Role of Membrane Cofactor Protein (CD46) in Regulation of C4b and C3b Deposited on Cells[1]

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C4b and C3b deposited on host cells undergo limited proteolytic cleavage by regulatory proteins. Membrane cofactor protein (MCP; CD46), factor H, and C4b binding protein mediate this reaction, known as cofactor activity, that also requires the plasma serine protease factor I. To explore the roles of the fluid phase regulators vs those expressed on host cells, a model system was used examining complement fragments deposited on cells transfected with human MCP as assessed by FACS and Western blotting. Following incubation with Ab and complement on MCP$^+$ cells, C4b was progressively cleaved over the first hour to C4d and C4c. There was no detectable cleavage of C4b on MCP$^-$ cells, indicating that MCP (and not C4BP in the serum) primarily mediates this cofactor activity. C3b deposition was not blocked on MCP$^+$ cells because classical pathway activation occurred before substantial C4b cleavage. Cleavage, though, of deposited C3b was rapid (<5 min) and iC3b was the dominant fragment on MCP$^-$ and MCP$^+$ cells. Studies using a function-blocking mAb further established factor H as the responsible cofactor. If the level of Ab sensitization was reduced 8-fold or if Mg^{2+}-EGTA was used to block the classical pathway, MCP efficiently inhibited C3b deposition mediated by the alternative pathway. Thus, for the classical pathway, MCP is the cofactor for C4b cleavage and factor H for C3b cleavage. However, if the alternative pathway mediates C3b deposition, then MCP's cofactor activity is sufficient to restrict complement activation. *The Journal of Immunology,* **2002, 168: 6298–6304.**

Proper regulation of the complement system prevents fluid phase component consumption and membrane damage to autologous tissue (reviewed in Refs. 1–3). Of the 11 plasma and membrane complement regulators, seven act on the C3 and C5 convertases. These convertase regulators have overlapping functional repertoires. The relative role of each in inhibiting complement activation is incompletely understood (1).

Membrane cofactor protein (MCP)[3] (CD46) is a widely expressed, intrinsically acting, 51–68-kDa regulator of the complement system (4–6). MCP and CR1 (CD35), as well as factor H (fH) and C4b-binding protein (C4BP) of plasma, serve as cofactors for the factor I-mediated cleavage of C4b and C3b. In contrast to MCP, CR1 has a limited tissue distribution, being expressed by peripheral blood cells and in tissues by B lymphocytes, follicular-dendritic cells, macrophages, and kidney epithelial cells (3, 7–9). MCP and CR1 are cofactor proteins for both C4b and C3b, while the plasma proteins fH and C4BP are specific for C3b and C4b, respectively. The resulting cleavage fragments are incapable of forming a convertase, thereby halting further complement activation. The proteolytic degradation of C4b leaves only the covalently attached C4d fragment on the target (a so-called immunologic scar as C4d possesses no known biologic activity). In contrast, the cleavage of C3b produces iC3b, which is a ligand for CR2 (CD21) (2, 10, 11), CR3 (CD11b/CD18) (12), and CR4 (CD11c/CD18) (13). These receptor-ligand interactions promote immune adherence, phagocytosis, Ag localization, and B cell signaling (12, 14).

Further dissection of these regulatory events may foster a better understanding of autoantibody-mediated tissue damage (15), ischemia-reperfusion injury (16), and apoptosis (17, 18). In these settings, complement activation occurs on host cells bearing regulatory proteins. Opsonization and clearance of immune-complexes and apoptotic cells by complement may be critical in preventing tissue deposition of proinflammatory complexes (15), and in maintaining tolerance to self-Ags (17–19). Thus, an enhanced understanding of these complement clearance mechanisms could lead to a means to reduce damage in immunopathologic reactions and to prevent loss of self-tolerance.

We previously developed a classical pathway (CP) activation model system in which C4 cleavage products were analyzed (20, 21). The goal of the present experiments was to investigate the role MCP plays vs that of the plasma regulatory proteins in modulating the deposition and degradation of C4b and C3b. To accomplish this, we activated complement on Chinese hamster ovary (CHO) cells transfected with human MCP. The results point out distinct and nonoverlapping roles for MCP vs that of the plasma regulators in the regulation of C4b and C3b deposited on host tissue.

Materials and Methods

Cell lines and Abs

CHO cells were stably transfected with the BC1 isoform of human MCP and cloned as previously reported (21). CHO cells, transfected with MCP cDNA in reverse orientation, served as a control (MCP$^-$). Abs were from standard sources (noted for each Ab in the sections below) except for the mAb to fH. This mouse mAb (MH10) was generated using purified human fH as the Ag (J. Lambris, unpublished observation).

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[3] Abbreviations used in this paper: MCP, membrane cofactor protein; C4BP, C4b-binding protein; fH, factor H; CHO, Chinese hamster ovary; CP, classical pathway; AP, alternative pathway; C7d, C7 deficient.

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Quantitation of MCP expression

The quantity of MCP expressed per cell line was assessed by FACS (21). Briefly, cells were trypsinized, harvested, and washed in 1% FCS-PBS before transfer to 96-well microtiter plates (5×10^5 cells/well). TRA-2-10 (a murine mAb to MCP; Ref. 22) was added (10 μg/ml) and incubated with the cells for 30 min at 4°C. Following centrifugation and washing, FITC-goat anti-mouse IgG was added (Sigma-Aldrich, St. Louis, MO). After a 30-min incubation at 4°C, cells were resuspended in 1% FCS-PBS and analyzed by FACS. These results were compared with an ELISA (21). Clones and expression levels were designated as follows: M1 (clone 23.10; 90,000 MCP/cell), M2 (clone 23.9; 200,000 MCP/cell), and M3 (clone 23.1; 234,000 MCP/cell).

Initiation of complement activation

Standard procedure for initiation of the CP has been described (21). Briefly, CHO cells were grown to 70–80% confluency and collected by trypsinization into 1% FCS-PBS. Rabbit anti-hamster lymphocyte IgG (8.5 mg/ml; Sigma-Aldrich) was added to the cells and incubated for 30 min at 4°C. Following two washes with 1% FCS-PBS, 100 μl of C7-deficient (C7d) serum (donated by P. Densen, University of Iowa, Iowa City, Iowa) in gelatin veronal buffer was added. To block the CP, gelatin veronal buffer was used containing 10 mM EGTA and 7 mM magnesium chloride (Mg^{2+}-EGTA). Cells were harvested at indicated time points and washed twice in 1% FCS-PBS before C4 and C3 fragment analyses. Antigenic levels of fH, C4BP, and factor I in C7d serum (measured at National Jewish Medical and Research Center, Denver, CO) were 117, 152, and 119% of normal values, respectively. C4BP functional activity in the C7d serum was comparable to that of normal human serum as assessed by monitoring cofactor activity for C4b. C6-depleted serum (Advanced Research Technologies, San Diego, CA) was also used.

FACS analysis of complement fragment deposition

Following complement deposition and washes, murine mAbs to the human complement component fragments C4c, C4d, C3c, C3d, or C5 (Quidel, San Diego, CA) were added (20 μg/ml). After a 30-min incubation at 4°C, FITC-conjugated goat anti-mouse IgG (preadsorbed with rabbit serum and rabbit-IgG agarose; both from Sigma-Aldrich) was added for 30 min at 4°C. Cells were then stained with propidium iodide and analyzed with a BD Biosciences FACSCalibur system, (BD Biosciences, Mountain View, CA). The anti-iC3b neoantigen mAb (Quidel) recognized both iC3b and C3b by FACS and Western blotting (data not shown); and thus, was not suitable for separating these fragments. C3 fragment deposition was also characterized by Western blotting.

Western blotting and fH blocking

For blocking studies, C7d serum was incubated at 4°C for 30 min with 1 mg/ml anti-fH mAb MH10 and this serum then incubated with sensitized CHO cells for 5 min. Cells were washed and then lysed with the nonionic detergent Nonidet P-40 (21) and the supernatants applied to gels containing 1×10^5 cell equivalents/lane. After SDS-PAGE, transfer to a nitrocellulose membrane, and blocking, a polyclonal goat anti-C3 Ab (Advanced Research Technologies) was added at a dilution of 1/7500 for 30 min at 37°C. This was followed by a 30-min 20°C incubation with rabbit anti-goat IgG-HRP at a dilution of 1/7500. Detection, using Super Signal West Pico Chemiluminescent substrate, was performed according to the manufacturer's directions (Pierce, Rockford, IL).

Statistical analysis

The kinetics of cleavage by the three cell lines expressing MCP vs that of control CHO cells was compared by a hierarchical linear model analysis of variance using the statistical software Stata (Release 7) (StataCorp, College Station, TX).

Results

The goal of this investigation was to assess the role of MCP as a cofactor for the factor I-mediated cleavage of C3b and C4b deposited on self-tissue. To this end, complement was activated on MCP^+ and MCP^- CHO cells and surface-bound C3b and C4b and their proteolytically derived fragments monitored by FACS and Western blot. In the first set of experiments we analyzed the CP, and in the second set of experiments we analyzed the alternative pathway (AP).

CP activation

For most experiments, CHO cells expressing human MCP were sensitized with 8.5 mg/ml of IgG and incubated in 10% human serum deficient in C7. The fluid phase complement regulators fH and C4BP were in the C7d serum.

MCP is the cofactor for the cleavage of deposited C4b. The appearance and processing of C4b was determined by FACS using mAbs to its C4c and C4d derivatives (Fig. 1). No C4b cleavage was detected on the MCP^- cells as deduced from the C4c:C4d ratio which was ~1.0. However, in the case of cells bearing MCP, a majority of the C4b was cleaved to C4c and C4d by 1 h. These findings indicate that MCP (and not C4BP) is responsible for most of the C4b cofactor activity. Comparable results were obtained with C6-depleted serum (data not shown) and with serum concentrations from 20 to 0.05%).

C4b deposition was rapid, with near maximal levels attained in $<$2 min (Fig. 2*A*). However, subsequent C4b cleavage was slow, being approximately linear over a 30-min period (Fig. 2, *A* and *B*). After 45 min, no further cleavage was observed, although 18–30% of the C4b remained intact (Figs. 1 and 2*B*). Cells expressing 234,000 copies of MCP/cell (cell line M3) produced more rapid C4b-cleavage compared with cells expressing 90,000 copies of MCP/cell (cell line M1) (Fig. 2*A*), but this trend was not statistically significant ($p = 0.46$).

C3b and C5b deposition are minimally affected by MCP. C3b deposition reached a maximum in $<$5 min under the standard CP activation conditions and 4- to 5-fold more C3b than C4b was deposited (Fig. 3). C3b and C5b deposition were similar in the MCP^- and MCP^+ cell lines. Thus, the presence of MCP did not influence the formation or stability of CP C3 or C5 convertases because the inactivation of C4b by MCP occurs too slowly to impact the activity of the CP C3 and C5 convertases.

fH mediates C3b cleavage on control and MCP-expressing cells. Additional experiments were performed to examine the processing of C3b in the CP-driven system. To that end, surface-bound C3b fragments were analyzed by Western blot (Fig. 4). First, the cleavage of C3b was much more rapid than that of C4b; that is, at 5 min the C3 α' fragment was barely detectable (*lanes* 6 and 7). Second, as also shown in *lane 6*, the α_1 and α_2 fragments of iC3b were

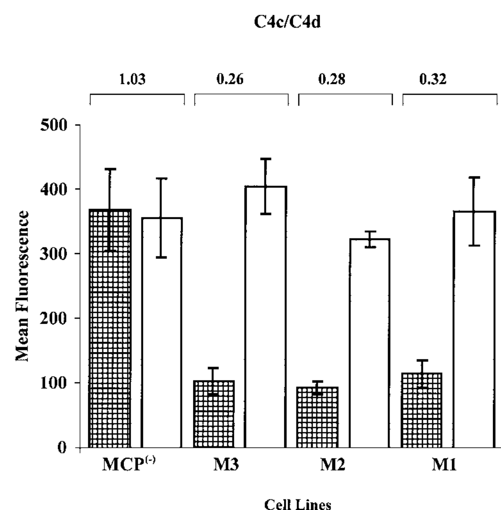

FIGURE 1. MCP and not C4BP cleaves deposited C4b. FACS analysis of C4 fragment deposition on sensitized cells (8.5 mg IgG/ml) exposed to C7d serum (10%) for 60 min. ▦, C4c; □, C4d. M1, M2, and M3 represent CHO cell lines expressing 90,000, 200,000, and 234,000 copies of MCP, respectively. Data represent mean ± 1 SE for five experiments.

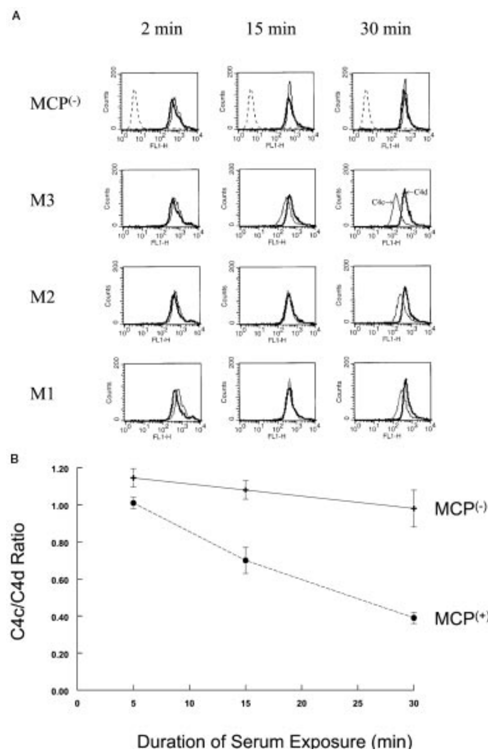

FIGURE 2. Cleavage of C4b by MCP-expressing cells. *A*, Kinetic analysis of C4b cleavage on MCP$^-$ and the three MCP$^+$ cell lines (see Fig. 1). Light line, C4c; heavy line, C4d; dashed line, control (sensitized cells not exposed to serum and unsensitized cells exposed to serum show the profile depicted). Ab and serum concentrations as per Fig. 1. Representative experiment of five. *B*, Cleavage of C4b by MCP (M3 cell line) is represented by the ratio of C4c:C4d. Data represent the mean ± 1 SE for three experiments.

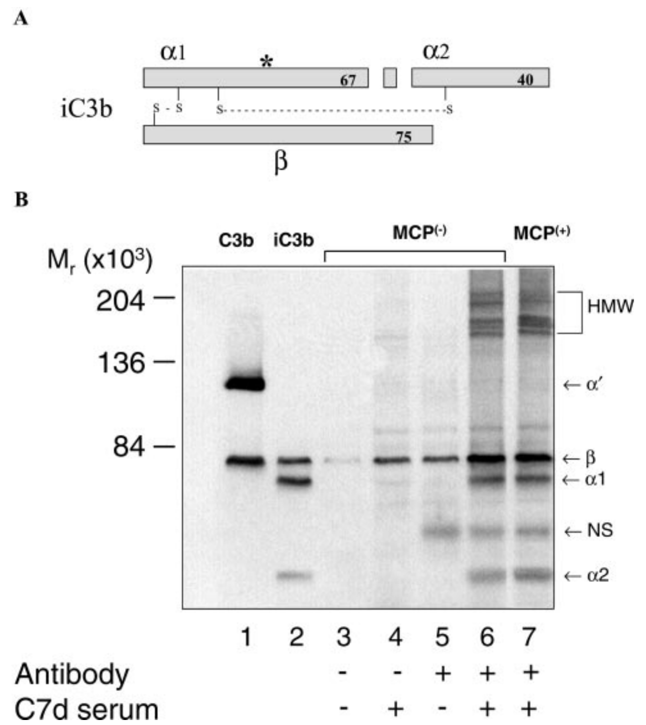

FIGURE 4. iC3b is the predominant fragment on MCP$^+$ and MCP$^-$ cells. *A*, Diagram of C3 fragments generated by cofactor activity (large asterisk is at site of thioester). *B*, Western blot of C3 fragment deposition on MCP$^-$ and the MCP$^+$ (M3 cell line). CHO cells were incubated under conditions shown, using the same Ab and serum concentrations described in Fig. 1. The solubilized cell preparations were analyzed on an 8% gel under reducing conditions. Following transfer, the blot was developed with a polyclonal anti-C3 Ab. *Lanes 1* and *2*, The fragment profile of C3b and iC3b, respectively. The fragments with an M_r greater than that of α'-chain (*lanes 6* and *7*; labeled HMW for high m.w.) represent α' and α_1 covalently bound to membrane constituents. The band at 75,000 consists primarily of the β-chain (as labeled) and, to a lesser extent, an unknown cell-derived cross-reacting protein (because it variably appears in conditions with cells alone as in *lane 3*). The band (labeled ns for "nonspecific") with a M_r of 55,000 (*lanes 5*, *6*, and *7*) represents a cross-reaction between the Ig H chain and the rabbit anti-hamster Ab. Representative experiment of four.

generated by the MCP$^-$ cells. Therefore, the generation of iC3b was likely due to cofactor activity of the serum protein fH. To test this, the effects of a mAb to human fH were examined. As observed in Fig. 5, *lanes 5* and *7*, the anti-fH mAb inhibits the appearance of iC3b fragments in the MCP$^-$ and MCP$^+$ cells, indicating the prime importance of fH in this process. Interestingly, under conditions in which fH is inhibited, some MCP-dependent processing is observed (compare α' in *lane 5* vs *7*).

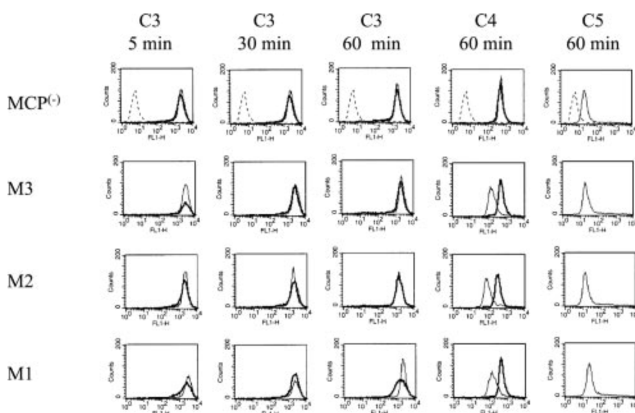

FIGURE 3. MCP does not inhibit C3b and C5b deposition by the CP. Kinetic analysis by FACS of complement deposition. Light line, C3c, C4c or C5; heavy line, C4d or C3d; dashed line, no serum exposure (see legend for Fig. 2). Ab, serum concentrations, and cell lines per Fig. 1.

iC3b/C3b fragments are attached to small molecules and membrane proteins. C3 carries a thioester moiety that becomes covalently attached to nearby molecules in the transition of C3 to C3b. That connection is retained by the α_1 fragment of iC3b. As visualized in Figs. 4 and 5, a substantial quantity of α_1 is present at its native m.w., indicating attachment to the cells either noncovalently or through small molecules. However, scans of these Western blots demonstrated 4-fold less native-sized α_1 than predicted from the amount of α_2, suggesting that a majority of α_1 is attached to higher m.w. membrane constituents. Lastly, the Western blot analysis indicates nearly identical quantities of C3b deposited on MCP$^+$ and MCP$^-$ cells (compare the β-, α_1-, and α_2-chains in *lanes 4* and *6* of Fig. 5). This finding confirms those made in the analyses by FACS.

MCP reduced C3b deposition at low serum concentrations. As the concentration of serum was reduced, C4b and C3b deposition also decreased (Fig. 6). C4b deposition was detectable at ≥0.05% serum, while C3b deposition was detectable at ≥0.25% serum. At serum concentrations of 1 and 0.5% (where the AP components are too dilute to be active), MCP$^+$ cells did reduce C3b deposition. We interpret this to mean that in the setting of limiting amounts of C4b deposition and C2 activation, the activity of CP C3 convertase can be inhibited by MCP.

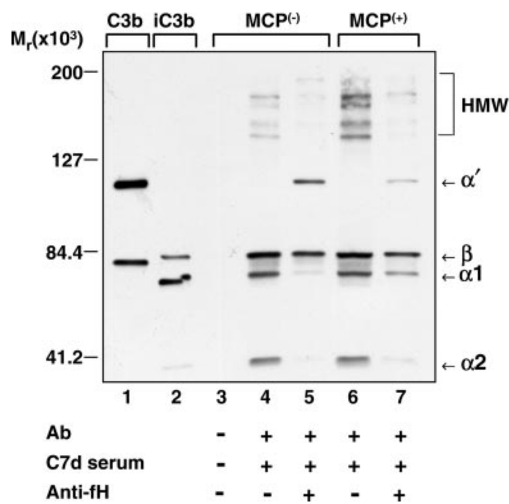

FIGURE 5. Blocking fH inhibits C3b cleavage. Western blot of C3 fragment deposition is shown. The sensitized cells (8.5 mg/ml) were incubated for 5 min with serum (10%) in the presence or absence of anti-fH mAb (1 mg/ml). These results were identical if the fH blocking mAb was decreased to 0.1 mg/ml and 4% serum was used. The misalignment of part of the α_1 band (*lane 2*) is secondary to a slight tear in the gel. Note the presence of the α' (*lane 5*) and only trace amounts of α_1 and α_2 in this same lane, indicative of the inhibition of fH. The lesser amounts of high m.w. (HMW) bands (*lanes 5* and *7* vs *4* and *6*) has two explanations. First, because α' remains bound in the fH-blocked lanes, the bands are of an even higher m.w. and do not all migrate into the gel. Second, based on the density of the β-chain band, there was substantial but somewhat less efficient complement activation in the lanes with an mAb to fH. The MCP-expressing cell line was M3.

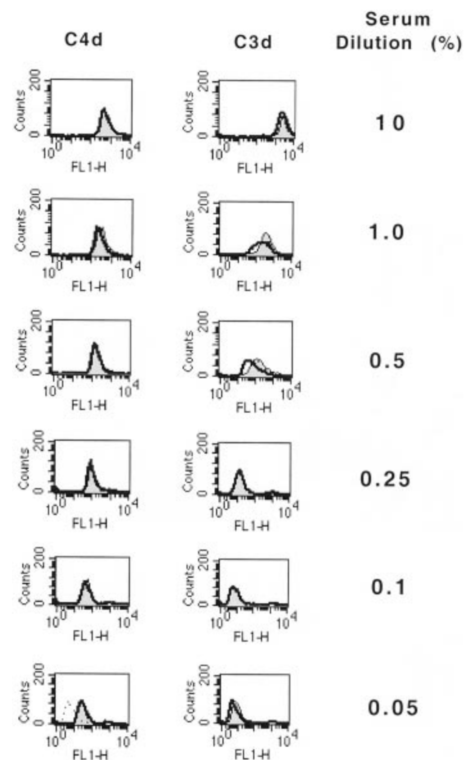

FIGURE 6. Effect of serum dilution on complement fragment deposition and cleavage. Concentration of the sensitizing Ab was 8.5 mg/ml. The cells were incubated with serum for 60 min at 37°C. The MCP$^+$ (cell line M3) is represented by the heavy line; MCP$^-$, by the filled area and control cells (no serum or no Ab added) by the dotted line shown on the last two panels. Representative experiment of three.

AP activation

In the following experiments, the concentration of sensitizing Ab was reduced until complement activation was largely mediated by the C3 convertase of the AP, or the CP was blocked altogether with Mg^{2+}-EGTA. Serum concentration was maintained at 10% to allow for AP activation.

MCP inhibits the AP C3 convertase. As the Ab used to sensitize the cells was reduced (Fig. 7), CP activation, as monitored by the quantity of C4d deposited, progressively decreased. At lower Ab concentrations (1 mg/ml IgG), the deposition of C3 fragments was inhibited >95% in the MCP$^+$ compared with the MCP$^-$ cells. Here C3d was monitored because like C4d, it reflects all of the parent C3b that had been deposited.

The CP is blocked in the presence of EGTA through its preferential ability to chelate calcium, which is required for C1 function. Interestingly, under these AP-driven activation conditions (Fig. 8, compare *A* and *C*), C3b was deposited on the MCP$^-$ cells to nearly the same extent as in the standard CP-driven activation condition. In contrast to the CP (Fig. 8*A*), AP activation was severely inhibited by MCP, as C3b deposition was 10-fold less on the MCP$^+$ cells (Fig. 8*C*). Thus, MCP is a potent inhibitor of the AP C3 convertase. Furthermore, at a lower level of sensitization (1 mg/ml), MCP inhibited C3b deposition by >90% (Fig. 8*B*). In this case, the feedback loop is responsible for most of the C3b deposition. If the CP is also blocked (Fig. 8*D*), there is no C3b deposition on MCP$^+$ cells, indicating complete inhibition of the AP by MCP at a lower level of cell sensitization.

Discussion

The most critical function of the complement cascade is arguably the deposition of C4b and C3b on a target surface. Clusters of these two opsonins and their cleavage products, in the case of C3, serve as ligands for complement receptors. Through such interactions, immune adherence and phagocytosis are mediated, B cell signaling is facilitated, and Ags are localized, processed, and retained. In this way, adaptive immunity is instructed by the complement system as to which Ags to target (reviewed in Ref. 23). C3 fragment deposition is also the gateway to C5 convertase formation and then assembly of the membrane attack complex. Furthermore, cleavage of C3 and C5 releases their anaphylatoxins to promote the local inflammatory response and to activate immunocompetent cells.

Regulation of the complement activation pathways is designed to allow unimpeded activation on an appropriate target, but to minimize attachment of C4b and C3b on self (1). Although the complement system's main evolutionary goal is to optimize interactions with a microbial target, activation on self-tissue occurs at low levels continuously and, more substantially, in the setting of autoantibodies, ischemia-reperfusion injury (16), apoptosis (17, 18), and inflammation. In these situations, the host membrane inhibitors are present.

The goal of this study was to enhance our understanding of the regulation and inactivation of C4b and C3b deposited on self-tissue. Therefore, following Ab-mediated activation of complement, the quantity and nature of complement fragments on cloned cell lines transfected with human MCP were analyzed. Cleavage of C4b by factor I releases C4c, while C4d remains surface-bound. This reaction is promoted by a cofactor, either MCP expressed on the same cell or C4BP in the serum or a combination thereof. Cleavage of cell-bound C3b by factor I requires MCP, fH, or both, and produces iC3b, which remains target bound. Because CHO cells display no intrinsic regulatory activity on their cell surface for

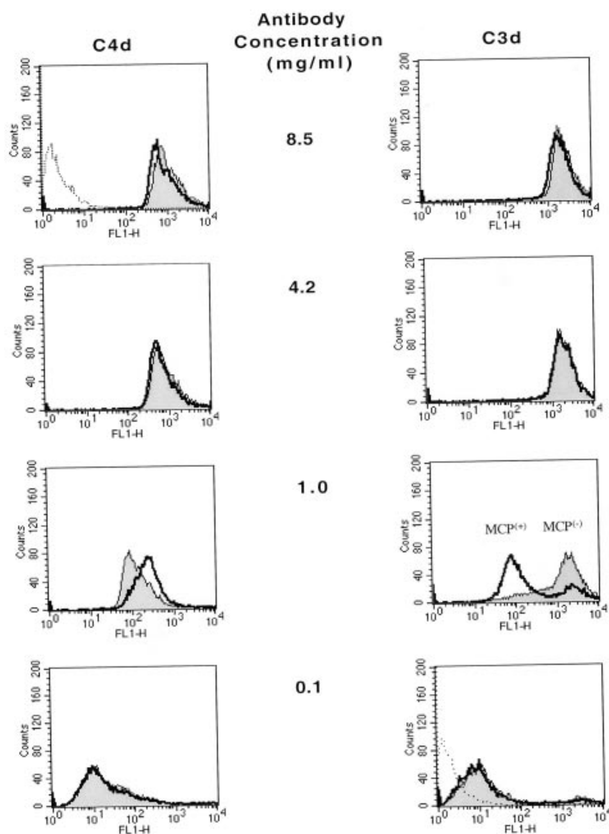

FIGURE 7. MCP inhibits deposition of C3b if the concentration of sensitizing Ab is reduced. FACS analysis of C4d and C3d deposition as a function of Ab dilution on MCP^+ cells (M3 cell line, dark line), MCP^- (filled), or control (dotted line as described in Fig. 2 and only shown in selected graphs). Cells were exposed to 10% C7d serum for 60 min at 37°C. Representative experiment of three. The reproducible decrease in detection of C4d deposition on MCP^- at 1 mg/ml is unexplained.

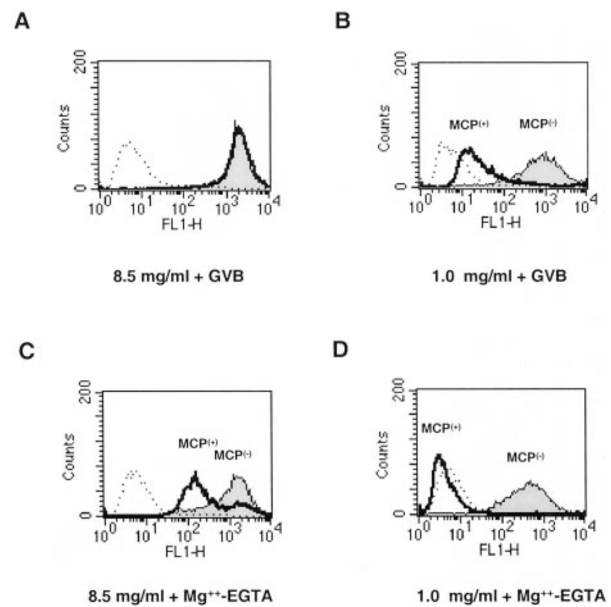

FIGURE 8. MCP inhibits C3b deposition by the AP. FACS analysis of C3d deposition using 8.5 or 1.0 mg/ml of sensitizing Ab with 10% C7d serum in presence or absence of Mg^{2+}-EGTA. Thick line, MCP^+ (cell line M3); thin line, MCP^-; and dotted line control as described in Fig. 2. Representative experiment of three.

human C4b or C3b, we could address which cofactor assists factor I in cleaving C4b and C3b.

C4b fragment deposition and degradation

First, there was no difference in the quantity of C4b deposited between the MCP^+ and MCP^- cells. This result was expected because MCP cleaves already deposited C4b (6). Second, on MCP^+ cells, C4b cleavage continued in a linear fashion for 30–45 min. The rate of C4b cleavage was relatively slow, especially compared with C3 and C5 activation by their CP convertases. Except in dilute serum, MCP did not influence the quantity of C3b and C5b deposited. Third, there was no detectable cleavage of C4b by the MCP^- cells. This result indicates that C4BP does not serve as a cofactor for cleavage of C4b in this system. Fourth, C4b cleavage was incomplete, even by the high MCP-expressing lines, as 18–30% remained after 2 h. How this deposited C4b escapes inactivation is under investigation. One possibility, as has been demonstrated for C3b and fH, is that such C4b may be attached to the sensitizing IgG, and thereby be less accessible to a cofactor protein (24).

Despite the presence of C4BP in the serum, as noted above, there was no cleavage of C4b on the surface of MCP^- cells. This was surprising because C4BP accelerates the decay of the CP C3 and C5 convertases and is a cofactor for the factor I-mediated cleavage of C4b (25–31).

Four possible explanations were considered and ruled out to account for this lack of an effect of C4BP on cell-bound C4b. First, in the serum source used, C4BP was present in the expected quantities and was appropriately active against fluid phase C4b. Thus, the protein was antigenically and functionally intact. In addition, experiments using a second (C6-depleted) serum source produced the same result. Second, factor I was present in the expected antigenic amounts and was functionally active, as indicated by its cleavage of C4b and C3b with other cofactors. Third, the quantity of C4b deposited as documented by FACS was of such a magnitude that C4BP binding should have occurred. Fourth, competitive inhibition by C2 (30, 31) was inconsequential, as there is 15–25% more C4BP than C2 in human serum and, in the presence of factor I, C4BP is not consumed in its reaction with C4b. Consequently, MCP, and not C4BP, was responsible for cleavage of C4b on CHO cells. We suggest that this is the case with most host cells.

C3b deposition and degradation by CP activation

Except for very dilute serum where a modest effect was observed, deposition of C3b was unaffected by MCP expression in the setting of CP activation. The reason for this is that the rate of C4b cleavage by MCP was too slow to alter the activity of the C3 convertases. This result is also in concert with those of Devaux et al. (32), who found no effect of MCP (5000 copies/cell) expression on CP-mediated lysis. In our experimental system, we used MCP cell lines expressing 90,000, 200,000, and 234,000 copies/cell, and also saw no effect. However, these results appear to be in conflict to earlier experiments in which an inverse relationship between MCP expression and C3b deposition in mouse fibroblast cells exposed to human serum (6) and the inhibition of CP-mediated lysis by MCP in several other experimental systems (6, 32). In one of these (6), cell lines were used with much more MCP (1×10^6 copies/cell), and lysis was monitored, but not C4b and C3b deposition. More importantly, normal human serum was used as a source of Ab compared with the high titer anti-CHO Ab used in this study (32). Using human serum in this manner, the feedback loop of the AP contributes to C3b deposition (see next section) and MCP has been shown by several groups to be efficacious in inhibiting the AP C3 and C5 convertases (32–35).

Western blots indicated that C3 fragments on the surface of MCP^- and MCP^+ cells consisted predominantly of iC3b. Therefore, fH and not MCP was the responsible cofactor. The experiments using a function-blocking mAb to fH further established this conclusion. These results parallel observations using a human melanoma cell line expressing moderate amounts of decay-accelerating factor and low amounts of MCP (5×10^3 copies/cell) in which the CP was activated by IgG and purified components served as the complement source (36, 37). In these studies, fH was responsible for iC3b generation. These data are also consistent with several reports that, once cells are heavily coated with C3b, fH binds efficiently (37, 38). We conclude that following deposition of relatively large amounts of C3b, fH is primarily responsible for the cleavage of C3b to iC3b on host tissue. Of note, this reaction occurs more rapidly than C4b cleavage by MCP. Our studies suggest that some of the higher m.w. fragments represent C3b α-chain fragments (α' or α_1) bound to the sensitizing IgG or other membrane constituents (39–41). Interestingly, a surprisingly large fraction of α' or α_1 were either not covalently attached or bound to small molecules.

Role of MCP in complement regulation: surveillance and inhibition of the AP and the feedback loop

In prior reports, MCP was shown to protect only the cell on which it is bound, so-called intrinsic regulatory activity (6, 35). MCP has a preferential ability to inactivate C3b bound to large protein complexes, including the AP C5 convertase (34, 35, 42). In studies reported herein, MCP was shown to be a potent inhibitor of AP activation and of the feedback amplification loop. Taken together, these data suggest a surveillance role for MCP on host cells. For example, we envision that MCP patrols the surface of host cells where it seeks out C4b and C3b deposited during an inflammatory/immune reaction or from the spontaneous turnover of C4 and C3 (1, 6). In this regard, MCP continuously monitors the cell surface to prevent the AP and its feedback loop from being engaged. MCP's role in the regulation of the AP has been repeatedly suggested (32–34), especially its ability to inhibit the feedback loop (32). A role for CP-mediated cytoprotection or inhibition of lysis by MCP also has been shown (6, 21, 34, 43, 44). However, our results indicate that this effect on the CP is likely to only be operative if the AP feedback loop is engaged or perhaps if very large quantities of MCP are expressed. In the present studies, we directly show that if the AP or feedback loop is required for C3b deposition, MCP is a potent inhibitor of this reaction.

A new role for MCP is suggested by these investigations; namely, that MCP is responsible for inactivating most of the C4b bound to self-tissue. In contrast, the plasma protein fH is the responsible cofactor if large quantities of C3b are deposited. However, as noted, our data also strongly support an important role for MCP in the inactivation of low-level C3b deposition to prevent AP engagement (32–35).

In summary, the striking frequency of autoimmunity, especially systemic lupus erythematosus, in early complement component deficiency states implies a critical role for these proteins in maintaining homeostasis in the immune system. The deposition and degradation of C4b and C3b may not only be pivotal in triggering the innate immune response and in instructing adaptive immunity, but also in the negative selection of self-reactive B cells (45). Consequently, further analysis will focus on experimental systems using both decay-accelerating factor and MCP with human autoantibodies and autoantigens. These types of studies should shed light on the role played by C4b and C3b and their limited degradation products in normal and aberrant immune responses.

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