Membrane Cofactor Protein (CD46) Protects Cells from Complement-mediated Attack by an Intrinsic Mechanism

By Teresa J. Oglesby,* Christopher J. Allen,* M. Kathryn Liszewski,* David J. G. White,‡ and John P. Atkinson*

*From the *Division of Rheumatology, Howard Hughes Medical Institute and Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110; and the ‡Department of Surgery, Cambridge University, Cambridge, United Kingdom*

Summary

The cleavage of C3 is a critical step for complement (C) activation in the classical and alternative pathways. This reaction is controlled by the regulators of C activation protein family. Membrane cofactor protein (MCP) is a cofactor for the factor I–mediated inactivation of C3b and C4b. As a widely distributed membrane protein, MCP may protect host cells from inadvertent C activation. Human MCP has recently been shown to protect transfected rodent cells from human C–mediated lysis. In this report the relationship of MCP expression to C3b deposition and cytoprotection was examined using NIH/3T3 cells transfected with human MCP and exposed to human serum as a source of C and naturally occurring anti–mouse antibody. MCP inhibited C3b deposition in a dose-dependent fashion and inhibited lysis of the mouse cells expressing it. MCP did not inhibit lysis on bystander cells. These results demonstrate the protective role of MCP, at the cellular level, by an intrinsic mechanism.

The human C system is an independent immune system that can separate self from nonself and function as a potent effector system (1). C components are spontaneously and continuously deposited on self tissue in small amounts (2), and in larger quantities during inflammatory reactions. Membrane cofactor protein (MCP;[1] CD46) and decay-accelerating factor (DAF; CD55) are membrane glycoproteins that protect host cells from damage by the C system (reviewed in references 3 and 4). MCP is a cofactor for the factor I–mediated cleavage of C3b and C4b, while DAF accelerates decay dissociation of the C3 and C5 convertases. Both proteins are widely distributed on hematopoietic, endothelial, and epithelial cells. They are also highly expressed on certain malignant cell lines (3–7) and reproductive tissues (8).

DAF is a glycolipid-anchored protein and functions intrinsically; that is, it protects only the cell on which it is expressed (9). The same mechanism has been postulated for MCP (3, 10, 11). Although experiments to date are consistent with this interpretation (11–15), the question has not been directly tested.

Oglesby et al. (14), Atkinson et al. (15), and Lublin and Coyne (13) have used nonhuman cell lines transfected with the human MCP or DAF gene to demonstrate the capacity of these proteins to protect cells from C-mediated damage. Seya et al. (12) have also shown that human T cell lines expressing MCP but no other C regulatory protein became susceptible to lysis by the alternative pathway if the activity of MCP was blocked by a mAb.

To further assess the mechanism of the inhibitory effect of MCP on C activation, mouse fibroblasts were transfected with human MCP and then exposed to human serum. Only those cells on which MCP was expressed were protected from lysis by human C. This intrinsic mechanism of cytoprotection by MCP and the quantitative aspects of its activity could be of interest to tumor, transplant, and reproductive immunologists.

Materials and Methods

Transfectant Cell Lines. NIH/3T3 cells (mouse fibroblast; American Type Cell Culture, Rockville, MD) were stably transfected with forward or reverse oriented full-length cDNA of an upper band isoform of MCP (16, 17). The cDNA was inserted into the EcoRI site of the pHβAPr-1-neo vector (18) and transfected into cells using Lipofectin® reagent (Bethesda Research Laboratories, Gaithersburg, MD). Transfected cells were selected for neomycin

[1] *Abbreviations used in this paper:* DAF, decay-accelerating factor; MCP, membrane cofactor protein; NHS, normal human serum.

resistance and maintained in DME supplemented with 10% calf serum and Geneticin® (0.4 mg/ml; Gibco BRL, Gaithersburg, MD).

MCP expression was confirmed by immunoprecipitation of ^{35}S-cysteine-labeled cell lysates with the anti-MCP mAb E4.3 (3, 6, 19), followed by SDS-PAGE on 10% mini-slab gels and autoradiography (6). An ELISA (20) and FACS® (Becton Dickinson & Co., Mountain View, CA) analysis of the transfectants and control cells (MCP-positive and -negative cell lines) allowed quantitation of surface MCP. Cells with low to average (<50,000 molecules/cell) or high (≥500,000) expression were chosen for further use. A low expressing line of 25,000 copies (MCP 1-3T3) was sorted three times by flow cytometry to obtain cells with intermediate (180,000) MCP expression (MCP 2-3T3). In addition, an unsorted transfectant with high (≥10^6) MCP expression was also used (MCP 3-3T3). Nontransfected cells or transfectants with the MCP construct in reverse orientation (reverse or R-3T3) served as controls and were interchangeable.

Quantitation of MCP. MCP in 1% NP-40 lysates of the transfectants was quantitated by a dual mAb ELISA (20) in which GB24 (6, 20, 21) was the capture Ab and TRA-2-10 (6, 22) was the detection Ab. MCP on the cell surface was also quantitated by radioassay using a single radiolabeled mAb (6). Standards for quantitation by ELISA, radioassay, and FACS® included the HeLa and HEp-2 cell lines, obtained from the Tissue Culture Support Center, Washington University School of Medicine.

Detection of C3 Fragment Deposition by FACS®. C6-deficient (C6D) normal human serum (Quidel, San Diego, CA) was diluted 1/6 in veronal-buffered saline containing 0.15 mM $CaCl_2$, 1 mM $MgCl_2$, 2.5% dextrose, 0.1% gelatin, and 75 mM NaCl ($DGVB^{2+}$) (final dilution, 1:9), and 600 μl was incubated with 300 μl of MCP-transfected cells (10^6/ml in $DGVB^{2+}$) at 37°C for 1 h. The cells were washed and C3 fragment deposition was determined by FACS® analysis using an anti-C3c mAb (Quidel) or MOPC 21 (Sigma Chemical Co., St. Louis, MO) followed by an FITC goat anti-mouse IgG (Cappel Laboratories, Durham, NC).

Cytotoxicity Assays. Cytotoxicity was assessed by ^{51}Cr release. Transfectant and control cells were labeled with ^{51}Cr (100 μCi/10^6 cells) at room temperature for 1.5 h.

Normal human serum (NHS) contains naturally occurring anti-mouse Ab and C. In some experiments, NHS diluted in $DGVB^{2+}$ served as a source of C and naturally occurring anti-mouse Ab. In other experiments, the NHS was absorbed twice at 4°C with mouse spleen cells and used as a C source. In these experiments the source of Ab was heat-inactivated (56°C for 30 min) NHS.

In duplicate or triplicate, 50 μl (10^6/ml) of ^{51}Cr-labeled cells was next incubated with 100 μl NHS (1:5 to 1:20 in $DGVB^{2+}$) at 37°C for 1 h. Cells were then pelleted by centrifugation, and 100 μl of supernatant was counted on a gamma counter (4000; Beckman Instruments, Inc., Palo Alto, CA). Percent specific ^{51}Cr release was calculated by the formula: (cpm − cpm buffer only [spontaneous release]/(cpm 100% lysis − cpm buffer only). In each experiment, the percent lysis of the MCP transfectants was compared with that of a nontransfected control, or the R-3T3 transfectant. Specificity of the cytoprotection was determined by addition of the mAb GB24 at a final concentration of 10 μg/ml.

The ability of human MCP to protect transfected murine cells by an intrinsic versus an extrinsic mechanism was assessed by performing the cytotoxicity assay above with the following modification: 50 μl of unlabeled cells (MCP 3-3T3 transfectant or control at 10^6 cells/ml) was added to 50 μl of ^{51}Cr-labeled cells (MCP 3-3T3 transfectant or control at 10^6 cells/ml), and then the mixture was incubated with NHS.

For cytotoxicity assays, each condition was performed in duplicate or triplicate and each experiment was performed three or four times.

Results

Quantitation of MCP on Transfected Cells. Three MCP transfectants, expressing 2.5 × 10^4 copies of MCP/cell (MCP 1), 1.8 × 10^5/cell (MCP 2), and 10^6/cell (MCP 3), were chosen for study. MCP 1, MCP 2, and MCP 3 express a copy number of MCP similar to PBL, HeLa, and HEp-2, respectively (6). Periodic FACS®, ELISA, and radioassay analyses of MCP expression were internally consistent and did not change during this investigation.

Protection of Murine Cells from Human C–mediated Lysis by Expression of Human MCP. Three transfectants, expressing low, intermediate, or high amounts of MCP, were exposed to NHS (the C and Ab source) (Fig. 1 *a*). A protective effect was observed over a fourfold range of serum dilution but was less pronounced at the higher serum concentrations. There was also a trend toward more lysis (less protection) in the low-expressing MCP 1 than in the MCP 2 or MCP 3 transfectants, whose lytic curves were similar.

In the assay system used in Fig. 1 *b*, the C source was NHS, absorbed with mouse spleen cells and diluted to a final concentration of 1:9. The source of Ab was heat-inactivated NHS, diluted as indicated. The classical pathway was predominantly responsible for lytic activity since there was no lysis in C2-deficient human serum or in EGTA-treated NHS. EDTA treatment or 56°C heat inactivation of NHS also abolished lysis (not shown).

Under these conditions, the MCP-expressing transfectant (MCP 3-3T3) was protected from lysis (Fig. 1 *b*). 60% of the mouse cells transfected with the MCP construct in reverse orientation were lysed while the lysis of the MCP 3-3T3 was not above background.

This protective effect was observed over a 16-fold range of Ab concentration. The mAb GB24, which blocks the binding of MCP to C3b (6), abrogated the protective effect.

Relationship of MCP Expression to C3 Deposition. Having established that human MCP can protect mouse cells from lysis by human C, the mechanism of this effect was explored. In these experiments, C6D human serum served both as the C and Ab source. The quantity of C3 fragment deposition by FACS® analysis was inversely related to MCP expression (Fig. 2). Compared with the nonexpressing control (R-3T3), the MCP 1 transfectant (2.5 × 10^4 MCP/cell) inhibited C3 deposition by 29%, MCP 2 (1.8 × 10^5/cell) by 62%, and MCP 3 (10^6/cell) by 82%. Thus, MCP protected cells from C3 deposition in a dose-dependent fashion, consistent with, and the likely mechanism for, MCP's ability to inhibit lysis.

Mechanism of Cytoprotection by MCP. This set of experiments, similar in design to those decribed in Fig. 1 *a*, was performed to address the question of the intrinsic versus extrinsic inhibitory profile of MCP. The labeled and unlabeled cells were mixed before the addition of the serum source of C and Ab.

The addition of ^{51}Cr-labeled non-MCP-expressing 3T3 cells with the unlabeled high MCP–expressing transfectant

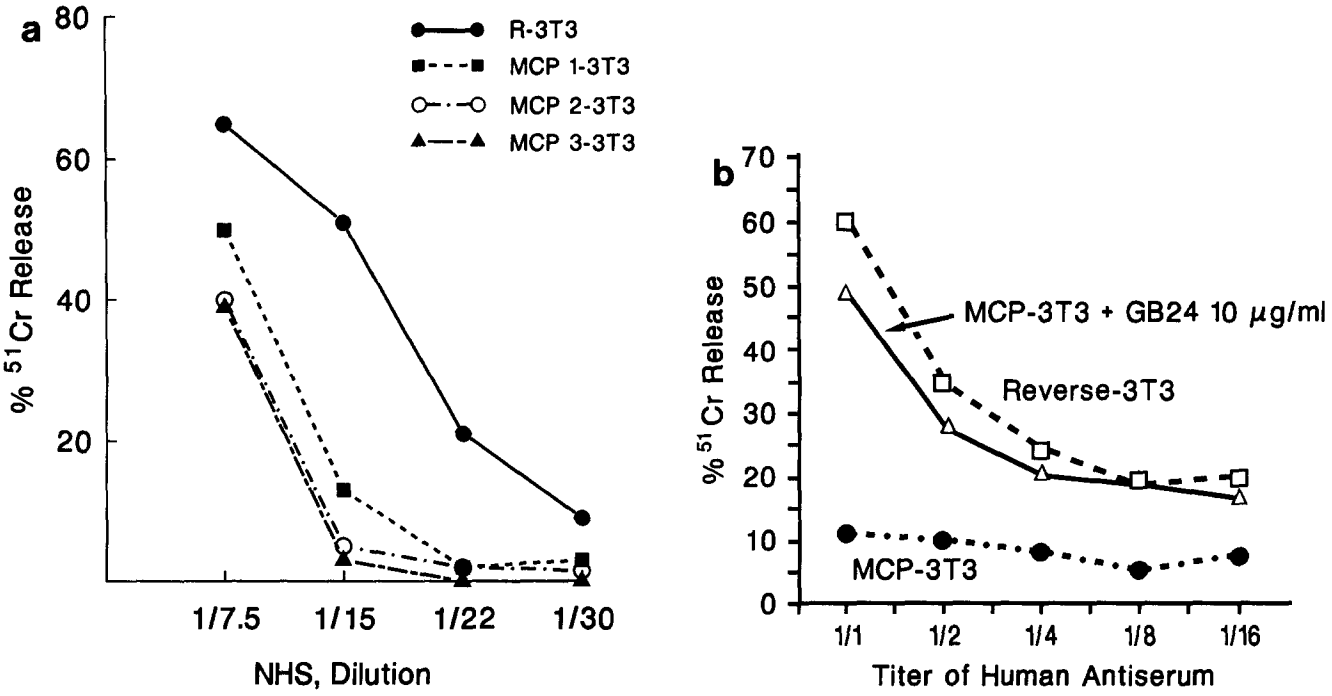

Figure 1. Protection of NIH/3T3 cells expressing human MCP from lysis by human C. The quantity of MCP expressed on each transfectant line is noted in Results and in Fig. 2. (*a*) ^{51}Cr release cytotoxicity assay. NHS was the source of both C and naturally occurring anti-mouse Ab. R-3T3 is cell line transfected with an MCP construct in the reverse orientation. (*b*) ^{51}Cr release cytotoxicity assay. C source was a 1:9 dilution of NHS absorbed with mouse spleen cells. Heat-inactivated NHS was the anti-mouse Ab source. The mAb GB24, which blocks the function of MCP, was used at a final concentration of 10 μg/ml. A figure similar to *b* was published in a supplement to the October 1991 issue of *Clinical and Experimental Immunology* (15), and represents a summary of an invited discussion given at the Complement in Disease Workshop, University of Wales, College of Medicine, Cardiff, September 21–23, 1991.

MCP 3 did not result in a reduction of lysis (Fig. 3, *open triangles*) of the labeled cells. The other combinations of labeled and/or unlabeled cells establish that there was no effect on lysis due to mixing of the cell populations or dilution.

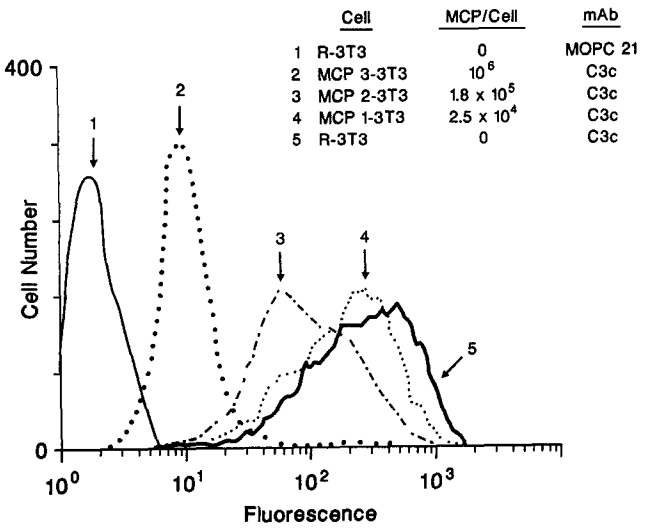

Figure 2. FACS® analysis of C3 fragment deposition on MCP-expressing transfectants. C6D NHS served as the C and anti-mouse Ab source. Anti-C3c mAb was added to detect C3 fragment deposition.

These results indicate that MCP protects cells in an intrinsic fashion.

Discussion

MCP was initially characterized as a C3b-binding protein of human peripheral blood cells (23). The subsequent demonstration of its broad tissue distribution and cofactor activity for both C3b and C4b led to the hypothesis that MCP was primarily a regulatory protein of the C system (1, 10).

Over the past 2 yr, reagents and cell lines have become available that have facilitated the study of the functional aspects of MCP. In particular, monoclonal and polyclonal Ab to MCP allowed quantitation by radioassay (5, 6), ELISA (20), and FACS® (5, 13, 14). Importantly, two mAbs were produced that block C3b/C4b binding and cofactor activity (5, 6, 20), allowing an analysis of the specificity of MCP's activity in cell lysates (6) and on intact cells (13–15). Likewise, the establishment of transient and stable transfectants expressing MCP was made possible by the cloning of the MCP cDNA (16, 17). Oglesby et al. (14), Atkinson et al. (15), and Lublin and Coyne (13) have shown that expression of MCP alone is sufficient to protect transfected mammalian cells from human C–mediated lysis by the classical pathway. The purpose of the present investigation was to analyze the mechanism by which MCP protects cells from C3 deposition and cell lysis,

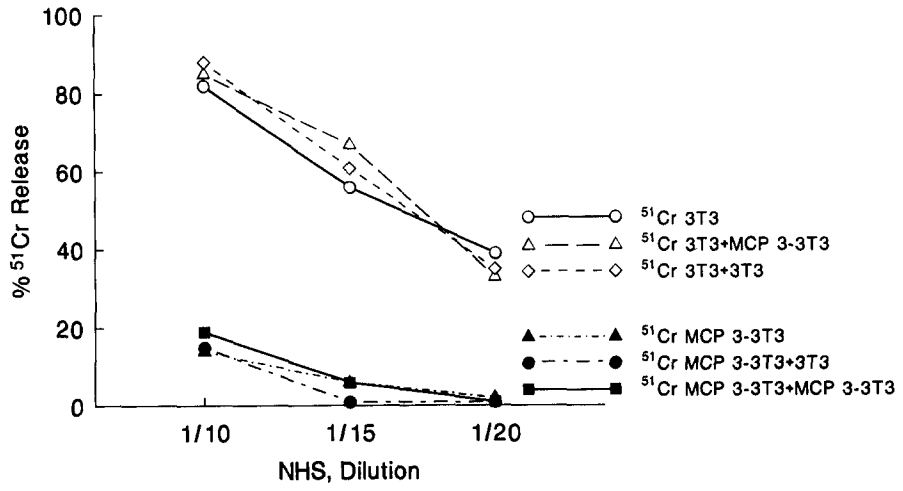

Figure 3. MCP protects cells by an intrinsic mechanism. Equal amounts of labeled (^{51}Cr) or unlabeled 3T3 and MCP 3-3T3 cells were mixed as indicated followed by the addition of NHS. The final dilution of NHS (same donor as in Fig. 1 *a*) is indicated.

and to determine how the level of MCP expression influences these parameters.

To investigate these points, mouse NIH/3T3 cells were transfected with human MCP. These transfectants, but not control 3T3 cells, were protected from lysis in a system in which naturally occurring human Ab activated the classical C pathway. This protective effect was abrogated by a mAb that blocks C3/C4 binding and cofactor activity of MCP (6, 20). The question was then asked if MCP expression on one cell population could protect against C-mediated lysis of a cell population not expressing MCP. Therefore, the non-MCP-expressing and MCP-expressing transfectants were mixed and exposed to human serum. Mouse cells expressing as many as 10^6 copies of MCP/cell did not protect bystander cells from C-mediated lysis. Therefore, MCP, like DAF (9), is an intrinsically acting membrane regulatory protein.

After exposure to human serum, MCP-expressing transfectants demonstrated a reduction in C3 fragment deposition that correlated with the number of copies of MCP expressed on the cell. However, there was not a close correlation between the inhibition of lysis and the decrease in C3 fragment deposition; the results illustrated in Fig. 1 *a* indicate only minor differences in lytic protection based upon MCP copy number. This may relate to several factors, including the cofactor activity of MCP for C4b and C3b cleavage, the enhanced ability of MCP to inactivate the C5 convertase (24), and a difference in the assay system used since C6D human serum was used for the FACS® assays. Further experiments are necessary to address the quantitative effects of MCP expression, the relative contribution to cell protection of its cofactor activity for C4b vs. C3b, and its interaction with DAF.

In vivo, C3b deposition on self-tissue to the extent observed in these experiments would not be expected. Thus, at the usual levels of expression, MCP should be able to protect host cells from its own C system. Previous studies have shown, for example, that peripheral blood cells possess ~10,000 MCP/cell (6). In contrast, hemopoietic and carcinoma cell lines can range from 2×10^4 to 10^6, respectively (6). The latter data suggest that high MCP expression on tumor cells could account for some of their resistance to destruction by antitumor Ab and C. In addition, in certain autoimmune disorders, Ab to cellular membrane constituents are produced. In these situations MCP would also be protective but, as in the model system used here, this could potentially be overcome with larger quantities of C-fixing Ab.

The results described in this report may have an additional and important ramification for transplantation immunology. Since human MCP and DAF protect foreign cells from human C, these two proteins are of special interest. Xenografts fail because of hyperacute rejection mediated by the recipient's C system and, in the case of discordant xenografts, the presence of anti-donor species Ab. In various animal models, either or both C pathways may be involved (reviewed in reference 25). The NIH/3T3 cells expressing MCP were of particular interest because each NHS tested contained C and naturally occurring anti-mouse Ab. Exposure of the 3T3 cells to NHS may be similar to what occurs during transplantation of a discordant xenograft. The expression of human MCP or DAF on a transplanted mammalian organ may protect the xenograft from attack by the human C system and thus prolong survival.

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Address correspondence to John P. Atkinson, Division of Rheumatology, Washington University School of Medicine, 660 South Euclid Avenue, Box 8045, St. Louis, MO 63110.

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References

1. Atkinson, J.P., and T. Farries. 1987. Separation of self from non-self in the complement system. *Immunol. Today.* 8:212.
2. Lachmann, P.J., and A.E. Nicol. 1973. Reaction mechanism of the alternative pathway of complement fixation. *Lancet.* i:465.
3. Liszewski, M.K., T.W. Post, and J.P. Atkinson. 1991. Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster. *Annu. Rev. Immunol.* 9:431.
4. Lublin, D.M., and J.P. Atkinson. 1989. Decay-accelerating factor: biochemistry, molecular biology, and function. *Annu. Rev. Immunol.* 7:35.
5. Seya, T., T. Hara, M. Matsumoto, and H. Akedo. 1990. Quantitative analysis of membrane cofactor protein (MCP) of complement. High expression of MCP of human leukemia cell lines, which is down-regulated during cell differentiation. *J. Immunol.* 145:238.
6. Cho, S.-W., T.J. Oglesby, B.-L. Hsi, E.M. Adams, and J.P. Atkinson. 1991. Characterization of three monoclonal antibodies to membrane cofactor protein (MCP) of the complement system and quantification of MCP by radioassay. *Clin. Exp. Immunol.* 83:257.
7. Medof, M.E., E.I. Walter, J.L. Rutgers, D.M. Knowles, and V. Nussenzweig. 1987. Identification of the complement decay-accelerating factor (DAF) on epithelium and glandular cells and in body fluids. *J. Exp. Med.* 165:848.
8. Hunt, J.S., and B.-L. Hsi. 1990. Evasive strategies of trophoblast cells: Selective expression of membrane antigens. *Am. J. Reprod. Immunol.* 23:57.
9. 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.
10. Holers, V.M., J.L. Cole, D.M. Lublin, T. Seya, and J.P. Atkinson. 1985. Human C3b- and C4b-regulatory proteins: A new multi-gene family. *Immunol. Today.* 6:188.
11. Seya, T., and J.P. Atkinson. 1989. Functional properties of membrane cofactor protein of complement. *Biochem. J.* 264:581.
12. Seya, T., T. Hara, M. Matsumoto, Y. Sugita, and H. Akedo. 1990. Complement-mediated tumor cell damage induced by antibodies against membrane cofactor protein (MCP, CD46). *J. Exp. Med.* 172:1673.
13. Lublin, D.M., and K.E. Coyne. 1991. Phospholipid-anchored and transmembrane versions of either decay-accelerating factor or membrane cofactor protein show equal efficiency in protection from complement-mediated cell damage. *J. Exp. Med.* 174:35.
14. Oglesby, T.J., D. White, I. Tedja, K. Liszewski, L. Wright, J. van den Bogaerde, and J.P. Atkinson. 1991. Protection of mammalian cells from complement-mediated lysis by transfection of human membrane cofactor protein (MCP) and decay accelerating factor (DAF). *Trans. Assoc. Am. Phys.* 104:164.
15. Atkinson, J.P., T.J. Oglesby, D. White, E.A. Adams, and M.K. Liszewski. 1991. Separation of self from non-self in the complement system: a role for membrane cofactor protein and decay accelerating factor. *Clin. Exp. Immunol.* 86:27.
16. Lublin, D.M., M.K. Liszewski, T.W. Post, M.A. Arce, M.M. LeBeau, M.B. Rebentisch, R.S. Lemons, T. Seya, and J.P. Atkinson. 1988. Molecular cloning and chromosomal localization of human membrane cofactor protein (MCP). Evidence for inclusion in the multi-gene family of complement-regulatory proteins. *J. Exp. Med.* 168:181.
17. Post, T.W., M.K. Liszewski, E.M. Adams, I. Tedja, E.A. Miller, and J.P. Atkinson. 1991. Membrane cofactor protein of the complement system: alternative splicing of serine/threonine/proline-rich exons and cytoplasmic tails produces multiple isoforms that correlate with protein phenotype. *J. Exp. Med.* 174:93.
18. Gunning, P., J. Leavitt, G. Museat, Ng, S.-Y., and L. Kedes. 1987. A human β-actin expression vector system directs high-level accumulation of anti-sense transcripts. *Proc. Natl. Acad. Sci. USA.* 84:4831.
19. Sparrow, R., and I.F.C. McKenzie. 1983. Hu Ly-m5: a unique antigen physically associated with HLA molecules. *Hum. Immunol.* 7:1.
20. Adams, E.M., M.C. Brown, M. Nunge, M. Krych, and J.P. Atkinson. 1991. Contribution of the repeating domains of membrane cofactor protein (MCP; CD46) of the complement system to ligand binding and cofactor activity. *J. Immunol.* 147:3005.
21. Hsi, B.-L., C.-J.G. Yeh, P. Fenichel, M. Samson, and C. Grivaux. 1988. Monoclonal antibody GB24 recognizes a trophoblast-lymphocyte cross-reactive antigen. *Am. J. Reprod. Immunol. Microbiol.* 18:21.
22. Andrews, P.W., B.B. Knowles, M. Parkar, B. Pym, K. Stanley, and P.N. Goodfellow. 1985. A human cell-surface antigen defined by a monoclonal antibody and controlled by a gene on human chromosome 1. *Ann. Hum. Genet.* 49:31.
23. 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.
24. Seya, T., M. Okada, M. Matsumoto, K. Hong, T. Kinoshita, and J.P. Atkinson. 1991. Preferential inactivation of the C5 convertase of the alternative complement pathway by factor I and membrane cofactor protein (MCP). *Mol. Immunol.* 28:1137.
25. Auchincloss, H., Jr. 1988. Xenogeneic transplantation. A review. *Transplantation (Baltimore).* 46:1.