The Lectin Pathway of Complement Regulation by the infectious *Fasciola hepatica* newly excysted juvenile (NEJs)

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The complement response is the first-line innate host defence against invading organisms and is activated via Classical, Lectin, and Alternative pathways. The Lectin pathway (LP) is initiated by the binding or recognition molecules to sugar arrays, e.g. mannose, on the pathogen's surfaces, which leads to the formation of C3-convertase, essential for propagation of the cascade. Recently, we showed that the invasive stage of *Fasciola hepatica*, newly excysted juveniles (NEJs), survives in normal human serum by inactivating the complement LP, despite being covered by glycans. Considering the rich glycan-coated surface displayed by *F. hepatica* NEJs, which includes both highly mannosylated and acetylated structures, it was surprising to observe that neither Mannose-binding lectin (MBL) nor Ficolin-2 (Fic-2) bind to live NEJs. To unveil how the parasite prevents the binding of these LP recognition molecules, we investigated the effect of NEJs' excreted-secreted (ES) molecules on recombinant human MBL and Fic-2. We discovered that 1 hour of incubation at 37°C of rhMBL or rhFic-2 with either live NEJs or ES alone results in their specific cleavage. Considering the presence of collagen-like domains in both MBL and Fic-2 and the well-known role of cathepsin L3 protease (FhCL3), highly secreted by the NEJs stage, we next assessed the ability of recombinant FhCL3 to digest rhMBL and rhFic-2. Co-incubation of the molecules at 37°C showed that rFhCL3 efficiently cleaves rhMBL and rhFic-2. Fascinatingly, our studies show that *F. hepatica* NEJs possess multiple and overlapping strategies to prevent LP activation, which also include the expression and secretion of serine protease inhibitors (Serpins; namely FhSrp1 and FhSrp2). We have shown that rFhSrp1 and rFhSrp2 inhibit the MBL-associated serine proteases (MASP-1 and MASP-2), the key initiators of the LP. Furthermore, rFhSrp1 and rFhSrp2 form complexes and inhibit rMASP-1/2, as shown by ELISAs, pull-down, SDS-Page, biochemical assays, and Mass spectrometry (MS). Similar to that demonstrated with live *F. hepatica* NEJs, incubation of either rFhSrp1 or rFhSrp2 with normal human serum leads to selective LP inhibition (>90%). Nevertheless, a time-course co-incubation of these serpins with rMASPs showed high efficiency of rFhSrp1 in binding and cleaving MASPs, and an interesting suicidal mechanism of inhibition between rFhSrp1 and MASP-1 and -2. The downstream effect of MASPs inhibition was verified by a proportional reduction in their ability to cleave complement C4, essential for forming C3-convertase. Here, we uncovered an array of novel mechanisms by which the invading *F. hepatica* NEJs circumvent the binding of MBL and Fic-2, and the activation of MASPs to become refractory to killing via the LP. The importance of such complement regulation during *F. hepatica* infection is stressed by the various strategies used to avoid an LP response.