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Regulation of Complement Classical Pathway by Association of C4b-Binding Protein to the Surfaces of SK-OV-3 and Caov-3 Ovarian Adenocarcinoma Cells[1]

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The role of fluid-phase regulators of complement is to inhibit excessive complement activation and maintain homeostasis in blood. By binding to and inactivating complement components on cell surfaces, they can also protect autologous cells from complement-mediated cytotoxicity and phagocytosis. In this study, we wanted to find out whether C4b-binding protein (C4bp), a fluid-phase regulator of the classical complement pathway, could directly bind to cell surfaces in a functionally active form. After screening several malignant cell lines, we observed that the ovarian adenocarcinoma cell lines SK-OV-3, Caov-3, and SW626 were capable of binding C4bp. Binding tests with recombinant deletion mutants suggested that the primary binding site on C4bp is located on the α-chain complement control protein 4 domain. Functional tests showed that tumor cell-bound C4bp retained its cofactor activity for factor I-mediated inactivation of C4b, thus increasing the control of classical complement pathway activation on the surfaces of these cells. These results demonstrate a novel mechanism of complement regulation on cell surfaces, particularly on those of malignant ovarian tumor cells. ***The Journal of Immunology*****, 2001, 167: 935–939.**

Activation of the complement system in the human body leads to responses that can potentially cause unnecessary inflammatory reactions and damage of autologous cells. To prevent excessive activation, the complement system is regulated by fluid-phase and membrane-bound proteins. Complement activation on cell surfaces is mainly controlled by membrane regulators, which include protectin (CD59), decay-accelerating factor (DAF,[3] CD55), membrane cofactor protein (MCP, CD46), and CR1 (CD35). CD59 regulates the terminal pathway by inhibiting formation of the membrane attack complex (1). MCP and CR1 act at the earlier steps of complement activation in both the classical and alternative pathway by acting as cofactors for factor I (fI)-mediated cleavage of C4b and C3b to their inactive products (2, 3). Because C4b and C3b are subunits of the C3/C5 convertase enzyme complexes (C4b2a in the classical, and C3bBb in the alternative pathway) in the complement activation cascades, MCP and CR1 thereby limit the number of convertases formed. DAF and CR1 promote directly the dissociation of the catalytic subunits C2a and Bb from the convertases (4, 5). The tissue distribution of CD59, MCP, and DAF is wide. These proteins are present on most circulating cells, endothelia, and on epithelial and mesenchymal cells of many organs. CR1 is found mostly on circulating cells, glomerular podocytes, and on cells of leukocytic origin in tissues.

Fluid-phase inhibitors of complement that control the C3/C5 convertases include factor H (fH), fH-like protein 1, and C4b-binding protein (C4bp). C4bp has a similar decay-accelerating activity as DAF on the C3/C5 convertase of the classical pathway, and it can act as a cofactor for fI-mediated cleavage of C4b (6, 7). fH and fH-like protein 1 have analogous functions in regulating the C3bBb enzyme complex (8–10). The primary role of these soluble complement regulators is to prevent excessive activation and depletion of complement in the fluid phase.

C4bp is a large plasma protein with an apparent molecular mass of 540–590 kDa in SDS-PAGE. It seems to be synthesized only in liver and is secreted to plasma to maintain an average concentration of 160 μg/ml. About half of C4bp in blood is bound to the anticoagulant protein S. This binding blocks the anticoagulant activity of protein S, but has no known effect on the complement-regulatory activity of C4bp (11). As protein S associates with negatively charged lipid membranes, it has been proposed that this interaction could direct C4bp to sites of cell injury on cell surfaces and thus protect tissues from excessive complement activation. C4bp is composed of six or seven 70-kDa α-chains and one or no 45-kDa β-chain, depending on the isoform (12). Electron microscopic analysis of C4bp has revealed a spider-like structure, in which the α-chains and the β-chain are linked together in the central core by their carboxyl-terminal domains (13). The α-chains consist of eight domains called either short consensus repeats or complement control protein (CCP) units. These domains are common to all regulators of complement activation, whose genes are encoded in the long arm of chromosome 1 (14, 15). In C4bp, the amino-terminal CCP1 and CCP2 in the α-chain are involved in the binding of C4b and are critical for the functional activity (16). As a cofactor for fI, C4bp promotes cleavage of the C4b molecule to C4c and C4d, thus preventing formation of the C4b2a enzyme complex. C4b is composed of α (93 kDa)-, β (75 kDa)-, and γ-chains (32 kDa) linked together with disulfide bonds. The fI-mediated cleavages occur in the α-chain between R^{937} and T^{938}

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[3] Abbreviations used in this paper: DAF, decay-accelerating factor; C4bp, C4b-binding protein; CCP, complement control protein; fH, factor H; fI, factor I; GVBS, 0.1% gelatin, Veronal-buffered saline; ^{125}I-C4b, ^{125}I-labeled C4b; ^{125}I-C4bp, ^{125}I-labeled C4bp; MCP, membrane cofactor protein.

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and between R_{1317} and N_{1318}. The fragment between these cleavage sites is C4d (45 kDa), and the remaining part of the molecule is called C4c (6, 17, 18).

Previously, we have observed that malignant glioblastoma cells (the H2 cell line) can bind the soluble complement alternative pathway regulator fH directly to their cell surfaces and thus inhibit complement activation (19). This mechanism is actually so efficient that even if all the known membrane complement regulators were blocked with mAbs, little or no lysis of the H2 cells could be observed after treatment with complement-activating Abs and serum. This was due to the fact that fH bound to the surface of H2 cells restricted the activation cascade at the C3 level. The principal mechanism whereby the fluid-phase regulators can protect cells from complement attack involves first, the binding of the regulator to C3b or C4b that have become covalently bound to the cell surface, and second, the reactions leading to the inactivation of the C3 convertases (cofactor and decay-accelerating functions). However, the ability of a regulator to directly bind to a cell surface can significantly increase the efficacy of complement inhibition, as the cell may become coated with a fluid-phase regulator also in the absence of initial complement activation. It has been demonstrated previously that in addition to H2 cells, fH can bind directly to a variety of other cells (20–22). Surface polyanions such as sialic acid have been shown to be important in promoting fH binding to cells, particularly after an initial deposition of C3b. Surface-bound fH thus directs subsequent events toward inhibition of complement activation (23–25).

In the present study, we screened malignant cell lines to find out whether also C4bp, a functional analogue of fH in the classical pathway, could bind to cell surfaces. As a model, we present SK-OV-3 and Caov-3 ovarian adenocarcinoma cell lines. We observed that C4bp binds to these cells via α-chain domains that are not involved in the complement-regulatory activity. Demonstration of C4bp binding to these cells in a functionally active form establishes a novel mechanism whereby human tumor cells can regulate complement activation.

Materials and Methods

Proteins and other reagents

Human C4bp was purified from plasma, as described previously (26). Protein S was removed from the molecule during purification. Polymeric recombinant mutant C4bp molecules lacking individual short consensus repeat domains were constructed and expressed essentially as described previously (27). The mutants, denoted as ΔCCP1, ΔCCP1–2, ΔCCP2, ΔCCP3, ΔCCP4, ΔCCP5, and ΔCCP6, lack either CCP1, both -1 and -2, -2, -3, -4, -5, or -6, respectively. Human C4b and fI were obtained from Calbiochem (La Jolla, CA). C4bp, the deletion mutants, and C4b were labeled with ^{125}I by the Iodogen method (28). A PD-10 gel filtration column (Amersham Pharmacia Biotech, Uppsala, Sweden) was used for separation of free iodine from the labeled protein, and Veronal-buffered saline (pH 7.4) was used as a buffer. Purities of the labeled proteins were verified by SDS-PAGE and autoradiography. Four mAbs against the α-chain of C4bp were used. mAbs 104 and 96 recognize CCP1–2, mAb 102 is against CCP1, and mAb 67 against CCP4. These Abs have similar affinities for the C4bp α-chain (29). The GB24 mouse mAb against MCP was a kind gift from J. Atkinson (Washington University School of Medicine, St. Louis, MO). A rabbit polyclonal Ab against the C4bp α-chain was from Calbiochem, and mouse polyclonal Ab against the C4bp β-chain was a kind gift from S. R. de Córdoba (Madrid, Spain).

Cell lines

The ovarian adenocarcinoma cell lines CaOV-3, SK-OV-3, and SW626, and a teratocarcinoma cell line PA-1 were obtained from American Type Culture Collection (Manassas, VA). The cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2 in RPMI 1640 medium (Life Technologies Laboratories, Paisley, U.K.) supplemented with 10% heat-inactivated FCS, 10 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. The cells were detached from the culture flasks with 0.02% (w/w) EDTA and washed before use.

Binding of radiolabeled C4bp to cells

Binding of ^{125}I-labeled C4bp (^{125}I-C4bp) to cells was analyzed by incubating 2×10^5 cells/sample with ^{125}I-C4bp (3–100 ng) in a 50-μl solution of GVBS (0.1% gelatin, Veronal-buffered saline, pH 7.4) at 37°C for 60 min. Diluted, undiluted, or concentrated GVBS was used as the sample buffer, depending on the experiment. Control samples for the background signal did not contain any cells, but were treated otherwise as cell samples. After incubation, the cell suspensions were transferred on top of a 250-μl column of 20% sucrose in GVBS (with the same dilution as in the sample buffer) in narrow 0.4-ml test tubes. The tubes were centrifuged for 5 min at $5600 \times g$ to separate the cells and free protein. The tubes were frozen and cut at the apex to separate the cell pellet and the sample supernatant. The radioactivities of both were measured. The amount of binding of ^{125}I-C4bp to cells was calculated from the radioactivity in the cell pellet after subtracting the mean background signal. All binding experiments were performed in duplicate or triplicate.

Cofactor activity test for cell-bound C4bp

To analyze the functional activity of cell-bound C4bp, its cofactor activity for fI-mediated cleavage of ^{125}I-labeled C4b (^{125}I-C4b) was tested. For C4bp binding, 3×10^6 cells were incubated with C4bp at a 50 μg/ml concentration in 300 μl RPMI 1640 medium (no FCS added) for 60 min at 37°C. The cells were then washed twice with PBS (pH 7.4) and divided into separate microtubes to have 3×10^5 cells/tube. To block the cofactor activity of cell surface MCP and/or C4bp, GB24 and mAb104 Abs, respectively, were added to the appropriate samples at a concentration of 33 μg/ml and incubated for 15 min at 37°C. Factor I (17 μg/ml) and ^{125}I-C4b (6 μg/ml) were added, and the samples were incubated in a final volume of 60 μl for 100 min at 37°C. A positive control sample for C4b degradation contained 10 μg/ml C4bp, 17 μg/ml fI, and 6 μg/ml ^{125}I-C4b in RPMI 1640. The negative control had 17 μg/ml fI and 6 μg/ml ^{125}I-C4b in RPMI 1640. After incubation, the cell samples were centrifuged and the supernatants were subjected to SDS-PAGE analysis under reducing conditions. In addition to the actual cofactor activity test, control samples for each cell line were prepared to monitor for possible cofactor activity of C4bp dissociated from the cells during the Ab and ^{125}I-C4b/fI incubations. These samples were treated similarly as the other samples until the ^{125}I-C4b/fI incubation step. However, at this point, no ^{125}I-C4b or fI was added, but the samples were incubated in RPMI 1640 with 33 μg/ml GB24 for 100 min under similar conditions as the actual test samples. The tubes were then centrifuged, and the supernatants of the control samples were removed and mixed with ^{125}I-C4b (6 μg/ml) and fI (17 μg/ml), and incubated for another 100 min.

Results

Binding of ^{125}I-C4bp to cells

To find out whether tumor cells could bind C4bp from the fluid phase, binding experiments with radiolabeled C4bp were performed. Of the various cell lines tested, SK-OV-3 adenocarcinoma cells bound C4bp the most, and PA-1 cells the least. Caov-3 and SW626 cells bound intermediately (Fig. 1). To further examine the nature of the binding, inhibition tests with unlabeled C4bp and BSA were performed. ^{125}I-C4bp binding to cells was inhibited by 40% when unlabeled C4bp was added within a concentration range of 80–120 μg/ml into a sample buffer that contained 0.3 μg/ml ^{125}I-C4bp. Under similar conditions, 100–500 μg/ml BSA had no significant effect (0–10% inhibition of binding). The binding of C4bp to cells was sensitive to an increasing salt concentration in the sample buffer. The binding of ^{125}I-C4bp increased 6-fold in GVBS containing 70 mM NaCl, and decreased by 50% at 400 mM, when compared with the binding in GVBS containing a physiological concentration of NaCl (150 mM).

Mapping the cell binding site on C4bp with Abs

Inhibition tests with Abs were performed to tentatively map the binding site on C4bp. Different concentrations of either polyclonal Abs or mAbs against C4bp were added to the sample buffer with ^{125}I-C4bp and incubated for 15 min at 20°C before the cells were

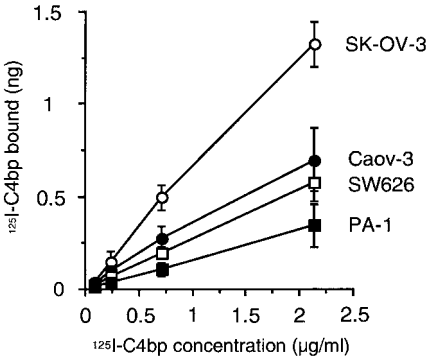

FIGURE 1. Binding of ^{125}I-C4bp to ovarian tumor cell lines. Cells were incubated with radiolabeled C4bp in GVBS with 150 mM NaCl. ^{125}I-C4bp concentration ranged from 0.1 to 2 μg/ml. The results are expressed as the amount of ^{125}I-C4bp bound to 2×10^5 cells (mean values $\pm$ SD from three experiments with duplicate samples in each). SK-OV-3 cells were the strongest binders of C4bp, Caov-3, and SW626 cells bound intermediately, and with the PA-1 teratocarcinoma cell line the ^{125}I-C4bp binding was weak.

added. As shown in Fig. 2*A*, a rabbit polyclonal Ab against the C4bp α-chain inhibited ^{125}I-C4bp binding to SK-OV-3 cells. When a polyclonal Ab against C4bp β-chain was used at similar concentrations, no inhibition of binding could be observed. Instead, the Ab increased C4bp binding to the cells, most likely because of oligomerization of C4bp. To further examine the binding site, the effect of various mAbs against C4bp α-chain was analyzed. mAb 102 (anti-C4bp α-chain CCP1), mAb 96 (anti-α-chain CCP1–2), and mAb 67 (anti-α-chain CCP4) all inhibited C4bp binding, but with mAb 67 the inhibition was clearly strongest, resulting in a 62% decrease in binding at a 30 μg/ml concentration. These results suggest that CCP4 of the C4bp α-chain plays a role in the binding of C4bp to SK-OV-3 cells (Fig. 2*B*).

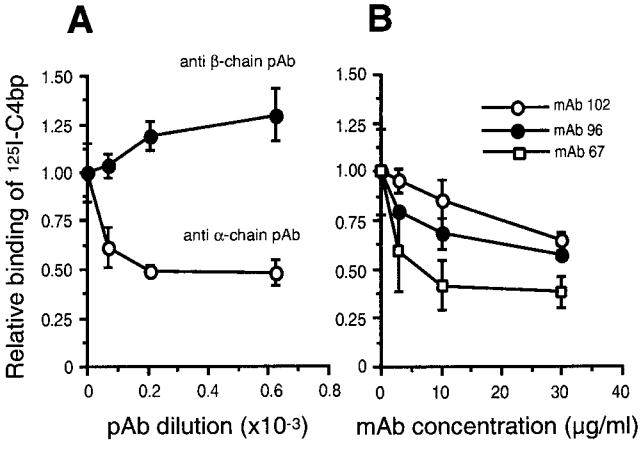

FIGURE 2. Inhibition of C4bp binding to cells with anti-C4bp Abs. *A*, Increasing amounts of polyclonal Abs against C4bp α- or β-chain were incubated in GVBS with ^{125}I-C4bp and SK-OV-3 cells. The binding results are expressed as mean $\pm$ SD relative to binding in samples without Abs. The addition of the anti-C4bp α-chain polyclonal Ab inhibited ^{125}I-C4bp binding to cells, whereas the β-chain polyclonal Ab induced a slight increase in the binding. *B*, Effect of mAbs against C4bp α-chain on ^{125}I-C4bp binding to SK-OV-3 cells. The mAbs 102, 96, and 67 bind to CCPs 1, 1–2, and 4, respectively. All three mAbs inhibited ^{125}I-C4bp binding, but mAb 67 (anti-CCP4) caused the strongest inhibition. Mean $\pm$ SD values are from two different experiments with duplicate samples.

Mapping the cell binding site on C4bp with rC4bp mutant proteins

To further characterize the interaction between C4bp and SK-OV-3 cells, a set of ^{125}I-C4bp α-chain deletion mutants was tested in the binding experiments (Fig. 3). These tests were performed with 90 mM NaCl in the sample buffer. The binding of ΔCCP1 and ΔCCP6 to cells was as strong or even slightly stronger than that of the wild-type C4bp (6% binding of offered ^{125}I-C4bp). The mutants that had CCP2, -3, -5, or both -1 and -2 removed bound intermediately (2–3% binding). The only mutant that bound clearly more weakly than any other protein was ΔCCP4 (0.8% binding). This suggests that CCP4 is the primary cell surface binding site on C4bp, which is in accordance with the mAb inhibition tests.

Cofactor activity of cell-bound C4bp

To analyze whether binding of C4bp had any functional consequences, cofactor activity tests for fI-mediated C4b degradation were performed with C4bp-preincubated and control cells. The cofactor activity of cell surface MCP (CD46) was blocked with the GB24 mAb. In these experiments, SK-OV-3, Caov-3, and SW626 cells induced fI-mediated cleavage of ^{125}I-C4b in the sample solution, also in the presence of GB24. This was seen in SDS-PAGE analysis of the sample supernatants as a characteristic fI-mediated cleavage pattern of C4b (Fig. 4*A*). In the samples that contained both GB24 and mAb104 that blocks the cofactor function of C4bp, no cleavage of ^{125}I-C4b was observed. PA-1 cells, preincubated with C4bp, had a weaker cofactor activity than the other cell lines, and it was completely inhibited by GB24 alone. To find out whether the cofactor activity seen in these cell samples was actually caused by cell-bound C4bp and not by C4bp that had become dissociated from the cells during the experiment, the supernatants of control cell samples were tested for their cofactor activity. SK-OV-3 and Caov-3 cells did not release any cofactor activity for C4b inactivation to the supernatants, whereas the SW626 cell supernatants had some cofactor activity (Fig. 4*B*).

Discussion

The present study shows that C4bp can bind to malignant ovarian tumor cells via its α-chain without prior complement activation

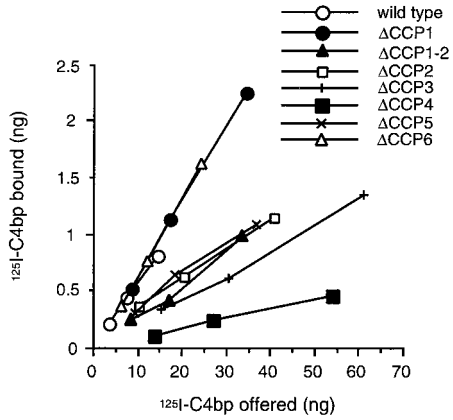

FIGURE 3. Binding of rC4bp deletion mutants to SK-OV-3 cells. Radiolabeled deletion mutants lacking individual CCP domains were allowed to bind to 2×10^5 cells in GVBS with 90 mM NaCl. As a control, the binding of plasma-purified ^{125}I-C4bp was tested under similar conditions. The results are expressed as mean amount of ^{125}I-C4bp per mutant bound to cells plotted against amounts of protein offered in the sample. ΔCCP1 and ΔCC6 bound as well as purified C4bp. ΔCCP4 bound only in very low quantities, confirming the importance of CCP4 to the interaction of C4bp and cells. All the other mutants bound with an intermediate efficiency.

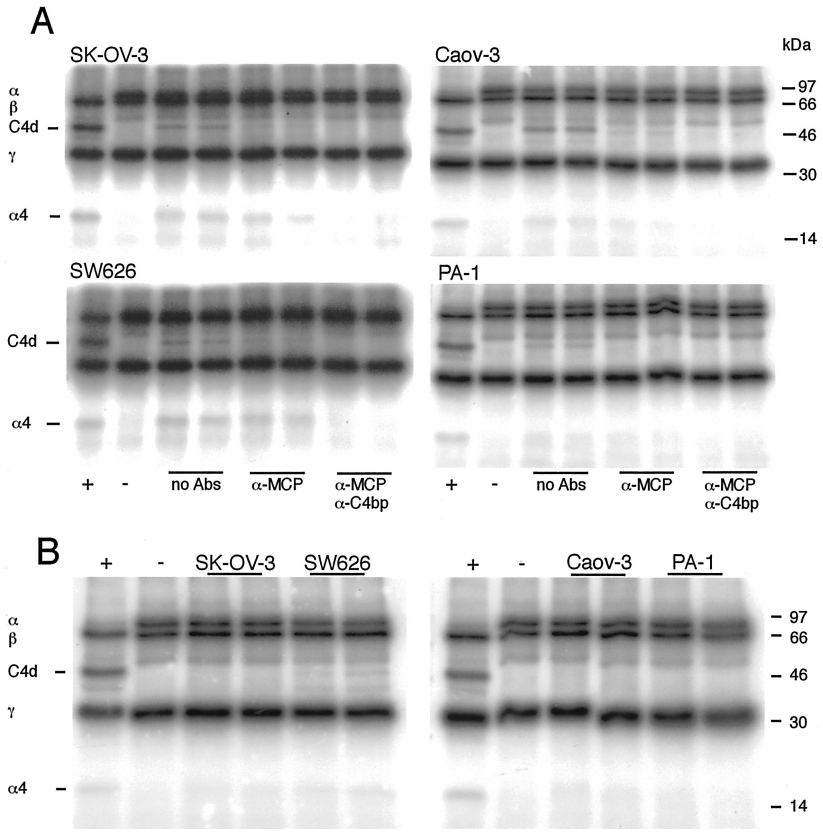

FIGURE 4. Cofactor activity test for cell-bound C4bp. *A*, Four indicated tumor cell lines were incubated with purified C4bp (50 μg/ml), washed carefully, and allowed to react with ^{125}I-C4b and fI to see whether the cell-bound C4bp retained its cofactor activity. The experiments were conducted in duplicate, either in the absence of Abs, or in the presence of GB24 (anti-MCP mAb) or both GB24 and mAb 104 (anti-C4bp) in the sample. In addition to cell samples, controls for fI-mediated C4b cleavage were examined. The positive control (+) had fI and purified C4bp with ^{125}I-C4b. The negative control (−) had only fI with ^{125}I-C4b. In the absence of mAbs, all the cell lines promoted C4b degradation. This can be seen as the appearance of the physiological cleavage fragments C4d (45 kDa) and α4 (15 kDa) in the SDS-PAGE run under reducing conditions. SK-OV-3, Caov-3, and SW626 cells retained cofactor activity under conditions in which the function of MCP was blocked with GB24. As the remaining cofactor activity could be blocked with the anti-C4bp mAb, the MCP-independent cofactor activity seen on these cells must be due to C4bp. The 50- to 60-kDa band seen in all samples is a nonspecific fragment, present in the original C4b batch, and should not be mixed with the 45-kDa C4d band. *B*, To confirm that the cell-bound C4bp was actually the cofactor in these tests, and not C4bp dissociated from the cells to the sample supernatant during the incubation, control experiments were performed. A duplicate set of cells was preincubated with C4bp and the α-MCP mAb GB24 and washed. After a 100-min incubation, the supernatants of the samples were removed and tested for C4bp-dependent cofactor activity by adding ^{125}I-C4b and fI. Neither the SK-OV-3 nor the Caov-3 supernatants showed any cofactor activity, suggesting that C4bp remained associated with the cells during the experiment. In contrast, the supernatants of SW626 cells showed some cofactor activity, which can be seen as the appearance of the 45-kDa C4d and 15-kDa α4 fragments.

and C4b deposition. The primary binding site on C4bp was found to be on CCP4 of the α-chain. The cofactor activity of cell-bound C4bp for C4b inactivation was retained, suggesting that this phenomenon directs complement-regulatory activity to the cell surfaces. Direct binding of C4bp to cell surfaces has not been reported before, and this finding suggests that in ovarian tumor tissue C4bp can bind to cell surface structures and lead to an increased control of the classical pathway activation.

Of the cell lines tested, the binding of C4bp was strongest to the SK-OV-3 adenocarcinoma cells. These cells bound appreciable amounts of C4bp under physiological ionic strength conditions. The other ovarian adenocarcinoma cells, Caov-3 and SW626, also bound C4bp, whereas with the PA-1 teratocarcinoma cells, the binding was negligible. The binding of C4bp to cells was sensitive to salt concentration in the sample buffer, suggesting that the interaction is of an electrostatic rather than of hydrophobic nature. Relatively high concentrations of unlabeled C4bp were required in the inhibition tests to compete out ^{125}I-C4bp binding to cells. This suggests a large number of binding sites on cell surface, and in contrast, a possibly relatively low affinity interaction. Initial ligand-blotting experiments with ^{125}I-C4bp have not revealed any protein ligand on the cell membranes (data not shown). Together these results suggest that the cell surfaces have diffusely distributed acceptors, such as carbohydrates or proteoglycans, which carry a large reservoir of negative charge, a possible attractant of C4bp molecules.

The cell surface binding sites on C4bp were found to be on the α-chain, as the polyclonal α-chain-specific Ab inhibited the binding, but the anti-β-chain Ab did not. Both the inhibition tests with mAbs and the binding tests with C4bp α-chain deletion mutants indicated that CCP4 of the α-chain is the most important domain for the binding. Removal of CCP1 or CCP6 did not interfere with the C4bp binding, ruling out these domains as possible binding sites. The small inhibiting effect of the mAb 102 (anti-CCP1) could have been due to steric hindrance. The other deletion mutants bound intermediately, making it difficult to judge the importance of CCP2, -3, and -5 for the binding. It is possible that these neighboring domains also contribute to the binding. In contrast, in deletion mutants, one cannot exclude changes in interdomain structure that may affect C4bp binding to cells. In conclusion, while multiple interactions between the cell surface and C4bp

α-chain CCPs 2 to 5 may exist, the CCP4 domain has the strongest activity.

The functional activity of cell-bound C4bp as a complement regulator was analyzed by testing its cofactor activity for fI-mediated inactivation of C4b. Cofactor activity tests showed that after binding of purified C4bp, SK-OV-3, Caov-3, and SW626 cells could degrade C4b under conditions in which the cofactor activity of MCP was blocked with the GB24 Ab. As all the cofactor activity for C4b degradation could be blocked by adding both GB24 and the anti-C4bp mAb104 to the sample buffer, the other cofactor in these samples must have been C4bp, and no other cofactors were present. To confirm that cell-bound C4bp was actually the principal cofactor and not free C4bp released into the sample supernatant, additional experiments were performed. The results did not show any cofactor activity in the supernatants of other than the SW626 cells. This suggests that with SK-OV-3 and Caov-3 cells, the binding interaction was strong enough to keep most C4bp associated with cells during the experiment, and that cell-bound C4bp retained its cofactor activity. With SW626, it is difficult to determine whether the cell-bound C4bp or dissociated C4bp is actually responsible for the cofactor activity. The results of the cofactor tests are in agreement with the binding results, as the release of cofactor activity to the SW626 sample supernatant suggests a weaker affinity of C4bp binding. Also, the PA-1 cells did not recruit any C4bp-mediated cofactor activity at all, in accordance with the weak binding of C4bp.

From the physiological point of view, the most obvious implication of C4bp binding to cells is an increased complement-regulatory activity on the cell surfaces. As C4bp is a regulator of the classical pathway C3 convertase, which acts early in the cascade, it can effectively control most of the complement activation events, including opsonization, cytolysis, C5a-mediated chemotaxis, and release of the anaphylatoxins C3a, C5a, and C4a. C4bp on the cell surface could also interfere with the local blood coagulation homeostasis, as the C4bp β-chain inactivates the anticoagulant protein S by a high affinity interaction. However, the physiological function of C4bp binding to protein S in the coagulation cascade is not known. As the cell lines used in this study are malignant, it is tempting to propose that C4bp binding is a complement-regulatory mechanism of some cancer cells. The tumor cells may use this property to increase their resistance of humoral and phagocytic immune responses. Ovarian adenocarcinomas, in particular, seem to use this mechanism, as the SK-OV-3 and Caov-3 cells were the strongest binders of C4bp when compared with other tumor cell lines. The SW626 cell line has been obtained from an ovarian tumor, but it has been suggested that it may actually have originated as a metastasis from a colon carcinoma (30). PA-1 is an ovarian teratocarcinoma cell line originating from an entirely different type of cells. In contrast, this phenomenon may occur with certain normal human cells as well. In this case, it would be interesting to characterize the natural receptors for C4bp and their distribution and physiological functions in tissues.

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