Investigation of immune-suppressive genes expressed by the *Cotesia rubecula* bracovirus (CrBV)

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Abstract

The hymenopteran endoparasitoid, *Cotesia rubecula*, employs integrated forms of active and passive immune-suppression in overcoming the defences of its host, *Pieris rapae*, a cosmopolitan pest of cruciferous crops. The immune-suppressive activity arises from a complex of maternally secreted proteins and polydnavirus (PDV) particles, which are injected in a host larva with the parasitoid egg at oviposition. The PDV associated with *C. rubecula* (CrBV) is unusual in that only four main viral genes (CrV1-CrV4) are expressed in *P. rapae* tissues and that expression is transient, remaining at high levels only in the period between four and eight hours post-parasitisation (hpp). Previously, CrV1 was characterised and found to inactivate host haemocytes by causing disruption of their cytoskeleton, leading to abrogation of immune-associated processes such as spreading. In this study, a cDNA library was constructed from parasitised *P. rapae* larvae and screened with total CrBV DNA, leading to isolation of CrV2 and CrV3. The open reading frame of each gene was cloned in a bacterial expression vector and the resultant recombinant proteins were used to produce antibodies against CrV2 and CrV3.

CrV2 has an open reading frame of 960 bp (with no introns) and encodes a glycoprotein of ≈ 40 kDa, which is secreted from infected haemocytes and fat body. Comparison of CrV2 deduced amino acid sequence with other known sequences revealed no significant homologies. CrV2 protein was detected in host larvae at 6 hpp, remaining in large amounts for at least a day and was declining by 48 hpp. A putative coiled-coil region at the C-terminus of CrV2 is suspected of involvement in formation of CrV2 trimers that were detected under non-denaturing conditions. CrV2 was visualised within haemocytes in large endosomes at 24 hpp. Although the function of CrV2 remains unclear, it appears to interact with host haemocytes presumably to suppress their immune function.

The CrV3 gene contained and intron and was found to encode a C-type lectin (CTL) homologue, which is secreted from infected host haemocytes and fat body into haemolymph. Two CrV3 monomers (of ≈ 14 and 17 kDa) were detected in parasitised larvae with the larger monomer being an N-glycosylated form of the smaller monomer. CrV3 dimers and tetramers were also detected *in vivo*. Recombinant CrV3 forms larger complexes and was shown to agglutinate ovine red blood cells, an activity that was Mn^{2+}- and Mg^{2+}-dependent but was independent of Ca^{2+}. CrV3-mediated hemagglutination was inhibited by EDTA but not by biological concentrations of 29 potential ligands tested. Interestingly, CrV3 is similar to invertebrate CTLs associated with humoral defence but not with previously isolated viral lectins. Further, CrV3 homologues were recently detected in bracoviruses from *C. ruficrus* and *C. karyai*, indicating that a novel CTL family is expressed by some *Cotesia*-associated PDVs. CrV3 probably interacts with a soluble host haemolymph component associated with host humoral immune defences.

CrV1 and Crp32 (an immune-suppressive *C. rubecula* calyx protein) were used to produce recombinant *Autographa californica* multiple nucleopolyhedrosis viruses (AcMNPVs), pathogens with putatively enhanced virulence in *P. rapae*. Bioassays were undertaken to investigate the pathogencity of wild-type AcMNPV in *P. rapae* (previously unreported) and the effect of insertion of Crp32. Although the proportion of larval deaths due to wild-type AcMNPV was significant, the slow rate of mortality indicated that *P. rapae* is only semi-permissive to AcMNPV. Crp32 insertion proved insignificant in terms of the proportion and rate of larval mortality. Given the semi-permissive nature of *P. rapae*, recombinant AcMNPVs expressing immune-suppressive and appropriate reporter genes may be useful for elucidating mechanisms of insect immunity and more specifically, how CrBV acts to subvert these mechanisms in *P. rapae*.