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Research paper

Interaction of extremophilic archaeal viruses with human and mouse complement system and viral biodistribution in mice

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ABSTRACT

Archaeal viruses offer exceptional biophysical properties for modification and exploration of their potential in bionanotechnology, bioengineering and nanotherapeutic developments. However, the interaction of archaeal viruses with elements of the innate immune system has not been explored, which is a necessary prerequisite if their potential for biomedical applications to be realized. Here we show complement activation through lectin (via direct binding of MBL/MASPs) and alternative pathways by two extremophilic archaeal viruses (*Sulfolobus* monocaudavirus 1 and *Sulfolobus* spindle-shaped virus 2) in human serum. We further show some differences in initiation of complement activation pathways between these viruses. Since, *Sulfolobus* monocaudavirus 1 was capable of directly triggering the alternative pathway, we also demonstrate that the complement regulator factor H has no affinity for the viral surface, but factor H deposition is purely C3-dependent. This suggests that unlike some virulent pathogens *Sulfolobus* monocaudavirus 1 does not acquire factor H for protection. Complement activation with *Sulfolobus* monocaudavirus 1 also proceeds in murine sera through MBL-A/C as well as factor D-dependent manner, but C3 deficiency has no overall effect on viral clearance by organs of the reticuloendothelial system on intravenous injection. However, splenic deposition was significantly higher in C3 knockout animals compared with the corresponding wild type mice. We discuss the potential application of these viruses in biomedicine in relation to their complement activating properties.

1. Introduction

Archaeal viruses constitute a group of morphologically and genetically unique viruses with exceptional life-cycle traits (Prangishvili et al., 2006; Peng et al., 2012). These viruses infect hyperthermophiles belonging to the crenarchaeal genera *Sulfolobus* and *Acidianus* or halophiles of the euryarchaeal genera *Haloarcula* and *Halorubrum* (Pietila et al., 2014). There are no reports of archaeal virus integration into human or any other eukaryotic genomes (Eckburg et al., 2003). Conceivably, it is unlikely for these viruses to trigger negative downstream effects in mammalian species due to their inability to proliferate.

All isolated archaeal viruses are extremophilic in nature and show adaptations to the extreme environments of their host. For instance, viruses of extreme halophiles show stability in solutions of high salt concentration (3–5 M) (Pina et al., 2011). Similarly, viruses infecting acidophilic hyperthermophiles are stable under very aggressive conditions comprising pH values < 3 and temperatures above 80 °C (Contursi et al., 2006). Archaeal viruses also appear in distinct morphologies to include bottle, spindle and droplet shapes (Pina et al., 2011). These unique features, together with their nano-size ranges, offer attractive traits for exploration of their potential in bionanotechnology and bioengineering arenas as well as for development of nanotherapeutics (Steinmetz et al., 2008; Uldahl et al., 2016b). However, to the best of our knowledge, there are no reports on interaction of archaeal viruses with elements of the mammalian innate immune system, and such studies would be of safety and performance importance for potential new therapeutic developments. It is also conceivable that unlike virulent pathogens and from an evolutionary point of view, archaeal viruses

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may have not developed strategies to combat the mammalian innate immune system. Here, we have selected two previously characterized spindle-shaped hyperthermostable and acid-resistant archaeal viruses (*Sulfolobus* monocaudavirus 1, SMV1 and *Sulfolobus* spindle-shaped virus 2, SSV2) (Uldahl et al., 2016a, 2016b) and studied their interaction with human and mouse complement system. We have further examined viral clearance and biodistribution on intravenous injection into wild type (WT) and C3 knockout (C3 KO) mice in C57/BL6J background.

2. Materials and methods

2.1. Production, purification and characterization of viruses

SSV2 was propagated in *S. solfataricus* strain 5E6, whereas SMV1 was propagated in *S. islandicus* CRISPR deletion mutant delta C1C2 as described in detail elsewhere (Gudbergsdottir et al., 2011; Okutan et al., 2013; Uldahl et al., 2016b). Procedures for cultures, ultrafiltration and viral purification by ultracentrifugation through a 10–40% (w/v) continuous Iodixanol gradient were as before (Uldahl et al., 2016b). The virus preparations were stored at 4 °C until used. Viral morphology was confirmed by transmission electron microscopy, whereas NanoSight LM20 Nanoparticle Tracking Analysis (Malvern Instruments, Malvern, UK), equipped with a sample chamber with a 405 nm blue laser and a Viton fluoroelastomer O-ring, was used for determination of the hydrodynamic size distribution and viral particle concentration as described in detail earlier (Uldahl et al., 2016b). The approximate virus titer was determined by a plaque assay as described previously (Uldahl et al., 2016b).

2.2. Sera preparation and treatment

Human serum was obtained from healthy Caucasian male volunteers (aged 25–40 years) and individuals genetically deficient in MBL according to approved local protocols. Purification and characterization of MBL/MASPs from a healthy human serum was in accordance with previous studies (Moghimi et al., 2006; Andersen et al., 2013). Serum concentration of mannan-binding lectin (MBL) and concentration of purified MBL was determined using MBL-C4 complex ELISA kit (HyCult Biotechnology, The Netherlands) (Andersen et al., 2013). Sera genetically deficient in MBL had MBL levels below 100 ng/mL. C1q-depleted serum was prepared and characterized as detailed previously (Andersen et al., 2013). Mouse sera deficient for C1q, ficolin A (FcnA), MBL-A/C, C1q/MBL-A/C, factor D (fD), MBL-A/C/fD and factor B (fB) were collected from corresponding maintained colonies of C57BL/6 homozygous mice at the University of Colorado Anschutz Medical Campus (Banda et al., 2007, 2010, 2011). Procedures for sera preparation, handling and use for complement activation studies were in accordance with the suggested guidelines of Lachmann (2010).

2.3. Complement activation studies

To measure complement activation in human sera, we determined viral-induced rise of complement activation products C4d, C5a, Bb and sC5b-9 using respective Quidel's (Quidel, CA, USA) kits according to the manufacturer's protocols as described previously (Andersen et al., 2013; Wibroe et al., 2017). For measurement of complement activation, the reaction was started by adding the required quantity of viruses to undiluted serum in Eppendorf tubes (in triplicate) in a shaking water bath at 37 °C for 30 min. Reactions were terminated by addition of "sample diluent" provided with assay kit or saline containing 25 mM EDTA. Viral-induced rises of serum complement activation products were then measured following virus removal. Control serum incubations contained saline (the same volume as viruses and other additions) for assessing background levels of complement activation products. In some studies complement activation was monitored following restoration of C1q and MBL/MASPs to depleted or genetically deficient serum. Zymosan, aggregated human IgG and mannan were used as positive control for complement activation. Complement activation by viruses was also monitored in the presence of either 10 mM EGTA/2.5 mM Mg^{2+} or 40 μM compstatin or its control peptide (a gift by Prof. T.E. Mollnes, University of Oslo, Norway). For quantification of complement activation products, standard curves were constructed using the assigned concentration of each respective standard supplied by the manufacturer and validated.

Superparamagnetic iron oxide (SPIO) nanoworms of 110 nm in size was prepared and characterized as described earlier (Chen et al., 2017). In some experiments, viral particles or SPIO nanoworms (1×10^{13} SMV1 or SPIO nanoworms/mL) were incubated with mouse serum or human serum (volume ratio 1:3) for 30 min at 37 °C. At the end of incubation, particles were washed 3 times with 1X phosphate-buffered saline (PBS) by ultracentrifugation at 100,000*g* for 10 min using Beckman Optima TLX ultracentrifuge. The pellets were re-suspended in 30 μL PBS, pH 7.0. For fB, factor H (fH) and C3 dot blot, 2 μL aliquots were applied in triplicates onto a nitrocellulose membrane. The membranes were blocked using 5% non-fat dry milk in 0.1% Tween® 20 in PBS at room temperature for 1 h, probed with corresponding primary antibodies (anti-human fB antibody and anti-human fH antibody were from Quidel, CA, USA; anti-mouse and anti-human polyclonal C3 antibodies were from MP Biomedicals, OH, USA) at room temperature for 1 h, followed by washing the membranes 3 times with PBS, and finally 1 h incubation with the corresponding IRDye 800CW labelled secondary antibodies (Li-COR Biosciences, NE, USA) against the primary antibody species. The membranes after immunoblotting were scanned using Li-COR Odyssey infrared imager. The integrated dot intensity in the scanned images was determined from 16-bit grayscale images using ImageJ software and plotted using Prism 6 software (GraphPad Software, Inc., CA, USA). The integrated density of fB, fH and C3 was determined by dot blot immunoassay against the standard dilutions of purified fB, fH and iC3b, respectively as described earlier (Chen et al., 2017; Wang et al., 2017).

2.4. Viral biodistribution studies

Wild type and $C3^{-/-}$ mice (Jackson Laboratories, B6;129S4-$C3^{tm1Crr}$/J) in C57/BL6J background were bred in house according to the approval by University of Colorado Animal Protocol Committee. Viral particles were labelled with 0.1 mM lipophilic near-infrared fluorescent DiIC18(7) (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide) (DiR) (Biotium Inc., CA, USA) for 1 h at 37 °C and washed twice with PBS to remove the unbound dye. DiR labelled viruses (1.7×10^{11}) were injected via tail vein into WT and C3KO mice (8 week age, females). Following the injection (1 min and 30 min), the blood was drawn via periorbital plexus using heparin as anti-coagulant. Plasma and washed cells were applied as dots on nitrocellulose membrane and scanned with Li-COR Odyssey scanner (Li-COR Biosciences, NE, USA) at $\lambda = 800$ nm. Organs from mice ($n = 3$ per group) were placed into petri dish and scanned with Li-COR Odyssey. The average fluorescence per organ was determined with ImageJ software by drawing a region of interest around the organ (16-bit gray image; background subtracted) and measuring average intensity with Measure function.

2.5. Statistical analysis

The results are presented either as mean ± s.d. or mean ± s.e.m, where applicable. Statistical analysis and comparison of different groups in relation to one or two factors were performed with one-way ANOVA or two-way ANOVA as appropriate. The Bonferroni method was subsequently used to correct *p* values after multiple comparisons to calculate statistical significance.