Complement-mediated Tumor Cell Damage Induced by Antibodies against Membrane Cofactor Protein (MCP, CD46)

By Tsukasa Seya, Tomoko Hara, Misako Matsumoto, Yuji Sugita,* and Hitoshi Akedo

*From the Department of Immunology, Center for Adult Diseases Osaka, Higashinari-Ku, Osaka 537; and the *Department of Physiological Chemistry, School of Pharmaceutical Sciences, Showa University, Shinagawa-ku, Tokyo 142 Japan*

Summary

We have developed polyclonal and monoclonal antibodies against human membrane cofactor protein (MCP) to use as tools to investigate the functions of MCP on intact nucleated cells. Two human T cell lines, CEM and TALL, are $CR1^-$ and DAF^-. Pretreatment of these cell lines with M177 and polyclonal anti-MCP, which inhibit cofactor activity almost completely, resulted in effective C3 deposition immediately following addition of these cells to Mg^{2+}/EGTA/human sera. The deposited C3 remained expressed partly on the cell surface and most of them were gradually converted to C3bi. Some of the deposited C3 were complexed with membrane proteins, since 140- and 250-kD bands became significantly accumulated on SDS-PAGE by treatment with the antibodies.

We next tested whether these C3-coated cells were damaged by complement-mediated cytolysis. p18, an inhibitor of membrane attack complex (MAC) formation, was negative in TALL but positive in CEM. TALL was lysed efficiently only by treatment with the polyclonal anti-MCP, while CEM showed only slight lysis with the same treatment. Monoclonal antibodies to MCP, including M177, caused only minimal cell destruction.

Based on these results, together with the fact that decay-accelerating factor (DAF) serves as a factor for preventing C3 attack on human cells, we conclude that MCP and DAF cooperatively protect host cells from C3 targeting and, in these T cell lines, MCP is sufficient for preventing C3 deposition even without DAF. After all, human cells undergo almost no autologous complement-mediated cytolysis if they express at least one of the functionally active inhibitors, MCP, DAF, or p18.

The deposition of C3 fragments is a critical step in complement activation and subsequent complement-mediated cell damage (1). In addition, bound C3 fragments serve as ligands for C3 receptors to participate in lymphocyte- and phagocyte-mediated cell damage (2, 3). Therefore activation of C3 must be kept under strict control (4). In the classical pathway, C3 can be activated only on cells where antibody deposits. In the alternative pathway, most human tissue is protected from autologous C3 deposition, whereas most foreign material is targeted by C3. The mechanism by which target sites are distinguished, however, remains to be clarified (5). Protection of host cells from C3 attack can be explained in part by the presence of a membrane complement inhibitor, decay-accelerating factor (DAF).[1] Because of the absence of DAF, paroxysmal nocturnal hemoglobinuria (PNH) erythrocytes amplify C3 deposition (6). In contrast, some human cell lines, including Raji and Daudi, permit deposition of human C3 on their surfaces (3, 7, 8), despite the fact that they express DAF (3, 9), a finding that is inexplicable. Furthermore, PNH leukocytes (10) and some human tumor cell lines (9, 11), although they lack DAF, have only minimal ability to trigger C3 deposition. Human nucleated cells must possess another factor for regulation of C3 deposition besides DAF.

Membrane cofactor protein (MCP) has been shown in the fluid phase to possess C3b-binding (12) and factor I–cofactor activities (13) which may play important roles in regulation of the alternative pathway. In the present study, we demonstrated that MCP actually prevents C3 deposition on nucleated host cells. We propose the evidence that on human nucleated cells, DAF and MCP jointly engage in protection of host cells, and in some cases, either one is sufficient to prevent complement-mediated cell damage.

[1] *Abbreviations used in this paper:* DAF, decay-accelerating factor; MAC, membrane attack complex; MCP, membrane cofactor protein; p18, an inhibitor for MAC formation, recently identified as CD59; PNH, paroxysmal nocturnal hemoglobinuria.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/90/12/1673/08 $2.00
Volume 172 December 1990 1673–1680

Materials and Methods

Proteins, Antibodies, and Cells. Human MCP (14) and C3 (15) were purified from a T cell line, HSB-2, and citrate phosphate-dextrose-plasma, respectively, as previously described. Protein labeling was performed with iodogen (16).

Rabbit polyclonal antibodies to human DAF (17) and MCP (18) were prepared as described. Monospecificity of these antibodies was further confirmed (19). Rabbit polyclonal antibody to human β_2-microglobulin was a gift from Dr. J. P. Atkinson (Washington University, St. Louis, MO). Mouse mAbs directed against human MCP (9, 20) and C3b/C4b receptor (CR1) (20) were produced as previously noted. These were purified using protein A-Sepharose (20). Mouse mAbs against human DAF, IA10 (21), and against p18 (22), an inhibitor of the formation of membrane attack complex (MAC), 1F1 (23), were generous gifts from Dr. T. Kinoshita (Osaka University, Osaka), and Dr. M. Tomita (Showa University, Tokyo), respectively. $F(ab')_2$ of these antibodies were prepared by treatment with pepsin, by the method of Nisonoff (24).

Mouse mAb to human C3bi (which is specific to a neoepitope for C3bi and C3dg, and does not react with C3, C3b, C3d, and C3c) and human C3c (which reacts with C3b, C3bi, and C3c, but not with C3d and C3dg) were purchased from Cytotech (San Diego, CA) and Genzyme Corp. (Boston, MA), respectively. Rabbit polyclonal antibody against human C3c was from Behringwerke (Marburg, FRG).

Human T cell lines CEM and TALL, and an erythroblastoid cell line, HEL, were gifts from the Japanese Cancer Research Resources Bank (JCRB, Tokyo). These were maintained in RPMI 1640 supplemented with 10% FCS (M.A. Bioproducts, Walkersville, MD) and antibiotics. Cultures were kept in a 5% CO_2/95% air atmosphere at 37°C.

Assay for Determination of Factor I-Cofactor Activity. A fluid phase assay system was used (13). The buffer used in this assay was 20 mM phosphate buffer/0.02% NP-40, pH 6.2. 30 ng of MCP was preincubated with various concentrations of mAbs to MCP, M75, M160, or M177, and then incubated with 0.5 μg of factor I and 10 μg of methylamine-treated fluorescent-labeled C3, f-C3(MA) or fluorescent-labeled C3b (f-C3b) (25) for 3 h at 37°C. The reaction was then stopped by the addition of 10 μl of 10% SDS and 3 μl of 2-ME. The samples were analyzed by SDS-PAGE and the percent α chain cleavage of the substrates was determined by spectrofluorometry as previously described (13, 14). The inhibitory effect of the antibodies on the cleavage of f-C3b was estimated assuming that the degree of cleavage of α chains in the absence of the antibody was 100%.

Analysis of C3 Deposition on Tumor Cells. Two methods were used to assess C3 deposition. In the first method, C3 fragments deposited on the cell-surface were analyzed by flow cytometry (9). Cells (10^6) were pretreated with about 25 μg of polyclonal antibodies to MCP or nonimmune rabbit IgG and incubated with 50 μl of Mg^{2+}/EGTA/human serum and 150 μl of EGTA/GVB^{2+} for 90 min at 37°C. As a control, EDTA/human serum was used instead of Mg^{2+}/EGTA/sera. The cells were washed twice in PBS containing 2% BSA, and then, 3 μg of anti-C3c or anti-C3bi mAbs were added as the first antibody. After 45 min, the cells were again washed twice in PBS/BSA, and 5 μg of the second antibody [(FITC-labeled-$F(ab')_2$ of goat anti-mouse IgG; Cappel Laboratories, Malvern, PA]) were added. The mixtures were allowed to stand for 45 min, washed twice, and analyzed by flow cytometry. When the mAbs to MCP were used as the pretreatment reagents, rabbit polyclonal anti-C3c and FITC-labeled $F(ab')_2$ of goat anti-rabbit IgG (Cappel Laboratories) were used as the first and second antibodies, respectively.

The other method employed was to use SDS-PAGE and autoradiography to analyze the deposited C3 fragments (9). Cells (10^6) were pretreated with 30 μg of M177 as described above. After washing, 50 μl of ^{125}I-labeled C3 (A_{280nm} = 3.0) were incubated with 50 μl of Mg^{2+}/EGTA/serum or EDTA/serum, and 100 μl of EGTA/GVB^{2+} or EDTA/GVB. The cells were washed thoroughly in PBS, solubilized with 1% NP-40/PBS containing 1 mM PMSF, 10 mM EDTA, and 25 mM iodoacetamide, pH 7.4. The samples were reduced, subjected to SDS-PAGE, and analyzed by autoradiography.

Flowcytometry Analysis for Assessment of Surface-expressed Complement Regulatory Proteins. Approximately 10^6 cells were incubated for 45 min with 10–20 μg of the primary antibody, such as anti-MCP (M177), anti-DAF (IA10), anti-CR1 (31R), or anti-P18 (1F1), together with 100 μl of EDTA plasma. 10 μg of mouse nonimmune IgG (Cappel) was used as a control. The cells were washed in PBS containing 2% FCS, and treated with 5 μg of FITC-conjugated second antibody in 100 μl of PBS/FCS and EDTA plasma. After 45 min, the cells were washed twice with PBS and fixed with paraformaldehyde. The samples were analyzed on a FACScan (Becton Dickinson, Mountain View, CA) (18) within 1 wk. The experiments were performed at room temperature.

SDS-PAGE and Autoradiography. SDS-PAGE using 8% slab gels was performed by the method of Laemmli (26). Gels were stained, destained, dried, and then exposed at −75°C. Cronex intensifier screens (DuPont Co., Wilmington, DE) and XAR-5 film (Eastman Kodak Co., Rochester, NY) were used for autoradiography (17).

Cytolysis Assay. Cells (10^7) were incubated with 200 μCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) in 400 μl of RPMI for 60–120 min at 37°C (9). Although labeling efficiency varied among the cell lines, 45,000–120,000 cpm were incorporated into 10^6 cells. The labeled cells were pretreated with antibodies to MCP or nonimmune IgG and incubated with 15–30% Mg^{2+}/EGTA/serum or EDTA/serum for 90 min at 37°C. Radioactivity in the supernatants was measured in a gamma counter.

Results

Cell Lines on Which C3 Deposition Was Induced by Treatment with Polyclonal Anti-MCP. 24 human leukemia cell lines (9) were treated with polyclonal anti-MCP and then human Mg^{2+}/EGTA/serum. The deposited C3 was assessed with anti-human C3c or anti-human C3bi mAb by flow cytometry. Two T cell lines, CEM and TALL, became C3-sensitive through treatment with anti-MCP (Fig. 1, *B–D*). C3 deposited only slightly with the same treatment of most of the other cell lines, including HEL (Fig. 1 *A*). The deposited C3 was expressed on the cell surface and was detected by both antibodies to C3c and C3bi (Fig. 1). As reported previously (9), little C3 was deposited on CEM and TALL unless the cells had been treated with anti-MCP (Fig. 1). Treatment of these cells with anti-β_2-microglobulin did not result in C3 deposition (not shown). In addition, no C3 was deposited if Mg^{2+}/EGTA/serum was substituted for EDTA serum. Therefore, the observed C3 deposition was specific for anti-MCP and the alternative complement pathway. The deposited C3 could be detected to a similar extent by both anti-C3c and anti-C3bi, suggesting that the main product expressed on the cells was C3bi (Fig. 1 *C, D*) (27).

Complement Regulatory Proteins Distributed on CEM and TALL. Using mAbs to CR1, DAF, and MCP, amounts

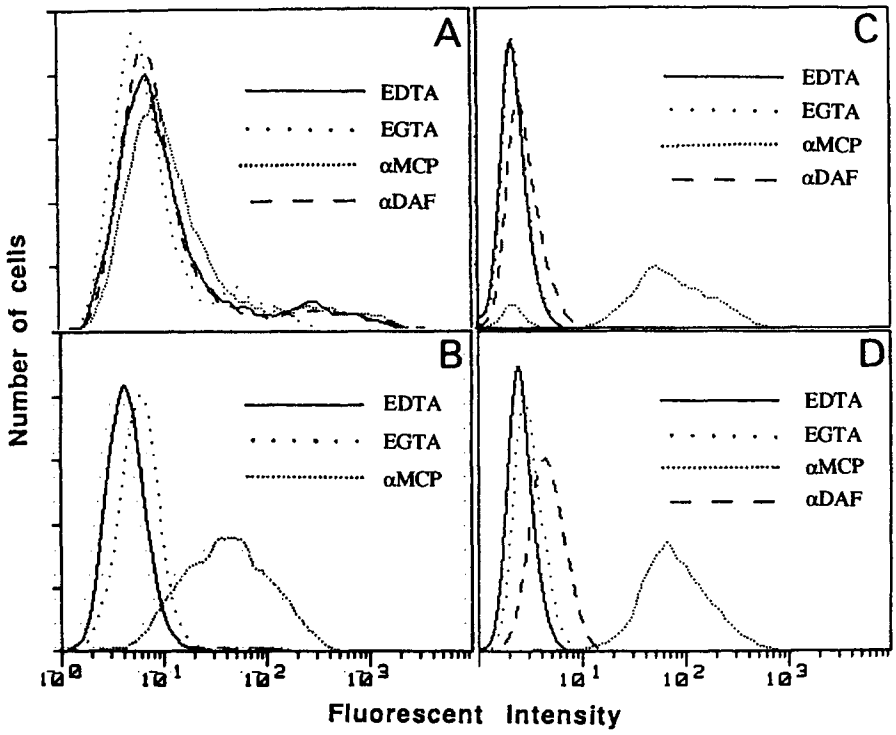

Figure 1. Deposition of C3-fragments on TALL and CEM by treatment with polyclonal antibody to MCP. HEL (*A*), CEM (*B*), and TALL (*C*, *D*) were pretreated with rabbit IgG (——, ····), polyclonal anti-MCP (- - -), or polyclonal anti-DAF (— — —), and incubated with human EDTA-serum (——) or Mg^{2+}/EGTA/serum (····, - - -, — — —). The deposited C3 was detected with mAbs against human C3c (*A*, *B*, *D*) or C3bi (*C*), and subsequent FITC-labeled goat anti-mouse IgG. After washing, the cells were analyzed by flow cytometry. Most of the other cell lines tested (9) exhibited similar profiles to those of HEL (not shown).

of surface-expressed C3 regulatory proteins were assessed by flowcytometry. CR1 and DAF were not detected in CEM and TALL (Fig. 2, *A*, *B*). The absence of CR1 and DAF in these cell lines was also supported by the protein A–rosette assay (20) and radiometric assay (9) (data not shown). Therefore, effective C3 deposition occurred specifically on the $DAF^-/CR1^-$ cells.

The level of p18, an inhibitor of the formation of MAC, was assessed in these T cell lines by flowcytometry. CEM possessed p18 whereas TALL did not (Fig. 2, *C*, *D*).

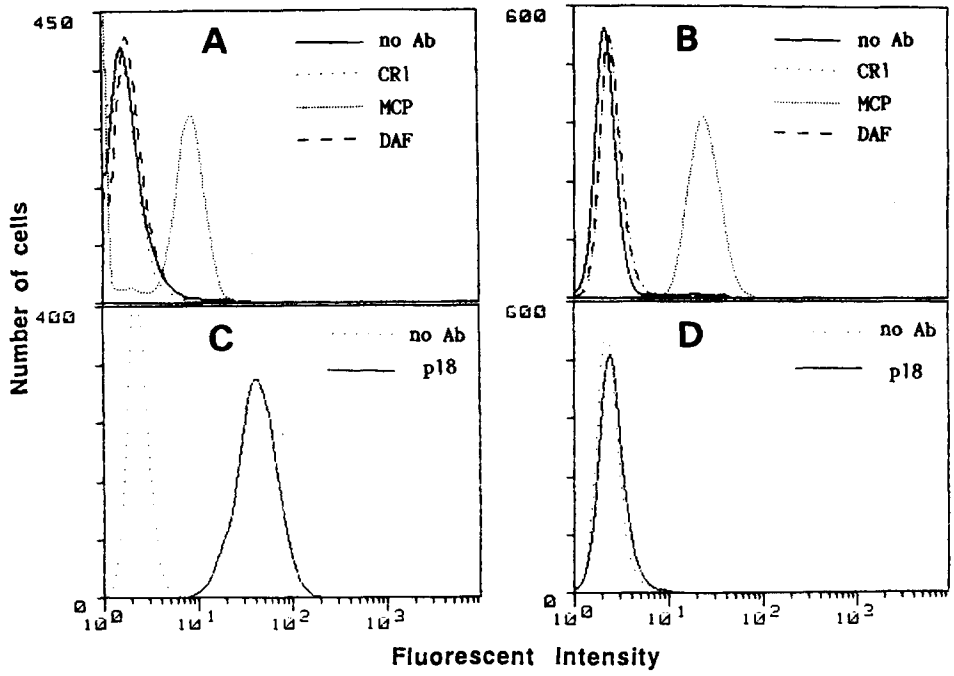

Figure 2. Levels of CR1, MCP, DAF, and p18 on TALL and CEM. TALL and CEM were incubated with mAbs to CR1 (31R) (···), MCP (M177) (- - -), DAF (IA10) (— — —), or p18 (1F1) (——). Mouse IgG was used as a control. The bound antibodies were detected with FITC-labeled goat anti-mouse IgG and flowcytometry. (*A*, *C*) CEM. (*B*, *D*) TALL.

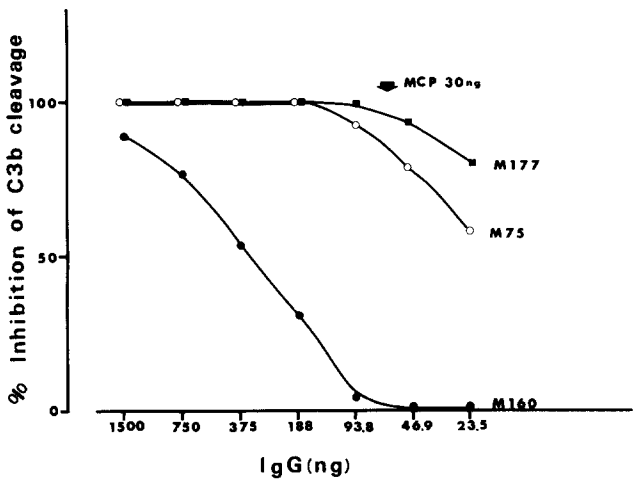

Figure 3. Inhibition of cofactor activity of MCP using mAbs against MCP. MCP (30 ng) was preincubated with various concentrations of M177 (■), M160 (●), or M75 (○). The mixtures were then incubated with factor I and f-C3b. All samples were analyzed under reducing conditions on SDS-PAGE. The percent cleavage of C3b was calculated based on the results of densitometric analysis of these gels (25). 30 ng of MCP are ~0.5 pmol. The equimolar point of each mAb is indicated as a closed arrow. Similar results were obtained using f-C3(MA) as a substrate (data not shown).

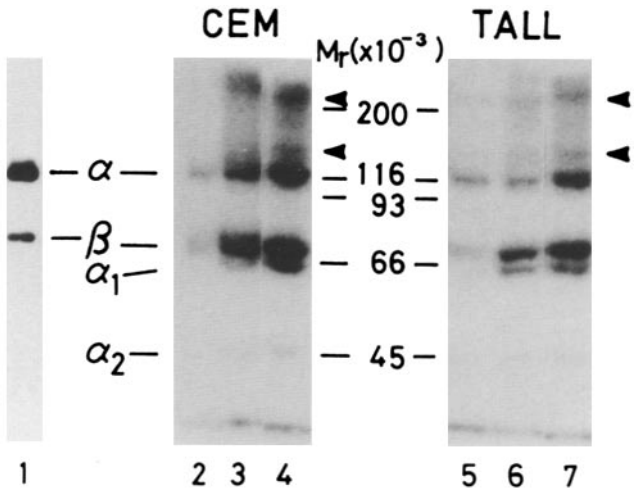

Figure 5. Analysis of the deposited fragments of C3 on CEM and TALL by SDS-PAGE followed by autoradiography. CEM (*left*) or TALL (*right*) was preincubated with M177 (lanes *2*, *4*, *5*, and *7*), and then incubated with EDTA/serum (lanes *2* and *5*) or Mg^{2+}/EGTA/serum (lanes *3*, *4*, *6*, and *7*) containing ^{125}I-labeled purified C3. Lane *1* is the labeled C3 used in this experiment. The arrowheads indicate C3 fragments complexed with membrane proteins. The C3 fragments were identified according to previous reports (9, 15). BioRad molecular weight marker was used.

Effect of mAbs on Factor I–Cofactor Activity of MCP and C3 Deposition on TALL. MCP acts as a cofactor for factor I to generate C3bi from C3b in the fluid phase (13, 28). We evaluated the inhibitory activity of the mAbs towards factor I–mediated cleavage of C3b. Purified MCP (30 ng) was pretreated with varying amounts of each mAb to MCP, and incubated with factor I and f-C3b. The inhibitory efficiency of these antibodies differed. The order of efficiency for inhibitory activity towards the factor I–cofactor activity of MCP was M177>M75>M160 (Fig. 3). More than 90% of MCP activity was blocked by equimolar M177.

We next examined the effect of $F(ab')_2$ of the mAb on C3 deposition in the T cell lines. The mAbs also had the ability to induce C3 deposition on these cells (Fig. 4). Amounts

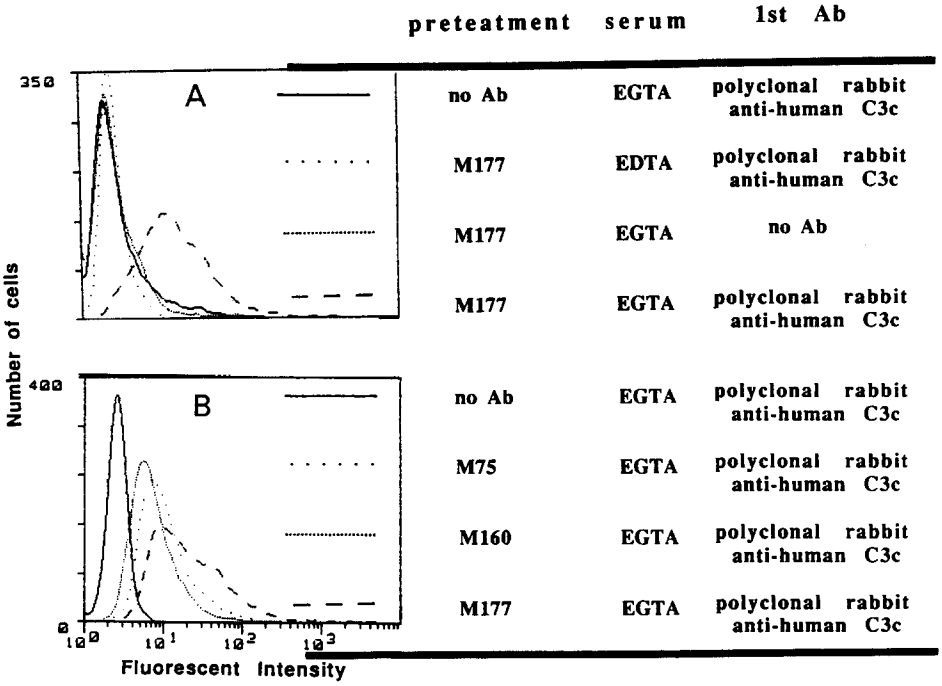

Figure 4. Deposition of human C3 on CEM and TALL using mAbs against MCP. CEM (*A*) and TALL (*B*) were pretreated with $F(ab')_2$ of the mAbs antibodies to MCP, and then incubated with EDTA- of Mg^{2+}/EGTA/serum (see the inset table). Bound C3 was detected with polyclonal rabbit anti-human C3c (see the Table) and FITC-labeled goat anti-rabbit IgG. The last antibody was added to all samples, although not shown in the table.

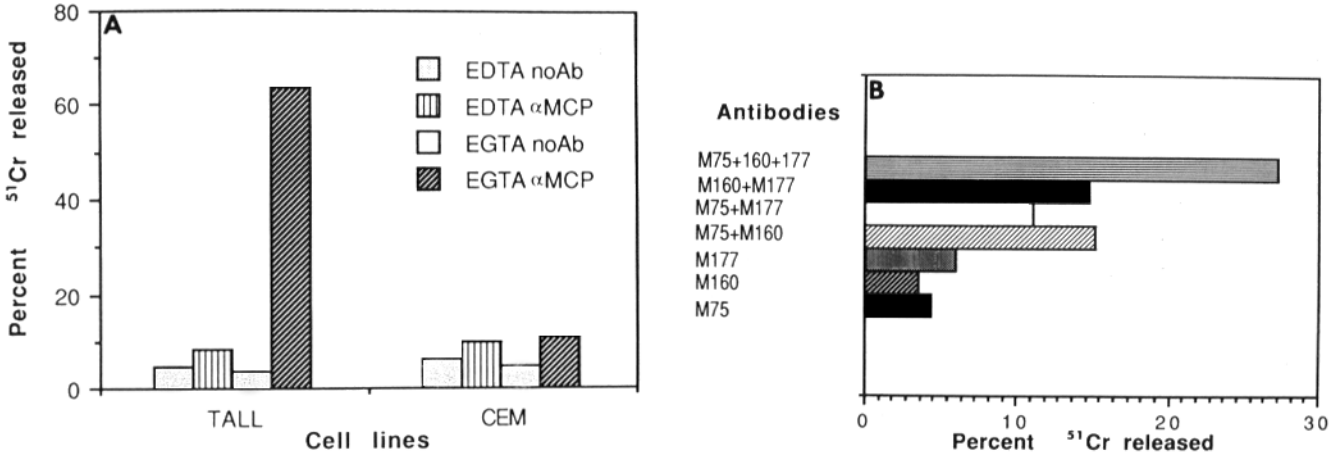

Figure 6. Complement-mediated cytotoxicity induced by antibodies against MCP. (*A*) Effect of polyclonal antibody. TALL and CEM were labeled with ^{51}Cr and pretreated with rabbit IgG or polyclonal rabbit anti-MCP. Thereafter, the samples were incubated with EDTA/serum or Mg^{2+}/EGTA/serum. The combinations of these reagents are shown in the figure. The liberated ^{51}Cr in the supernatants was recovered and assayed using a gamma counter. (*B*) Effect of mAbs. TALL were labeled with ^{51}Cr and incubated with antibodies, as indicated in the figure. The samples were then incubated with Mg^{2+}/EGTA/serum. The released ^{51}Cr was estimated as in *A*.

of deposited C3 were compared using the three antibodies and TALL. The greatest amount was deposited on cells pretreated with M177. The order of efficiency for induction of C3 deposition was M177>M75>M160, which is the same order as that of the inhibitory activity for factor I–mediated C3b cleavage. Similar results were obtained with CEM (not shown).

Analysis of the Deposited C3 on the DAF⁻/CD1⁻ T Cells. To identify the C3 fragments deposited on M177-treated CEM and TALL, Mg^{2+}/EGTA/serum containing ^{125}I-labeled C3 was incubated with the M177-treated cells. EDTA/serum and untreated cells were used as controls. The deposited fragments of ^{125}I-C3 were solubilized, reduced, and subjected to SDS-PAGE followed by autoradiography. In the sample with M177 and Mg^{2+}/EGTA/serum, prominent high molecular mass bands of 250 and 140 kD together with α/α', $\alpha 1$, $\alpha 2$, and β chains of C3 (Fig. 5) were accumulated. Although it is unknown whether these C3 fragments were expressed on the cell surface, the results from electrophoresis suggested that C3/C3b and C3bi were the major forms detected in the NP-40 extract of the anti-MCP–treated cells (Fig. 5).

Complement-mediated Killing of TALL which was Pretreated with the mAbs to MCP. The effect of mAbs to MCP on complement-mediated cytolysis was examined by ^{51}Cr-release assay. All antibodies, including M177, killed <10% of TALL (Fig. 6 *B*). A combination of the antibodies yielded about 30% ^{51}Cr-release. On the other hand, polyclonal anti-MCP released >60% of the ^{51}Cr (Fig. 6 *A*). CEM showed <10% release by pretreatment with either polyclonal or monoclonal antibodies (Fig. 6, *A* and *B*).

Discussion

This paper is the first to document that MCP actually protects host cells from C3 targeting. This functional property was revealed using the DAF⁻/CR1⁻ human T cell lines, which triggered efficient C3 deposition via the alternative pathway by treatment with polyclonal or monoclonal anti-MCP. Little C3 was deposited on the cells that had been treated with anti-β_2-microglobulin. C3 deposition was also induced by two $F(ab')_2$ of the mAb to MCP belonging to IgG_1 subclass (which lack the ability to activate complement), suggesting that C3 deposition is independent of the complement-activating effect of IgG. Of the mAbs tested, M177 caused the most efficient C3 deposition. The order of efficiency of these antibodies in the induction of C3-deposition was M177>M75>M160, which is the same as that for inhibition of MCP-mediated C3b-C3bi conversion. These results suggest that the blocking of MCP cofactor activity leads directly to deposition of C3 on DAF⁻/CR1⁻ cells via activation of the alternative pathway.

MCP generally interacts with C3b on the same cell membrane and not with C3b on other cells, and is said to be an intrinsic factor I–cofactor protein, in contrast with the extrinsic cofactor protein, CR1 (14, 28). DAF has been characterized as an intrinsic complement regulator; although it does not serve as a cofactor, it accelerates the decay of the convertase complex (6, 29, 30). Some human nucleated cells and cell lines become susceptible to autologous C3 if DAF is functionally blocked (11). Of the three cell-associated complement regulatory molecules, DAF and MCP have now been shown to be engaged in protection of nucleated host cells from C3 attack. We favor the interpretation that the protective function of DAF and MCP is closely in conjunction with their intrinsic activities but not with their mode of inhibition. MCP is distributed on all nucleated blood cells and cell lines (18, 31). This may partly explain the well-characterized high resistance of nucleated cells to complement attack (32).

Extensive C3 amplification occurs on the DAF⁻/CR1⁻ T cell lines CEM and TALL if the MCP activity is blocked;

the C3b-accepting sites on TALL and CEM should be susceptible to MCP. This means that the DAF$^-$/CR1$^-$ T cell lines come to express acceptor molecules for autologous C3, thereby allowing deposition of C3b. Some of the important C3-accepting molecules are likely to be membrane proteins forming complexes with fragments of labeled C3 that migrate at 250 and 140 kD on SDS-PAGE.

It has been generally accepted that C3 deposition occurs on foreign material but not on "self" tissue since the latter does not express on its surface "activator" that can accept C3b molecules and activate the alternative pathway (33, 34). If the activator represents acceptor molecules for C3b, as Mold et al. suggested (35), the anti-MCP-treated CEM and TALL must have activator, which accepts the initial C3b and assists in extensive amplification of C3 deposition. According to preceding work (33–35), together with the results of our present study, most foreign cells effectively accept C3b via their activator, which eventually results in cell damage; on self cells, although most of them express activator on their surface, the C3b initially deposited on it is essentially MCP- and/or DAF-sensitive, thereby preventing activation of subsequent C3, as well as its anchoring onto the cells. In other words, since most C3b deposited on molecules of human cells are not resistant to the inhibitors, they fail to amplify C3 activation. If this is the case, lack of inhibitors or a high expression of activator resistant to the action of inhibitors may be representative conditions under which C3 deposition occurs effectively on the cells. Indeed, C3 is able to deposit on human Raji cells (3, 7–9) because they express CR2 (35) which is known to evade the action of both MCP and DAF at least on B cell lines (8, 35, 36), and on PNH erythrocytes because they lack MCP and DAF (10, 21, 30, 37). Based on this hypothesis, autologous cells can be converted to target cells by the function of activators or by modulating the inhibitors.

Deposited C3b appears to be largely converted to C3bi, since antibodies specific to C3bi and C3c could recognize the deposited C3 fragments to a similar extent (28). These results are only in partial agreement with those of the autoradiographic analysis of the deposited C3 (Fig. 5), for reasons unknown. As discussed in a previous paper (9), this discrepancy may be partly due to the fact that some C3/C3b is unexpressed, as a result of internalization. Kinetic analysis indicated that more than 30 min is required to reveal full C3bi sites (Seya, T., manuscript in preparation), suggesting that the C3 deposition and following C3b-C3bi conversion occur gradually on the cell surface under conditions in which MCP is not functional. MCP is 50 times more active as a cofactor than factor H (13) and is known to inactivate C3b instantly, even in the fluid phase (14, 28). Most likely, this slow inactivation of the deposited C3b is not due to the surviving MCP but due to serum factor H. The fact that this slow inactivation of C3b did not impair amplification of C3 suggests the inability of the fluid phase regulatory factors to provide adequate control of cell surface activation of C3.

Many reports have suggested that efficient C3-deposition is induced on some tumor cells (7–9, 38–40). In most cases, however, sensitizing antibody was needed for induction of C3 deposition. This means that extensive C3 activation would be required to allow acceptor molecules to deposit C3b overcoming regulatory factors. In this study, the blocking of MCP facilitated extensive C3 deposition even without sensitizing antibodies. As shown in a previous paper (9), most tumor cells and immature nucleated cells predominantly express MCP, but little or no CR1. Furthermore, some lack DAF (9, 11). Therefore, in complement-mediated cytotoxicity, a combination of anti-MCP with antibodies against tumor-associated antigens may be more effective.

It has been reported that target cells could be eliminated by CR1-mediated clearance (41), phagocytosis (2, 42, 43), lymphocyte-mediated cytotoxicity (3, 44), and/or C9-mediated immune-cytolysis (32). The last has been defined traditionally as complement-mediated cytolysis. We next tested the effect of anti-MCP on complement-mediated cytolysis. Only polyclonal antibody–treated TALL were killed efficiently by complement. Monoclonal anti-MCP also induced cell destruction, but only by 30% at most. The reasons for this discrepancy between polyclonal and monoclonal antibodies remain unknown. Nucleated cells appear to have unique systems to protect against MAC formation (32, 45–47). This may explain in part the incomplete lysis of the monoclonal anti-MCP-treated cells. CEM was barely killed by anti-MCP. This finding seems to reflect the fact that TALL, but not CEM, lacked p18.

DAF and p18 are both phosphatidylinositol (PI)-anchored proteins (48–51). A number of tumor cell lines are reported to lack DAF, although it is unknown whether they lack p18. A coincidental deficiency of these proteins may frequently occur on tumor cells. These DAF$^-$/p18$^-$ cells are to be damaged only by anti-MCP treatment, which causes little or no damage to normal cells expressing DAF and p18 (Matsumoto, M., and T. Seya, unpublished data). Therefore, the coincidental dysfunction of the C3 regulatory/activating system and the C9 regulatory system would lead to cell damage by autologous complement via the alternative complement cascade resulting in immune cytolysis of human nucleated cells. We have postulated that C3 deposition on autologous cells takes place more frequently than cytolysis (9). The role and effect of the C3 fragments deposited on autologous cells, in relation to other three mechanisms of cell destruction, should prove to be of great importance in the elimination of tumor cells.

We are grateful to Drs. M. Tomita (Showa University, Tokyo), T. Kinoshita (Osaka University, Osaka), and J. P. Atkinson (Washington University, St. Louis, MO) for their gifts of the reagents. We thank

JCRB for sending us CEM and TALL and Kyowa Hakko Co. (Tokyo) for supporting this work. Thanks are also due to Ms. Akiko Itoh for secretarial assistance.

This work was supported in part by grants from the Mochida Memorial Foundation, the Naito Memorial Foundation, Sagawa Cancer Research Foundation, and the Cell Science Research Foundation.

Address correspondence to Dr. T. Seya, Center for Adult Diseases, Osaka, Higashinari-ku, Osaka 537, Japan.

Received for publication 13 July 1990.

References

1. Ross, G.D., and M.E. Medof. 1985. Membrane complement receptors specific for bound fragments of C3. *Adv. Immunol.* 37:217.
2. Schreiber, R.D., M.K. Pangburn, A.B. Bjornson, M.A. Brothers, and H.J. Muller-Eberhard. 1982. The role of C3 fragments in endocytosis and extracellular cytotoxic reactions by polymorphonuclear leukocytes. *Clin. Immunol. Immunopathol.* 23:335.
3. Ramos, O.F., M. Patarroyo, E. Yefenof, and E. Klein. 1989. Requirement of leukocytic cell adhesion molecules (CD11a-c/CD18) in the enhanced NK lysis of iC3b-opsonized targets. *J. Immunol.* 142:4100.
4. Hourcade, D., V.M. Holers, and J.P. Atkinson. 1988. The regulators of complement activation (RCA) gene cluster. *Adv. Immunol.* 45:381.
5. Atkinson, J.P., and T.C. Farries. 1987. Separation of self from non-self in the complement system. *Immunol. Today.* 8:212.
6. Medof, M.E., T. Kinoshita, and V. Nussenzweig. 1984. Inhibition of complement activation on the surface of cells after incorporation of decay-accelerating factor (DAF) into their membranes. *J. Exp. Med.* 160:1558.
7. Praz, F., and P. Lesavre. 1983. Alternative pathway activation by human lymphoblastoid B and T cell lines. *J. Immunol.* 131:1396.
8. McConnell, I., G. Klein, M. Macanovic, M.T. Gorpman, and J. Raniwalla. 1981. Malignant cells isolated from Burkitt's lymphoma but not other forms of leukemia activate the alternative complement pathway in human serum. *Eur. J. Immunol.* 11:132.
9. Seya, T., T. Hara, M. Matsumoto, and H. Akedo. 1990. Quantitative analysis of membrane cofactor protein (MCP) of complement: High expression of MCP on human leukemia cell lines, which is down-regulated during cell differentiation. *J. Immunol.* 145:238.
10. Nicholson-Weller, A., D.B. Spicer, and K.F. Austen. 1985. Deficiency of the complement regulatory protein "decay-accelerating factor" on membranes of granulocytes, monocytes, and platelets in paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* 312:1091.
11. Cheung, N.V., E.I. Walter, W.H. Smith-Mensah, W.D. Ratnoff, M.L. Tykocinski, and M.E. Medof. 1988. Decay-accelerating factor protects human tumor cells from complement-mediated cytotoxicity in vitro. *J. Clin. Invest.* 81:122.
12. Cole, J.L., G.A. Housley, Jr., T.R. Dykman, R.P. MacDermott, and J.P. Atkinson. 1985. Identification of an additional class of C3-binding membrane proteins of human peripheral blood leukocytes and cell lines. *Proc. Natl. Acad. Sci. USA.* 82:859.
13. Seya, T., J.R. Turner, and J.P. Atkinson. 1986. Purification and characterization of a membrane protein (gp45–70) that is a cofactor for cleavage of C3b and C4b. *J. Exp. Med.* 163:837.
14. Seya, T., M. Okada, H. Nishino, and J.P. Atkinson. 1990. Regulation of proteolytic activity of complement factor I by pH: C3b/C4b receptor (CR1) and membrane cofactor protein (MCP) have different pH optima for factor I-mediated cleavage of C3b. *J. Biochem.* 107:310.
15. Nagasawa, S., and R.M. Stroud. 1977. Mechanism of action of the C3b inactivator: requirement for a high molecular weight cofactor (C3b-C4b INA cofactor) and production of a new C3b derivative (C3b'). *Immunochemistry.* 14:749.
16. Markwell, M.A.K., and C.F. Fox. 1978. Surface-specific iodination of membrane proteins of viruses and eucariotic cells using 1,3,4,6-tetrachloro-3a,6a-diphenylglycerol. *Biochemistry.* 17:4807.
17. Seya, T., T.C. Farries, M.W. Nickels, and J.P. Atkinson. 1987. Additional forms of human decay-accelerating factor (DAF). *J. Immunol.* 139:1260.
18. Seya, T., L. Ballard, N. Bora, V. Kumar, W. Cui, and J.P. Atkinson. 1988. Distribution of membrane cofactor protein of complement on human peripheral blood cells: An altered form is found on granulocytes. *Eur. J. Immunol.* 18:1289.
19. Seya, T., S. Nagasawa, and J.P. Atkinson. 1990. C4b-binding protein (C4bp) and a 60,000-dalton plasma protein share antigenic determinants with membrane cofactor protein of complement. *J. Immunol.* 144:2312.
20. Seya, T., T. Hara, A. Uenaka, E. Nakayama, and H. Akedo. 1990. Application of protein A-rosette assay for screening of monoclonal antibodies to human complement regulatory proteins. *Complement Inflammation.* 7:78.
21. Kinoshita, T., M.E. Medof, R. Silber, and V. Nussenzweig. 1985. Distribution of decay-accelerating factor in the peripheral blood of normal individuals and patients with paroxysmal nocturnal hemoglobinuria. *J. Exp. Med.* 162:75.
22. Sugita, Y., Y. Nakano, and M. Tomita. 1988. Isolation from human erythrocytes of a new membrane protein which inhibits the formation of complement transmembrane channels. *J. Biochem.* 104:633.
23. Sugita, Y., Y. Yamagishi, T. Tobe, N. Choi, Y. Nakano, and M. Tomita. 1989. Quantitation and characterization of soluble forms of MACIF (membrane attack complex inhibitor factor) in serum and urine. *Proc. Jpn. Soc. Immunol.* 19:264.
24. Nisonoff, A., F.C. Wissler, L.N. Lipman, and D.L. Woernley. 1960. Properties of the major component of a peptic digest of rabbit antibody. *Science (Wash. DC).* 132:1770.
25. Seya, T., and S. Nagasawa. 1982. A fluorometric method for determination of C3b inactivator. *Clin. Chim. Acta.* 119:189.
26. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature (Lond.).* 227:680.

27. Newman, S.L., and L.K. Mikus. 1985. Deposition of C3b and iC3b onto particulate activators of the human complement system: quantitation with monoclonal antibodies to human C3. *J. Exp. Med.* 161:1414.
28. Seya, T., and J.P. Atkinson. 1989. Functional properties of membrane cofactor protein of complement. *Biochem. J.* 264:581.
29. Nicholson-Weller, A., J. Burge, D.T. Fearon, P.F. Weller, and K.F. Austen. 1982. Isolation of a human erythrocyte membrane glycoprotein with decay-accelerating activity for C3 convertases of the complement system. *J. Immunol.* 129:184.
30. Pangburn, M.K., R.D. Schreiber, and H.J. Muller-Eberhard. 1983. Deficiency of an erythrocyte membrane protein with complement regulatory activity in paroxysmal nocturnal hemoglobinuria. *Proc. Natl. Acad. Sci. USA.* 80:5430.
31. McNearney, T., L. Ballard, T. Seya, and J.P. Atkinson. 1989. Membrane cofactor protein of complement is present on human fibroblast, epithelial and endothelial cells. *J. Clin. Invest.* 84:538.
32. Ohanian, S.H., and S.I. Schlager. 1981. Humoral immune killing of nucleated cells: mechanisms of complement-mediated attack and target cell defense. *CRC Crit. Rev. Immunol.* 1:165.
33. Pangburn, M.K., and H.J. Muller-Eberhard. 1984. The alternative pathway of complement. *Springer Semin. Immunopathol.* 7:163.
34. Fearon, D.T. 1979. Activation of the alternative complement pathway. *CRC Crit. Rev. Immunol.* 1:1.
35. Mold, C., G.R. Nemerow, B.M. Bradt, and N.R. Cooper. 1988. CR2 is a complement activator and the covalent binding site for C3 during alternative pathway activation by Raji cells. *J. Immunol.* 140:1923.
36. Seya, T., T. Hara, M. Matsumoto, and A. Uenaka. 1989. Quantitative analysis of membrane cofactor protein (MCP) among tumor cell lines with monoclonal antibodies. *Complement Inflammation.* 6:400. (Abstr.)
37. Parker, C.J., T. Wiedmer, P.J. Sims, and W.F. Rosse. 1985. Characterization of the complement sensitivity of paroxysmal nocturnal hemoglobinuria erythrocytes. *J. Clin. Invest.* 75:2074.
38. Morgan, B.P., D.K. Imagawa, J.R. Dankert, and L.E. Ramm. 1986. Complement lysis of U937, a nucleated mammalian cell line in the absence of C9: effect of C9 on C5b-8 mediated cell lysis. *J. Immunol.* 136:3402.
39. Yefenof, E., and I. McConnell. 1985. Interferon amplifies complement activation by Burkitt's lymphoma cells. *Nature (Lond.).* 313:684.
40. Bara, S., and T.F. Lint. 1987. The third component of complement (C3) bound to tumor target cells enhances their sensitivity to killing by activated macrophages. *J. Immunol.* 138: 1303.
41. Cornacoff, J.B., L.A. Hebert, W.L. Swead, M.E. Vanaman, D.J. Birmingham, and F.J. Waxman. 1983. Primate erythrocyte-immune complex-clearing mechanism. *J. Clin. Invest.* 71:236.
42. Pommier, C.G., S. Inada, L.R. Fries, T. Takahashi, M.M. Frank, and E.J. Brown. 1983. Plasma fibronectin enhances phagocytosis of opsonized particles by human peripheral blood monocytes. *J. Exp. Med.* 157:1844.
43. Wright, S.D., L.S. Craigmyle, and S.C Silverstein. 1983. Fibronectin and serum amyloid P component stimulate C3b- and C3bi-mediated phagocytosis in cultured human monocytes. *J. Exp. Med.* 158:1338.
44. Ramos, O.F., C. Kai, E. Yefenof, and E. Klein. 1988. The natural killer sensitivity of targets carrying surface attached C3 fragments require the availability of the iC3b receptor (CR3) on the effectors. *J. Immunol.* 140:1239.
45. Morgan, B.P. 1989. Complement membrane attack on nucleated cells: resistance, recovery, and non-lethal effects. *Biochem. J.* 264:1.
46. Zalman, L.S., L.M. Wood, M.M. Frank, and H.J. Muller-Eberhard. 1987. Deficiency of the homologous restriction factor in paroxysmal nocturnal hemoglobinuria. *J. Exp. Med.* 165:572.
47. Haensch, G.M., S. Schoenrmark, and D. Roelcke. 1987. Paroxysmal nocturnal hemoglobinuria type III: lack of an erythrocyte membrane protein restricting the lysis by C5b-9. *J. Clin. Invest.* 80:7.
48. Davitz, M.A., M.G. Low, and V. Nussenzweig. 1986. Release of decay-accelerating factor (DAF) from the cell membrane by phosphatidylinositol-specific phospholipase C (PIPLC). *J. Exp. Med.* 163:1150.
49. Harada, R., N. Okada, T. Fujita, and H. Okada. 1990. Purification of 1F5 antigen that prevents complement attack on homologous cell membranes. *J. Immunol.* 144:1823.
50. Sugita, Y., T. Tobe, E. Oda, M. Tomita, K. Yasukawa, N. Yamaji, T. Takemoto, K. Furuichi, M. Takayama, and S. Yano. 1989. Molecular cloning and characterization of MACIF, an inhibitor of membrane channel formation of complement. *J. Biochem.* 106:555.
51. Davies, A., D.L. Simmons, G. Hale, R.A. Harrison, H. Tighe, P.J. Lachmann, and H. Waldmann. 1989. CD59, an Ly-6 like protein expressed in human lymphoid cells, regulates the action of the complement membrane attack complex on homologous cells. *J. Exp. Med.* 170:637.