In Vivo Characterization and Therapeutic Efficacy of a C5-specific Inhibitor from the Soft Tick Ornithodoros moubata*

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The complement (C) system is a crucial arm of innate immunity. It is activated by foreign or antibody-coated surfaces via various different pathways that converge at the formation of the C3 convertase enzyme. This cleaves C3 to C3b, which binds covalently to the target surface. Surface-bound C3b acts as an opsonin, leading to cell death via phagocytosis, or contributes to further progression of the C cascade through formation of the C5 convertase and subsequent cleavage of C5 to C5a and C5b. C5a has chemotactic and anaphylactic properties, thereby triggering inflammation and aiding pathogen clearance. C5b binds components of the terminal pathway that culminates in formation of the membrane attack complex (MAC), a pore through the cell membrane that can result in osmotic lysis of the target cell. In addition to cell killing, the C system has other key roles such as in solubilization and clearance of immune complexes (1), and it also has important links to adaptive immunity, drastically lowering the threshold concentration of antigen required to stimulate an effective antibody response (2).

Thus C, and activated C3 in particular, plays key roles in health and prevention of infection.

The propensity for C activation products to “drift” from the site of activation and deposit on self-cells puts these cells at risk. However, damaging effects of C are prevented by expression of multiple C regulators (CReg) on the membrane of self-cells (3, 4). Unfortunately, however, this protection can be overwhelmed, particularly in autoimmune diseases where non-C induction mechanisms, such as auto-reactive T cells and antibodies, trigger disease (5). C acts as a destructive effector mechanism that damages tissue and drives inflammation. C has been implicated in pathology of various diseases such as multiple sclerosis, rheumatoid arthritis, hemolytic uremic syndrome, paroxysmal nocturnal hemoglobinuria, and myasthenia gravis. C is also responsible for much of the damage occurring in ischemia-reperfusion injury, such as that seen in transplantation, stroke, cardiopulmonary bypass, and myocardial infarction. As a consequence, controlling inappropriate activation of C is a therapeutic strategy in these pathologies (6, 7). Numerous approaches to the development of agents that regulate C activation have evolved, including development of soluble, recombinant CReg. The best characterized of these, soluble CR1 (sCR1) has been tested in humans for acute respiratory distress
syndrome and myocardial infarction (8). A different approach has been to use antibodies to specific components to arrest the C cascade at specific stages. A humanized antibody to C5 has shown promise in clinical trials for treatment of myocardial infarction and paroxysmal nocturnal hemoglobinuria (9, 10).

The stage at which the C system should be inhibited using therapeutics is of considerable importance. Agents that cause systemic inhibition of C in vivo may cause iatrogenic infections or immune complex disease, likely limiting their use to acute situations. The problems of systemic inhibition of C might be overcome either by targeting the agent to a specific site or by choosing an agent that permits the physiological actions of C to proceed unimpeded. Inhibition at the C5 stage is an attractive proposition as C3b opsonization of pathogens and immune complex solubilization proceed unaffected while C5a-mediated inflammation and tissue damage or cell activation caused by MAC formation are prevented. Individuals deficient in components of the MAC are healthy apart from an increased susceptibility to meningococcal disease. We and others have shown in animal models that the terminal pathway is involved in pathology of various diseases and that therapeutic intervention at the later stages of the C cascade will be of benefit. A lack of C6, a C component involved only in the terminal pathway, prevents both demyelination and axonal injury in ADEAE (antibody-mediated demyelinating experimental allergic encephalomyelitis) and damage to neuromuscular endplates in experimental autoimmune myasthenia gravis (EAMG) (11, 12). We have also demonstrated that intra-articular injection of a membrane-targeted form of CD59 inhibits disease progression in mono-articular antigen-induced arthritis (13). Furthermore, we have shown that mice lacking CD59, a membrane CReg specifically inhibiting MAC, develop enhanced severity of experimental allergic encephalomyelitis, EAMG, and arthritis compared with wild-type animals (14–16).

Various C-inhibitory proteins have been identified in the saliva of ticks (17, 18). The recent cloning and expression from the soft tick Ornithodoros moubata of a protein that prevented C5 activation raised the possibility that this protein could be used to inhibit therapeutically the terminal pathway of C (19). The mature protein, termed OmCI, is a 150- amino acid (17-kDa) non-glycosylated protein belonging to the lipocalin family of β barrel-forming proteins and has no similarity to any known CReg. We have expressed recombinant OmCI and here characterize further its inhibitory function in vitro and in vivo in rodents. We also describe its efficacy in the rat model of myasthenia gravis, a disease in which the terminal pathway has been shown to have a key role in pathology.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals unless otherwise stated were from Sigma Aldrich or Fisher Scientific UK Ltd (Loughborough, UK). Polyclonal goat anti-rat C3c was from Nordic Laborataries (Copenhagen, Denmark) and was used at a dilution of 1:400. Mouse anti-rat CD68 mAb (EDI) was from Serotec (Oxford, UK) and was used at a final concentration of 2 µg/ml. Polyclonal rabbit anti-OmCI and polyclonal sheep anti-human C9 were prepared in-house using standard immunization techniques and were used at 1/1000 and 1/400, respectively. Donkey anti-mouse IgG fluorescein isothiocyanate and donkey anti-goat/sheep IgG fluorescein isothiocyanate were from Jackson ImmunoResearch (Stratech Scientific Ltd, Soham, UK) and were used at 1/200. α-Bungarotoxin-rhodamine conjugate (T-1175) was from Molecular Probes (Invitrogen). Goat anti-rabbit Ig horseradish peroxidase was from Jackson ImmunoResearch and was used at 1/10,000. The rat hybridoma TIB-17, secreting the anti-AChR mAb35 (20), was obtained from the American Tissue Culture Collection (TIB-175; ATCC); antibody was purified as described previously (12).

Expression and Purification of pOmCI—Native mature OmCI, also known as EV576, is not glycosylated. The asparagines of the NX(S/T) glycosylation sites (Asn-78 and Asn-102) were mutated into glutamines using PCR to prevent the hyper-glycosylation seen when expressed in Pichia methanolica (19). The double mutant N78Q/N102Q Pichia-expressed protein is termed pOmCI. A single pOmCI-positive colony, grown overnight in BDMY (buffered complex dextrose) medium (Invitrogen) was used to seed a 10-liter fermentation vessel housed on a Bioflow 3000 benchtop fermentor (New Brunswick, NJ). The vessel contained 3 liters of sterile Fermentation Basal Salts Medium supplemented with PTM1 trace metal solution. Controls were set to: temperature 30 °C, pH 5.0, agitation 250 rpm, and DO₂ 30% maintained within an agitation range of 250–1000 rpm. After 24 h of growth the initial glucose was consumed and glucose feeding begun: 20 h of 50% glucose containing 12 ml/liter of PTM1; 60 ml/h, followed by a 24-h mixed glucose (19.5 ml/h) -methanol (24 ml/h) feed; and a final 24-h methanol feed (24 ml/h for the first 6 h and then 48 ml/h for 18 h). The fermentation broth (6.5 liters, 220 g/liter of wet cells) was clarified (4500 × g, 20 min, 4 °C) and the supernatant concentrated and exchanged to 20 mM sodium phosphate, pH 7, by tangential flow filtration (Centramate, 5000 MWCO membrane; Pall Corp.). The sample was fractionated on a XK 26/20 Q-Sepharose HP 80-ml column (GE Healthcare) using a 0–0.5 M NaCl gradient and further purified over a Mono Q 5/5 column (GE Healthcare) using a 0–0.3 M NaCl gradient. After concentration of active fractions (Vivaspin 20, 5000 MWCO; Sartorius), the sample was loaded onto a Superdex 75 HiLoad 26/60 column equilibrated in 20 mM sodium phosphate, pH 7.2, 150 mM NaCl. Positive fractions were analyzed by SDS-PAGE, pooled, and concentrated to 8 mg/ml by ultrafiltration. A predicted A₂₈₀,0.1% 1 cm of 1.3 was used to estimate the protein concentration. The final protein was estimated to be >96% pure by reducing SDS-PAGE (data not shown).

Hemolysis Assays and Serum Hemolytic Activity—Inhibition of human or rat C by pOmCI was assessed by hemolysis assay using antibody-coated sheep erythrocytes and inhibition of mouse C using antibody-coated rabbit erythrocytes as target cells as described previously (21, 22). Final dilutions of sera were as follows: human serum 1/240, rat serum 1/600, mouse serum 1/21.

To determine in vivo C-inhibitory activity in rats (normal and EAMG-induced), 15 mg/kg pOmCI was administered through the tail vein to female Lewis rats (average animal weight 180 g), and blood was collected at specific time points by venesection; a pre-bleed was taken to assess normal hemolytic activity. Serum was harvested and CH50 was determined as...
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Described previously (22). Values are expressed as percent activity compared with pre-bleed. To determine C-inhibitory activity in DBA/1OlaHsd mice (C5-sufficient), the same serum samples taken for the clearance study (below) were used. Restoration of hemolytic activity in mouse serum was assessed as follows: 50-µl aliquot of antibody-coated sheep erythrocytes was incubated with 10 µl of 50% (v/v) C5-depleted human serum (in-house) and 50 µl of 10% (v/v) mouse serum. Cells were incubated at 37 °C for 30 min. Percent lysis was calculated as described previously (22), and values were expressed as percent activity compared with pre-bleed.

Clearance of pOmCI in Vivo—pOmCI (1 mg) was labeled with 125I (MP Biomedicals, Stretton Scientific Ltd, Stretton, UK) using Iodobeads (Pierce) according to the method of Fraker and Speck (23). Labeled protein was isolated from free iodine by gel filtration into PBS using PD10 columns (GE Healthcare, Amersham Biosciences). Five Lewis rats (Charles River, average weight 180 g) were injected through the tail vein with −12 × 106 cpm radiolabeled protein. Blood was collected at specific time points following administration, and protein-bound radioactivity was determined by trichloroacetic acid precipitation as previously described (22). For each time point protein-bound counts/ml of blood were calculated and expressed relative to protein-bound counts at 5 min post-administration as follows: % of 5-min level = 100 × protein-bound counts at time (t)/protein-bound counts at 5 min.

Log of the percentage counts (y-axis) were plotted against time in hours (x-axis). The half-life was calculated from the slope of the linear region of the line: β-half-life = 0.693/−slope (24). Clearance in mice was determined as follows: six 8-week-old DBA/1OlaHsd mice (C5-sufficient) and six DBA/2 OlaHsd mice (C5-deficient; Harlan UK, Bicester, UK) were given 320 µg of pOmCI through the tail vein. Blood (50 µl) was collected by venesection at specific times post-administration. Presence of pOmCI in serum (1 µl) was assessed by Western blot following electrophoresis on a 15% polyacrylamide gel. pOmCI was detected using rabbit polyclonal anti-pOmCI followed by anti-rabbit Ig horseradish peroxidase; blots were developed using ECL (Amersham Biosciences) and captured on autoradiographic film (Kodak).

Surface Plasmon Resonance—All analyses were carried out on a BiaCore T100 (Biacore International AB, Uppsala, Sweden). pOmCI (20 resonance units) was immobilized to a CM5 chip (carboxymethylated dextran surface; Biacore) using amine coupling according to the manufacturer’s instructions (NHS/EDC coupling kit; Biacore). For kinetic analysis the flow rate was maintained at 30 µl/min, and data were collected at 25 °C. Data from a reference cell were subtracted to control for bulk refractive index changes. The R_max was kept low and the flow rate high to eliminate mass transfer. C5 was purified using classical chromatography (25); protein was “polished” by size exclusion chromatography to ensure removal of any aggregates prior to kinetic analysis. Samples were injected using the KINJECT command to ensure accurate association kinetics. Interactions were analyzed in HEPES-buffered saline (10 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% surfactant P20). Data were evaluated using Biaevaluation software (Biacore International AB).

Passive Induction of EAMG—To test therapeutic efficacy of pOmCI in EAMG, 11 female Lewis rats (160–200 g) were obtained from Charles River Laboratories (Margate, UK) and allowed to acclimatize for 1 week. Disease was induced on day zero using 1 mg/kg mAb35 in PBS (intraperitoneal). MAb35, produced against Electrophorus electricus electric organ muscle-type nicotinic AChR, binds the main immunogenic region of the ε subunit of the AChR and cross-reacts with chicken, rat, mouse, and human AChR. Animals were assessed as described previously (16). Six animals were also given pOmCI at a dose of 15 mg/kg intravenously.

Collection and Processing of Tissues for Immunohistochemical Analysis—Soleus muscle was removed, frozen, and sectioned as described previously (12). Sections were fixed in aceton for 5 min and were stained for 1 h at room temperature with primary antibodies (anti-C3c, anti-C9, and anti-CD68) at the stated dilutions in block buffer (PBS, 1% bovine serum albumin). Sections were washed and incubated with appropriate secondary antibodies for 40 min at room temperature. Sections were washed and mounted in VectorShield for analysis using a fluorescent microscope. The number of BuTx-reactive AChR in each section was measured using density slicing in an image analysis system (Openlab Improvisation, Coventry, UK). Twenty fields were captured from comparable regions of muscle in each sample at the same exposure and magnification. For hematoxylin and eosin staining, muscle was post-fixed in 10% formaldehyde in PBS and embedded in paraffin for sectioning and staining. Inflammatory infiltrate was scored blind using a semiquantitative method: 0, no infiltration; 1, up to 20% of whole muscle infiltrated; 2, up to 40% of whole muscle infiltrated; 3, up to 60% of whole muscle infiltrated; 4, over 80% of muscle fibers infiltrated.

RESULTS

Cross-species Activity of pOmCI—Initial analysis of pOmCI indicated that it inhibited the terminal C pathway in both human and guinea pig serum (19). We extended this study of cross-species activity to include rat and mouse serum; comparison with SCR1 was also made. Using classical pathway-mediated lysis of antibody-coated erythrocytes, we show that pOmCI efficiently protected cells against lysis mediated by either rat, mouse, or human serum in vitro (Fig. 1).

Circulating Half-life—pOmCI was labeled with 125I and administered as a single bolus intravenously to Lewis rats. Plasma was harvested at specific times following administration, and protein-bound counts were measured. The calculated β-phase half-life of pOmCI was 30 h (Fig. 2). We have previously shown that small (~30-kDa) recombinant CReg given intravenously to rodents have half-lives of ~20 min3 (22). This

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prolonged half-life of pOmCI suggested that it circulated in plasma as a complex with C5. To determine whether this was the case, pOmCI was given to mice that were either sufficient or deficient in C5. At specific times following administration, plasma was harvested and analyzed for the presence of pOmCI by Western blot (Fig. 3). In C5-deficient animals, pOmCI was rapidly excreted and only trace amounts were apparent in plasma 30 min after administration; thereafter it was not detectable. In the C5-sufficient animals, the pOmCI signal was very strong at 30 min and was still apparent 48 h later, demonstrating that C5 dramatically increased the half-life of this reagent.
**Effect on C Activity in Rodents in Vivo**—pOmCI was administered to rats and mice at a dose of 15 mg/kg. Serum was harvested at specific times, and total C activity (rats) or C5 activity (mice) was determined by hemolysis assay as described under “Experimental Procedures.” Results are expressed as percent activity compared with pre-administration levels. Results are means of determinations from five animals, and vertical bars represent S.D.

**Binding of pOmCI to C5**—The clearance data in C5-deficient and C5-sufficient mice indicated that binding between C5 and pOmCI was of high affinity. To confirm this, surface plasmon resonance was used to characterize the on- and off-rates of the interaction. pOmCI was immobilized on the sensor chip surface and human C5 was flowed across. The $K_D$ of the interaction was $1.85 \times 10^{-8}$ M, and binding kinetics were characterized by a very slow off-rate (Fig. 5). This indicates that, following binding of pOmCI in vivo, a stable complex with C5 will be formed.

**Induction of EAMG and Effect of pOmCI on Clinical Disease**—Rats were given mAb35 at 1 mg/kg, and control animals began to lose weight consistently by 24 h post-induction. In contrast, animals given an initial bolus of pOmCI were stable or continued to gain weight over the time course of the experiment (Fig. 6). Control animals began to display clinical symptoms comprising limp tails, pilo-erection, and reduced grip strength by 30 h post-induction, and all exhibited severe disease with hind limb weakness and/or partial paralysis by 52 h. In contrast, all animals given pOmCI were protected from any clinical manifestations of EAMG for the duration of the experiment. There was no measurable decrease in grip strength, and the general condition remained unaltered (Fig. 6). Control animals were sacrificed at 52 h in accordance with Home Office regulations, and tissue sections were prepared. pOmCI-treated animals...
were monitored for a further 131 h, although one animal was sacrificed at 52 h for comparative histology with the control group. At the end of the experiment, tissue sections were taken from all remaining animals treated with pOmCI.

Hemolytic activity in control and OmCI-treated groups was monitored during the course of the experiment. This had increased in control animals by 2 h post-disease induction, presumably due to the acute phase response, and rose to 175% pre-induction levels by 24 h. In OmCI-treated animals, hemolytic activity was absent for 4 h following disease induction and had started to return by 6 h (14.8%). Levels rose to ~120% of pre-induction levels and remained constant (Table 1).

**TABLE 1**

| Time post-disease induction | Hemolytic activity in control animals | Hemolytic activity in treated animals |
|----------------------------|---------------------------------------|---------------------------------------|
| h | % preinduction level | % preinduction level |
| 0 | 0 | 0 |
| 2 | 113 ± 20.7 | 0.0 ± 0.0* |
| 4 | 129 ± 35.9 | 0.2 ± 0.1* |
| 6 | 143 ± 35.5 | 14.8 ± 5.2* |
| 24 | 175 ± 58.5 | 123.3 ± 18.7 (ns) |
| 52 | 156 ± 45.2 | 126.2 ± 11.1 (ns) |

**Effect of pOmCI on Endplate Integrity and Cellular Infiltration in EAMG**—Following sacrifice, AChR in muscle sections were stained with α-BuTx-rhodamine and receptor numbers were quantitated in an automated system in 20 different representative fields. The number of AChR was significantly decreased in control animals compared with pOmCI-treated animals (Fig. 7a). Immunohistochemical analysis of C3 and C9 deposition at the endplate demonstrated marked deposition at the AChR in control animals at 52 h (Table 2; Fig. 7b). Immunohistological analysis and hematoxylin and eosin staining also demonstrated high levels of cellular infiltration in untreated animals. In contrast, whereas C3 and C9 deposition was visible in treated animals at sacrifice, there was less staining of activated C and cellular infiltration was minimal (Table 2). Analysis at 52 h indicated that C3 was deposited at the neuro-muscular junction (NMJ) but only trace C9 at this time point; there was no evidence of cellular infiltration.

**DISCUSSION**

We describe here the cross-species activity of recombinant soft tick-derived *O. moubata* C5 inhibitor (pOmCI) and examine its potential as an anti-C therapeutic agent. Using hemolysis assays we have demonstrated that pOmCI is a potent inhibitor of rat, mouse, and human C (Fig. 1). This opened up the possibility to use the reagent in rodent models of C-mediated disease. Inhibition of C by pOmCI differed markedly from other currently available agents, such as...
sCR1, in that the inhibition curve was very steep. Plasma C5 is not a limiting agent in C activation, and the kinetics of inhibition by pOmCI indicated that effective inhibition was achieved at a threshold concentration where available C5 was saturated with reagent. Following correction for the dilution of serum used in the human serum hemolytic assay (Fig. 1a), 100% inhibition was obtained at an equivalent of 0.76 μM pOmCI (14.0 μg/ml) and 92% inhibition obtained at 0.5 μM (9.4 μg/ml). This concentration range correlates very well with known human C5 plasma concentration of ~0.38 μM, suggesting tight 1:1 binding between C5 and pOmCI. The broad species cross-reactivity indicates that pOmCI binds to a site that is well conserved between C5 and pOmCI. The broad species cross-reactivity and either activated C3 or C9. Deposition of C3 and C9 was scored as follows: 0, no infiltration; 1, up to 20% of whole muscle infiltrated; 2, up to 40% of whole muscle infiltrated; 3, up to 60% of whole muscle infiltrated; 4, over 80% of muscle fibers infiltrated.

We have previously shown that small (~35-kDa), recombinant CReg have a very short half-life in vivo, ~20 min (22). When we administered radiolabeled pOmCI intravenously to rats, following an initial rapid elimination of protein, the β phase half-life was measured as 30 h (Fig. 2). This was far longer than agents of similar size and compared well to other agents with deliberately extended half-lives such as CReg-Ig fusion proteins that have a half-life of 30–40 h (22, 26). We reasoned that the prolonged half-life was due to stable binding to C5 in the circulation, and this was confirmed in mice by analyzing clearance in C5-sufficient and -deficient animals (Fig. 3). In the absence of C5, pOmCI was rapidly cleared from plasma. In the presence of C5, the half-life was prolonged and reagent was detectable 48 h later. The data suggest more rapid clearance in mice compared with rats, perhaps indicating a weaker affinity of pOmCI for murine C5. In vitro kinetic analysis of the binding interaction between human C5 and pOmCI using surface plasmon resonance (Biacore) supported a 1:1 binding interaction and demonstrated an affinity comparable with that of antibody/antigen interactions with a very slow decay (Fig. 5). This indicated that once the complex formed in plasma it would be very stable and its half-life would be influenced by that of native C5, reported in humans as 63 h (27). The rapid elimination of pOmCI immediately post-administration was probably due to clearance of uncomplexed reagent (Fig. 3, top panel). The effect of pOmCI on plasma hemolytic activity was dramatic, with no hemolytic activity being demonstrable for several hours following administration (Fig. 4). However, hemolytic activity was detected 4–6 h after administration and was back to normal levels 24–48 h later. As C5 is not limiting in the C cascade, the return of hemolytic activity must represent production of new C5; the synthesis rate in humans is 90 μg/kg/h (27). As unbound pOmCI was cleared rapidly from the circulation, there was no free agent remaining to bind newly synthesized C5.

The therapeutic effect of pOmCI in rat EAMG was dramatic, with treated rats continuing to gain weight during the normal course of disease and demonstrating no clinical score. This dramatic effect, despite the fact that C was inhibited for less than 24 h, correlates with previous data using scFv and confirms that most of the damage in this model occurs in the first few hours post-induction (28). The initial bolus of pOmCI was effective at protecting animals during the most aggressive stage of disease and prevented disease perpetuation presumably as tissue was not damaged. At sacrifice (52 h), control animals had marked C3 and C9 deposition at the NMJ and marked inflammatory infiltrate (Fig. 7b, Table 2). One treated animal was also analyzed at this time point. There was no inflammatory infiltrate, but there was evidence of C3 deposition and trace C9 deposition. At sacrifice (183 h), four of the remaining five treated animals had trace C3 and C9 deposition at the NMJ and very little inflammatory infiltrate. Significantly, at 52 and 183 h AChR numbers were high in treated animals, indicating no loss or internalization of receptor. pOmCI inhibits the terminal pathway, and C3 deposition was therefore expected in treated animals. However, C3 deposition levels were lower in pOmCI-treated animals than controls, indicating that tissue damage caused by MAC formation and cellular infiltrates may have contributed to C activation and subsequent deposition of C3. Previous data demonstrate that pOmCI does not bind C3, and C activation as a result of tissue damage is well described (19, 29). pOmCI inhibits production of C5a, a powerful chemoattractant, and should therefore decrease cellular infiltration, an effect that we observed here (Table 2). C9 deposition was evident in treated animals, again reflecting restoration of the terminal pathway following C5 synthesis; this was not sufficient to produce clinical disease. During the course of EAMG we measured CH50 in control and treated animals (Table 1). Because of the acute phase response, C activity increased dramatically compared with pre-disease levels; at 6, 24, and 52 h post-disease induction CH50 in control animals was 143, 175, and 156% that of pre-induction levels, respectively, with the peak of activity being at 24 h. Despite the acute phase response pOmCI was effective at preventing clinical disease.

These data illustrate the therapeutic potential of pOmCI in acute disease. There is a huge market for short-term C inhibition, such as in stroke, myocardial infarction, and cardiopulmonary bypass (7). Anti-C5 therapy using scFv derived from antibody reactive with C5 has shown enormous potential in these

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**TABLE 2**

**Histological analysis of NMJ in soleus muscle postmortem**

Sections were double-stained for AChR (rhodamine-conjugated α-bungarotoxin) and either activated C3 or C9. Deposition of C3 and C9 was scored as follows: −, negative; +/−, trace; +, weak; ++, strong. Infiltration was assessed following hematoxylin and eosin staining, and infiltrating macrophages were detected by immunofluorescence using ED1 (anti-CD68). Infiltration was scored as follows: 0, no infiltration; 1, up to 20% of whole muscle infiltrated; 2, up to 40% of whole muscle infiltrated; 3, up to 60% of whole muscle infiltrated; 4, over 80% of muscle fibers infiltrated.

| Group               | C3 deposition | C9 deposition | Infiltration (hematoxylin and eosin) | Infiltration (ED1 staining) |
|---------------------|---------------|---------------|--------------------------------------|----------------------------|
| PBS PBS control (52 h) |
| 1                   | −/−           | +             | 2                                    | 2–3                       |
| 2                   | ++            | −             | 2                                    | 2–3                       |
| 3                   | ++            | +             | 3                                    | 3                         |
| 4                   | ++            | +/−           | 3                                    | 2                         |
| 5                   | +             | +/−           | 3                                    | 2–3                       |
| PBS PBS control (52 h) |
| 1                   | −/−           | +             | 1                                    | 0–1                       |
| 2                   | +             | −             | 1                                    | 0–1                       |
| 3                   | +/−           | +             | 1                                    | 0–1                       |
| 4                   | +/−           | +/−           | 1                                    | 0–1                       |
| 5                   | +             | +/−           | 1                                    | 0–1                       |
| PBS PBS control (52 h) |
| 1                   | −/−           | +             | 0                                    | 0–1                       |

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areas with decreased patient mortality (9, 30, 31). Long-term inhibition using intact anti-C5 antibody has shown promise in paroxysmal nocturnal hemoglobinuria, with hemolysis and transfusion dependence being dramatically reduced (10, 32). A possible drawback to long-term use of pOmCI is immunogenicity of the foreign protein in rodents or humans. However, tick salivary proteins have evolved to be poorly immunogenic; it is not in the individual or collective interests of the tick if an immune response is mounted. It takes 2 weeks to complete a blood meal and in larger animals, such as cattle, thousands of ticks can infest a single animal. In support of this, we have administered pOmCI daily to rats (intravenously) and monitored their specific IgM levels after 7 days. No immune response was mounted in six animals tested (data not shown).

Therapeutic inhibition of the C system at the terminal pathway stage is an attractive proposition as important roles of C in immune complex solubilization and opsonization are not inhibited. Humans who lack a functional terminal pathway because of a genetic defect are usually healthy, although they can show an increased tendency for Neisserial infections (33). In addition to acute conditions such as myocardial infarction and cardiopulmonary bypass, the terminal pathway has been implicated in pathology of various chronic diseases, including paroxysmal nocturnal hemoglobinuria, multiple sclerosis, and myasthenia gravis. The therapeutic efficacy of pOmCI that we have demonstrated here highlights its potential in acute disease. Further development of pOmCI by engineering a molecule with an extended circulating half-life to bind and inactivate C5 as it is synthesized will permit its use in both acute and chronic disease and will be of major therapeutic potential in the clinic.

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