Targeting of Functional Antibody-Decay-accelerating Factor Fusion Proteins to a Cell Surface*  

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Recombinant soluble complement inhibitors hold promise for the treatment of inflammatory disease and disease states associated with transplantation. Targeting complement inhibitors to the site of complement activation and disease may enhance their efficacy and safety. Data presented show that targeting of decay-accelerating factor (DAF, an inhibitor of complement activation) to a cell surface by means of antibody fragments is feasible and that cell-targeted DAF provides significantly enhanced protection from complement deposition and lysis compared with soluble untargeted DAF. An extracellular region of DAF was joined to an antibody combining site with specificity for the hapten dansyl at the end of either C1q or C1r/C1s Ig regions. The recombinant IgG-DAF chimeric proteins retained antigen specificity and bound to dansylated Chinese hamster ovary cells. Both soluble C1q-DAF and C1r/C1s-DAF were effective at inhibiting complement-mediated lysis of untargeted Chinese hamster ovary cells at molar concentrations within the range reported by others for soluble DAF. However, when targeted to a dansyl-labeled cell membrane, C1q-DAF was significantly more potent at inhibiting complement deposition and complement-mediated lysis. Cell-bound C1q-DAF also provided cells with protection from complement lysis after removal of unbound C1q-DAF. Of further importance, the insertion of a nonfunctional protein domain of DAF (the N-terminal short consensus repeat) between C1q and the functional DAF domain increased activity of the fusion protein. In contrast to C1q-DAF, C1r/C1s-DAF was not significantly better at protecting targeted versus untargeted cells from complement, indicating that a small targeting vehicle is preferable to a large one. We have previously shown that for effective functioning of soluble complement inhibitor CD59, binding of CD59 to the cell surface close to the site of complement activation is required. Significantly, such a constraint did not apply for effective DAF function.

The complement pathway can be divided into two distinct phases. The activation phase is an enzymatic amplification cascade in which complement proteins are cleaved, resulting in the generation of cell-bound opsonins (e.g. C3b, iC3b, and C3dg) and bioactive soluble fragments (C3a and C5a). These complement activation fragments are important for host defense and immune complex catabolism. The terminal phase of the complement pathway is initiated following cleavage of C5 and consists of the nonenzymatic assembly of the complement proteins C5b, C6, C7, C8, and C9 to form the cytolytic membrane attack complex (MAC),1 usually on the activating surface. Host cells are normally protected from the proinflammatory and cytolytic effects of complement activation by membrane-bound complement inhibitors that inhibit either the activation phase or the terminal phase and MAC assembly.

Membrane inhibitors of complement activation include complement receptor 1, membrane cofactor protein, and decay-accelerating factor (DAF). These proteins regulate complement activation by inhibiting formation of C3 (and C5) convertase, a central enzyme complex involved in the amplification of the complement cascade. Inhibitors of complement activation all have a protein structure that consists of varying numbers of repeating units of about 60–70 amino acids termed short consensus repeats (SCR) (1, 2). DAF is composed of four contiguous SCRs at its N terminus that are connected to a serine-threonine-rich region linked to the membrane via a glycosylphosphatidylinositol (GPI) anchor. The active site of DAF (C3/C4 binding site) is located within SCRs 2–4 (3, 4). Control of the terminal complement pathway and MAC formation occurs principally through the activity of CD59, a membrane protein that binds to C8 and C9 in the assembling MAC (5–7).

There is interest in developing membrane complement inhibitors as recombinant soluble proteins for therapy of disease states in which complement activation and deposition is involved in pathogenesis. In diseases in which the MAC is involved in pathogenesis, CD59-based inhibitors offer a potential advantage over inhibitors of complement activation because CD59 will only block MAC formation and not the generation of the earlier complement activation products that are important for host defense and immune complex catabolism. Although soluble CD59 is not an effective inhibitor of MAC formation, we have shown previously that the effectiveness of soluble CD59 can be significantly enhanced when it is targeted to the site of MAC formation (8). Nevertheless, in many disease states early complement activation products are involved in pathogenesis, and only an inhibitor of complement activation would have

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1 The abbreviations used are: MAC, membrane attack complex; DAF, decay-accelerating factor; SCR, short consensus repeat; GPI, glycosylphosphatidylinositol; CHO, Chinese hamster ovary; NHS, normal human serum; Dansyl, dansyl 5-dimethylaminonaphthalene-1-sulfonyl; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate.
potential therapeutic value. In this context, recombinant soluble complement inhibitors based on membrane inhibitors of complement activation, including DAF, have been shown to be effective at attenuating complement-dependent pathology in a variety of animal models of autoimmune and inflammatory disease and at considerably prolonging the survival of transplanted xenografts (for reviews on the subject of therapeutic intervention in the complement system see Refs. 9–15). However, unresolved questions regarding the clinical use of systemic inhibitors of complement activation remain. Complement plays a crucial role in both innate and adaptive immunity to infection, and it is not clear what effect systemic complement inhibition may have on the ability of the host to control infection, particularly if the patient is immunocompromised. In addition, complement is required for the effective catabolism of immune complexes, and the administration of systemic complement inhibitors would probably exacerbate preexisting clinical features of immune complex disease, a potential candidate disease for complement inhibitor therapy.

The efficacy and perhaps safety of inhibitors of complement activation may be improved by targeting them to sites of complement activation and disease. Local targeting of complement activation inhibitors may allow a much lower effective serum concentration and result in reduced levels of systemic complement inhibition. In this study we address the feasibility of targeting an inhibitor of complement activation to a cell surface and site of complement activation by means of antibody fragments containing an antigen binding site.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—The TWS2 cell line was produced by transfecting the Ig nonproducing mouse myeloma cell Sp2/0 with a chimeric light chain composed of murine α-anti-dansyl variable domain joined to human Cε constant domain (16). TWS2 were cultured in RPMI medium (Life Technologies, Inc.) containing 10% fetal calf serum. Chinese hamster ovary (CHO) cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

**DNA, Antibodies, and Reagents**—DAF cDNA and anti-DAF polyclonal antigen were kindly provided by Dr. V. Nussenzweig (New York University School of Medicine, New York, NY). Anti-DAF monoclonal antibodies IIH6 (epitope on SCR 4) and IH4 (epitope on SCR 3) were the gifts of Drs. T. Kinoshita (Osaka University, Osaka, Japan) and D. Lublin (Washington University, St. Louis, MO), respectively. Normal human serum (NHS) was obtained from the blood of healthy volunteers in the laboratory and stored in aliquots at −80 °C. Rabbit anti-CHO cell membrane antigen was prepared by inoculation with CHO cells. Control antibody was generated by standard techniques (17). Dansyl (3-dimethylaminoethylaminonaphthalene-1-sulfanyl) (dansyl) and all conjugated secondary antibodies (unless otherwise indicated) were purchased from Sigma.

**Construction and Expression of Antibody-DAF Fusion Proteins**—cDNA encoding a soluble DAF functional unit (SCRs 2–4) lacking the N-terminal SCR, serine-threonine-rich C-terminal region, and the GPI anchor addition signal was generated by polymerase chain reaction amplification (for DAF sequence information, refer to Refs. 18 and 19). The polymerase chain reaction product contained a blunt 5′ end and an EcoRI site at its 3′ end and was blunt end-ligated (Stul restriction site) in frame to the 3′ end of a (SG,SG,SG,S) linker in a cloning vector. The linker-DAF sequence was then inserted at the 3′ end of human IgG Cε1 domain (Cε1-DAF), the hinge region (H-DAF), or the Cε3 domain (Cε3-DAF). The vector and cloning strategy is as described for the generation of IgG-CDS9 constructs (8). For expression, the gene constructs were subcloned into the expression vector pAG4882, which contains the murine heavy chain anti-dansyl variable region (20, 21). The antibody constant region sequences in pAG4882 were replaced by the Cε1-DAF, H-DAF, or Cε3-DAF coding sequence using unique BamHI and SalI sites (20, 21). For the Cε3-DAF construct, the human IgG heavy chain constant region was truncated at the Cε3 domain by replacing the 3′ end region of human IgG4 using the SalI and NsiI sites present in both genes. An additional Cε1-DAF construct containing the N-terminal SCR of DAF and termed Cε1-DAF(1–4) was generated as described above except that the N-terminal primer for polymerase chain reaction amplification was at the beginning of the mature protein sequence. Transfection of TWS2 cells and clone selection were performed as previously described (8).

**ELISA and Protein Assays**—Detection of IgG-DAF fusion proteins and determination of their relative concentrations were achieved using a standard ELISA technique (17). The capture antibody was dansylated bovine serum albumin, and anti-DAF mAb IIH6 or IH4 was the primary detection antibody. Details of the assay have been described previously (8). The protein concentration of IgG-DAF fusion proteins was determined either by UV absorbance or by using a Coomassie protein assay kit (Pierce).

**Fusion Protein Purification**—IgG-DAF proteins were purified from culture supernatant by anti-DAF affinity chromatography using IIH6 mAb. The purified IIH6 mAb was coupled to HiTrap NHS-activated affinity columns (Amersham Pharmacia Biotech) as described by the manufacturer. Culture supernatants containing DAF were adjusted to pH 7.5 and applied to affinity columns at a flow rate of 0.5 ml/min. The column was washed with 10 column volumes of PBS, and the fusion protein was eluted with 2–3 column volumes of 0.1 M glycine, pH 2.6. Fractions containing fusion protein were collected into tubes containing 1 0 Tris buffer, pH 8 for neutralization, and samples were then dialyzed against PBS.

**Western Blotting**—Samples were separated on 4–15% acrylamide SDS-polyacrylamide gel electrophoresis gels (Bio-Rad) under nonreducing conditions by standard procedures (22) and transferred to a nitrocellulose membrane. The membrane was blocked with 5% dried milk in Tris-buffered saline (TBS), and transferred DAF constructs were detected with anti-DAF mAb IIH6 or IH4 at 10 μg/ml in Tris-buffered saline-Tween 20 buffer (Bio-Rad). After washing with Tris-buffered saline-Tween 20, the membrane was incubated with horseradish peroxidase-conjugated anti-mouse IgG at a 1:2500 dilution in Tris-buffered saline-Tween 20. The membrane was developed using 5-bromo-4-chloro-3-indolyl phosphate nitroblue tetrazolium substrate tablets (Sigma).

**Flow Cytometry and Measurement of C3 Deposition**—To detect IgG-DAF binding to targeted CHO cells, the cells were labeled with the hapten dansyl as previously described (8). Control unlabeled or dansyl-labeled CHO cells were incubated with fusion proteins at a 160 nM final concentration (30 min/4 °C). Following incubation, cells were washed twice in Dulbecco's modified Eagle's medium and further incubated with anti-DAF mAb IIH6 (10 μg/ml) (30 min/4 °C). After washing, FITC-conjugated anti-mouse IgG was added (1:100 (30 min/4 °C). Cells were then washed, fixed with 2% paraformaldehyde in PBS, and analyzed using a Becton Dickinson FACScan. Dansyl fluorescence and FITC fluorescence displayed distinct excitation and emission spectra, and machine gating ensured that only FITC fluorescence was recorded (8).

To measure the effect of the IgG-DAF fusion proteins on C3 deposition, labeled or unlabeled CHO cells were first incubated with IgG-DAF proteins at a 100 nM final concentration for 15 min at 37 °C. The cells were then sensitized with anti-CHO cell membrane antiserum (10%), and NHS depleted of complement protein C9 (Quidel, San Diego, CA) was added to a final concentration of 10%. The use of C9-depleted serum prevents the formation of the MAC and cell lysis following complement activation. After a 45 min/37 °C incubation, the cells were washed, and FITC-conjugated anti-human C3 antibody (Accurate Chemical Corp., Westbury, NY) was added (30 min/4 °C). After washing, cells were fixed with 2% paraformaldehyde and analyzed.

**Complement Lysis Assays**—CHO cells at between 60 and 80% confluence were detached with versene (Life Technologies, Inc.), washed once, and resuspended to 1 × 10⁶/ml in Dulbecco's modified Eagle's medium. Cells were then labeled with dansyl or treated under control conditions (8) and sensitized to complement by adding rabbit anti-CHO cell membrane antiserum to a 10% final concentration. Following addition of antiserum, an equal volume of NHS diluted in Dulbecco's modified Eagle's medium was then added, and cells were incubated for 45 min at 37 °C. Cell viability was determined either by trypan blue exclusion (both live and dead cells counted) or by a fluorescence-based assay that measures release of fluorescence from cells pre-loaded with calcine-AM (23). Results obtained from either assay were similar. Cells were lysed with 0.01% saponin for 100% lysis controls, and heat-inactivated NHS was used for background lysis. Cell lysis assays were typically performed in 1.5-ml microtube tubes in a final volume of 10 μl. A 100% lysis control at 30% resulted in 10% cell viability. Anti-CHO antibodies were generated by standard techniques (17). Dansyl fluorescence and FITC fluorescence displayed distinct excitation and emission spectra, and machine gating ensured that only FITC fluorescence was recorded (8).

**Fluorescence-activated Cell Sorting**—Labeled cells were analyzed on a Becton Dickinson FACScan fluorescence-activated cell sorting instrument. Data were collected in the linear region of the amplification, and the linear range was characterized by the number of events in the lower 10% of the log linear region. Data were analyzed using the Lysys software (Becton Dickinson) on a microcomputer.
RESULTS

Construction of IgG-DAF Fusion Proteins—The extracellular portion of decay-accelerating factor is composed of four SCR units at its N terminus followed by a serine-threonine-rich sequence. Neither the N-terminal SCR nor the serine-threonine-rich region is required for function (3, 4), and IgG-DAF constructs were prepared using cDNA encoding SCRs 2–4 of DAF. The DAF-encoding regions were inserted at the 3′ end of the coding sequence for IgG heavy chain fragments, after the CH1-, hinge-, or CH3-encoding regions. The two fusion partners were joined by a Ser-Gly linker. The predicted set of fusion proteins is shown in Fig. 1. The constructs contain murine variable regions with specificity for the hapten dansyl, linked to human IgG constant regions. The CH3-DAF fusion was constructed with a human IgG4 Fc region (CH2 and CH3), because human IgG4 does not activate complement.

Expression and Characterization of IgG-DAF Fusion Proteins—Cells stably expressing an anti-dansyl light chain were transfected with expression vectors containing the heavy chain-DAF fusion constructs. Following selection and limiting dilution, transfectoma clones secreting IgG-DAF proteins with specificity for dansyl were identified by assaying culture supernatant by ELISA. Fusion proteins were purified from culture supernatant by means of anti-DAF affinity chromatography, and clones secreting CH1-DAF and CH3-DAF at ~0.3 and 0.1 µg/ml culture supernatant, respectively, were isolated. We failed to isolate a clone or stable population expressing a useful quantity of CH1-, hinge-, or CH3-encoding regions. The two fusion partners were joined by a Ser-Gly linker. The predicted set of fusion proteins is shown in Fig. 1. The constructs contain murine variable regions with specificity for the hapten dansyl, linked to human IgG constant regions. The CH3-DAF fusion was constructed with a human IgG4 Fc region (CH2 and CH3), because human IgG4 does not activate complement.

Affinity-purified IgG-DAF fusion proteins (25 ng) were separated on 4–15% acrylamide nonreducing SDS-polyacrylamide gels. The targeting activity of CH1-DAF and CH3-DAF was analyzed by measuring the effect of the fusion proteins on both cellular C3 deposition and complement-mediated cell lysis. To determine the functional activity of CH1-DAF and CH3-DAF, the binding of IgG-DAF proteins to washed cells was detected by flow cytometry by means of anti-DAF monoclonal antibody IIH6 and FITC-conjugated secondary antibody. Dansylated CHO cells were incubated in PBS (a), and unlabeled or dansylated CHO cells were incubated with CH1-DAF (b) or CH3-DAF (c). The figure shows FITC fluorescence, which has a distinct excitation/emission spectrum from dansyl (8). The numbers next to histogram peaks indicate mean fluorescence. A representative experiment is shown.
CHO cells were incubated with either C11-DAF or C13-DAF in the presence of human serum. The concentration of human serum used in these experiments (10%) resulted in ∼90% lysis of unprotected CHO cells. Fig. 4 shows that both C11-DAF and C13-DAF protected both targeted and untargeted CHO cells from complement-mediated lysis. However, C11-DAF provided targeted cells with significantly better protection; 50% inhibition of lysis required a C11-DAF concentration of ∼35 nM for targeted cells and ∼100 nM for untargeted cells. The specific activity of untargeted C11-DAF is within the range previously reported for recombinant soluble DAF (24–26). C13-DAF and C11-DAF had similar molar activities in protecting untargeted cells from complement lysis, even though each C13-DAF construct contained two DAF molecules. However, in contrast to C11-DAF, there was little difference in the protection C13-DAF provided to targeted cells compared with untargeted cells, with 50% inhibition of lysis requiring a concentration of ∼80 nM for targeted cells and ∼95 nM for untargeted cells. C13-DAF was constructed using the IgG4 Fe region, and we have shown previously that anti-dansyl IgG4 has no effect on complement-mediated lysis of unlabeled or dansylated CHO cells (8). Dansylation of CHO cells also has no effect on their susceptibility to complement (8).

These data indicate that the increased potency of C11-DAF at protecting targeted cells is due to cell-associated DAF. To confirm this, dansyl-labeled and unlabeled cells were incubated in the presence of C11-DAF and then washed before exposure to sensitizing antibody and complement. As expected, unlabeled CHO cells were not protected from complement-mediated lysis (Fig. 5). However, C11-DAF did protect washed dansyl-labeled CHO cells from complement lysis, showing that cell-bound C11-DAF functions effectively. The level of protection was less than when unbound C11-DAF was not removed, and >60% protection from lysis could not be achieved.

To determine the effect of the IgG-DAF fusion proteins on C3 deposition, unlabeled or dansyl-labeled CHO cells were incubated with either C11-DAF or C13-DAF in the presence of C8-deficient serum (to prevent complement-mediated lysis). Fig. 6 shows that both fusion proteins inhibited C3 deposition on both targeted and untargeted cells. Targeted (dansyl-labeled) cells were better protected from C3 deposition than untargeted cells by C11-DAF, but there was little difference between C3 deposition on targeted and untargeted cells with C13-DAF. With regard to targeted CHO cells, C11-DAF and C13-DAF resulted in a respective 70% and 45% approximate reduction in C3 deposition (mean relative fluorescence) compared with controls. For untargeted CHO cells, C11-DAF and C13-DAF both resulted in a 40–45% reduction in C3 deposition. The inhibition of C3 deposition by the IgG-DAF constructs correlates with their activity at inhibiting complement-mediated lysis.

Comparison of Two Different C11-DAF Constructs—IgG-DAF proteins were constructed using only SCR 2–4 of DAF, because the N-terminal SCR of DAF does not form part of its active site (3, 4). Although the complement-regulatory function of the C11-DAF construct reported above is within the range of activity previously reported for recombinant soluble DAF (24–26), we subsequently prepared a C11-DAF construct containing all four SCR units of DAF, designated C11-DAF(1–4). Western blot analysis of affinity-purified C11-DAF(1–4) revealed a protein of about 105 kDa. The molecular mass is slightly greater than predicted from the amino acid sequence, but there is an N-glycosylation site in the region linking SCRs 1 and 2 (not present in the other constructs). To perform a functional comparison, C11-DAF and C11-DAF(1–4) were titrated by dansyl-capture ELISA as described above, and flow cytometry showed that at similar input concentrations of C11-DAF and C11-DAF(1–4), similar relative levels of DAF bound to the cell surface (data not shown). When the activity of the two constructs was compared in complement lysis assays, the inclusion of the N-terminal SCR in the C11-DAF(1–4) construct was found to result in a small, but statistically significant (p < 0.05) increase in complement-inhibitory function in both cell targeted and untargeted assays (Fig. 7).

**DISCUSSION**

Both C11-DAF and C13-DAF were effective inhibitors of complement in an untargeted system, and the activity of both constructs (50% inhibition of cell lysis at ∼100 nM) is within the range reported by others for the activity of recombinant soluble DAF in vitro. However, the potency of C11-DAF at protecting cells from complement was significantly enhanced when the construct was targeted to the cell surface. In contrast, C13-DAF was not significantly better at protecting targeted versus...
untargeted cells and human serum was added to a final concentration of 10%. Cell lysis was determined after 45 min at 37 °C. Background lysis (cells incubated and sensitized to complement with anti-CHO cell membrane antiserum, full-length extracellular DAF and SCR units 1–4). Cells were then incubated with 100 nM CH1-DAF or CH3-DAF (15 min/37 °C) and then fusion proteins.

Control unlabeled or dansyl-labeled CHO cells were incubated with 40 nM CH1-DAF (containing SCR units 2–4) or CH1-DAF(1–4) (containing SCR units 1–4). Cells were then sensitized to complement with anti-CHO cell membrane antiserum, and human serum was added to a final concentration of 10%. Following incubation (45 min, 37°C), cells were washed, and C3 deposition was measured by flow cytometry using FITC-conjugated anti-C3 antibody. Histograms show relative fluorescence, with mean relative fluorescence indicated next to each peak. The data are representative of four experiments.

Cell-associated CH1-DAF was effective at inhibiting complement-mediated lysis of the cells to which it was bound. However, even at saturation, cell-bound CH1-DAF alone was unable to achieve the level of protection that could be provided to cells when unbound CH1-DAF was also present. There are various factors that could influence the activity of cell-associated CH1-DAF, the most obvious being surface antigen density and characteristics of the targeting vehicle. The targeting vehicle can affect the positioning of cell-associated DAF, either with regard to its orientation or distance from the site of C3 convertase formation on the membrane. Data presented here indicate that a small targeting vehicle is preferable to a large one. In addition, in the case of antibody targeting, the location of the epitope relative to the cell membrane may influence the activity of a cell-targeted complement inhibitor. Addressing these considerations may result in further enhancing the effectiveness of targeted complement inhibitors of activation, and such considerations may result in further enhancing the effectiveness of a cell-targeted complement inhibitor.

Although the removal of unbound DAF from our assay system resulted in the protection of only about 60% of targeted cells from complement, it is important to note that targeted DAF was still a significantly better inhibitor than untargeted DAF when unbound DAF is not removed. This is a therapeutically relevant observation because untargeted inhibitors of complement activation have shown promise in animal models of inflammatory disease and are in clinical trials. Rapid clearance from the circulation may be a concern untargeted cells. However, there are some important distinctions to be made. DAF containing its GPI anchor will insert into any membrane and is not targeted to a specific cell type. Furthermore, GPI-DAF is ineffective in the presence of serum due to its affinity for lipoproteins and hydrophobic serum proteins (24, 27), making it unsuitable for clinical application. CH1-DAF is not affected by the presence of serum lipoproteins, and it is straightforward to change the binding specificity of the variable region, potentially providing protection to any cell population recognized by a specific antibody.

In this study, DAF was joined to the C terminus of IgG CH1 and CH3 domains via a [S(Gly)4]3 linker, and one factor that could affect function is the type of linkage or spacer between the IgG domains and DAF. The N-terminal SCR of DAF is not required for function (3, 4), and the increase in activity of CH3-DAF when the N-terminal SCR is included in the construct may be related to it functioning as a spacer. The additional SCR increased the function of both targeted and untargeted CH1-DAF(1–4), possibly by permitting better access of the DAF active site to its site of interaction on the C3 convertase, and perhaps reflecting some degree of steric hindrance by the antibody domains in CH1-DAF. With regard to cell-bound CH1 fusion proteins, the additional spacer linker linked to the flexible serine-glycine linker may allow the active site of DAF to be positioned in a more favorable orientation (distance?) with respect to the site of C3 convertase formation on the cell membrane. Similar considerations may explain why the smaller CH1-DAF was more effective than CH3-DAF at inhibiting complement on targeted cells. Also of note, there was no difference between the complement-inhibitory activity of CH3-DAF and CH1-DAF for untargeted cells, even though each CH3-DAF contained two DAF molecules. Steric hindrance may explain this observation.

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for both targeted and untargeted constructs, but probably less so for targeted complement inhibitors because they are also cleared from the circulation by binding to their target tissue. The presence of antibody fragments may improve the half-life of constructs, particularly if the Fc region is present. Although current data did not show a significant improvement of targeted over untargeted C_{H1}3-DAF (i.e. Fc-containing construct), engineering of the constructs and choice of target epitope (see “Discussion” above) could potentially improve the effectiveness of a targeted C_{H1}3-DAF construct.

We have previously shown that functional IgG-CD59 fusion proteins can be targeted to a cell surface (8). CD59 is an inhibitor of the terminal complement pathway and cytolytic MAC formation. However, aside from the fact that DAF and CD59 inhibit different parts of the complement pathway, there are important differences in how the IgG-CD59 and IgG-DAF constructs function. Most significantly, unlike IgG-DAF constructs, untargeted IgG-CD59 constructs were not effective inhibitors of complement. In fact, IgG-CD59 only provided effective protection from complement-mediated lysis when bound to the cell surface, and data from us (8) and others (28) indicate that CD59 must be positioned close to the membrane at the site of MAC formation to be effective. Such a constraint does not seem to apply for effective DAF function, and this is an important difference. A C_{H1}1-CD59 construct was also the most effective type of IgG-CD59 construct at inhibiting complement-mediated lysis but was about 5-fold less effective than C_{H1}1-DAF (50% inhibition of lysis required ~35 nM C_{H1}1-DAF and 160 nM C_{H1}1-CD59 (8)). When unbound C_{H1}1-DAF was removed, the complement-inhibitory activity of cell-bound C_{H1}1-DAF was comparable with the activity of C_{H1}1-CD59, in which cell-associated CD59 accounts for almost all observed complement-inhibitory function.

Selectively blocking different parts of the complement pathway may allow the generation of beneficial complement activation products but inhibit the generation of complement activation products involved in disease pathogenesis. For example, in disease conditions where the MAC or C5a are important mediators of inflammation and tissue damage, CD59-based inhibitors and antibodies to C5 (29–31) may be advantageous, because they do not inhibit the generation of C3a and C5b-9.

To summarize, recombinant soluble complement inhibitors (including soluble DAF) have been shown to be effective for the treatment of inflammatory disease in various rodent models. Targeting complement inhibitors to the site of complement activation and disease offers the potential of enhancing therapeutic efficacy and of reducing the level of systemic complement inhibition during treatment. This study demonstrates that targeting DAF to a cell surface by means of an antibody-combining site is feasible and that targeted DAF provides cells with enhanced protection from complement. The data justify the preparation of antibody-complement inhibitor constructs with antibody specificities to disease-relevant antigens for testing in animal models. Finally, the enhanced functioning of targeted versus untargeted DAF has implications for a targeting approach in general and is not limited to antibody-mediated targeting of complement inhibitors.

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