies as it has been shown that rabbit DAF has no effect on the human alternative pathway convertase (9, 10), E rab do not express MCP (11), and the cells classically function as potent “activators” of the human alternative pathway. Using this system, we found that DAF and MCP synergize each other’s actions in preventing alternative pathway-mediated C3b deposition. We further found that the complexing of Bb, the factor B activation fragment that generates the C3 convertase, with cell-associated C3b inhibits the ability of MCP to catalyze the cleavage of C3b and, that by dissociating Bb from the alternative pathway C3 convertase, DAF restores MCP’s activity.

Materials and Methods

Buffers and reagents

Isotonic Veronal-buffered saline (DGVB2+) consisted of 2.5 mM Veronal, 73.7 mM NaCl, pH 7.5, 0.1% gelatin, 2.5% dextrose, and 1 mM MgCl2/0.15 mM CaCl2. Isoionic Veronal buffer (GVB2+) contained 145 mM
Preparation of GPI-anchored MCP protein

The cDNA encoding GPI-anchored MCP was prepared by cutting MCP-BC1 cDNA (GenBank accession no. X59405) cloned into the EcoRI site of pSc65 vector at MCP’s membrane proximal FokI site and pSc65’s EcoRI site, A PCR-amplified sequence encoding the 30 aa of DAF’s C-terminal signal peptide flanked by FokI and EcoRI sites then was ligated into the FokI- and EcoRI-digested digest.

For the preparation of transfectants, 10 μl of DNA was preincubated for 30 min at 25°C with 15 μl of lipofectin reagent (Life Technologies, Gaithersburg, MD) suspended in 1 ml of Opti-MEM (Life Technologies). The mixture then was added to 6×10⁶CHO-K1 cells grown to 60% confluence on 100-mm culture plates and the plates incubated for 4 h at 37°C. The cells subsequently were incubated for 18 h in complete medium, after which methionine sulfoximine (25 μM) was added and selection carried out over the next 4–6 wk.

Surviving colonies were isolated with cloning cylinders and tested for expression of recombinant MCP by staining with anti-MCP mAb GB24 (14). CHO cells expressing native MCP transmembrane protein, derived from CHO cells transfected with full-length MCP were counted. The concentration of MCP of each sample was calculated by comparing the cpm of each well with that of MCP-BCl1 standards of known concentrations (14).

DAF protein concentrations were quantitated by the DAF IRMA in the same fashion using anti-DAF mAb IA10 for capture, [125I]-labeled anti-DAF mAb III6 for detection of bound protein, and human erythrocyte DAF of known concentration as standards (16).

Western blot analyses

Proteins were electrophoresed on 7.5% SDS-polyacrylamide gel under nonreducing conditions, transferred onto 0.45-μm Immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA) for 1 h at 100 mV in a Transblot apparatus (Bio-Rad, Hercules, CA), and blocked for 15 h at 4°C with 5% BSA.Blocked membranes then were incubated with 10 ng/ml anti-MCP GB24 for 2 h at 4°C followed by incubation with HRP-conjugated sheep anti-mouse mAb for 1 h at room temperature. After washing, the blots were developed using an electrochemoluminescence (ECL) Western blotting reagents kit (Amersham, Chicago, IL).

Cytotoxicity assays

CHO K-1 parental cells or CHO cell transfectants were grown to 70% confluence on 100-mm culture plates. Cells were removed with 4 ml Versene (Life Technologies) and washed three times with PBS. A total of 1×10⁵ cells then were suspended in 1 ml PBS containing 500 μCi ³¹Cr, and the cell suspensions were incubated for 2 h at 37°C with occasional shaking. After washing three times with GVB-E, 5×10⁶ labeled cells were incubated for 15 min at 4°C with rabbit anti-hamster lymphocyte serum 1:2 (Sigma) in 10 μl of GVB-E. Cells were washed three more times with GVB-E, resuspended to 10⁶ cells/ml in GVB-E, and 100-μl aliquots of the cell suspension added to the wells of 96-well V-bottom plates. Then serial dilutions of normal human serum were added in 100-μl volumes in triplicate wells for each cell type. A volume of 100 μl of 1% Triton X-100 and buffer alone were included as controls for 100% release and for spontaneous release, respectively. The percent specific release was calculated from the formula: % specific release = [(measured release – spontaneous release)/(100% release – spontaneous release)].

C3 deposition and clumping studies

For uptake and clumping studies, C3b was deposited onto E⁶⁺⁺ using purified alternative pathway components. A convertase was formed by first incubating 600 μg/ml C3, 400 μg/ml B, and 10 μg/ml F for 5 min at room temperature. The resulting convertase complexes then were added to 8×10⁵ Erab and 240 μg/ml C3 in GVB/Ni²⁺ buffer, and the mixture was incubated for 20 min at 37°C. The resulting E⁶⁺⁺C3b cells were washed, resuspended to 10⁶ cells/ml in GVB-E, and stored on ice.

For C3 deposition studies, MCP and DAF were incorporated for 45 min at 30°C as previously described (8, 17, 18). Briefly, 10⁶ E⁶⁺⁺C3b were incubated with MCP-GPI or detergent buffer control to generate cells with or without incorporated MCP. Washed C3b-bearing cells were incubated with 100 μl of DGVB²⁺ containing 55 μg/ml B and 4 μg/ml D, and the mixtures were incubated for 10 min at 37°C. The cells bearing C3bBb then were quickly pelleted, resuspended in 100 μl DGVB²⁺ containing 400 μg/ml C3, and incubated for another 10 min at 37°C. The resulting E⁶⁺⁺ bearing large amounts of C3b were incubated with DAF or detergent buffer control. The amplification procedure then was repeated a second time in which 40 μg/ml [125I]-C3 was added together with 400 μg/ml C3. In some tubes, factor I (3.4 μg/ml) together with factors B and D were added to the cell suspension.

For fluid-phase C3b cleavage assays, C3b (Advanced Research Technologies, San Diego, CA) was biotinylated by incubating it with NHS-LC-biotin (10 μg/ml) for 30 min at room temperature. The biotinylated C3b was extensively dialyzed. The biotinylated C3b then was incubated with varying concentrations of MCP and factor I or buffer alone for 1 h at 37°C. Then 15 μl of each reaction mixture was mixed with reducing buffer, boiled, and loaded onto 10% SDS-PAGE gels. The electrophoresed proteins were transferred into nitrocellulose, the blots were incubated with streptavidin-conjugated HRP, and the C3b proteins revealed using the enhanced chemiluminescence (ECL) Western blotting reagents kit (Amersham, Chicago, IL).
Cells were washed, pelleted, and lysed by the addition of 1.5 ml H$_2$O for 15 min at 4°C. Following lysis, erythrocyte stroma were collected by centrifugation at 10,000 × g for 30 min at 4°C, the pellet resuspended in reducing buffer and the sample electrophoresed on 7.5% SDS-PAGE gels. Separated proteins were blotted onto polyvinylidene difluoride membranes, and, following blocking, the membranes were incubated for 1.5 h at room temperature with HRP-conjugated streptavidin and developed using the ECL kit.

Separated proteins were blotted onto polyvinylidene difluoride membranes, reducing buffer and the sample electrophoresed on 7.5% SDS-PAGE gels. Bands corresponding to the bound tropheosis documentation and analysis system 120 (Rochester, NY). ECL kit.

The abilities of the recombinant GPI-anchored MCP and native MCP to inhibit complement-mediated lysis then were compared. As shown in Fig. 2, when tested in cytotoxicity assays, cells expressing equivalent copy numbers (see Fig. 1) of the recombinant GPI-anchored protein were protected from complement-mediated lysis as efficiently as cells expressing the transmembrane form of the protein.

Lastly, the recombinant protein was examined in the isolated state (Fig. 3). When analyzed by Western blotting, the extracted MCP-GPI displayed an apparent $M_r$ of 55 kDa (Fig. 3A). The recombinant protein bound to C3b-coated plates and C3b-Sepharose beads with an affinity comparable to that of the extracted native protein MCP-BC1 (data not shown). Also, when mixed with factor I (Fig. 3B), it promoted cleavage of fluid-phase C3b with efficiency equivalent to that of extracted native (BC1) transmembrane MCP protein.

**MCP and DAF work synergistically in preventing C3b deposition**

As described previously, we used E$_{493}^{ab}$ and purified alternative pathway components to determine whether and how MCP and DAF cooperate in regulating C3 convertase activity. In initial studies, the activity of each protein individually following its incorporation was assessed. As shown in Fig. 4A (lanes 5 and 6), when incorporated into E$_{493}^{ab}$C3b (after the first amplification step), DAF inhibited the further deposition of C3b on the cell surface in a dose-dependent manner. Similarly, when MCP-GPI was incorporated (prior to the amplification step) (lanes 1–4), and the cells resuspended in factor I, it also displayed a dose-dependent inhibitory effect on C3b deposition. Interestingly, in the absence of factor I, incorporated MCP-GPI had no effect on the amount of C3b deposition (see Discussion).

Next, the proteins were studied together. For these analyses, pretitrations were performed to determine the threshold concentrations of DAF and MCP-GPI that had barely discernible effects on C3b uptake. In the first set of experiments, the predetermined

![Fluorescent Intensity](image1)

**Figure 1. PI-PLC sensitivity of GPI-anchored MCP.** A total of $1 \times 10^6$ K562 (A and B) or CHO cell transfectants (C and D) were treated with buffer alone (A and C) or with 10 U/ml PI-PLC (B and D) for 30 min at 37°C. Washed cells were stained with anti-MCP mAb GB24 or non-relevant isotype-matched control and analyzed as described in Materials and Methods. The MCP epitope was markedly reduced on cells expressing MCP-GPI upon treatment with PI-PLC as compared to K562 cells expressing native conventionally anchored MCP.

![% NHS](image2)

**Figure 2.** Inhibition of CHO cell lysis by GPI-anchored MCP. CHO cell transfectants MCP-GPI and MCP-BC1 with equal copy numbers ($10^5$ molecules/cell) (34) as assessed by flow cytometry with the same high affinity anti-MCP mAb (GB24) were studied. A total of $5 \times 10^6$ cells of each transfectant or the parental line were labeled with $^{51}$Cr. Cells then were sensitized with rabbit anti-hamster cell antisemur (see Materials and Methods). Sensitized cells were incubated with increasing dilutions of normal human serum for 1 h at 37°C. Cells were pelleted, and the number of counts released was measured for each sample. Shown are the mean values of three replicate tubes with error bars representing SD of the means. Transfectants expressing either MCP-BC1 or MCP-GPI had a reduced amount of specific release at all serum concentrations tested.
limiting amount of MCP-GPI was incorporated into the cells followed by the incorporation of varying limiting amounts of DAF. As shown in Fig. 4B, MCP-GPI alone on the cell surface conferred minimal inhibition of C3b deposition (5.8%), as did a range of limiting DAF concentrations (0–7.3% inhibition). In contrast, inhibition increased dramatically when both proteins were incorporated into the cells (up to 43.3%). With the fixed limiting dose of MCP (20 incorporated molecules/cell), inhibition was again dependent on the concentration of the DAF added.

Next, the converse experiment was performed in which cells were incubated with a range of limiting MCP-GPI concentrations followed by a fixed limiting dose of DAF (45 incorporated molecules/cell) (Fig. 4C). Once again, inhibition dramatically increased in the presence of both proteins (up to 64.1%) as compared to each protein separately (from 0 to 8.2%). As above, either in the presence or absence of DAF, MCP-GPI inhibited C3b deposition in a dose-dependent fashion.

DAF is required for MCP’s function in the presence of excess factors B and D

Previous studies of cell-associated MCP function have relied principally on cytotoxicity assays of transfectants in whole serum (2, 19, 20). As an initial approach for directly assaying MCP’s function on the cell surface, biotinylated C3b was deposited during the last amplification step (see Materials and Methods) on E40C3b.
that contained incorporated MCP-GPI. As shown in Fig. 5A, upon incubation of the resulting cells with factor I, incorporated MCP-GPI cleaved the cell-bound C3b to iC3b (as evident by the appearance of the characteristic 43-kDa α’ fragment of iC3b. The cleavage did not occur in the absence of factor I or MCP (not shown). In pretitrations, a mAb against factor H exhibited the ability to completely block H cofactor activity (data not shown). Control studies in which this mAb was included in the reaction mixture

**FIGURE 5.** Cleavage of cell-bound C3b by incorporated MCP-GPI, inhibition by B and D, and restoration of cleavage by DAF. A total of 1 × 10⁷ E striker E were incubated with MCP-GPI as before. Cells then were incubated with B and D in DGVB⁺⁻⁺, washed, resuspended DGVB⁻⁻ containing C3 and biotinylated C3 and incubated for 15 min at 37°C. The resulting intermediates were incubated with factor I where noted. A, In the presence of MCP-GPI, C3b was cleaved to yield the band marked 43 kDa (lane 1). No cleavage occurred in the absence of I (lane 2). Anti-factor H was added to exclude cleavage due to contaminating factor H (lane 3). Addition of 470 ng/ml factor H was included as a positive control (lane 4). B, Increasing concentrations of MCP-GPI were incubated with the cells during the incorporation step. As indicated by the proportion of the 43-kDa band, the amount of C3b cleavage was dependent on the concentration of MCP-GPI added. The identity of the extra band in lane 4 is unknown. C, Factors B and D were added to E striker E (MCP) prepared with 25 ng/ml MCP during the factor I incubation. In their presence, the cleavage of C3b was abolished. D, DAF was incorporated into the MCP-containing cells after the deposition of biotinylated C3 but before incubation with factors B, D, and I. DAF restored the cleavage of C3b at all DAF:MCP ratios tested. E, Quantification of the percent of restoration of MCP-dependent cleavage by DAF. The percentage of DAF restoration of cleavage was quantified by densitometry as described in Materials and Methods. The values shown are the mean values of independent experiments (two in addition to the one shown in D) with the bars giving the SD. Percent restoration of cleavage was dependent on the amount of DAF added.
excluded the possibility that the cleavage was mediated by contaminating factor H. Additionally, no cleavage was seen in the absence of the MCP-GPI. The factor I-mediated cleavage of cell-bound C3b was dependent on the amount of MCP-GPI incorporated into the cells (Fig. 5B).

To test whether the presence of factors B and D would influence the ability of MCP to cleave cell-bound C3b, the two components were added to the E\(^{ab}\)C3b (MCP) cells at the same time as factor I. As shown in Fig. 5C, the addition of B and D and consequent assembly of C3b into C3bBb markedly reduced the amount of cell-bound C3b cleavage. Further studies demonstrated that this inhibition of cleavage was dependent on the amount of B added together with factors D and I (data not shown).

Finally, the effect of the presence of DAF on the B- and D-mediated inhibition of MCP function was assessed. As shown in Fig. 5D, when DAF was incorporated into the cells, the inhibition of MCP-GPI/factor I-mediated cleavage of cell-bound C3b to iC3b in the presence of factors B and D was reversed. The restoration of cleavage was dependent on the amount of DAF incubated with the cells (Fig. 5E). A similar extent of cleavage restoration was seen at higher DAF levels.

Discussion

In the present study, a recombinant GPI-anchored MCP was generated so as to permit its incorporation into erythrocyte complement intermediates and thereby allow analysis of how it interacts functionally with DAF in an easily manipulated system. Control studies showed that the MCP-GPI retained the ability to bind to and catalyze the cleavage of C3b with an efficiency comparable to that of native MCP protein. Incorporation of the recombinant MCP-GPI and DAF into E\(^{ab}\) in studies with [\(^{125}\)I]-C3 showed that, when present in the same cell membrane, the two regulators work synergistically to prevent C3b deposition. In addition, when incorporated into E\(^{ab}\)C3b cells, the recombinant MCP-GPI protein functioned to catalyze the factor I-mediated cleavage of cell-bound C3b. In the presence of B and D, this cleavage was reversed but was restored when DAF was incorporated into the same cell surface.

It was previously reported by Lublin and Coyne (21) that changing the cell attachment mechanisms of DAF and MCP between GPI and conventional transmembrane anchors does not alter either protein’s regulatory efficiency in CHO cell cytotoxicity assays. These studies indicated that neither differences in surface mobility conferred by the two anchoring mechanisms nor localization of GPI-anchored proteins in nucleated cell “rafts” markedly affects either protein’s regulatory function. Here we confirmed that an MCP molecule that is attached to the cell surface with a GPI anchor functions with an efficiency essentially equivalent to that of the native MCP molecule. Studies with isolated MCP-GPI protein showed that the replacement of the transmembrane anchoring mechanism with a GPI anchor had no effect on the protein’s ability to bind to C3b-coated plates or C3b-Sepharose beads when compared to the extracted native protein. In addition, the change of the C-terminal anchor did not affect the protein’s ability to catalyze the factor I-mediated cleavage of fluid-phase C3b. Based on the above, it is unlikely that replacement of MCP’s anchor with a GPI in the present study had a significant influence on the results that were obtained. The use of erythrocytes rather than nucleated cells for the assays precluded any involvement of “rafts.”

The initial functional system that we used was designed to study the ability of DAF and MCP either individually or together to inhibit the deposition of C3b on the cell surface. Recent studies by others (20) found that MCP does not affect the initial deposition of C3b resulting from the “tickover” of C3 but rather that it works to prevent the amplification of C3b deposition by inhibiting the alternative pathway C3 convertase C3bBb. Previous studies have shown that the same is true for DAF (4) because DAF works to accelerate the decay of already formed convertases (22, 23). We found that the presence of this small amount of C3b had little to no effect on the amount of MCP-GPI that incorporated into the cells. However, if MCP-GPI was incubated with cells after C3b amplification, a much larger amount of MCP associated with the cells compared to control cells (730 vs 230 molecules of MCP per cell) presumably due to the binding of MCP-GPI to cell-bound C3b. In our system, following the incorporation of MCP-GPI into E\(^{ab}\), C3b deposition next was amplified on the cell surface for one or more rounds and then DAF was incorporated into the cells (as earlier incorporation did not allow sufficient C3b deposition). A subsequent round of amplification in the presence of factor I constituted the final readout step for analysis. Because DAF was not incorporated until after amplification, MCP exhibited an apparent greater inhibitory effect when the proteins were studied individually in the C3b uptake assays (Fig. 4A).

Although DAF and MCP each have been studied extensively, no investigations have focused on whether the two proteins interact in providing optimal protection of self cells from autologous complement despite the fact that nearly all cells express both proteins. Using the above experimental system with incorporated MCP-GPI and DAF in E\(^{ab}\), we demonstrated that the two regulators work synergistically on the cell surface in preventing alternative pathway-mediated C3b deposition. The magnitude of the inhibition of C3b uptake in the presence of the two proteins compared to each protein individually was striking. At higher concentrations of the proteins, this cooperative inhibition reached 43–64% as compared to 0–8% for each protein when the proteins were incorporated individually at the same concentrations.

In previous studies using soluble MCP added to C3b-bearing cells (24) in which it was found that (in the absence of factor I) the uptake of the soluble (fluid-phase) molecule was enhanced by C3b, it was hypothesized that MCP by itself may function to stabilize alternative pathway C3 convertase complexes. However, the data presented here clearly show that when MCP is in the cell membrane and factor I is absent, C3b uptake is not affected. In contrast, in the presence of factor I, a large decrease in the amount of C3b deposited on the cell surface is observed.

To our knowledge, the data presented here provide the first direct demonstration in well-defined erythrocyte intermediates that when present on the same surface, MCP functions to catalyze the cleavage of cell-bound C3b. A recent study, using Abs directed against iC3b in conjunction with CHO cell transfecants incubated with factor I, obtained similar results regarding MCP cofactor activity (25). A number of earlier studies strongly suggested this using fluid-phase C3b cleavage assays and cytotoxicity protection assays (5, 14, 26). However, several factors complicated these previously established systems. In the course of our work using some of these other systems, we found that MCP-mediated cleavage of cell-bound C3b is not easily observed when using serum depleted of C5 or subsequent components. Because a low dilution of serum (1:2 to 1:8) must be used, high background cleavage is observed, presumably due to serum factor H, which masks the effect of MCP. To eliminate background cleavage, purified complement components must be used. Some previous systems (26) have used purified components in fluid-phase cleavage assays, but such methods do not directly relate to the cell surface, and low ionic strength buffer (20 mM NaCl) is needed. Although, as mentioned, a GPI-anchored form of MCP was previously generated, this protein was used in transfected cell lines with whole serum containing factor H. By isolating and purifying MCP-GPI, we were able to incorporate the
protein into erythrocyte (i.e., E^{ab}) intermediates that could be easily used for cell-associated complement studies using purified components.

In our second experimental system designed to assess MCP’s function directly, i.e., cofactor activity, we added biotinylated C3 in the final C3 amplification step because this probe labels the sequential 43/41-kDa fragment of the C3b α’ chain that is generated by the cleavage reaction. We found that in the presence of factor I, MCP promoted the formation of this band. We further found that the ability of incorporated MCP-GPI to catalyze this cleavage was inhibited when B and D were added at the same time as factor I. Our finding in the C3b uptake studies that MCP alone was able to inhibit C3b deposition (Fig. 4A) is not inconsistent with this because not all bound C3b molecules are involved in the formation of C3 convertases and because much higher concentrations of MCP were used in those studies. Moreover, of possibly greater relevance, the studies were done in the absence of properdin and the spontaneous decay rate of non-properdin-stabilized C3bBb complexes is faster than the incubation times used.

Incorporation of DAF into the E^{ab}C3b intermediates in the presence of B and D showed that the presence of DAF on the cell surface restored MCP-catalyzed cleavage of cell-bound C3b. Presumably, this is due to its ability to accelerate the decay of Bb from C3b and render the bound C3b molecules susceptible to MCP-dependent factor I-mediated cleavage. In our experimental system, complete restoration of C3b cleavage was not seen at the concentrations of DAF we added. This could be due to the fact that insufficient amounts of DAF were added or to the inability of DAF to act on nonactivated convertases (C3bB) (23). Because B associates with C3b prior to D-mediated cleavage to Bb and Ba, it is likely that some C3b molecules are bound to factor B zymogen, preventing MCP-mediated dissociation.

As found in the C3b deposition studies, within the range of concentrations studied the maximal effect on restoration of C3b cleavage in the presence of B and D was observed at a ratio of DAF to MCP of 3:1. However, because saturation was not achieved, the precise ratio of the two proteins providing for optimal synergism remains to be determined. A possible mechanism of this synergism is proposed in Fig. 6. Initially, C3b, deposited by natural “tickover,” is bound by factor B. The binding of both DAF (23, 27) and MCP is prevented. B is then cleaved by D to form the alternative pathway C3 convertase, C3bBb, which then prevents MCP from associating with C3b and factor I cleaving it to iC3b.

When MCP is present on the target cell surface, it acts to decay Bb from C3b, thereby rendering C3b susceptible to MCP-supported factor I-mediated cleavage. Because C3b complexed with B (C3bB) is not affected by DAF, such C3b molecules are consequently protected from MCP and factor I-mediated cleavage. However, this complex cannot amplify C3b deposition. By this formulation, DAF would incompletely restore MCP/factor I activity (i.e., act on C3bBb but not C3bB) and, at the same time, synergistically inhibit further C3b deposition.

Other C3 regulatory proteins exist that contain both DAF’s decay accelerating and MCP’s cofactor functions. These include factor H, C4BP, and CR1. It is likely that these proteins evolved from DAF and MCP and that by combining the two activities into close proximity to each other provide for further enhancement of the overall efficiency of the two reactions. Likewise, our results explain the ability of recombinantly produced MCP-DAF hybrid proteins (28, 29) to have greater inhibitory activity than either protein alone for alternative pathway activation and are relevant to the production of future variants of such pharmaceuticals.

A growing interest in xenotransplants (cross-species transplants) has brought attention to ways of circumventing the hyperacute graft rejection by the recipient, which is in large part complement mediated. In this regard, transgenic animals are being produced that express one or more complement regulatory proteins. With respect to DAF and MCP, our findings suggest that both proteins must be present together and that a DAF to MCP ratio of 2:1 to 3:1 confers a high level of synergism.

Finally, numerous studies (30–33) have shown that many tumor cells express relatively high levels of both proteins. Our findings further suggest that the above-defined synergism would enhance their ability to evade endogenous humoral immune responses as well as responses to exogenously administered complement-activating antitumor Abs.

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References

FIGURE 6. Proposed mechanism of DAF/MCP cooperative activities. A. C3b is deposited on the cell surface followed by the binding by factor B. This complex C3bB cannot be dissociated by DAF nor can the C3b be cleaved by I and MCP. B. Once D cleaves B and releases the three-CCP-containing fragment Ba, the newly formed convertase, C3bBb, can be dissociated by CCCPs 2–4 of DAF. C. Once Bb is dissociated from C3b, DAF no longer is associated with the residual C3b molecule. MCP can now catalyze the factor I-mediated cleavage of the cell-bound C3b to iC3b.


