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Inhibition of complement activation on a model biomaterial surface by streptococcal M protein-derived peptides

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Abstract

The aim of this study was to evaluate a new approach to inhibit complement activation triggered by biomaterial surfaces in contact with blood. In order to inhibit complement activation initiated by the classical pathway (CP), we used streptococcal M protein-derived peptides that specifically bind human C4BP, an inhibitor of the CP. The peptides were used to coat polystyrene microtiter wells which served as a model biomaterial. The ability of coated peptides to bind C4BP and to attenuate complement activation via the CP (monitored as generation of fluid-phase C3a and binding of fragments of C3 and C4 to the surface) was investigated using diluted normal human serum, where complement activation by the AP is minimal, as well as serum from a patient lacking alternative pathway activation. Complement activation (all parameters) was significantly decreased in serum incubated in well surfaces coated with peptides. Total inhibition of complement activation was obtained at peptide coating concentrations as low as 1-5 µg/mL. Successful use of *Streptococcus*-derived peptides shows that it is feasible to control complement activation at a model biomaterial surface by capturing autologous complement regulatory molecules from plasma.

Keywords

Blood compatibility; Complement; C4b-binding protein (C4BP); *In vitro* test; Regulator of Complement Activation (RCA); Streptococcal M proteins

1. Introduction

When implanted into blood or tissue, biomaterials rapidly adsorb plasma proteins, which then trigger activation of the cascade systems of the blood, *i.e.*, the complement, contact, and coagulation systems. This process initiates and propagates an inflammatory reaction that may cause serious harm to the patient and to the equipment used for treatment. We have previously reported that the initial protein film consists of a monolayer and that complement activation occurs on top of this layer [1,2].

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The aim of this project was to create a self-regulatory surface, *i.e.*, one that actively down-regulates the cascade systems of the blood, by immobilizing peptides with affinity for natural inhibitors present in human blood. The approach that we took to accomplish this goal was to examine microbial surface proteins that bind human inhibitors and thereby allow the microbe to evade host defense, identifying binding sequences for the ligands involved and, based on this knowledge, designing new, synthetic peptides that retain ability to bind the human inhibitor. In the present study we have used complement as a model system to obtain proof of principle for this new concept for regulation.

The complement system, which plays a primary role in host defense, consists of at least 30 plasma or cell-bound proteins. Its activation takes place in three steps comprising: i) recognition of non-self compounds by three different pathways; ii) initiation of activation of C3 into C3a and bound C3b by two multi-molecular enzyme complexes called C3 convertases; iii) amplification loop by the alternative pathway (AP) which leads to the vast majority of C3 activation (Figure 1). The anaphylatoxin C3a (together with a second anaphylotoxin, C5a) activate and recruit phagocytes, while target-bound C3 fragments facilitate binding to and further activation of the recruited cells. Assembly of the classical pathway (CP) convertase C4bC2a is triggered either by the formation of antigen-antibody complexes which are recognized by C1q or by activation of the lectin pathway (LP) through the binding of certain plasma proteins, mannan-binding lectin (MBL) or ficolins, to carbohydrates. Several groups, including ours, have shown that the complement activation that occurs at a biomaterial surface is often initiated by the CP [1,3-5]. The therapeutic importance of this initiation is highlighted in the study of Lhotta *et al.* who report of delayed complement activation in a C4 deficient patient undergoing hemodialysis [5]. Assembly of the AP convertase C3bBb may be initiated by properdin binding to alien substances, such as microorganisms [6] or by biomaterials which do not provide adequate down-regulation of the AP convertase C3bBb [1,7-11]. The AP also serves as a major amplification loop, so that a weak stimulus initiated by any of the pathways can be markedly enhanced [3,12]. Research has indicated that in a model in which complement activation was initiated exclusively by the CP, the AP can contribute more than 80% of the C5 activation [13].

In vivo, the complement system is controlled by several soluble and membrane-bound regulators, most of which regulate the CP and AP convertases. The plasma proteins C4b-binding protein (C4BP), which controls the CP convertase, and factor H, which controls the AP convertase, as well as the membrane proteins CR1 (CD35), DAF (CD55) and MCP (CD46) are all members of the regulators of complement activation (RCA) protein superfamily.

A variety of mechanisms have evolved in microorganisms to enable them to survive host complement attack. Variola and vaccinia viruses encode RCA proteins, which are functional homologues of mammalian factor H and C4BP and cause inactivation of C3b and C4b (reviewed in [14]). An alternative mechanism for surviving attack by host complement has been identified in certain bacteria, *e.g.*, *Streptococcus pyogenes* and *Borrelia burgdorferi*, which both have surface proteins with affinity for human RCAs. Many strains of *Streptococcus pyogenes* express M proteins with an affinity for C4BP, the main fluid-phase inhibitor of the CP and LP [15-17]. It is of particular interest that the C4BP-binding regions of M proteins are hypervariable, allowing the bacteria to evade host antibodies while retaining the ability to bind C4BP [18]. Similarly, *Borrelia* express bacterial proteins termed complement regulator-acquiring surface proteins (CRASPs) that bind factor H, the main inhibitor of the AP [19,20].

Here, we have explored the concept that a self-regulatory surface can be created by immobilizing peptides (designated MPs) derived from the hypervariable, C4BP-binding domains of several different M-proteins from *S. pyogenes*.

2. Materials and Methods

2.1. Reagents

Unless stated otherwise, all chemicals were purchased from Sigma (St. Louis, MO, USA) or BioRad (Hercules, CA, USA).

2.1.1. Peptides and proteins—Peptides M2-N, M4-N, and M22-N were derived from the N-terminal hypervariable regions of the streptococcal M2, M4, and M22 proteins, respectively [18,21]. These MPs had a length of 47-53 amino acid residues and contained a C-terminal cysteine residue that is not present in the parental M proteins, which was used to dimerize the peptides by the introduction of a disulfide bond [21]. The three MPs were synthesized at the Department of Clinical Chemistry, Malmö General Hospital (Malmö, Sweden). Recombinant forms of the M4-N and M22-N peptides were purified after expression in *E. coli* as described [22]. The dimerized MPs have previously been demonstrated to specifically bind human C4BP [21,22]. The synthetic peptides were dissolved in 10 mM Tris-HCl, pH 8.0, supplemented with NaCl (0.4 M) and CuCl₂ (20 µM). The recombinant peptides were stored in double-distilled H₂O. Each experiment was performed using synthetic M2-N and both synthetic and recombinant M4-N and M22-N.

The intact M4 (Arp4) protein was purified after expression in *E. coli*, as described elsewhere [23]. C4BP was purified from human plasma as previously described [24].

2.1.2. Antisera against the MPs and C4BP—Rabbit antisera against the individual synthetic MPs were raised as described [21]. Rabbit antiserum against human C4BP was raised by standard procedures, using complete Freund's adjuvant for the first immunization and incomplete Freund's adjuvant for the booster injections. The antibodies were purified by affinity chromatography on protein G-Sepharose. Biotinylation of these antibody preparations and of commercial polyclonal anti C3c and anti C4c antibodies (Dako A/S, Glostrup, Denmark) was accomplished using biotin-amidocaproate N-hydroxysuccinimide ester.

2.1.3. Detection of anti-MP antibodies in serum by enzyme immunoassay (EIA)—Serum from 70 healthy blood donors was analyzed for anti MP antibodies by EIA. Wells of microtiter plates were coated with 1 µg/mL of one of the synthetic peptides M2-N, M4-N, or M22-N, or with intact M4 protein as a positive control, since this protein is known to bind IgG, albeit in a non-antigen-dependent fashion [25]. Human serum, diluted 1:10 in PBS (10 mM phosphate buffer, pH 7.4, with 0.15 M NaCl and 10 mM EDTA) containing 0.05% (v/v) Tween 20 (PBS-Tween) and 1% (w/v) bovine serum albumin (BSA) was incubated in the coated wells and in uncoated control wells. Bound IgG was detected using horseradish peroxidase (HRP)-conjugated rabbit anti-human IgG (Dako). Blood donors who were positive for anti-peptide antibodies for any of the peptides (>50% of the tested individuals; only 10% of the tested individuals were negative for all three peptides) were excluded from the experiments.

2.1.4. Serum pool—Pools of serum were prepared from blood collected from three donors, all with very low titers of anti-MP antibodies, and allowed to clot at room temperature (RT, approximately 25°C) for 45 min in 7-mL Vacutainer™ glass tubes without additives (Becton, Dickinson and Co., Plymouth, UK). The supernatants were collected and pooled after centrifugation at 3450 × g for 25 min at 4°C. The serum pool was stored at -80°C prior to use. In addition, EDTA plasma and serum samples were collected from a patient who was not able to activate complement via the AP.

2.2. Coating of MPs onto polystyrene surfaces

Wells of polystyrene microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated overnight at 4°C with peptides serially diluted from 10 µg/mL to 0.125 µg/mL in PBS. The wells were then washed three times with PBS-Tween to remove unbound peptides and block the plates. Peptide binding was assessed with the M4-N peptide as an example, and checked by EIA using rabbit anti-M4-N antibodies in conjunction with HRP-conjugated anti-rabbit antibody (Dako). To investigate whether the coated peptide was desorbed, experiments were performed where surfaces coated with the M4-N peptide were washed extensively and then exposed to PBS-Tween for up to 28 h, during which the buffer was replaced 3 times. No desorption of the peptide was seen under these conditions. Furthermore, to investigate whether the coated peptide might become masked by albumin upon exposure to serum, separate experiments were performed in which coated plates were incubated with PBS or PBS-Tween, with or without the addition of 1% (w/v) human serum albumin (HSA), followed by detection of the M4-N peptide by EIA. Identical levels of M4-N peptide were detected whether or not Tween and/or HSA were present (data not shown), demonstrating that the peptide was not blocked by Tween or by the layer of deposited HSA.

2.3. Exposure of surface-coated MP to plasma or serum

To monitor complement activation via the CP, normal human serum was diluted 1:8 (a concentration at which the activity of the AP is minimal [3,11,13]) in veronal-buffered saline, pH 7.4, containing 0.15 mM Ca²⁺ and 0.5 mM Mg²⁺ (VBS²⁺), and 100 µL of this diluted serum was added to each coated well. The diluted serum was incubated for 30 min at 37°C and then transferred to polypropylene microtiter plates containing EDTA at a final concentration of 10 mM and frozen at -80°C until further analysis. In addition, the coated wells were incubated with EDTA-chelated serum (in which ≈ 5% of the total amount of C4 had been activated to C4b) to visualize the deposition of C4b (onto the C4BP bound to the MPs) in the absence of an active complement system. Undiluted serum from a patient with a nonfunctional AP but active CP due to a C3 dysfunction [26] was incubated and analyzed under identical conditions. In all experiments, the wells were washed with PBS-Tween and then treated as described below.

Activation of complement was monitored by measuring the generation of C3a in the fluid phase and the binding of C3 fragments (C3b/iC3b) and C4 fragments (C4b/iC4b) to the surface of the microtiter wells. The activity of the bound peptides, i.e., the ability to bind C4BP in an active conformation, was monitored by quantitating the depletion of plasma C4BP from the fluid phase, the binding of C4BP to the surface, and the binding of C4b in the absence of an active complement system (*i.e.*, in the presence of EDTA).

2.4. Detection of surface-bound and fluid-phase proteins by EIA

For all EIAs, PBS-Tween was used as the washing buffer, and PBS-Tween supplemented with 1% (w/w) BSA was used as the working buffer. In the C3a EIA, the working buffer also contained 10 mM EDTA. All incubations were performed at RT. The plates for all assays were stained with σ -phenyldiamine dihydrochloride (0.25 mg/mL) dissolved in citrate-phosphate buffer (0.1 M, pH 5) containing H₂O₂ (0.5 µL/mL buffer), and the absorbance was measured at 490 nm.

2.4.1. Fluid-phase and surface-bound C4BP—To measure the depletion of C4BP as a result of its capture by the MPs, a sandwich EIA was used to determine the amount of remaining C4BP in the 1:8 diluted serum that had been subjected to complement activation by incubation in the peptide-coated microtiter wells. Purified C4BP was used as a standard. Polyclonal anti-C4BP was used both for capture and detection (biotinylated), followed by HRP-conjugated streptavidin (GE Healthcare, Uppsala, Sweden). Direct binding of C4BP to surface-bound M-peptides was visualized in an analogous way.

2.4.2. Deposition of fragments of C3 and C4—After the incubation described above, the serum was removed, and the wells of the original plate were carefully washed. Detection of the bound fragments of C3 (C3b/iC3b) and C4 (C4b/iC4b) was achieved with biotinylated anti-C3c and anti-C4c (Dako), followed by HRP-conjugated streptavidin.

2.4.3. C3a—C3a was detected by a sandwich EIA using mAb 4SD17.3 as the capture antibody and biotinylated anti-human C3a, followed by HRP-conjugated streptavidin for detection. In order to remove high molecular weight fragments of C3, iC3 (*i.e.*, hydrolyzed C3 or C3H₂O), which are also detected by the C3a EIA, the sera were precipitated by mixing them with an equal volume of 25 % PEG 6000 in VBS²⁺ and incubating them on ice for 30 min. Before being analyzed, the samples were centrifuged at 3000 × g for 30 min at 4°C, and the supernatants were collected and analyzed using the C3a EIA. Zymosan-activated serum calibrated against a solution of purified C3a served as a standard; values are given in ng/mL, and pooled zymosan-activated serum from blood donors was diluted 1:500 and used as a control [27].

2.5. Statistics

All experiments were performed four to eight separate times (as stated in the figure legends), each time in duplicate, and data are presented as mean values ± SEM. Two of the three peptides, M-4 and M-22, were analyzed in both synthetic and recombinant forms, with identical results. Significance was determined by one-way ANOVA analysis, and significant results, as compared to the uncoated controls, are indicated with * (p<0.05), ** (p<0.01) or *** (p<0.001). For assessment of correlations, simple regression analysis was used.

2.6. Ethics

This study was approved by the Medical Ethical Committees at the Universities of Linköping, and Uppsala, Sweden, and informed consent was given by the patient with complement dysfunction.

3. Results

3.1 Binding of functionally active C4BP to immobilized MPs

Prior to their use in the *in vitro* tests for complement inhibition, the functionality of the immobilized MPs was ascertained; C4BP was detected on the surface of coated wells after incubation with EDTA-plasma, *i.e.*, under conditions in which complement activation was prevented (data not shown).

To determine the extent to which C4BP was captured by the peptides on the PS surface in the presence of an active complement system, we measured the decrease in C4BP in serum samples (diluted 1:8) after incubation on an MP-coated surface. Our results showed that at a coating concentration of 10 µg/mL, both the M4-N and M22-N peptides were able to capture approximately 25% of the C4BP present in 100 µL of the diluted serum. The M2-N peptide showed a somewhat lower level of binding, with only about 12% of the C4BP being removed from the serum (Figure 2A).

The depletion of C4BP from incubated serum was significant at 1 µg/mL (p<0.05) to 10 µg/mL (p<0.001) for the M4-N peptide, whereas the M2-N and M22-N peptides showed significant decreases in the binding of C4BP at 10 µg/mL (p<0.01) and at 5 µg/mL and 10 µg/mL (p<0.01), respectively. Since the area in contact with the fluid in each well represented a 0.95 cm² surface area, the density of bound C4BP on the surface of each well coated with peptide was estimated as ~300-625 ng/cm² (~0.5-1 pmol/cm²).

To ascertain that the peptides were coated onto the polystyrene wells in a conformation that would allow them to bind C4BP, and subsequently C4b, we incubated EDTA-treated serum from healthy blood donors and from the AP-deficient individual in coated wells. In these sera, $\approx 5\%$ of the total C4 had been activated to C4b, the natural ligand for C4BP. A dose-dependent binding of C4b was observed (Figure 2B).

3.2 Complement inhibition in serum by bound MPs

After a 30-min incubation of diluted normal serum (in which mainly the CP was operative) on a polystyrene surface with or without coating, we measured the amount of surface-bound fragments of C4 and C3. The results indicated a significant depression in the complement activation, as demonstrated by a decrease in the amount of bound C4 fragments with increasing concentrations of MP in the coating solution (Figure 3). Already at 1 $\mu\text{g/mL}$ MP, the decrease in bound C4 fragments was significant ($p < 0.001$) for all the tested peptides, and their levels had decreased to background level by 5 $\mu\text{g/mL}$ (1 $\mu\text{g/mL}$ for M2-N). Similar attenuation was seen for the activation of C3, as monitored by the binding of C3 fragments to the surface and the generation of C3a in the fluid phase (Figure 4A, B). In addition, strong inhibition was observed in the serum from the patient with an AP dysfunction. In this case, the serum was tested undiluted to compensate for the lack of the amplification loop that activates C3 via the AP. In contrast to these data demonstrating complement inhibition, we saw complement activation, proportional to the concentration of the bound peptide, when the serum used contained antibodies against the MPs (data not shown).

Our conclusion that C4BP recruited to immobilized MPs can inhibit complement deposition was further supported by our analysis demonstrating an inverse correlation between the decline in C4BP levels in serum samples after contact with each coated peptide and the degree of complement activation, as monitored by the binding of C3 and C4 fragments. (R^2 -values for C3 ranged from 0.823 to 0.968, while those for C4 ranged from 0.644 to 0.970 for the three peptides.) An approximately linear correlation ($R^2 = 0.941$) was found when the values for the deposition of C3 and C4 were plotted against each other.

4. Discussion

The present study was undertaken to test a new concept designed to reduce complement activation at the interface between blood and an artificial surface. In our system, an artificial surface was coated with a synthetic peptide derived from a streptococcal virulence factor that is known to bind to a human complement inhibitor. Upon contact with blood, this peptide was expected to bind that inhibitor in the blood sample, thereby inhibiting complement activation and deposition onto the peptide-coated surface. This concept was tested using peptides derived from the hypervariable, C4BP-binding region of three different streptococcal M proteins. After the peptides were used to coat a polystyrene model biomaterial surface, we measured the ability of the peptides to bind C4BP from serum and subsequently inhibit CP activation induced by the surface. To verify that the activation we detected was due to the CP alone, without any contribution from or amplification by the AP, we used normal human serum from healthy blood donors diluted 1:8, a condition in which complement activation by the AP is minimal [3,11, 13]. Furthermore, we conducted corroborative experiments using serum from a patient who was unable to activate complement via the AP. A functional relationship between complement inhibition and the C4BP-binding capacity of the coated peptides was demonstrated by these experiments, since for each peptide an inverse correlation was found between the decline in serum C4BP and complement activation. In this context it is of interest to observe that one of the peptides, M2-N, was shown to bind slightly less C4BP from the serum, compared to the two other peptides. In contrast, the levels of bound C4b were shown to be slightly higher for M2-N, as well as the capacity to inhibit complement activation. The discrepancy between C4BP

binding and effect the on complement activation might be explained by a more physiological and flexible conformation of the C4BP which was captured by this peptide.

Based on the results reported here, we propose the model illustrated in Figure 5. Panel B shows dimerized MPs immobilized on a polystyrene surface that serves as a model biomaterial. Upon addition of blood, there is an initial rapid adsorption of a protein film corresponding to a monolayer [1], here depicted as consisting of mainly albumin, with protruding MPs. Plasma C4BP binds to these peptides and inhibits CP convertase activity. The net result of this inactivation is lower complement activation (monitored by measuring the generation of C3a and binding of C3 fragments to the protein film on the surface) as that produced by control surfaces without MPs (panel A).

In two previous studies, we have employed a strategy for controlling complement activation at a biomaterial surface that involved conjugating intact purified factor H via different surface chemistries [28,29]. In both cases, we created surfaces that were complement-inhibitory when tested in serum and in whole blood models. This inhibitory effect was postulated to be due to the ability of factor H to inhibit the AP amplification loop, which strongly enhances the activation that is initiated via all activation pathways.

In our previous studies, the density of surface-bound factor H necessary to accomplish full inhibition was demonstrated to be between 2 and 4 pmol/cm², whereas in our current system, inhibition of the CP required a density of C4BP of up to 1 pmol/cm². Since under physiological conditions the plasma concentrations of C3 and C4 are approximately 5.5 and 1.8 μM (a 3:1 ratio), respectively, it seems logical to expect that the ratio between the corresponding inhibitors needed for inhibition at a surface in contact with serum would be in the same range (approximately ratio 2-4:1). It should be noted that the C4BP molecule comprises seven α-chains, each of which can bind one C4b molecule. The binding sites on the α-chain for C4b and M-proteins are overlapping, though not identical [30], and some of these sites will, undoubtedly, be occupied in binding the molecule to the coated plate. However, since each C4BP has multiple binding sites, it might be expected that each bound C4BP molecule would still show a higher binding efficacy than would each bound monomeric factor H molecule, which binds C3b in a 1:1 complex.

In our hands, the binding of full-length RCAs to model biomaterial surfaces [28,29] and the approach using RCA binding peptides (the present study) have worked well on a laboratory scale. Since RCAs are naturally occurring proteins present in all humans, the risk of immunization of an individual, even after repeated exposure to surface-bound RCA molecules, is expected to be negligible. However, conjugation of a full-length RCA molecule may not be a realistic approach when it comes to designing a commercial biomaterial, since large amounts of purified protein would be needed, and it is highly likely that the immobilization procedure *per se* would cause a large fraction of the RCA to lose its biological activity. Using the technology available today, it would not be realistic to expect to be able to express and purify recombinant RCAs in the required quantities. The shortage of human blood for industrial use, the potential risk of transmission of blood-borne diseases, and the limited shelf-life of a product that consists in part of a native protein are all limiting factors. The alternative approach using RCA-capturing structures investigated here would avoid several of these concerns. From an economic point of view, the present approach would be an attractive method because RCA-binding peptides can be produced in recombinant form, allowing access to large amounts of material. The production of such peptides using expression systems would, obviously, circumvent the risk of transmitting blood-borne disease.

A potential limitation of the approach described here is the antigenicity of microorganism-based peptides. Most adults have had streptococcal infections, and 90% of those in our pool

of healthy blood donors had antibodies against at least one of the three peptides used in the present study. When present, either initially or as a result of immunization during treatment with a peptide-coated biomaterial, such antibodies may induce complement activation by the CP. In addition, the MPs inhibit the initiation of complement activation but do not affect the amplification driven by the AP.

However, the antigenicity issue just raised need not be a major concern in future use of this technology because our long-term goal is not to use peptides with naturally occurring sequences, but rather to use the bacterial peptides as a starting material to design artificial peptides containing RCA-binding domains not encountered in nature. In this context, it is encouraging that site-specific mutagenesis of a streptococcal MP results in peptides that retain the ability to bind C4BP, while strongly altering the antigenicity of the peptide [18]. To dampen complement activation in whole blood, it is most likely necessary to also target the amplification of complement activation that is accomplished by the AP in an analogous way, using peptides with affinity for factor H.

5. Conclusion

In the present work we demonstrate that a model biomaterial coated with peptides that specifically bind a human RCA is protected against complement attack. This concept was tested by investigating the ability of three different streptococcal M4-derived peptides to bind C4BP and to modulate complement activation via the CP on a material surface, using diluted normal human serum, where complement activation by the AP is negligible, and serum from a patient lacking AP activation. Our study shows that it is feasible to render an artificial surface inherently autoprotective by coating it with RCA-binding molecules.

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Figure 1.

Schematic overview of the complement system with emphasis on the targets for recognition (C1q and MBL/ficolins), formation of the two C3 convertases (C4bC2a and C3bBb), and activation of C3 by the proteolytic cleavage into C3a and C3b. The alternative pathway is responsible for a powerful amplification loop, for complement activation initiated by all three pathways. Membrane- and fluid-phase inhibitors of the complement system are assigned in boxes.

Figure 2.

Binding properties of the streptococcal MPs. Panel A: Residual C4BP in serum (diluted 1:8) after incubation in polystyrene microtiter wells coated with synthetic MPs at the indicated concentrations (similar results were obtained with recombinant peptides). Panel B: Deposition of C4b on a polystyrene surface coated with synthetic MPs after contact with EDTA-chelated serum (in which $\approx 5\%$ of the total C4 was activated to C4b). Data are expressed as means $\pm$ SEM; $n = 5-8$ experiments, each performed in duplicate. Significant results compared to the non-coated control surface are indicated with * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

Figure 3.

Inhibition of complement activation in human serum diluted 1:8, monitored as the deposition of C4 fragments on polystyrene surfaces coated with synthetic streptococcal MPs (similar results were obtained with recombinant peptides). Data are expressed as means $\pm$ SEM (n = 4-6). Significant results compared to the control surface (0 μ g MP/ml) are indicated with *** (p<0.001).

Figure 4.

Inhibition of complement activation by synthetic MPs coated on polystyrene (similar results were obtained with recombinant peptides). Human serum diluted 1:8 (all three peptides) and undiluted serum from a patient deficient in activation via the AP (peptide M4-N only) were each incubated on coated polystyrene microtiter wells. Complement activation was monitored as surface-bound C3 fragments (Panel A) and generation of C3a (Panel B). Data are expressed as means $\pm$ SEM; $n = 4-6$ (all three peptides, normal serum 1:8) or $n=5$ (M4-N, AP-deficient patient). Significant results compared to the control surface ($0 \mu\text{g MP/mL}$) are indicated with * ($p<0.05$) and *** ($p<0.001$).

Figure 5. Model of the attenuation of complement activation by surface-bound MPs. Panel B shows an artificial surface coated with MPs. Upon blood contact, these MPs bind serum C4BP and thereby indirectly also C4b, one of the building blocks in the classical C3 convertase (C4bC2a). This will result in lower complement activation via the CP. An uncoated surface is shown in panel A. See text for details.