Protein tyrosine phosphatase-H2 from a polydnavirus induces apoptosis of insect cells

Richard J. Suderman, Andrea J. Pruijssers and Michael R. Strand

Correspondence
Michael R. Strand
mrstrand@uga.edu
Department of Entomology and Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602, USA

The family Polydnaviridae is a large group of immunosuppressive insect viruses that are symbiotically associated with parasitoid wasps. The polydnavirus Microplitis demolitor bracovirus (MdBV) causes several alterations that disable the cellular and humoral immune defences of host insects, including apoptosis of the primary phagocytic population of circulating immune cells (haemocytes), called granulocytes. Here, we show that MdBV infection causes granulocytes in the lepidopteran Spodoptera frugiperda to apoptose. An expression screen conducted in the S. frugiperda 21 cell line identified the MdBV gene ptp-H2 as an apoptosis inducer, as indicated by cell fragmentation, annexin V binding, mitochondrial membrane depolarization and caspase activation. PTP-H2 is a classical protein tyrosine phosphatase that has been shown previously to function as an inhibitor of phagocytosis. PTP-H2-mediated death of SF-21 cells was blocked by the pan-caspase inhibitor benzyloxycarbonyl-Val-Ala-(O-methyl) Asp-fluoromethylketone (Z-VAD-FMK), but cells maintained in this inhibitor still exhibited a suppressed phagocytic response. Mutagenesis experiments indicated that the essential catalytic cysteine residue required for the phosphatase activity of PTP-H2 was required for apoptotic activity in SF-21 cells. Loss of adhesion was insufficient to stimulate apoptosis of SF-21 cells. PTP-H2 expression, however, did significantly reduce proliferation of SF-21 cells, which could contribute to the apoptotic activity of this viral gene. Overall, our results indicate that specific genes expressed by MdBV induce apoptosis of certain insect cells and that this activity contributes to immunosuppression of hosts.

INTRODUCTION

The innate immune system of insects serves as a critical defence against invading organisms. Reciprocally, many pathogens and parasites of insects are successful because they have developed strategies for evading the immune defences of their hosts. Among the most potent immunosuppressive pathogens of insects are viruses in the family Polydnaviridae. Polydnaviruses (PDVs) are the only viruses with segmented, double-stranded DNA genomes and are currently divided into two genera, ichnoviruses (IV) and bracoviruses (BV), on the basis of their association with approximately 40 000 species of parasitoid wasps in the families Ichneumonidae and Braconidae (Kroemer & Webb, 2004; Webb & Strand, 2005; Dupuy et al., 2006). Each PDV is genetically unique and persists as a stably integrated provirus in the genome of its associated wasp species. Transmission of the virus to parasitoid offspring is vertical through the germ line, while viral replication is restricted to specialized calyx cells in the ovaries of females. Virions accumulate to high densities in the reproductive tract and adult wasps inject a portion of this virus along with one or more eggs when they oviposit into host insects. The virus then infects immune cells (haemocytes) and other host tissues. Several viral gene products are expressed in infected host cells, but no viral replication occurs. In the absence of viral infection the host immune system usually recognizes and eliminates the parasitoid’s offspring by encapsulation, a conserved defence response in which host haemocytes bind to the wasp egg and form a multicellular sheath. In the presence of virus, however, encapsulation and other host immune defences are disrupted, which allows the parasitoid’s offspring to develop (Schmidt et al., 2001; Webb & Strand, 2005; Pennacchio & Strand, 2006). Thus, a true mutualism exists, with transmission of the virus depending upon survival of the wasp and survival of the wasp depending upon the immunosuppressive activity of the virus in its host.

Microplitis demolitor is a braconid wasp that parasitizes the larval stage of several moth species (Lepidoptera) and carries M. demolitor bracovirus (MdBV). In addition to encapsulation, MdBV suppresses other host immune defences including phagocytosis, melanization of haemolymph and inducible expression of other humoral defence molecules (Strand & Pech, 1995a, b; Beck & Strand, 2005; Theokliattikul et al., 2005; Strand et al., 2006). The MdBV genome is divided into 15 circular DNA segments with an
aggregate size of 189 kb (Webb et al., 2006). However, coding densities are extremely low with only 61 open reading frames (ORFs) encoding predicted proteins of >100 aa. The majority of these ORFs consist of related variants that form four gene families: (i) cell surface mucins (Glc genes, two members), (ii) protein tyrosine phosphatases (PTP genes, 13 members), (iii) inhibitor kB-like proteins (IkB-like genes, 12 members) and (iv) epidermal growth factor-motif-containing proteins (Egf genes, four members). One member of the Glc family, Glc1.8, blocks adhesion of immune cells to large foreign targets, like parasitoid eggs, and phagocytosis of small targets like bacteria (Beck & Strand, 2003, 2005). A catalytically active member of the PTP gene family, PTP-H2, has also been found recently to localize to focal adhesions and to reduce the ability of insect immune cells to phagocytize foreign targets (Prujssers & Strand, 2007). Together these two genes appear to be the key virulence factors responsible for disrupting the capsule-forming and phagocytic activity of virus-infected immune cells. Other MdBV virulence factors, including the IkB-like and Egf gene families target humoral defences involving NF-kB signalling and activation of the phenoloxidase cascade (Thoetkiattikul et al., 2005; Beck & Strand, 2007).

Despite severe interference with host immune cell functions, the fate of MdBV-infected haemocytes is far from clear. In lepidopterans, granulocytes are the primary haemocyte type that phagocytizes foreign intruders, whereas encapsulation requires cooperation between granulocytes and a second class of haemocytes called plasmatocytes (Pech & Strand, 1996; Nardi et al., 2006; Strand et al., 2006). The primary role of granulocytes in capsule formation appears to be the recognition of foreign targets and release of cytokines that recruit plasmatocytes. Plasmatocytes then bind and form an overlapping sheath around the target. In the host Pseudoplasia includens, both granulocytes and plasmatocytes lose the capacity to bind foreign surfaces or phagocytize small foreign targets 4–8 h after infection by MdBV (Strand & Noda, 1991; Strand, 1994; Strand et al., 2006). Thereafter, a large proportion of granulocytes, but not plasmatocytes, die by apoptosis (Strand & Pech, 1995b). Transcriptionally inactive MdBV has no apoptotic activity, suggesting that expression of one or more viral gene products is required for this response (Strand & Pech, 1995b). Here, we report that PTP-H2 directly induces apoptosis of SF-21 cells derived from the lepidopteran host Spodoptera frugiperda. Expression of PTP-H2 triggered mitochondrial membrane depolarization and caspase-dependent apoptosis, suggesting that this virulence factor induces apoptosis through a mitochondria-dependent death pathway.

METHODS

Insects and cell lines. M. demolitor, P. includens and S. frugiperda were reared as described previously (Strand & Noda, 1991). IPLB-SF-21 (SF-21) cells derived from S. frugiperda were maintained in TC-100 medium (Invitrogen) with 10% fetal bovine serum (Atlanta Biologicals) and 1% antibiotic cocktail (Gibco). Cells were maintained and passaged as adherent cells in Corning 25 cm² tissue-culture flasks. Most experiments were conducted in 24- or 96-well culture plates (Corning).

Virus collection and injection into S. frugiperda larvae. MdBV was collected from wasps and injected into larvae as described previously (Strand et al., 1992; Beck & Strand, 2003). As is convention in the PDV literature, the amount of MdBV collected from the reproductive tract of a single adult female is defined as one wasp equivalent. MdBV packages only one genomic segment per virion and the 15 genomic segments that comprise the genome are non-equmolar in abundance (Beck et al., 2007). Quantitative data indicate that one wasp equivalent on average equals 1 × 10⁹ virions and that wasps inject 0.01–0.05 wasp equivalents per host during oviposition, which equals 1 × 10⁸–5 × 10⁹ virions (Beck et al., 2007). For this study, we injected 0.05 wasp equivalents of MdBV into fourth instar S. frugiperda larvae as described previously (Beck et al., 2007). Negative controls were larvae injected with 0.05 wasp equivalents of MdBV that had been inactivated by treatment with psoralen and UV light (Strand et al., 1992).

Plasmid expression constructs and cell transfections. ORFs for the MdBV genes Glc1.8, ptp-H1, ptp-H2, ptp-J1, egf0.4, egf1.0, ikB-H4 and IkB-N5, have been cloned previously into the vector pIZT/V5-His (Invitrogen) to produce the expression constructs pIZT/Glc1.8, pIZT/PTP-H1, pIZT/PTP-H2, pIZT/PTP-J1, pIZT/Egf0.4, pIZT/Egf1.0, pIZT/IkB-H4 and pIZT/IkB-N5 (Beck & Strand, 2003; Thoetkiattikul et al., 2005; Prujiessers & Strand, 2007). pIZT/V5-His constructs contains the OpIE2 early promoter from the Orgyia pseudotsugata baculovirus (OpMNPV) for constitutive expression of the gene of interest and encodes a Zeocin–green fluorescent protein (GFP) gene fusion under the control of the OpIE1 promoter. We have also produced previously an expression construct of PTP-H2 that lacks PTP activity (pIZT/PTP-H2C236A) by replacing the essential catalytic cysteine with an alanine residue (Prujssers & Strand, 2007). Empty pIZT/V5-His vector was used as a negative control. Expression constructs (2 µg ml⁻¹) were transiently transfected into SF-21 cells seeded at 70–80% confluency in 12- or 24-well culture plates using Lipofectin (Invitrogen) as described previously (Beck & Strand, 2003). In some experiments, the pan-caspase inhibitor benzoxyl-carbonyl-Val-Ala-(O-methyl) Asp-fluoromethyl ketone (Z-VAD-FMK; Promega) (20 µM) was added to cultures in fresh medium 5 h post-transfection.

Assessment of apoptosis by S. frugiperda haemocytes and SF-21 cells. Apoptosis of haemocytes was measured by bleeding S. frugiperda larvae from a cut proleg 3, 18 and 30 h post-infection. Cells from individual larvae were rinsed twice in physiological saline and placed into wells of 24-well culture plates (Pech et al., 1994). The four major haemocyte types in circulation (granulocytes, plasmatocytes, spherule cells and oenocytoids) were identified by morphology and staining with haemocyte type-specific antibodies (Gardiner & Strand, 2000). Haemocytes exhibit several classical symptoms during MdBV-mediated apoptosis including DNA fragmentation, alterations in mitochondrial membrane potential and cell blebbing (Clem et al., 1991; Clem & Miller, 1993). In the current study, apoptotic haemocytes were identified by staining with fluorescein-conjugated annexin V (BD Biosciences), which detects phosphatidylserine on the surface of apoptosing cells, and the mitochondrial membrane potential marker JC-1 (5,5′,6,6′-tetrachloro-a,a′,3′,3′-tetraethyl-benzimidazolylcarbocyanine iodide, excitation wavelength (λex)=490 nm, emission wavelength (λem)=580 nm; Biotium) (10 µg ml⁻¹). Annexin V staining was performed as recommended by the manufacturer. Haemocytes were stained with JC-1 as outlined previously, with healthy cells fluorescing red due to aggregation of
the dye on mitochondria with high membrane potential and apoptosing cells fluorescing green due to the dye forming monomers on mitochondria with reduced membrane potential (Gorman et al., 1997). The percentage of apoptotic cells for each haemocyte type was determined by counting 200 cells using an epifluorescence, phase-contrast microscope (Leica DM IRB). The proportion of apoptotic cells for each haemocyte type was then compared between larvae injected with viable and inactivated MdBV by two-tailed binomial test using JMP statistical software (SAS Institute).

Since Sf-21 cells undergo extensive blebbing in association with apoptosis (Clem et al., 1991; Clem & Miller, 1993), we initially screened for apoptosis in this cell line using a cell-survival assay in which we counted the proportion of living cells 20 h after a given treatment. This was done by counting the number of intact (non-blebbled) cells present in three randomly selected fields of view at \( \times 400 \) magnification both 2 and 20 h post-treatment. Percentage cell survival was determined by dividing the number of intact cells present at the end of the experiment by the number of cells at the beginning of the experiment, with values greater than 100% indicating that the number of cells per well increased during the 20 h assay. Sf-21 cells were also stained with annexin V and JC-1 as described above. Proportional data were arcsin transformed and analysed by one-way ANOVA with treatments compared with the negative empty vector control using Dunnett’s multiple comparison procedure. Images were captured using Q-imaging and Adobe Photoshop software.

**Caspase activity assay.** Protein extracts for caspase activity assays were prepared by lysing approximately \( 1.5 \times 10^5 \) cells at 24 h post-transfection by three cycles of freeze–thawing in an activity buffer (50 mM HEPES, pH 7.5, 0.1% CHAPS, 10% sucrose, 1 mM EDTA, 0.1 M NaCl, 5 mM DTT), followed by removal of insoluble material by centrifugation at 16 000 \( \times g \) at 4°C for 5 min. The supernatants were assayed for protein concentration by modified Bradford assay (Pierce). Concentrations were normalized and 17 \( \mu g \) total protein was incubated in 0.2 ml caspase assay buffer containing 100 \( \mu M \) acetyl-Asp-Glu-Val-Asp-7-Amino-4-trifluoromethylcoumarin (Ac-DEVAD-afc, fluorescent caspase 3 substrate; Biomol International). Fluorescence due to enzymic release of AFC [7-amino-(trifluoromethyl)coumarin] from the substrate was measured using a spectrofluorimeter (BMC) over 1 h at 37°C (\( \lambda_{ex} = 405 \text{ nm, } \lambda_{em} = 520 \text{ nm} \)). Experiments were carried out in triplicate.

**Phagocytosis assays.** We assessed the ability of Sf-21 cells to phagocytes heat-killed *Escherichia coli* labelled with rhodamine (Pruijssers & Strand, 2007). Cells were first transfected with plZT/PTP-H2 or the plZT empty vector and then maintained for 20 h in the presence of Z-VAD-FMK. Cells were then transferred to new 12-well culture plates in serum-free medium plus Z-VAD-FMK at a density of \( 1 \times 10^5 \) cells per well. After a 1 h preincubation period, bacteria were added to each culture well at a ratio of 15:1. Cells were allowed to phagocyte for 90 min at 27°C followed by transfer of the culture plate to ice. We then scored the percentage of cells with one or more ingested bacteria by counting 200 cells per well from four randomly selected fields of view using a Leica TCS inverted epifluorescence microscope. Bacteria were red, while cells expressing a gene of interest were green. Each treatment was replicated a minimum of five times using independently prepared samples. The data were then analysed by Student’s t-test.

**Immunofluorescence microscopy.** Sf-21 cells transfected with plZT/PTP-H2 or other expression constructs were processed for immunofluorescence microscopy as described previously (Pruijssers & Strand, 2007). Briefly, cells cultured in the presence of Z-VAD-FMK were washed in PBS 20 h post-transfection and then fixed for 20 min in 4% paraformaldehyde in PBS. Fixed cells were permeabilized with PBS–0.1% Triton X-100 (PBT), blocked in 5% dried milk in PBS, and then incubated overnight at 4°C with anti-V5 antisera (1:5000) that recognizes recombinant PTP-H2. After washing in PBT, cells were incubated with Alexa fluor 568-conjugated goat anti-mouse (1:2000) (Invitrogen). Incubation of cells with secondary antibody alone served as the negative control. Samples were examined on a Leica-TCS microscope with images captured as described above.

**Cell proliferation assays.** Proliferation of Sf-21 cells transfected with plZT/PTP-H2 and plZT (empty vector) in the presence of Z-VAD-FMK was assessed by seeding 5000 cells per well into 96-well culture plates. The mock controls were cells transfected with empty vector maintained in medium without caspase inhibitor. At selected times post-transfection, cells were resuspended by gentle pipetting and the number of cells per well was determined using a Neubauer haemocytometer. The numbers of cells present per well for each treatment over a 6 day assay period were then compared by conducting a repeated-measures analysis and by using the general linear model and JMP software.

**RESULTS**

**MdBV infection induces apoptosis of granulocytes in *S. frugiperda* larvae**

*P. includens* haemocytes lose the capacity to bind foreign surfaces and phagocytize small targets as early as 4 h post-infection by MdBV followed by apoptosis of granulocytes by 24 h, as measured by DNA fragmentation, annexin V staining and cell blebbing (Strand & Pech, 1995b; Strand et al., 2006). MdBV-infected haemocytes from the related host *S. frugiperda* similarly lose the capacity to bind foreign surfaces and form capsules (Trudeau & Strand, 1998), but whether infected granulocytes undergo apoptosis has not, to our knowledge, been investigated previously. During the current study we determined that, as in *P. includens*, MdBV infection caused *S. frugiperda* granulocytes but not other haemocyte types to apoptose, as measured by annexin V binding. Almost no haemocytes bound annexin V at 3 h post-infection but by 18 h significantly more granulocytes from MdBV-infected larvae were stained by annexin V compared with those from control larvae infected with UV-inactivated virus (Fig. 1a, b). Additional evidence that *S. frugiperda* granulocytes undergo apoptosis included nuclear condensation, as visualized by propidium iodide staining, and loss of mitochondrial membrane potential, as visualized by JC-1 staining (data not shown). By 30 h post-infection, numerous apoptotic bodies from fragmenting granulocytes were observed in the haemolymph, resulting in a significant reduction in the number of granulocytes per microlitre of haemolymph compared with larvae injected with inactive MdBV (Fig. 1c).

**PTP-H2 induces apoptosis of Sf-21 cells**

Since MdBV and other PDVs do not replicate outside of female wasps, it is currently not possible to produce, maintain or screen PDV mutants. Therefore, to assess whether a specific MdBV gene product functions as an apoptosis inducer, we conducted an expression screen in
the Sf-21 cell line using plasmid constructs of viral genes that previous studies indicated are preferentially expressed in host insect haemocytes (Strand et al., 1997; Trudeau et al., 2000; Beck & Strand, 2005; Thoetkiattikul et al., 2005; Pruijssers & Strand, 2007; ). We reasoned that Sf-21 cells were a good choice for this screen because preliminary experiments indicated that MdBV infection caused Sf-21 cells to undergo apoptosis similar to granulocytes (data not presented). Sf-21 cells are also well known to undergo high levels of apoptosis following infection by baculovirus mutants deficient in anti-apoptotic gene activity (Clem et al., 1991; Clem & Miller, 1993; Hershberger et al., 1994). Our screen indicated that Sf-21 cells transfected with pIZT/PTP-H2 exhibited a significant reduction in survival, whereas the other MdBV genes we tested had no effect on survival compared with cells transfected with the empty vector that expressed only GFP (Fig. 2a). A large proportion of cells expressing PTP-H2 fragmented into apoptotic bodies, resulting in a loss of intact cells and the accumulation of small membrane blebs in assay wells (Fig. 2b). PTP-H2 expression also resulted in significantly increased binding of annexin V and a pronounced spectral shift from red to green following staining with the mitochondrial marker JC-1 (Fig. 3). Together, these symptoms suggested that PTP-H2 induced apoptosis of Sf-21 cells rather than necrosis.

PTP-H2 promotes apoptosis by caspase activation

To determine whether caspases were involved in PTP-H2-mediated cell death, we conducted enzymic assays using the fluorogenic caspase-3 substrate Ac-DEVD-AFC. These experiments indicated that effector caspase activity was significantly higher in lysates from cells expressing PTP-H2 than in cells transfected with empty vector (Fig. 4a). Reciprocally, addition of the pan caspase inhibitor Z-VAD-FMK blocked PTP-H2-mediated death, as measured by our cell survival assay (Fig. 4b). Sf-21 cells expressing PTP-H2 in the presence of Z-VAD-FMK were morphologically similar to control cells when examined by phase-contrast microscopy, with almost no cell blebbing and more than 90% of cells remaining viable, as measured by exclusion of propidium iodide. Depolarization of mitochondria in cells expressing PTP-H2 was also apparently related to caspase activation, because we did not observe any change in JC-1 fluorescence of cells transfected with pIZT/PTP-H2 and maintained in the presence of Z-VAD-FMK (data not shown). Immunostaining of Sf-21 cells cultured in the presence of Z-VAD-FMK indicated that PTP-H2 preferentially localized to focal adhesions, as reported previously for haemocytes and haemocyte-like cell lines, such as High Five cells from Trichoplusia ni and S2 cells from Drosophila (Trudeau et al., 2000; Beck & Strand, 2005; Pruijssers & Strand, 2007) (data not shown). This observation indicated that Z-VAD-FMK did not affect the cellular distribution of PTP-H2 and led to the question of whether Sf-21 cells expressing PTP-H2 lost the capacity to phagocytize foreign targets when prevented from apoptosing, as previously found for High Five and S2 cells (Pruijssers & Strand, 2007). Our results indicated
that PTP-H2 had precisely this effect (Fig. 5a). Since the anti-phagocytic activity of PTP-H2 depends on an intact catalytic core and associated phosphatase activity (Pruijssers & Strand, 2007), we also assessed whether the phosphatase-inactive mutant PTP-H2^{C236A} lacked apoptosis-inducing activity. Unlike wild-type PTP-H2, pIZT/PTP-H2^{C236A} had no apoptotic activity in Sf-21 cells (Fig. 5b).
PTP-H2 expression reduces cell proliferation

In addition to disrupting phagocytosis, MdBV also disrupts the ability of immune cells to bind to foreign surfaces, which effectively disables the ability of infected haemocytes to bind and encapsulate large foreign targets, like parasitoid eggs, or to internalize small foreign targets, like bacteria (Strand, 1994; Strand & Noda, 1991; Strand et al., 2006). We thus considered the possibility that PTP-H2-mediated apoptosis of SF-21 cells could involve alterations in adhesion, since this triggers apoptosis in some...
mammalian cells through a response called anoikis (Gilmore, 2005). Like many insect cells (Pech & Strand, 1996; Iavine & Strand, 2003), Sf-21 cells are unable to bind or spread on the surface of culture plates coated with agarose. Sf-21 cells transfected with pIZT/PTP-H2 in agarose-coated wells underwent the same high level of apoptosis as cells on non-coated plates, yet cells transfected with empty vector exhibited no increase in apoptosis (Fig. 6a). This result suggested that simple loss of adhesion was insufficient to induce Sf-21 cell death. Other factors associated with apoptosis, however, are proliferation- and cell-cycle-arrest. To assess whether PTP-H2 affected proliferation, we compared the growth of Sf-21 cells maintained in the presence of Z-VAD-FMK following transfection with pIZT/PTP-H2 or empty vector with mock control cells transfected with the empty vector but maintained in medium lacking caspase inhibitor. Our repeated-measures analysis revealed a significant interaction between treatment and time, indicating that cells expressing PTP-H2 proliferated significantly less over the 6-day assay period than cells transfected with the empty vector (Fig. 6b). Empty-vector-treated and mock-treated cells increased from an average of 5.0 × 10⁴ cells per well to more than 4.0 × 10⁵ cells per well over 6 days, whereas cells expressing PTP-H2 increased from 5.0 × 10⁴ cells per well to only 12.3 × 10⁴ cells per well during the same period (Fig. 6b). As expected in a transient expression assay, PTP-H2 expression thereafter declined, as monitored by the GFP marker in the pIZT vector. Concurrent with this decline, cell proliferation increased to levels similar to those of cells transfected with the empty vector and mock-transfected controls (data not shown).

![Fig. 6. Apoptosis of Sf-21 cells is unaffected by culture on agarose-coated plates but PTP-H2 expression reduces cell proliferation. (a) Mean ± SD percentage survival of Sf-21 cells cultured on agarose-coated plates. Most cells transfected with the empty vector survive despite being unable to adhere to the plate’s surface, whereas cells expressing PTP-H2 apoptose (t = 8.3, P = 0.001, n = 4 replicates per treatment). (b) Proliferation of cells transfected with pIZT/PTP-H2 or pIZT empty vector and maintained in the presence of Z-VAD-FMK (+ Z-VAD) compared with cells transfected with pIZT and maintained in medium without Z-VAD-FMK (Mock). Repeated-measures analysis indicated a significant interaction between day and treatment (F = 3.1, d.f. = 8, P = 0.04), with cells expressing PTP-H2 proliferating less than the control cells.](http://vir.sgmjournals.org/1417)

DISCUSSION

We determined previously that MdBV suppresses several components of the cellular and humoral immune response of permissive hosts, including S. frugiperda (Strand & Noda, 1991; Strand & Pech, 1995b; Trudeau & Strand, 1998; Beck & Strand, 2003, 2005; Pruijssers & Strand, 2007; Thoetkiattikul et al., 2005; Beck & Strand, 2007). In the present study we took advantage of the Sf-21 cell line, which is well known to undergo apoptosis in response to different stimuli (Clem et al., 1991), to conduct a screen for candidate apoptosis-inducing genes encoded by MdBV. Our results identified only one gene, ptp-H2, that induced apoptosis, suggesting that apoptosis in Sf-21 cells is not a generalized response to infection by MdBV or viral transcriptional activity. It is also unlikely that apoptosis is due to overexpression of PTP-H2 in Sf-21 cells given that: (1) granulocytes and Sf-21 cells exhibit a similar response following natural infection and (2) real-time PCR analyses indicate that PTP-H2 transcript abundance is actually higher in MdBV-infected haemocytes than in Sf-21 cells transfected with pIZT/PTP-H2 (A. Pruijssers and M. R. Strand, unpublished).

In mammalian cells, terminal caspases are activated by either the extrinsic pathway, which requires death receptors like the tumour necrosis factor (TNF) receptor, or the intrinsic pathway, which involves mitochondrial disruption and release of factors like cytochrome c that stimulates caspase activation (Li et al., 2000; Daniel & Korsmeyer, 2004). In insects, an extrinsic pathway involving the TNF-α superfamily member Eiger and receptor Wengen has been described in Drosophila that stimulates apoptosis through the c-Jun N-terminal kinase (JNK) pathway. An intrinsic apoptotic pathway requiring RHG-family proteins like Rpr, Hid and Grim that promote mitochondrial disruption and terminal caspase activation has also been reported (Olson et al., 2003; Abdelwahid et al., 2007; Kuranaga & Miura, 2007; Kuranaga & Miura, 2007).
2007). Rescue from the apoptotic effects of PTP-H2 by Z-VAD-FMK, combined with high levels of JC-1 monomer fluorescence (green), implicate caspase activation and loss of mitochondrial membrane potential in PTP-H2-induced apoptosis. Our results with the mutant PTP-H2C363A combined with those from previous studies (Prujssers & Strand, 2007) further suggest that PTP-H2-associated phosphatase activity is required for both the apoptotic and anti-phagocytic functions of this protein. Recent studies in a related bracovirus, CpBV, also implicate PTPs in disabling haemocyte function (Ibrahim et al., 2007), while studies with Taxoneuron nigriceps bracovirus (TnBV) suggest that other bracovirus gene products induce caspase activation without causing apoptosis (Lapointe et al., 2005).

We have reported elsewhere that PTP-H2 inhibits phagocytosis by haemocyte-like High Five and S2 cells as well as by primary immune cells like granulocytes and plasmatocytes in caterpillar hosts (Strand & Noda, 1991; Beck & Strand, 2005; Prujsiers & Strand, 2007). Unlike SF-21 cells and granulocytes (Strand & Pech, 1995b), however, PTP-H2 does not induce apoptosis of these cell types. This finding indicates that the anti-phagocytic activity of PTP-H2 extends across a diversity of insect cell backgrounds but its apoptosis-inducing activity is restricted and must depend in part on intrinsic differences between cell types. Analogously, TN-368 cells, which served as the parental cell line for development of High Five cells, are similarly resistant to a variety of apoptotic stimuli, including baculoviruses deficient in the apoptotic inhibitor P35 (Clem & Miller, 1993; Clem et al., 1994). With diverse pathways regulating apoptosis in insect and mammalian cells, it is currently, to our knowledge, not known why PTP-H2 and other apoptotic stimuli trigger apoptosis in one cell type but not another. Intriguingly though, studies with both SF-21 and S2 cells implicate depletion of inhibitor of apoptosis proteins (IAP) in caspase activation (Huang et al., 2000; Muro et al., 2002). This suggests the possibility that the different apoptotic responses of insect cells to PTP-H2 could reflect differences in IAP abundance or a differential effect of PTP-H2 on IAP depletion.

Given the anti-adhesive and anti-phagocytic activities of PTP-H2 in combination with Gc1.8, we initially hypothesized that PTP-H2-mediated apoptosis of SF-21 cells could reflect a form of anoikis (Gilmore, 2005). However, SF-21 cells cultured on non-adhesive surfaces like agarose do not undergo apoptosis in the absence of PTP-H2 expression, suggesting that loss of adhesion alone is insufficient to induce an apoptotic response. As in haemocytes, expression of the MdBV virulence gene gcl1.8 also causes ~30–50% of SF-21 cells to detach 20 h post-transfection (R. J. Suderman, A. J. Prujsiers & M. R. Strand, unpublished) but this gene product had no apoptotic-inducing activity (Fig. 2). In contrast, our results indicate that PTP-H2 significantly reduces proliferation of SF-21 cells under conditions in which apoptosis is inhibited by the presence of Z-VAD-FMK. It is well known that perturbations in the cell cycle can trigger apoptosis and that several caspases involved in apoptosis also function as cell-cycle regulators. This includes effector caspases like caspase 3, which inhibits proliferation of certain mammalian cell types (Woo et al., 2003; Wells et al., 2006; Kuranaga & Miura, 2007). Thus, while the presence of a caspase inhibitor could itself affect cell proliferation, our results indicate this is not the case, since SF-21 cells transfected with empty vector and maintained in Z-VAD-FMK proliferated similarly to cells maintained in the absence of caspase inhibitor. Taken together, these results suggest that PTP-H2 may induce apoptosis by directly or indirectly perturbing the cell cycle.

PTP-H2 is a classical PTP whose closest homologues include PTPs encoded by other PDVs and cytosolic PTPs from mammals, like PTP1B (Prujssers & Strand, 2007). Although, to our knowledge, no studies in insects implicate PTPs in apoptosis, several phosphatases including PTP1B have been implicated in apoptosis of mammalian cells (Takada et al., 2002; Yousefi & Simon, 2003; Gu et al., 2004). We also note interesting parallels between PTP-H2, the classical, cytosolic phosphatase YopH from the bacterium Yersinia pestis and PTP-PEST from mice that each induce apoptosis of certain cells, while also localizing to focal adhesions and disrupting functions like phagocytosis and cell division that require the actin cytoskeleton (Bruckner et al., 2005; Halle et al., 2007; Prujsiers & Strand, 2007). Like Yersinia sp., PDVs appear to have acquired and co-opted PTPs for use as immunosuppressive virulence factors, while PTP1B and PTP-PEST function as endogenous regulators of related functions.

Our results to date collectively indicate that MdBV encodes a diverse repertoire of virulence factors that synergistically interact to disable host immune defences. Elimination of granulocytes by apoptosis is likely to be beneficial to the parasitoid because of the essential role these cells play in non-self recognition, capsule formation and the production of different effector molecules (Strand, 2008). However, apoptosis has also been suggested to function as an antiviral defence by hosts by eliminating infected cells that serve as sites for viral replication. Given the absence of replication by MdBV in lepidopteran hosts, the observation that only certain host cells infected by MdBV apoptose, and that a single viral gene triggered cell death of SF-21 cells, our results overall favour the hypothesis that MdBV induces apoptosis as part of a larger strategy to disable the host immune system and facilitate successful development of its associated wasp.

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