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Review Article

Venom-Induced Immunosuppression: An Overview of Hemocyte-Mediated Responses

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Parasitic wasps are important natural enemies of several insect pests. They use a variety of methods to modulate their insect host for their progeny to develop. For example, the female wasp needs to avoid or suppress the host immune responses by introducing venom with or without virus like particles and/or polydnaviruses. The aim of this paper is to provide a synthesis of current knowledge regarding the immunosuppression of host immunity with venom in parasitoids that are devoid of symbiotic viruses. Special emphasis is given through disabling host hemocytes by venom of the endoparasitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae) with comparisons of venoms from other parasitoid species.

1. Introduction

Insects and other invertebrates defend their lives against foreign materials with their effective immune systems. The immune system is commonly divided into two major branches named innate (or natural) and adaptive (or acquired) immunity [1–3]. Although invertebrates have long been categorized as possessing only innate immunity, cumulative experimental data from invertebrates indicate that specificity and memory might exist in invertebrates [4]. However, functional evidence for this is still scarce [1]. The innate immune system of insects is divided into humoral and cellular defense responses [2, 5, 6]. Insects are known to possess an innate immune system capable of recognizing foreign (i.e., nonself) materials like parasitic wasp eggs and larvae. Innate immune responses include phagocytosis, nodulation, encapsulation, melanization, blood coagulation, and release of stress-responsive proteins and molecules [1, 2, 5, 6].

Once pathogens and/or invading organisms gain entry into the hemocoel of the host, they encounter innate defense mechanisms involving cellular and humoral responses [1]. Humoral defenses include the production of antimicrobial peptides (AMPs), reactive intermediates of oxygen or nitrogen, and the complex enzymatic cascades that regulate clotting or melanization of hemolymph [1, 5–7]. In contrast, a cellular immune response that involves different types of hemocytes, which participate in pathogen clearance by phagocytosing microorganisms, trapping them in hemocyte aggregates nodules, or encapsulation of larger microorganisms and cytotoxic reactions is also triggered [1–3, 8]. In fact, there is an overlap between humoral and cellular defense, since many humoral factors affect hemocyte function and hemocytes are an important source of many humoral molecules [2, 8].

Parasitic wasps have evolved a variety of strategies in avoiding host-cell-mediated immune responses [5, 6, 9–11]. Endoparasitoids have probably coevolved with their hosts and use host milieu both for nutrition and as regulatory signals [12]. To develop successfully in the hemocoel of their hosts, endoparasitoids suppress, modify, or regulate the host immune/defense system by maternally derived secretions by female wasp during oviposition [5, 9]. These secretions include endosymbiotic viruses (e.g., polydnaviruses (PDVs), entomopoxvirus), virus-like particles (VLPs), ovarian fluids, teratocytes (derived from injected eggs), and venoms [5, 9, 13–18].

Either alone or in combination with other maternal factors, parasitoid venom is known to have distinct functions, including inhibition or reduction of the hemocyte responses [5, 19]. In most cases, venom enhances the effects of PDVs or calyx fluid rather than serving as separate immunological suppressants [16, 19–21]. However, in parasitoid species that are devoid of PDVs or other symbiotic viruses [9, 22–26], venom would alone perturb host immune defenses and may complement or replace the functions of other maternal factors [24].

Examples of such parasitoids include *Pimpla hypochondriaca* Retzius (Hymenoptera: Ichneumonidae) [9, 22], *Pteromalus puparum* L. (Hymenoptera: Pteromalidae) [23, 24], *Nasonia vitripennis* Walker (Hymenoptera: Pteromalidae) [26, 27], *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) [25], *Asobara citri* (Hymenoptera: Braconidae) [28], *Asobara tabida* (Hymenoptera: Braconidae) [29], and *Asobara japonica* (Hymenoptera: Braconidae) [30].

The purpose of this paper is to summarize the general patterns of changes in host immunity after parasitism by parasitoid species. Emphasis is placed on studies in parasitoids that are devoid of PDVs. We will also discuss this subject with other parasitoid species that lack PDVs in their venoms.

2. Mode of Action of Venom That Lacks PDVs

In endoparasitoid and ectoparasitoid species that are devoid of PDVs and virus-like particles, venom appears to play a major role in suppression of host immunity [9, 23, 25, 26]. Venoms of parasitoids are notably known to disrupt host physiology, biochemistry, and development by evoking paralysis, through inhibition of host molting, and by disrupting calcium homeostasis in specific host tissues in some cases [9, 22, 25, 31–39]. The composition of venom from Hymenoptera may vary within groups or even species [40–42]. Several studies have revealed the composition of parasitoid wasp venoms to be a complex cocktail of low- and high-molecular-weight compounds such as amines, peptides, proteins, enzymes, and glycoprotein [22, 39, 40, 43–61]. This complexity of venom enables parasitoids to avoid variations in the susceptibility of different hosts to a single component and to adapt for a wide range of hosts [60].

The physiological effects of wasp venoms vary depending on the host species and stage attacked [21, 35, 37, 62]. For example, idiobiont parasitoids possess venom that paralyzes or kills the host [63, 64], whereas koinobiont venoms cause an arrest or slow growth and development [22, 34, 37, 65, 66]. Moreover, qualitative and quantitative changes in venom content arise with changes in the physiological state of the wasp associated with age and source of food available for adult females [67].

3. Alterations in Total and Differential Hemocyte Count

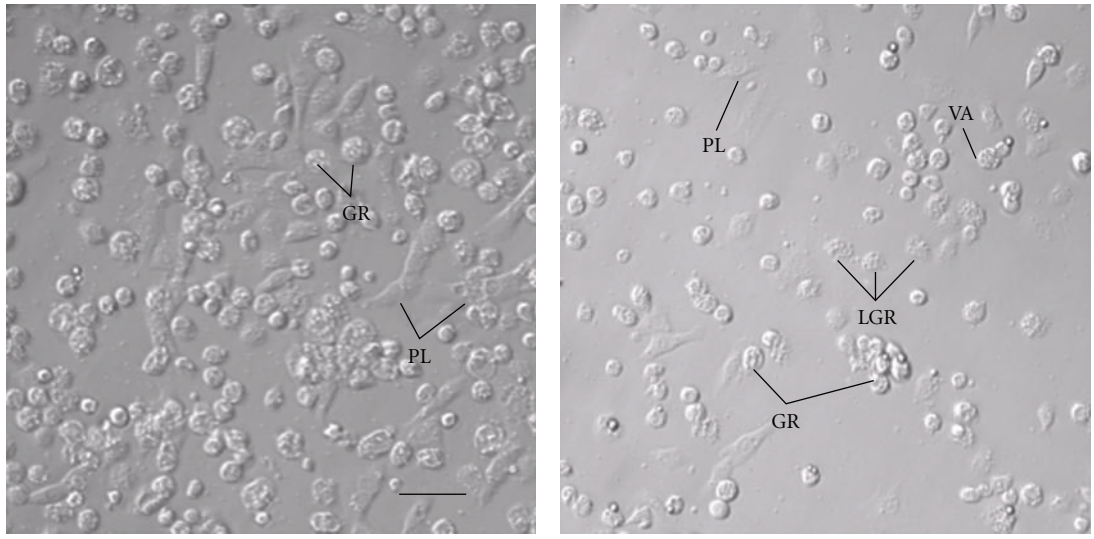
In endoparasitic wasps not transmitting PDVs, venom has been shown to paralyze [22, 25], castrate the host [68], have an antibacterial effect [69], or affect the host immune system [10, 23, 70–72]. Inhibition of host immune responses is critical for endoparasitic species to ensure that eggs and larvae are not recognized and eliminated by the cellular arm of the immune defenses. Protection for wasp progeny generally is achieved through disabling host hemocytes [71].

Several authors have reported on the effects of parasitism on total and differential hemocyte counts in different insect hosts. Recently, studies have been conducted to examine the effects of parasitism and venom from *P. turionellae* on total and differential hemocyte numbers in two developmental stages of the host, *Galleria mellonella* (Lepidoptera: Pyralidae) [71]. Total hemocyte count indicated a considerable decline in the number of circulating hemocytes in *G. mellonella* pupae and larvae exposed to *P. turionellae* or any dose of wasp venom below the LD_{99} calculated for *G. mellonella* pupae and larvae [25] injected experimentally [71]. Also, significant variations in the number of differential hemocyte counts of *G. mellonella* occurred among parasitization and venom treatments in vivo [71]. Significant decreases in the percentage of granulocytes and increases in the percentage of plasmatocytes were observed at different time intervals at pupal and larval stages of *G. mellonella* [71]. In vitro assays with isolated *G. mellonella* hemocytes revealed that addition of an LC_{99} dose of venom (0.001 VRE/μL) induced some vacuole formation in both plasmatocytes and granular cells within 15 min of treatment [71]. However, the degree of vacuole formation was much more extensive in granular cells at later time points than for plasmatocytes, and granular cells seemed much more susceptible to venom as evidenced by cell death (Figure 1) [71].

The drop in cell numbers in venom-treated and parasitized hosts appeared to be due to hemocyte death. *P. turionellae* displays a broad host range [73] and can successfully oviposit in multiple life stages of the same hosts. Similarly, isolated venom has been shown to be toxic to a broad range of insects, including multiple developmental stages and cell types [25]. However, *P. turionellae* females select pupae over larvae for oviposition when given a choice and pupal hemocytes are more susceptible to parasitism and venom injection [71].

In several lepidopteran hosts, successful parasitization by parasitic wasps leads to a reduction in the total number of hemocytes in circulation [74, 75]. Studies suggested that the number of hemocytes remaining in the hemolymph is an essential factor of the host immune defense reactions especially in encapsulation reactions around the parasitoid eggs. Decreases in hemocyte numbers and increases in hemocyte damage also occurred in experimentally envenomated insects [70, 76, 77]. In contrast, Zhang et al. [78] reported that parasitism by *P. puparum* resulted in a noticeable increase in total hemocyte numbers of its two hosts for a defined period. The same trend was also observed before by Eslin and Prevost [29] who reported an increase of hemocyte counts in larvae from six *Drosophila melanogaster* Fabr. (Diptera: Drosophilidae) subgroup species after parasitism by *A. tabida*.

A major part of our knowledge concerning the immune suppressive effects of parasitoids on their hosts comes from



(a) (b)

Figure 1: Phase-contrast micrographs of adhesive hemocytes from the last instars of *G. mellonella* cultured in vitro with isolated crude venom (0.001 VRE/μL) from *P. turionellae*. Hemocytes were incubated in vitro at 27°C for 1 h in TC-100 medium with 10% FBS before the addition of phosphate isolation buffer (a) or venom (b). Photomicrographs were taken at 45 min after treatment. GR: granular cells; PL: plasmatocytes; LGR: lysed granular cells; VA: vacuoles. Bar = 20 μm *(Effects of parasitization and envenomation by the endoparasitic wasp *Pimpla turionellae* (Hymenoptera: Ichneumonidae) on hemocyte numbers, morphology and viability of its host *Galleria mellonella* (Lepidoptera:Pyralidae) [71]. Copyright [2010] Entomological Society of America).

PDVs or VLPs. In certain host parasitoid systems, action of venom components is necessary to enhance the effects of PDVs [79–81]. However, a limited number of studies suggest that venom from idiobiont endoparasitoids devoid of symbiotic viruses may alone affect total and differential hemocyte counts and hemocyte morphology as a part of perturbing host immune defenses (Table 1).

In certain host parasitoid systems, the reduction of hemocytes in circulation was caused by cell death [27, 63, 82–85]. Apoptosis and/or oncosis appear to be necessary means to manipulate the host to ensure successful development of parasitoid larvae [27, 63, 82–85]. Recently, studies have been conducted to examine the ability of *P. turionellae* venom to induce cell death in the circulating hemocytes of the natural host *G. mellonella* at the larval and pupal stage [82]. The occurrence of apoptosis in venom-treated, parasitized, and untreated host larvae and pupae was detected using acridine orange/ethidium bromide double staining method (Figure 2) [82]. This method of detecting apoptosis is based on the loss of plasma membrane integrity as cells die [86]. Cells were identified as viable (green nucleus with red-orange cytoplasm with an intact membrane), early apoptotic (cell membrane still continuous but chromatin condensation and an irregular green nucleus are visible), late apoptotic (ethidium bromide penetrates through altered cell membrane and stains the nuclei orange, while fragmentation or condensation of chromatin is still observed), and necrotic (orange nucleus with intact structure) [86, 87]. Also venom-induced apoptosis was detected using an Annexin V-FITC and propidium iodide apoptosis detection kit [82]. The kit relies on cells undergoing early apoptosis translocating membrane phosphatidylserine (PS) to the cell surface [82].

Acridine orange/ethidium bromide double staining indicated that parasitism and experimental envenomation of *G. mellonella* by *P. turionellae* resulted in markedly different effects on the ratio of apoptotic hemocytes circulating in hemolymph depending on the host developmental stages [82]. The ratio of early and late apoptotic hemocytes increased more than 100% compared to untreated, null-, and PBS-injected controls for host pupae and larvae at higher doses of venom and after parasitization for pupae. Venom-induced apoptosis was also observed in vitro using hemocytes from the last instar larvae of *G. mellonella* double stained with an annexin-V-sensitive probe (conjugated to FITC) and propidium iodide (Figures 3 and 4) [82]. Staining of hemocytes with annexin V-FITC revealed green fluorescent "halos" along the plasma membranes of venom-treated cells within 15 min following exposure to venom. By 1 h after venom treatment, the majority of hemocytes displayed binding of this probe, indicative of early stage apoptosis. These same hemocytes also displayed a loss of plasma membrane integrity at the same time points as evidenced by accumulation of propidium iodide in nuclei [82].

The most common feature shared in the action of wasp secretions and viral products is the induction of cell death in selected tissues of the insect host [85]. Apoptosis and/or oncosis appear to be necessary means to manipulate the host to ensure successful development of parasitoid larvae

Table 1: Changes in total and differential hemocyte counts and hemocyte morphology induced by parasitism and venom from the parasitoids devoid of symbiotic viruses.

Parasitoid	Host	Treatments	Effects observed			Ref.
			THC	DHC	Hemocyte Morph.	
Pimpla turionellae	*Galleria mellonella*	Parasitized Venom	Reduced Reduced	GR increased PL reduced GR increased PL reduced	(i) Vacuole formation (ii) Membrane blebs	[71]
Pimpla turionellae	Cells derived from *Trichoplusia ni* and *Aedes aegypti*	Venom			(i) Rounded (ii) Plasma membranes Swelled	[25]
Nasonia vitripennis	Cells derived from *Trichoplusia ni*	Venom			(i) Retract cytoplasmic extensions (ii) Rounded (iii) Vacuole formation	[27]
Nasonia vitripennis	*Sarcophaga bullata*	Parasitized Venom	Reduced Reduced	PL reduced PL reduced	(i) Retract pseudopods (ii) Rounded	[26]
Asobara citri	*Drosophila melanogaster*	Parasitized	Reduced	PL reduced LM reduced		[28]
Asobara tabida	6 *Drosophila* species	Parasitized	Increased	PL increased LM increased		[29]
Asobarajaponica	*Drosophila melanogaster*	Parasitized Venom + ovarian extract Ovarian extract	Reduced Reduced No changes	PL reduced PL reduced No changes	No effect	[30]
Pimpla hypochondriaca	*Lacanobia oleracea*	Venom	Reduced		Extensive damage disintegration	[10]
Pteromalus puparum	*Pieris rapae*	Parasitized Venom	Increased	PL reduced GR increased	Rounded	[23]

[63, 76, 89]. Apoptosis, triggered by symbiotic viruses of parasitoid wasps has already been reported in previous studies. In *Pseudoplusia includens* Walker (Lepidoptera: Noctuidae) parasitized by *Microplitis demolitor* Wilkinson (Hymenoptera: Braconidae), infection with PDV induces host granulocytes to undergo apoptosis characterized by cell surface blebbing, fragmentation of DNA, and chromatin condensation, while plasmatocytes lose their capacity to adhere to foreign surfaces [90]. In *Diachasmimorpha longicaudata* Ashmead (Hymenoptera: Braconidae)/*Anastrepha suspensa* Loew (Diptera: Tephritidae) system, the entomopoxvirus of the parasitoid caused hemocyte apoptosis [91]. In *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) larvae parasitized by *Toxoneuron nigriceps* (Hymenoptera: Braconidae), total number of hemocytes decreased and the hemocytes showed different structural damages which suggested the occurrence of apoptosis and these hemocyte alterations selectively induced in granulocytes [92]. In *Pseudaletia separata* Walker (Lepidoptera: Noctuidae) parasitized by *Cotesia kariyai* (Hymenoptera: Braconidae), the hemocytes increased in number and PDVs induced apoptosis in the circulating hemocytes and hematopoietic organs [83]. Suzuki and Tanaka demonstrated that injection of *Meteorus pulchricornis* Wesmael (Hymenoptera: Braconidae) virus like particles into *P. separata* induced apoptosis in hemocytes, particularly granulocytes [93]. The authors suggested that induction of apoptosis could be triggered directly or indirectly by the viral gene products expressed in host cells [93]. It is speculated that the envelopes of VLPs seem to contain some ligands for penetration against receptors on the host cell surface and specificity between such ligands and receptors relates to susceptibility of host tissues to VLPs function [93]. Relatively, little is known about the mechanisms involved in hemocyte apoptosis induction triggered by endosymbiotic viruses or VLPs. Cytoplasmic bleb formation is frequently associated with plasma membranes of apoptotic cells and involves disruption of the cytoskeletal membrane interactions and is speculated to depend on the activation of Ca^{2+}-dependent proteases [85, 94, 95]. Protease activation is dependent on an elevation of intracellular calcium and involvement of phospholipases [85, 96]. Influx of Ca^{2+} through L-type calcium channels on the plasma membrane and mobilization of calcium from intracellular stores are primary effects of several animal viruses [85, 97].

Despite the lethal action of parasitic wasp venoms on their hosts, the molecular mechanisms caused by venom in suppressing host immunity and inducing death are partly unknown. In most cases, venom enhances the effects of PDVs or calyx fluid rather than serving as separate immunological suppressants [16, 19–21]. However, in parasitoid species that are devoid of PDVs or other symbiotic viruses venoms alone perturb host immune defenses. In the *P. puparum/Pieris*

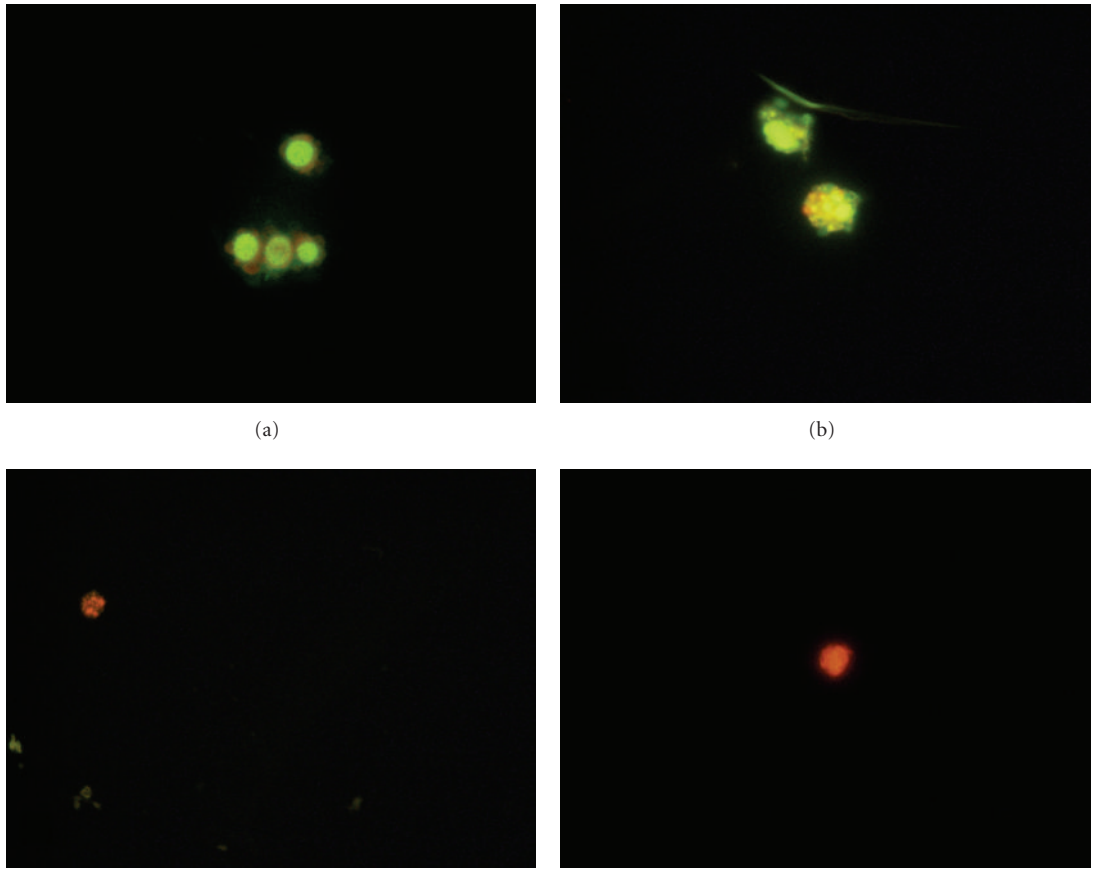


Figure 2: Acridine orange/ethidium bromide double staining of *G. mellonella* hemocytes with characteristic symptoms of apoptosis. (a) Normal hemocytes from untreated larvae, (b) early apoptosis, (c) late apoptosis, and (d) necrosis from parasitized larvae of *G. mellonella*. Scale bar 10 μm *(Effects of parasitism and application of venom from the endoparasitoid *Pimpla turionellae* on hemocytes of the host *Galleria mellonella* [82]. Copyright [2010] Blackwell Verlag, GmbH).

rapae Linnaeus (Lepidoptera: Pieridae) system venom alone was shown to prevent spreading and encapsulation of hemocytes; however, staining of filamentous actin showed that the cytoskeleton of host hemocytes was not visibly affected by venom treatment [23]. Parasitization and high doses of venom from *P. turionellae* induced hemocyte apoptosis in the larval and pupal stage of the host *G. mellonella* [82]. Also observations revealed that when envenomation experiments are performed in media lacking a source of calcium, PI accumulates in the nucleus but annexin V does not bind to the hemocytes. These findings indicate that venom from *P. turionellae* induces apoptosis in hemocytes by a pathway dependent on extracellular calcium influx [82]. However information is lacking on how venom operates at the cellular level or interactions that occur between venom proteins and target hemocytes. Similarly, venom from *P. hypochondriaca* that lacks PDV and VLP kills the *L. oleracea* hemocytes by apoptosis in a dose-responsive manner [84]. Another study indicates that venom from *P. hypochondriaca* triggers apoptotic pathways leading to cell death in some cell types and active phenoloxidase in venom triggers apoptosis in cultured insect cells [98]. Venom from the ectoparasitic wasp *N. vitripennis* causes the host hemocytes to die by an oncotic mechanism largely due to the induced cellular swelling [26]. However, further studies suggested that apoptotic and/or nonapoptotic programmed cell death is the primary mechanism of hemocyte death evoked by *N. vitripennis* venom [27]. The identification of calreticulin in both *P. hypochondriaca* and *N. vitripennis* makes it the candidate in venom to trigger apoptotic pathways [27, 99, 100]. Calreticulin is a Ca^{+2}-binding protein that modulates calcium levels in both endoplasmic reticulum (ER) and mitochondria, and, hence, once in the intracellular environment, this protein could conceivably stimulate the venom-induced mobilization of intracellular calcium, which in turn would trigger numerous cellular changes including movement of the cytoskeletal filaments, swelling, and death by oncosis and apoptosis [98, 101]. Also, laccase in venom from *N. vitripennis* has phenoloxidase activity, which could evoke disruption of plasma membrane integrity in susceptible cells, blebbing, rounding, and swelling, and it was suggested that, together with calreticulin, they could be involved in venom-mediated mobilization of intracellular calcium that ultimately leads to cell death [61, 102]. The cytotoxic effects triggering apoptosis

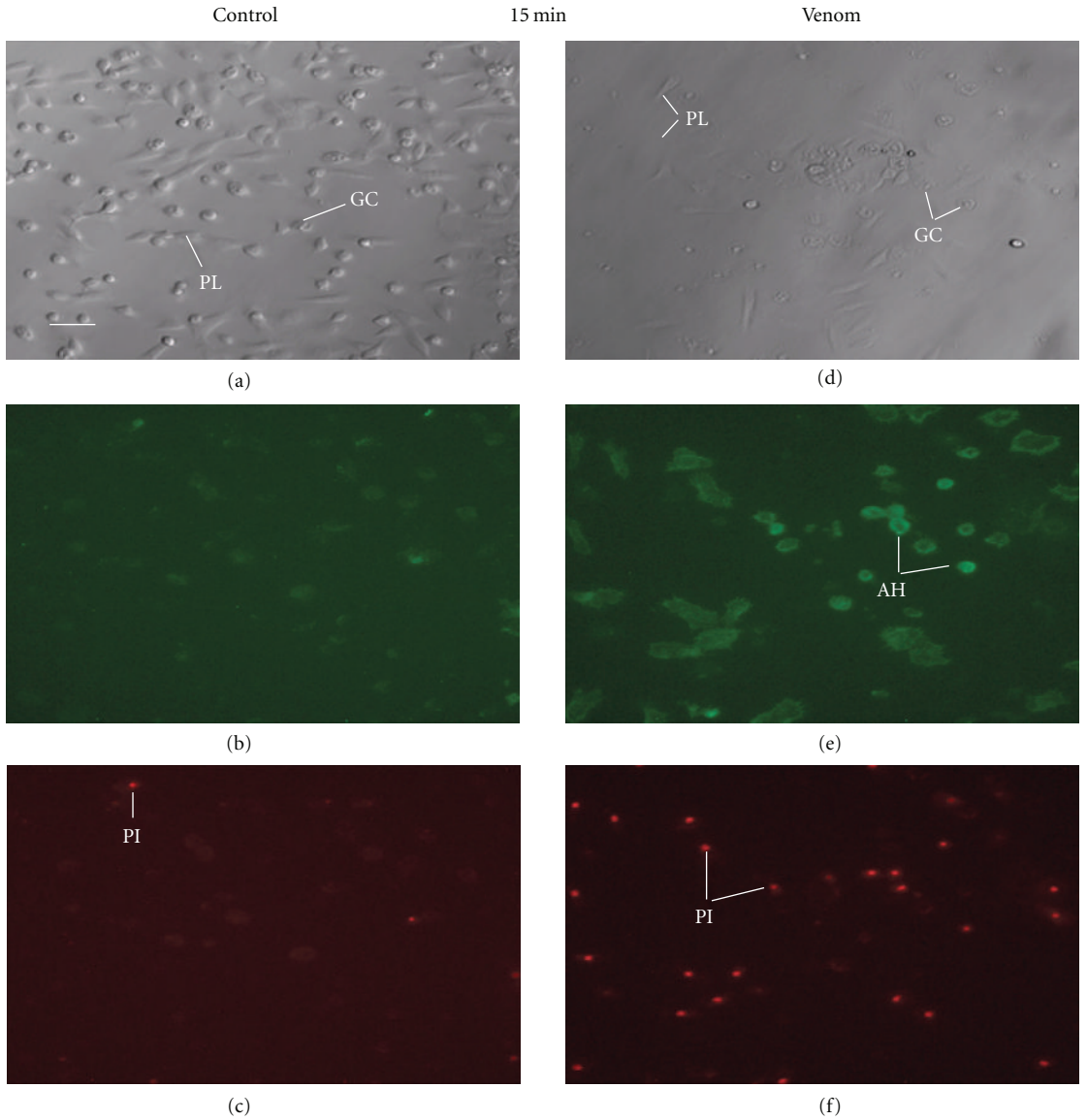


Figure 3: Fluorescence microscopy of hemocytes collected from *G. mellonella* incubated with crude venom from *P. turionellae* and double stained with an annexin-V-sensitive probe (conjugated to FITC) and propidium iodide. Qualitative labeling of annexin V in the plasma membrane (b and e) or cellular uptake of propidium iodide (c and f) was monitored 15 min after exposure to wasp venom. Cells exposed to PBS served as controls (a)–(c), and a 0.25 VRE dose of *P. turionellae* venom was used for toxicity assays (d)–(f). PL: plasmatocyte; GC: granular cell; AH: annexin halo; PI: propidium iodide. The bar corresponds to 18 μm. *(Effects of parasitism and application of venom from the endoparasitoid *Pimpla turionellae* on hemocytes of the host *Galleria mellonella* [82]. Copyright [2010] Blackwell Verlag, GmbH).

of parasitoid venoms that are not working with symbiotic viruses synergistically could also be attributed to the component metalloproteinases which were identified in wasp venom [53, 82, 100]. Previously apoptosis caused by snake venom metalloproteinases has been characterized in human endothelial cells [103, 104].

Another possible mechanism that is responsible for variations in the hemocyte numbers that is associated with parasitism in several host-parasitoid systems could be the suppression of cell cycle via parasitoid-derived secretions. It is known that the maintenance of circulating hemocytes is supplied by the mitosis of circulating hemocytes itself and from hematopoietic organs [105, 106]. In *G. mellonella*, *Bombyx mori* Linnaeus (Lepidoptera: Bombycidae), and *Euxoa declarata* Walker (Lepidoptera: Noctuidae) mitosis in circulating hemocytes has been observed and it was confirmed that 1–8% of the population of circulating hemocytes is in the mitotic phase [105, 107–109]. Er et al. [82] recently showed that both parasitization and envenomation by *P. turionellae* venom resulted with a considerable decline in mitotic hemocytes in circulation of *G. mellonella*. Though there are few studies on the effect of endoparasitoid venom or parasitization on mitosis of host hemocytes, it was revealed that mitosis of circulating hemocytes halted after the injection of

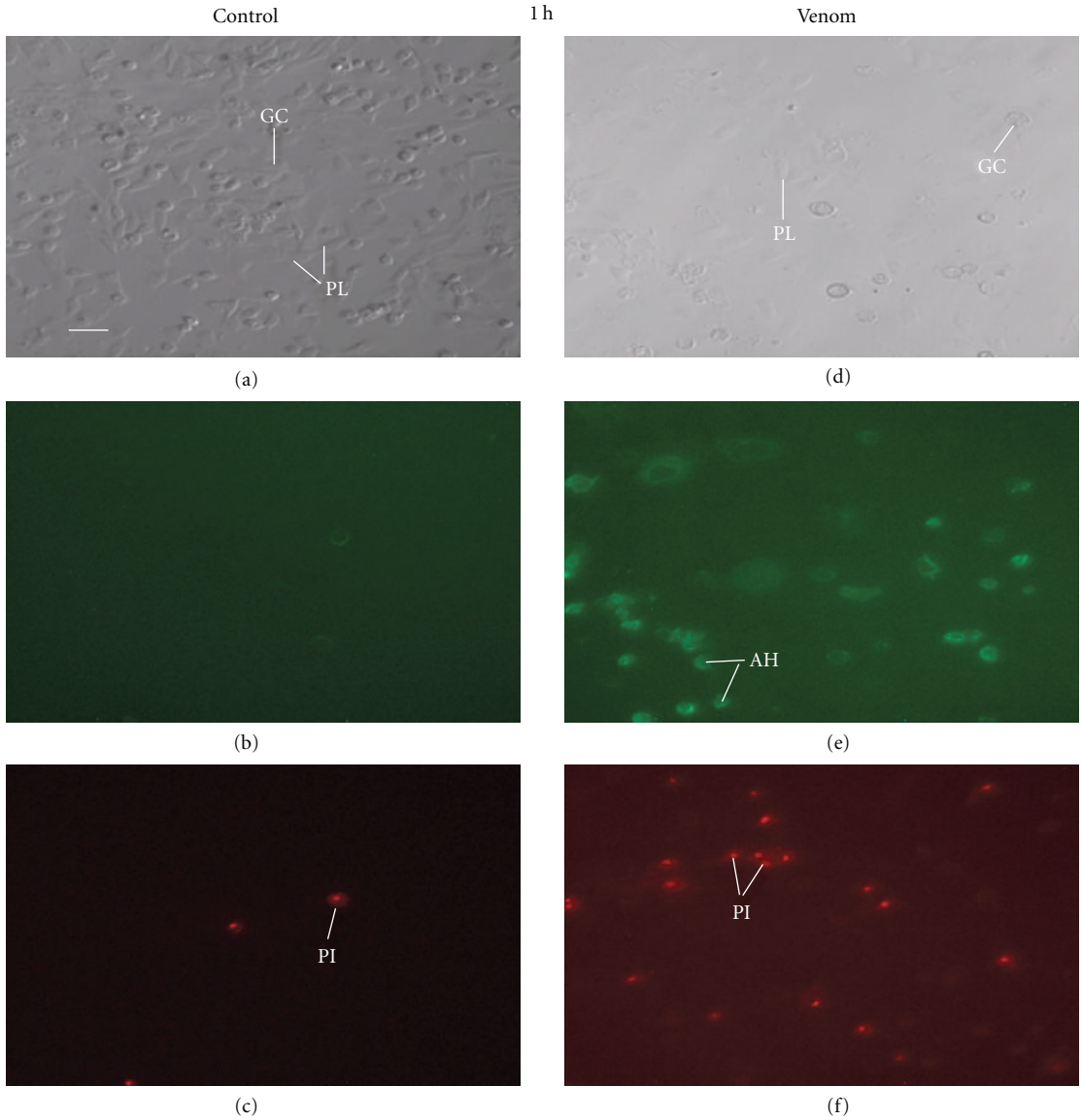


Figure 4: Fluorescence microscopy of hemocytes collected from *G. mellonella* incubated with crude venom from *P. turionellae* and double stained with an annexin-V-sensitive probe (conjugated to FITC) and propidium iodide. Qualitative labeling of annexin V in the plasma membrane (b and e) or cellular uptake of propidium iodide (c and f) was monitored 1 h after exposure to wasp venom. Cells exposed to PBS served as controls (a)–(c), and a 0.25 VRE dose of *P. turionellae* venom was used for toxicity assays (d)–(f). PL: plasmatocyte; GC: granular cell; AH: annexin halo; PI: propidium iodide. The bar corresponds to 15 μm. *(Effects of parasitism and application of venom from the endoparasitoid *Pimpla turionellae* on hemocytes of the host *Galleria mellonella* [82]. Copyright [2010] Blackwell Verlag, GmbH).

C. kariyai PDV plus venom into *P. separata* [83]. The authors demonstrated that the PDV plus venom caused the disappearance of the 4C and 8C ploidies, and PDV alone produced the humoral plasma factors that suppress the cell cycle [83]. Further investigation is needed concerning the mechanisms involved in cell cycle arrest as there are only a few studies on venom-induced changes in mitotic indices of the host.

4. Encapsulation

The major immune response towards internal parasites and other foreign entities that enter the insect's hemocoel is encapsulation [72, 90, 110, 111]. The sequence of how different hemocyte types are engaged in encapsulation, including recognition, opsonization, recruitment of cells, and formation of a multilayer sheath, has also been described [2, 72, 112, 113]. Encapsulation begins when host granulocytes attach to the surface of a foreign target. The attached granulocytes lyse or degranulate, releasing the contents of their granules over the foreign object. This is assumed to attract and allow the plasmatocytes to attach. Termination of capsule formation occurs when a subpopulation of granulocytes adheres in a monolayer around the periphery of the capsule [5, 14, 72, 114–116]. The process is ultimately

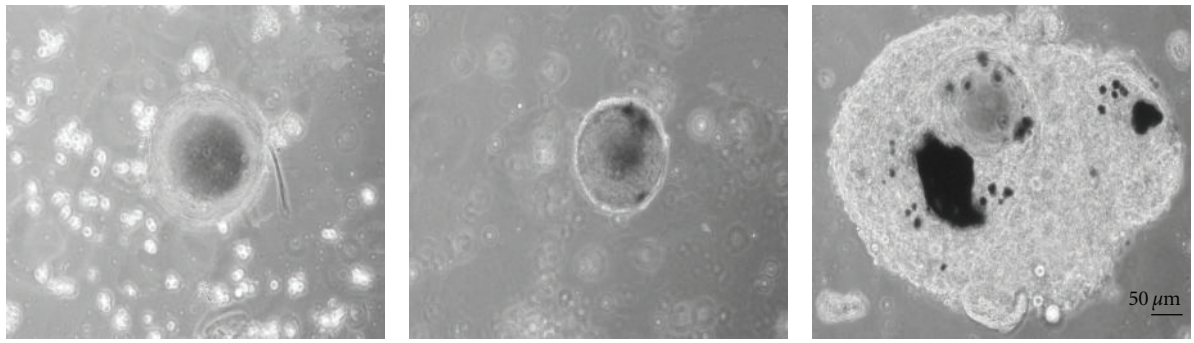


Figure 5: Encapsulation of Sephadex A-25 beads in *G. mellonella* larvae. (a) Negative (no or only a few beads attached to the bead), (b) weak (2–10 layers of hemocytes around the bead), AND (c) Strong (more than ten layers of hemocytes around the bead). *(Levels of encapsulation and melanization in *Galleria mellonella* (Lepidoptera: Pyralidae) parasitized and envenomated by *Pimpla turionellae* (Hymenoptera: Ichneumonidae) [72]. Copyright [2009] Blackwell Verlag, GmbH).

accompanied by blackening of the capsule because of melanization and finally the encapsulated organism almost always dies [2, 5]. Several factors, including asphyxiation, the local production of cytotoxic quinones or semiquinones via the proPO activation cascade during melanization, free radicals, and antibacterial peptides have been suggested to function as killing agents [1, 2, 110, 117, 118].

An important point of the encapsulation response in insects is associated with host-parasitoid relationships. Endoparasitoid species that lay their eggs into insect hosts must avoid encapsulation responses of the host hemocytes. According to Strand and Pech [115], the most direct way of preventing encapsulation is to destroy, deplete from circulation, or alter the behavior of the hemocytes that mediate encapsulation. Many parasitoids do this by introducing fluids at the time of oviposition that contain antihemocytic and/or immunosuppressive factors [119]. Some of these factors may be derived from PDVs or VLPs while others from venom produced by the adult female parasitoids. The role of PDVs, VLPs, and other ovarian fluids in suppression of host encapsulation ability in many parasitoid-host systems has been well documented [13, 75, 79, 93, 120, 121]. However, a limited number of studies suggest that venom from endoparasitoid species devoid of symbiotic viruses may alone suppress encapsulation reactions of the host hemocytes.

We recently described how parasitism and venom from *P. turionellae* that lacks symbiotic viruses affected the encapsulation and melanization rates of *G. mellonella* larvae and pupae [72]. In the experiments, DEAE-Sephadex A-25 beads were used as encapsulation targets. The removed beads from the insects scored as negative (no, or only a few hemocytes attached to beads), weak (2–10 layers of hemocytes around beads), and strong (more than 10 layers of hemocytes around beads) (Figure 5) [72, 119]. The analysis of the Sephadex A-25 beads injected in different developmental stages of *G. mellonella* revealed that a strong encapsulation reaction can occur in the pupal stage but the encapsulation material was less compacted compared to larvae [72]. Our investigation revealed that the number of beads strongly encapsulated and melanized was reduced by more than 50% at 4 and 24 h after injection of venom in pupae (0.05 VRE) and in larvae (0.5 VRE) [72]. Similar results were also obtained when beads were recovered from parasitized pupae indicating that parasitization by *P. turionellae* suppressed hemocyte-mediated encapsulation in *G. mellonella* [72]. Our results are similar to those reported in the host parasitoid systems that lack symbiotic viruses where venom was shown to prevent encapsulation (Table 2). The efficacy of encapsulation response is known to be influenced by a number of parameters, including the number of hemocytes available and their ability to spread [115, 120, 121]. Thus changes in the spreading ability of hemocytes caused by venom are also shown in Table 2. Parasitism by an ectoparasitoid *N. vitripennis* was showed to have an impact on host hemocytes that plasmatocytes and granulocytes lost the ability to spread [26]. Similarly venom from the endoparasitoid *P. hypochondriaca* affects the spreading of plasmatocytes [10]. In the *P. puparum/P. rapae* and *P. puparum/P. xuthus* systems the spreading of hemocytes was greatly inhibited with venom resulting from the inhibition of plasmatocyte pseudopod formation [23, 78].

Since *P. turionellae* and other endoparasitoid species specially emphasized on in this paper are devoid of any VLPs or PDVs, it was expected that components of the wasp venoms could contribute towards avoidance of encapsulation by the parasitoids. However, limited numbers of endoparasitoid venom proteins have been reported to affect the hemocyte behavior of insects [122]. For example, a single venom protein Vn.11 with a mean of 24.1 kDa in size has been isolated from *P. puparum* venom [24]. The protein was identified as an immune suppressive factor, and was suggested to affect the spreading and encapsulation ability of host hemocytes [24]. *P. puparum* venom was also shown to influence gene expression in host hemocytes and fat body [123]. Venom treatments led to reductions in expression of a large number of genes acting especially in immunity [123]. Biochemically isolated venom proteins Vpr1 and Vpr3 from *P. hypochondriaca* were shown to suppress encapsulation

Table 2: Changes in the encapsulation and spreading ability of hemocytes induced by parasitism and venom from the parasitoids devoid of symbiotic viruses.

Parasitoid	Host	Treatments	Effects observed		Ref.
			Hemocyte encapsulation	Cell spreading	
Pimpla turionellae	*Galleria mellonella*	Parasitized Venom	Reduced Reduced		[72]
Pimpla turionellae	Cells derived from *Trichoplusia ni* and *Aedes aegypti*	Venom		Reduced	[25]
Nasonia vitripennis	*Sarcophaga bullata*	Venom		Reduced	[26]
Asobara citri *Asobara tabida*	*Drosophila melanogaster* *Drosophila melanogaster*	Parasitized Parasitized	Reduced No effect		[28]
Asobara tabida	*Drosophila sechellia* *Drosophiala melanogaster*	Parasitized	Slight ability to encapsulate		[29]
	Drosophila mauritiana Drosophila yakuba	Parasitized	Medium ability to encapsulate		
	Drosophila teissieri Drosophila simulans	Parasitized	High ability to encapsulate		
Asobara japonica	*Drosophila melanogaster*	Parasitized	Reduced		[30]
Pimpla hypochondriaca	*Lacanobia Oleracea* (larval stage)	Venom	Reduced	Reduced	[10]
Pimpla hypochondriaca	*Lacanobia Oleracea* (pupal stage)	Venom	Reduced		[54]
Pteromalus puparum	*Pieris rapae*	Venom	Reduced	Reduced	[23]
Pteromalus puparum	*Pieris rapae* *Papilio xuthus*	Venom		Reduced	[78]
Pteromalus puparum	*Pieris rapae*	Venom	Reduced		[88]

responses in a lepidopteran larva in vivo and to inhibit the spreading and aggregation of insect hemocytes maintained in vitro [119, 122]. These works also represent for the first time that the genes for such proteins (i.e., Vpr1 and Vpr3) have been identified from *P. hypochondriaca* and that a function can be applied to proteins produced from the Vpr1 and Vpr3 genes [119, 122]. In *C. rubecula* venom that contains PDV, a 58 kDa calreticulin-like protein was found to inhibit the spreading behavior of the host hemocytes and thus preventing the encapsulation of the developing parasitoid in the host [124]. Despite the considerable attention given to determine the role of venoms in suppression of host encapsulation response, the modes of action or molecular target sites of parasitoid venom components in host-endoparasitoid systems is still not well known and is in need of further investigation.

5. Conclusion Remarks

In certain host parasitoid systems, venom that is injected prior to oviposition can elicit a diverse range of host responses including suppression of host immune responses. As discussed in this paper, venom can lead to a reduction of hemocytes in circulation, and this phenomenon was thought to be caused by cell death via apoptosis. Also, we have provided an overview of current knowledge on the effects of venom on hemocytic encapsulation responses in different developmental stages of the host insects. Because endoparasitoid species specially emphasized on in this paper are devoid of any VLPs or PDVs, it was expected that components of the wasp venoms could contribute to avoidance of encapsulation by the parasitoids. A series of potential candidates in venom that has obvious potential roles in venom-mediated immunosuppression have been identified in several wasp venoms. However the information presented in this section lacks many mechanistic details of how venom components suppress hemocyte-mediated immune responses at the cellular level. The characterization of venom proteins and comparative genomic approaches should provide insights into their possible mechanisms of action in host-parasite interactions at molecular level. This represents an area of new studies associated with host regulatory factors in parasitoid venom that needs further investigations.

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