A soft tick *Ornithodoros moubata* salivary protein OmCI is a potent inhibitor to prevent avian complement activation

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Abstract (221 words)

Complement is a key first line innate host defense system in the blood of vertebrates. Upon activation, this powerful defense mechanism can elicit inflammatory responses, lyse non-self-cells, or mark them for opsonophagocytic removal. Blood-feeding arthropods thus require the ability to block host complement activation in the bloodmeal to prevent undesired cell or tissue damage during feeding. The soft tick *Ornithodoros moubata* produces a complement inhibitory protein, OmCI. This protein binds to a mammalian complement protein C5 and blocks further activation of complement cascades, which results in the prevention of complement-mediated bacterial killing through membrane attack complex. Interestingly, the amino acids involved in OmCI binding are highly conserved among mammalian and avian C5, but the ability of this protein to inhibit the complement from birds remains unclear. Here we demonstrated that OmCI is capable of preventing quail complement-mediated erythrocyte lysis, inhibiting the capability of this animal's complement to eliminate a serum-sensitive Lyme disease bacterial strain. We also found that the ability of OmCI to inhibit quail complement-mediated killing of Lyme disease bacteria can be extended to different domestic and wild birds. Our results illustrate the utility of OmCI to block bird complement. These results provide the foundation for further use of this protein as a tool to study the molecular basis of avian complement and pathogen evasion to such a defense mechanism.

Key words: Bacterial killing; Lyme borreliae; OmCI; Avian complement

1. Introduction

The complement system is composed of a group of proteins, that function as the first line of host defense in the blood of vertebrates in response to invading pathogens (Meri, 2016; Trouw et al., 2017; Zipfel and Skerka, 2009). Complement is activated on the surface of the pathogen by three pathways: the classical, lectin, and alternative pathways (Meri, 2016; Trouw et al., 2017; Zipfel and Skerka, 2009). Activation of the classical pathway (initiated by antibody-bacterial antigen complexes) and the lectin pathway (initiated by lectin-microbial carbohydrate complexes) results in the formation of C3 convertase, C4b2a. Activation of the alternative pathway (initiated by the interaction of C3b with the microbial surface) triggers the formation of C3 convertase, C3bBb. These C3 convertases recruit the complement protein C3b; addition of a C3b molecule to C3 convertases results in formation of the C5 convertases, C4b2a3b, and C3bBb3b. The C5 convertases then cleave C5 to C5a and C5b to release the anaphylatoxin C5a that attracts neutrophils, monocytes, and mast cells to induce inflammatory responses. C5b assembles with C6, C7, C8, and C9, resulting in the formation of a pore-forming membrane attack complex (MAC or C5b-9) on the pathogen surfaces to cause lysis.

When complement is not properly controlled, those complement complexes can be formed on the surface of host cells, leading to tissue destruction (Sjoberg et al., 2009). Numerous complement inhibitory molecules including synthetic compounds, monoclonal antibodies, and prokaryotic or eukaryotic proteins inhibit different steps of the complement cascades (Morgan and Harris, 2015; Ricklin et al., 2016). Some of these molecules have been used or are under development as therapeutics to treat diseases caused by unregulated complement activation (Morgan and Harris, 2015; Ricklin et al., 2016). However, because of variation of complement proteins across different hosts, most of the complement inhibitory molecules developed to target

human complement are human- or primate-specific (Jore et al., 2016; Kai et al., 1983). With the exception of mice, the investigation of complement-associated mechanisms in non-mammalian hosts is very limited.

One such complement inhibitory molecule is OmCI (also known as conversin), a 17-kDa protein that was initially isolated from the saliva of a soft tick species, *Ornithodoros moubata* (Nunn et al., 2005). This protein is believed to protect tick cells/tissues from destruction by host complement attack during blood feeding and promote survival of the pathogens carried by ticks at the stage of transmission (Fredslund et al., 2008). OmCI binds to C5 and prevents its cleavage by C5 convertases (Fredslund et al., 2008; Hepburn et al., 2007; Jore et al., 2016), which renders tissue damage or pathogen killing by complement of mice, rats, guinea pigs, pigs, and humans (Barratt-Due et al., 2013; Barratt-Due et al., 2011; Garcia et al., 2013; Hepburn et al., 2007; Jore et al., 2016; Nunn et al., 2005). Therefore, OmCI is currently under clinical trials for its use in human complement inhibitory therapy and has been commonly utilized to investigate the C5-mediated complement activation in mammalian hosts (Barratt-Due et al., 2013; Berends et al., 2015; Blom et al., 2014; Fluiter et al., 2014; Garcia et al., 2013; Kuhn et al., 2016; Macpherson et al., 2018; Pischke et al., 2017). Interestingly, the residues in human C5 involved in OmCI binding bear close identity to C5 from avian and mammalian hosts (Jore et al., 2016). This finding raises the possibility that OmCI can inhibit the complement from non-mammalian hosts such as Aves. In this study, we tested the ability of OmCI to inhibit avian complement-mediated hemolysis and have used the Lyme disease bacterium as an example to study the consequences of blocking avian-mediated bacterial killing.

2. Material and Methods

2.1 Birds and bacterial strains

American robins (*Turdus migratorius*) and Gray catbirds (*Dumetella carolinensis*) were mist netted on Block Island, RI from June-August, 2016. Serum was collected from 14 birds (*n* = 6 robins; *n* = 8 catbirds) using BD Microtainer Capillary Blood Collector tubes (Fisher Scientific, Hampton, NH). The *Borrelia burgdorferi sensu stricto (B. burgdorferi)*, *Borrelia duttonii*, and *Escherichia coli* strains used in this study are described in Table S1. The *E. coli* strain BL21 (DE3) was grown in Luria-Bertani broth or agar (BD Bioscience, Franklin lakes, NJ), supplemented with antibiotics as described previously (Nazarova and Avaeva, 1973). All *B. burgdorferi* and *B. duttonii* strains were grown in BSK-II completed medium with no antibiotics (Barbour, 1984).

2.2 The production of OmCI.

The recombinant OmCI protein was purified in a similar manner as previously described (Blom et al., 2014). Briefly, the open reading frames lacking the putative signal sequences of *omcI* with TEC-His6 sequences were codon optimized and synthetized (gift of Dr. Strömberg, SOBI, Sweden) and cloned into pET16b vector (Novagen, Merck). The resulting plasmid was transformed into *E. coli* strain BL21 (DE3), which was then induced with IPTG for 3h at 30°C. The harvested cells were then suspended into PBS buffer containing lysozyme and then lysed by sonication. Subsequently, the inclusion bodies were isolated by centrifugation and then solubilized in 6 M guanidine hydrochloride in 20 mM Tris-HCl, pH 8.0 and 10 mM reduced glutathione. The protein was then dialyzed overnight against 20 mM Tris-HCl pH 8.0. The refolded OmCI was then applied to Ni-NTA column (Qiagen) and eluted using 20 mM Tris,

pH 7 with 700 mM imidazole. The pooled fractions with OmCI were then dialyzed against 50 mM Tris-HCl at pH 8.0 with 150 mM NaCl, and stored at −70 °C.

2.3 Three-dimensional structure modeling of quail C5d

The structure of C5d from *Coturnix* quail was modelled according to the crystal structure of Human C5d (PDB ID: 5I5K) using the Swiss-Model protein modelling server as described (Arnold et al., 2006). The structure of C5d from *Coturnix* quail is considered as a high-quality model as the amino acid identity of C5d from human and quail is 55.54 %.

2.4 Hemolytic assays.

The procedure for hemolytic assays was modified from a previously described protocol (Jore et al., 2016). Sheep red blood cells previously sensitized by incubating with anti-sheep red blood cell stroma antibody in $GVB2^{+}$ buffer (142 mM NaCl, 50 mM Sodium 5,5-diethylbarbiturate, 0.1 % gelatin, 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.35) were obtained from Diamedix (Miami Lakes, FL). Next, the cell suspension was incubated with 5 % of serum from humans (Complement technology, Inc, Tyler, TX) or *Coturnix* quail (Canola poultry market, Brooklyn, NY) as well as different concentrations of OmCI (2×10^{-11} to 1.6×10^{-6} M) at 37 °C for 1 h. Subsequently, 100 µl of PBS was added, and the supernatant collected by centrifugation was transferred to a 96-well ELISA plate. Plates were read at 405 nm using a Tecan Sunrise Microplate reader. The supernatant from the cells with $GVB2^{+}$ buffer instead of serum was included as control (no lysis). The supernatant from the cells suspended in water was a positive control used for normalization (100 % lysis). To determine the concentration of OmCI that inhibits 50 % levels of erythrocyte lysis (IC_{50}) in the presence of serum, the data

points were fitted using nonlinear regression methods by GraphPad Prism software (Version 7, La Jolla, CA).

2.5 Serum resistance assays.

Serum resistance of *B. burgdorferi* strains B313 and B31-5A4 and *B. duttonii* strains V and LA1 was determined as described previously (Hart et al., 2018; Marcinkiewicz et al., 2019). Briefly, the mid-log phase of *B. burgdorferi* or *B. duttonii* strains were cultivated in triplicate. The resulting spirochete culture was diluted to a final concentration of 5×10^6 bacteria per milliliter into BSKII medium without rabbit serum. The cell suspensions were then mixed with 40 % of the normal serum from human, *Coturnix* (quail), *Gallus gallus* (chickens) (Biowest, Riverside, MO), *Anser anser* (geese) (BioIVT, Hicksville, NY), *Meleagris gallopavo* (turkey) (BioIVT), *Turdus migratorius* (American robins) or *Dumetella carolinensis* (Gray catbirds) in the presence or absence of OmCI. We also included spirochetes mixed with 40 % heat-inactivated (55 ºC for 2 h) serum from these animals as negative controls for complement-mediated killing. OmCI was used from 0.07 to 6.66 µM for human and quail serum and at 2 µM for sera from chickens, geese, turkey, American robins, or Gray catbirds. Note that the concentrations of OmCI were greater in this assay, compared to that in hemolytic assays described in section 2.4 because the amount of sera applied to this assay (40 % of sera) is higher than that in the hemolytic assays (5 %). At 0 and 4 h after the incubation with serum, an aliquot was taken from each condition and counted using a Petroff-Hausser counting chamber (Hausser Scientific, Horsham, PA) and a Nikon Eclipse E600 darkfield microscope (Nikon, Melville, NY). We determined the percentage of surviving spirochetes by two independent approaches, motile spirochete measuring and Live/Dead staining. For motile spirochete

158 measuring, the number of motile spirochetes at 0 and 4 h post incubation with sera was counted
159 under microscopy as described (Hart et al., 2018). For Live/Dead staining, spirochetes
160 immediately after mixed with sera or incubated with sera for 4h were treated for 15 min with
161 1× SYBR Green I (ThermoFisher) and 6 μM of propidium iodide (ThermoFisher) in 0.5 %
162 BSA in PBS as described (Feng et al., 2014; Marcinkiewicz et al., 2019). The live (green) and
163 dead (red) spirochetes were visualized by overlaid FITC and Texas Red filters using an
164 Olympus BX51 fluorescence microscope (Olympus Corporation, Waltham, MA). We then
165 calculated the number of live spirochetes at 4 h post incubation normalized to that immediately
166 after incubation with serum. Additionally, we determined the concentration of OmCI required
167 to inhibit killing by human or quail serum via 50 % (IC_{50}) fitting the data points using
168 nonlinear regression methods from GraphPad Prism 7.0 software (GraphPad Software, Inc.,
169 San Diego, CA).
170
171 **2.6 Statistical analysis.**
172 Significant differences between samples were determined using a one-way ANOVA with
173 post hoc Dunn's test using GraphPad Prism 7.0 Software. P-values were determined for each
174 sample. A P-value < 0.05 (*) was considered to be significant.
175
176 **3. Results**
177 **3.1 OmCI inhibits the ability of quail complement to lyse red blood cells.**
178 The high resolution structure of OmCI with human C5 indicates the amino acids on C5d
179 fragment involved in the binding to OmCI, and these residues are conserved across different
180 mammalian species (Fig. 1A) (Jore et al., 2016). We observed that these amino acids are also

181 highly conserved in C5 from multiple avian hosts including quail and chicken (Fig. 1A).
182 Similarly, when building the tertiary structure modelling of quail C5d using human C5d as a
183 template, the topography of the amino acids from human C5d critical for OmCI-binding is
184 nearly identical to that of the corresponding residues from quail C5d (Fig. 1B). These results
185 raise the possibility that OmCI may inhibit avian complement. We thus used quail serum as a
186 model to evaluate this protein's ability to inhibit quail complement-mediated erythrocyte lysis.
187 Antibody-sensitized sheep erythrocytes were incubated with sera from quail (or humans, as a
188 positive control) containing different concentrations of recombinant OmCI. In the absence of
189 OmCI, close to 100 % of the erythrocytes incubated with human serum were lysed whereas red
190 blood cells in PBS buffer (control) displayed undetectable lysis (Fig. 2). The addition of OmCI
191 to serum reduced the percent lysis of those cells in a dose dependent manner (IC_{50}= 6.80 ± 1.07
192 nM) (Fig. 2), consistent with the ability of OmCI to inhibit human complement (Nunn et al.,
193 2005). Quail serum added to erythrocytes in the absence of OmCI resulted in more than 90 %
194 lysis (Fig. 2). However, OmCI inhibited sheep red blood cell lysis in a dose-dependent manner,
195 suggesting that this protein inhibits quail complement (IC_{50}= 7.15 ± 0.61 nM) (Fig. 2).
196
197 **3.2 OmCI reduces the capability of the quail serum to eradicate a serum sensitive *B.***
198 ***burgdorferi* strain.**
199 We next verified the ability of OmCI to inhibit human complement-mediated bacterial
200 killing. Calculating the number of motile cells and determining the live cells by Live/Dead
201 staining using dark field microscope are two most common approaches to measure
202 complement's ability to kill bacteria. We thus initially utilized a Lyme disease causing
203 bacterium, *B. burgdorferi*, as a model bacterium to verify the ability of OmCI in inhibiting

spirochete killing by complement. These sera at a final concentration of 40 % containing different concentrations of OmCI were incubated with a high passage, non-pathogenic, and serum sensitive *B. burgdorferi* strain B313. We also included a low passage, pathogenic, and serum resistant *B. burgdorferi* strain B31-5A4 as a control. When measuring spirochete viability by counting the percentage of motile bacteria under microscopy, we found that more than 95 % of strain B31-5A4 survives in untreated or heat-treated human sera, independent on the presence of OmCI (Fig. S1). Similarly, close to 95 % of strain B313 was detected motile in heat-treated human sera, in the presence or absence of OmCI (Fig. S1). Though greater than 95 % of this strain was motile in untreated human sera in the presence OmCI, merely 30 % of strain B313 were motile in untreated human sera in the absence of OmCI (Fig. S1). These results indicate that OmCI inhibits human complement-mediated bacterial killing. Further, there was no statistical difference in percent survival determined by Live/Dead staining, compared to that by calculating motile spirochete using dark field microscopy (Fig. S1). Though there is a caveat that non-motile bacteria may still be alive, our results suggest that the viability and motility of Lyme borreliae in this experimental setup are nearly equivalent. As calculating the percentage of motile spirochete was a state-of-the-art approach to quantitatively determine Lyme borreliae viability (Garcia et al., 2016; Hart et al., 2018; Marcinkiewicz et al., 2017; Marcinkiewicz et al., 2019; Wang et al., 2002), we evaluated spirochete survival in the following work using this approach.

We next compared the efficacy of OmCI in inhibiting bacterial killing mediated by quail and human sera (control) by mixing spirochetes with each of these sera in the presence of different concentrations of OmCI. In the absence of OmCI, more than 90 % of strain B31-5A4 survived in either untreated or heat-treated human serum, consistent with previous studies (Hart

et al., 2018; Marcinkiewicz et al., 2019) (Fig. 3A, C, and E). The presence of OmCI in sera did not impact survival (Fig. 3A, C, and E). Similarly, strain B313 showed approximately 98 % survival in heat-treated human serum, which was not altered by the addition of OmCI (Fig. 3A, C, and E). However, less than 20 % of this strain survived in untreated human serum in the absence of OmCI; survival of B313 increased in the presence of increasing concentrations of OmCI (IC_{50}= 3.78 ± 1.71 μM) (Fig. 3A, C, and E). These results indicate that OmCI can block human serum-mediated killing of a serum sensitive Lyme borreliae strain. Furthermore, close to 95 % of strain B31-5A4 was found to survive in either untreated or heat-treated quail serum in the absence of OmCI, and this survival was independent of the presence of OmCI (Fig. 3B, D, and F). Close to 100 % of strain B313 was viable in the heat-treated quail serum whether or not OmCI was present (Fig. 3B, D, and F). Conversely, only 25 % of this strain remained motile in untreated quail serum in the absence of OmCI, in agreement with previous findings (Hart et al., 2018; Marcinkiewicz et al., 2019) (Fig. 3B, D, and F). The addition of OmCI increased survival of strain B313 in a dose dependent manner. (Fig. 3B, D, and F). These findings indicate that OmCI prevents killing of a serum sensitive Lyme borreliae strain by quail complement.

3.3 OmCI inhibits the killing of a serum sensitive *B. burgdorferi* strain by the sera from different domestic and wild birds.

To examine if OmCI can block complement-mediated bacterial killing of a serum sensitive *B. burgdorferi* strain by sera from additional avian hosts, we tested sera from domestic birds including chicken, geese, and turkey, and wild Aves including American robins and Gray catbirds. Sera samples from these species were serologically verified for non-infectious status

of Lyme disease bacteria as described (data not shown) (Hart et al., 2018; Marcinkiewicz et al., 2019). We then incubated each of these avian sera with *B. burgdorferi* strains B31-5A4 or B313 in the presence or absence of OmCI. In the absence of OmCI, greater than 90 % of the strain B31-5A4 survived in either untreated or heat-treated serum from all tested avian hosts (Fig. 4A to F, left panel); survival was not altered by the presence of OmCI (Fig. 4A to F, left panel). The results derived from quail sera in Fig. 3 were included as control (Fig. 4A). Though close to 100 % of strain B313 survived in heat-treated avian sera, less than 20 % of this strain was found motile in the untreated sera from each tested avian host. These results suggest the inability of strain B313 to evade avian complement (Fig. 4, right panel). The presence of OmCI in untreated sera permitted greater than 90 % survival of strain B313 (Fig. 4, right panel). As expected, bacteria survived greater than 90 % in heat treated sera and were not affected by the presence of OmCI. Note that the relapsing fever spirochete *Borrelia duttonii* can be transmitted from *Ornithodoros moubata* ticks to humans or potentially to chicken (Elbir et al., 2013; McCall et al., 2007). We thus also included two *B. duttonii* strains in this study and found that both strains survived in human or chicken sera at levels of nearly 100 %, independent of heat or OmCI treatment (Fig. S2). As no serum sensitive *B. duttonii* strains are currently available, our finding strongly suggests the need of using a serum sensitive *Borrelia* strain (e.g. *B. burgdorferi* B313) as a tool to examine OmCI-mediated inhibition of bacterial killing. Taken together, using *B. burgdorferi* B313 as a model, we showed that OmCI can block complement-dependent bacterial killing in the sera of a variety of Aves.

4. Discussion

Complement is a pivotal host innate immune defense mechanism in the vertebrates' blood to eradicate the non-self-cells (Meri, 2016; Trouw et al., 2017; Zipfel and Skerka, 2009). Blood-feeding arthropods such as ticks generate a variety of proteins in their saliva to prevent damage to their own cells caused by attack from host complement in a blood meal (Chmelar et al., 2016a; Chmelar et al., 2016b; Francischetti et al., 2009; Nuttall, 2019; Valenzuela et al., 2000). One of these proteins, OmCI, inhibit complement from diverse mammalian species, which prevents tissue damage caused by complement activation (Fluiter et al., 2014; Pischke et al., 2017; Roversi et al., 2013). Unlike other tick salivary proteins that display mammalian or primate-specific complement inhibition (Jore et al., 2016), we found that OmCI prevents erythrocyte lysis mediated avian complement. OmCI is produced by *O. moubata* ticks, which feed on humans and domestic and wild swine, as well as poultry such as chickens (Elbir et al., 2013; McCall et al., 2007). Our results thus support the possibility that OmCI prevents potential tissue damage during blood feeding of ticks on avian hosts, illustrating the co-evolution between a tick salivary protein and the hosts that the tick parasitizes. Further, *O. moubata* can carry and transmit pathogens, including *B. duttonii* and African Swine Fever viruses (Burrage, 2013; Dixon et al., 2019; Dworkin et al., 2008; Talagrand-Reboul et al., 2018). Thus, there is a possibility that OmCI facilitates the transmission of these pathogens from ticks to vertebrates by inhibiting complement-mediated bactericidal activity (Nunn et al., 2005). However, no serum sensitive and *O. moubata*-transmitted pathogens were reported, indicating the limitations of using these pathogens to test such a possibility. Furthermore, some of *O. moubata*-transmitted pathogens including *B. duttonii* use OmCI-independent strategies to evade complement (e.g. producing complement inhibitory proteins) (Arias et al., 2017; Hovis et al., 2004; Meri et al., 2006;

294 Rossmann et al., 2007). This caveat increases the complexity to delineate the phenotypes of these
295 pathogens' tick-to-host transmission conferred by OmCI, which warrants further investigations.
296 Numerous anti-complement proteins have been identified to inhibit mammalian complement,
297 allowing them to be used as tools to tackle the molecular mechanisms of human complement
298 activation or a pathogen's complement evading strategies (Morgan and Harris, 2015; Ricklin et
299 al., 2016). However, the sequences of complement proteins varying among different vertebrates
300 often render the possibility in using those anti-complement proteins to investigate the functions
301 of non-mammalian complement (Kai et al., 1983; Kai et al., 1985; Mavroidis et al., 1995; Vogel
302 and Muller-Eberhard, 1985). Many microbes such as Lyme borreliae survive in bird sera or can
303 be carried by Aves, highlighting the need to identify a tool to study avian complement evasion of
304 these microorganisms (Kurtenbach et al., 2002; Tufts et al., 2019). We demonstrated that OmCI
305 blocks complement from avian hosts and promotes the survival of a serum sensitive Lyme
306 borreliae strain in the sera from different domestic and wild birds. These findings thus illustrate
307 an innovative use of OmCI and this bacteria strain as a model to perform mechanistic study of
308 avian complement activation and pathogen evasion to that complement.
309
310 **5. Conclusions**
311 In this study, we demonstrated that a tick salivary protein, OmCI, can inhibit not only
312 human but also avian complement-mediated hemolysis. We further used Lyme disease bacteria
313 as a model to demonstrate that OmCI-mediated avian complement inhibition facilitates bacteria
314 evading the complement-mediated killing. It is noteworthy that *B. burgdorferi* is transmitted by
315 the hard tick species *Ixodes* rather than the soft tick *O. moubata*, from which OmCI was
316 derived (Radolf et al., 2012; Steere et al., 2016). Thus, our observation here does not suggest

the role of this protein to facilitate the transmission of Lyme disease bacteria from *O. moubata* ticks to avian hosts. Instead, the information derived from this study provides a useful tool to inhibit avian complement, which could contribute to the novel insights into bird innate immune defense mechanisms and pathogens' ability to evade bird complement.

Abbreviations

OmCI: *Ornithodoros moubata* complement inhibitor; B31-5A4: *Borrelia burgdorferi* strain B31-5A4; B313: *Borrelia burgdorferi strain* B313; MAC: Membrane attack complex; IC_{50}: 50 % levels of lysis or bacterial killing.

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Ethics statement

The experiments involved in collecting serum from wild birds (American robins and catbirds) were performed in strict accordance with all provisions of the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the PHS Policy on Humane Care and Use of Laboratory Animals. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Columbia University (Protocol number AC-AAAS5402). The bleeding and banding permits were approved by US Fish and Wildlife (Permit number MB122969-1). All efforts were made to minimize animal suffering.

Declarations of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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514 **Figure captions**

515 **Figure 1. Comparison of primary and tertiary structure of OmCI-binding site on C5**
516 **among avian and mammalian hosts. (A)** Shown is the alignment of partial amino acid
517 sequences of C5 from *Peromyscus leucopus* mouse (white-footed mouse), *Mus musculus*
518 mouse (house mouse), rat, human, rabbit, horse, pig, chicken, and quail analyzed by ClustalW.
519 The amino acids of human C5 that make contact with OmCI in the crystal structures shown
520 previously (Jore et al., 2016) are highlighted by red squares. **(B)** The crystal structure of human
521 C5d (5I5K, residues 932-1372 of human C5) is shown in gray ribbons, and the amino acids
522 involved in binding to OmCI are shown and labeled. The modelled structure of *Coturnix* quail
523 C5d is indicated (residues 930-1370 of quail C5) as purple ribbons. The quail C5d amino acids
524 corresponding with the residues of human C5d that bind to OmCI are shown and labelled.
525 Graphics were generated using Swiss PDB Viewer (Guex and Peitsch, 1997). The amino acids
526 labelled in yellow are conserved between human and quail C5, whereas the amino acids that
527 vary between these animals' C5 are shown in red.

528

529 **Figure 2. OmCI inhibits the ability of human and quail complement to initiate antibody-**
530 **mediated erythrocyte lysis.** Sheep erythrocytes previously treated with anti-erythrocyte
531 antibody were incubated with indicated concentrations of OmCI as well as human or quail
532 serum with a final concentration as 5 % or PBS buffer (control) for 1 h. The levels of
533 erythrocyte lysis were evaluated by measuring the absorbance at 405 nm of the supernatant
534 from the reactions. The absorbance values at 405 nm of each reaction were normalized to that
535 obtained from incubating these erythrocytes with water in the absence of OmCI. The work was
536 performed on three independent experiments; within each experiment, samples were run in
537 triplicate. The result shown here is the experiment **(A)** 1, **(B)** 2, and **(C)** 3 from the average

538 percent lysis ± standard deviation of three replicates in each experiment. The concentration of
539 OmCI to inhibit 50 % of erythrocyte lysis (IC_{50}) in the presence of human (blue) and quail
540 (red) serum was obtained by fitting the data points using nonlinear regression methods (IC_{50}=
541 6.80 ± 1.07 nM for human serum, IC_{50}= 7.15 ± 0.61 nM for quail serum).
542
543 **Figure 3. OmCI inhibits the ability of human and quail serum to kill a serum sensitive *B.***
544 ***burgdorferi* strain in a dose dependent manner.** A low passage, infectious, and serum
545 resistant *B. burgdorferi* strain B31-5A4 ("B31-5A4") or a high passage, non-infectious, and
546 serum sensitive *B. burgdorferi* strain B313 ("B313") was incubated for 4h with indicated
547 concentrations of OmCI as well as **(A, C, and E)** human or **(B, D, and F)** quail serum with a
548 final concentration of 40 %. Heat-inactivated human or quail serum was included as controls.
549 The number of motile spirochetes was then assessed microscopically. The percentage of
550 survival for those *B. burgdorferi* strains was calculated using the number of mobile spirochetes
551 at 4 h post incubation normalized to that prior to the incubation with serum. The work was
552 performed on three independent experiments; within each experiment, samples were run in
553 triplicate. The result shown here is the experiment **(A and B)** 1, **(C and D)** 2, and **(E and F)** 3
554 from the average survival percentage ± standard deviation of three replicates in each
555 experiment. The concentration of OmCI to inhibit 50 % levels of serum killing (IC_{50}) was
556 obtained by fitting the data points using nonlinear regression methods (IC_{50}= 3.78 ± 1.71 μM
557 for human serum, IC_{50}= 2.94 ± 0.83 μM for quail serum).
558
559 **Figure 4. OmCI reduces the ability of serum from domestic and wild avian hosts to**
560 **eradicate a serum sensitive *B. burgdorferi* strain.** A low passage, infectious, and serum
561 resistant *B. burgdorferi* strain B31-5A4 ("B31-5A4") or a high passage, a non-infectious, and

562 serum sensitive *B. burgdorferi* strain B313 ("B313") was incubated for 4h with the serum from
563 **(A)** *Coturnix* quail ("quail"), **(B)** chicken, **(C)** geese, **(D)** turkey, **(E**) American robins
564 ("robins"), or **(F**) Gray catbirds ("catbirds") at a final concentration of 40 % in the presence
565 ("OmCI-serum") or absence ("serum") of 2 μM of OmCI. Note that the results from quail were
566 derived from Figure 3. The heat-inactivated serum from the above-mentioned animals was
567 included as a control ("heat-treated"). The number of motile spirochetes was assessed
568 microscopically. The percentage of survival for those *B. burgdorferi* strains was calculated
569 using the number of mobile spirochetes at 4 h post incubation normalized to that prior to the
570 incubation with serum. The experiments were performed on three independent experiments;
571 within each experiment, samples were run in triplicate, and the survive percentage for each
572 experiment was calculated by averaging the results from triplicate experiments. The result
573 shown here is the average ± standard deviation of the survival percentage from three
574 independent experiments. (*), the significant difference ($P < 0.05$) of the percent survival of
575 spirochetes between indicated groups was determined using the one-way ANOVA with post
576 hoc Dunn's test.
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586 **Appendix: Supplementary data**

587 **Supplementary Figure captions**

588 **Supplemental Figure 1. No difference of Lyme borreliae survival in human sera**
589 **determined by motile spirochete measuring and Live/Dead staining.** A low passage,
590 infectious, and serum resistant *B. burgdorferi* strain B31-5A4 ("B31-5A4") or a high passage, a
591 non-infectious, and serum sensitive *B. burgdorferi* strain B313 ("B313") was incubated for 4h
592 with the human sera at a final concentration of 40 % in the presence ("OmCI-serum") or
593 absence ("serum") of 2 μM of OmCI. The heat-inactivated human sera were included as a
594 control ("heat-treated"). The number of motile spirochetes was assessed microscopically
595 ("Motile spirochete measuring") or using Live/Dead staining. The percentage of survival for
596 those *B. burgdorferi* strains was calculated using the number of live spirochetes at 4 h post
597 incubation normalized to that prior to the incubation with serum. The work was performed on
598 three independent experiments; within each experiment, samples were run in triplicate, and the
599 survive percentage for each experiment was calculated by averaging the results from triplicate
600 experiments. The result shown here is the average ± standard deviation of the survival
601 percentage from three independent experiments. (*), the significant difference ($P < 0.05$) of the
602 percent survival of spirochetes between indicated groups was determined using the one-way
603 ANOVA with post hoc Dunn's test.
604

605 **Supplemental Figure 2. *B. duttonii* survives in human and chicken sera independent on**
606 **heat or OmCI treatment.** *B. duttonii* strains V ("V") or LA1 ("LA1") was incubated for 4h
607 with the serum from **(A)** human or **(B)** chicken at a final concentration of 40 % in the presence
608 ("OmCI-serum") or absence ("serum") of 2 μM of OmCI. The heat-inactivated sera from the
609 above-mentioned animals were included as a control ("heat-treated"). The number of motile

spirochetes was assessed microscopically. The percentage of survival for those *B. burgdorferi* strains was calculated using the number of mobile spirochetes at 4 h post incubation normalized to that prior to the incubation with serum. The work was performed on three independent experiments; within each experiment, samples were run in triplicate, and the survive percentage for each experiment was calculated by averaging the results from triplicate experiments. The result shown here is the average ± standard deviation of the survival percentage from three independent experiments using the one-way ANOVA with post hoc Dunn's test, no statistical difference ($P > 0.05$) were observed between groups.

633 **Supplementary Tables**

634 **Supplemental Table 1. Bacteria strains used in this study**

Strain or plasmid	Genotype or characteristic	References or Sources
B. burgdorferi strains		
B31-5A4	*B. burgdorferi* strain B31 clone 5A4	(Purser and Norris, 2000)
B313	High-passage *B. burgdorferi* B31 missing lp5, lp17, lp21, lp25, lp28-1, lp28-2, lp28-3, lp28-4, lp36, lp38, lp54, lp56, cp9, cp32-4, cp32-6, cp32-8, cp32-9	(Sadziene et al., 1993)
B. duttonii strains		
V	*B. duttonii* strain isolated from a patient with relapsing fever	This study
LA1	Passaged from a *B. duttonii* strain La, isolated from an Ethiopian patient with relapsing fever	(Cutler et al., 1999)
E. coli strains		
BL21(DE3)	F–, *ompT*, *hsdSB* (rB–, mB–), *dcm*, *gal*, λ(DE3)	Promega
BL21(DE3)/pET16-*omCI*	BL21 producing histidine tagged residue OmCI	(Blom et al., 2014)

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