Antiviral Role of Apoptosis-Dependent Phagocytosis of Virus-Infected Cells in Drosophila

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Summary

To ensure host survival, virus-infected cells are targeted for elimination by host immune mechanisms. While vertebrates successfully accommodate such a task to their sophisticated immune system, the mechanism how invertebrates, being equipped with simple innate immunity, accomplish this vital responsibility remains unknown. Our laboratory previously reported that influenza virus-infected cells are engulfed by macrophages in an apoptosis-dependent manner, resulting in the inhibition of virus growth in mice. Owing to the similarity of phagocyte's characteristics and functions between mammals and insects, I anticipated that a similar response is also employed in insects as a part of innate immunity. To assess this, I established an infection model system using *Drosophila melanogaster* as a host and *Drosophila* C virus as a pathogen. Infection of *Drosophila* S2, an embryonic cell-derived cell line, was characterized by massive production of progenitor virus accompanied by an elevated level of cells undergoing apoptosis. Such cells were phagocytosed by l(2)mbn, a larval phagocyte-derived cell line, in a manner inhibitable by the treatment with a caspase inhibitor or phosphatidylserine-containing liposomes, and by the RNA interference knockdown of engulfment receptors in phagocytes. Adult flies showed an indication of apoptosis upon infection with *Drosophila* C virus, and their hemolymph contained hemocytes that had phagocytosed virus-infected cells. Furthermore, flies succumbed to viral infection more severely than control flies when they were manipulated for the inhibition of phagocytosis by the injection with latex beads, the depletion of engulfment receptors, or the expression of a phosphatidylserine-binding protein. Taken together, these results indicate that the apoptosis-dependent, phosphatidylserine-mediated phagocytic removal of virus-infected cells is evolutionarily conserved from insects to mice as an anti-viral innate immune mechanism.

Dissertation Abstract

Viral infection is one of the most significant maladies, giving a life-threatening pressure to all living organisms. For affected species to survive, successful adaptation to such biological pressure is, therefore, indispensable. One of means available to achieve this purpose is by the active engagement of various host defense mechanisms against incoming viral threat. Host strategies to fight against viral attack is represented by the immune system that recognizes pathogens, alerts the body of invasion, evokes and amplifies biological reactions, kills and eliminates virus, and cures damaged tissues. Failure to perform such tasks may lead to impairment in cellular homeostasis, development of infectious disease, and, to the worst, lethality to the infected host.

In response to foreign viral particles, vertebrates, including mammals, employ an extensive array of cellular defenses, starting from the production of interferon either by a classical Toll-like receptor-mediated pathway or a recently defined pathway involving mitochondrial antiviral-signaling protein and cyclic GMP-AMP synthase-stimulator of interferon genes, which are categorized into built-in or innate arms of the immune system, to more sophisticated and specific reactions mediated by antigen receptors in an adaptive part of defense mechanisms. In addition to these, a self-consumption process, termed autophagy, as well as an RNA interference pathway that targets viral genomes have been added to a list of major innate antiviral arsenals. Similar protection systems, based on RNA interference and autophagy, have also been described in evolutionarily lower organisms such as invertebrates. However, unlike vertebrates, invertebrates, including insects, are only equipped with an innate part of the immunity system, leaving them with fewer options to resist infection compared to higher organisms.

In addition to virus entities, virus-infected host cells are targeted for elimination by host immune responses. In vertebrates, the involvement of cellular innate and adaptive immunity, owing to the actions of natural killer cells, dendritic cells, macrophages, and T cell subsets, in the removal of virus-infected cells has been widely appreciated. However, most of antiviral cellular effectors are unavailable in invertebrates, despite the fact that those organisms also suffer from a range of virus infection. Our laboratory previously reported that mammalian host cells undergo apoptosis upon viral infection and are

subsequently eliminated by apoptosis-dependent phagocytosis. Since phagocytes are main immune cells present in most invertebrates, including insects, I hypothesized that the phagocytic elimination of apoptotic virus-infected cells may also play an antiviral role in insects. Using *Drosophila melanogaster* as a model animal, I assessed such possibility.

I started to establish an *in vitro* infection system using S2, an embryonic cell-derived *Drosophila* cell line, and *Drosophila* C virus (DCV), a natural pathogenic virus of *Drosophila*. Morphological and biochemical examinations revealed that DCV-infected S2 cells undergo apoptosis in a caspase-dependent manner (Fig. 1A) and subsequently

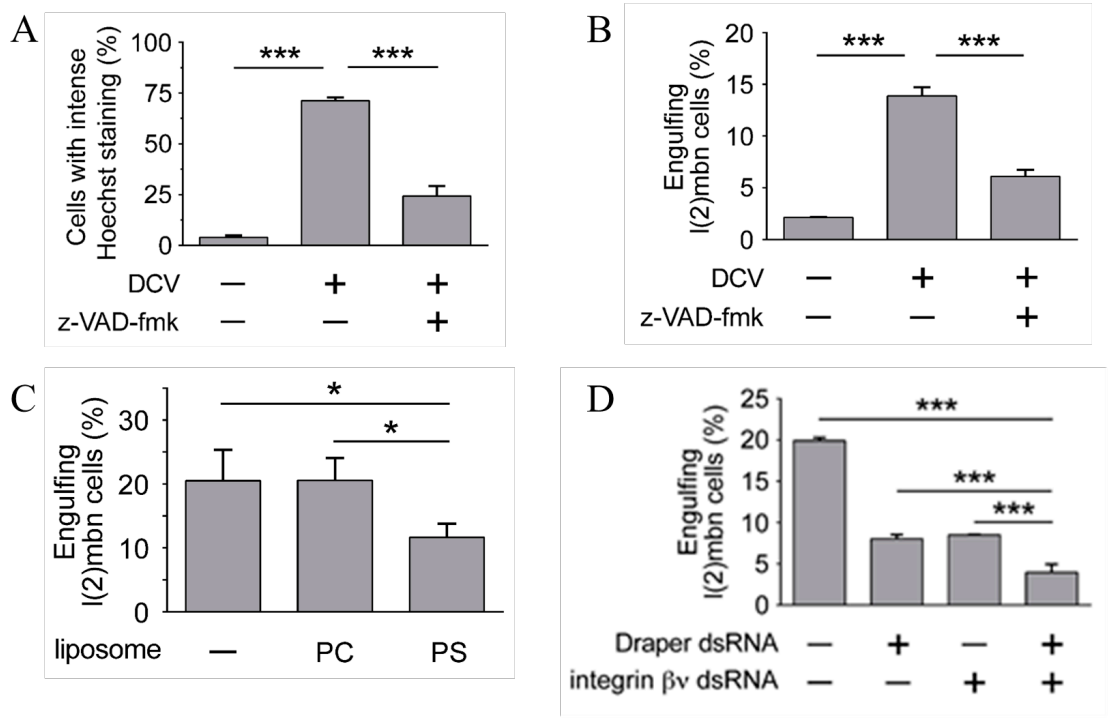

Fig. 1. PS-dependent, engulfment receptors-mediated phagocytosis of apoptotic DCV-infected S2 cells
(**A**) S2 cells were infected with DCV for 3 days in the presence or absence of the caspase inhibitor z-VAD-fmk and stained with Hoechst 33342 prior to examination under a fluorescence microscope. (**B**) Similar cells were subsequently used as target cells in an assay for phagocytosis by 20-hydroxyecdysone-treated l(2)mbn cells. The ratio of l(2)mbn cells that had accomplished phagocytosis were determined. (**C**) S2 cells infected with DCV for 3 days were subjected to an assay for phagocytosis with ecdysone-treated l(2)mbn cells as phagocytes in the presence and absence of liposome (1 mM). (**D**) dsRNA-treated l(2)mbn cells were tested in an assay for phagocytosis with S2 cells infected with DCV for 3 days as targets. Mean ± standard deviations of the data obtained from three independent experiments are presented. * $p < 0.05$ and *** $p < 0.001$.

phagocytosed by l(2)mbn, a cell line established from larval hemocytes, in a manner inhibitable by a caspase inhibitor (Fig. 1B) or phosphatidylserine-containing liposomes (Fig. 1C), leading to the inhibition of virus production. Further analysis demonstrated the independent requirement for two engulfment receptors of *Drosophila*, Draper and integrin αPS3βν, in the recognition and phagocytosis of DCV-infected S2 cells by l(2)mbn cells

(Fig. 1D).

To confirm whether my *in vitro* findings are manifestable in living animals, I next examined the occurrence of apoptosis and the phagocytosis of DCV-infected cells in *Drosophila* that had been infected with DCV. Apoptosis was successfully observed in DCV-infected adult flies based on the cleavage of a caspase target protein, and hemocytes containing engulfed DCV-positive cells were found. When flies were injected with latex beads, a procedure to inhibit phagocytosis, prior to DCV infection, the survival rate of infected flies decreased. Furthermore, flies with mutation in a gene coding for either Draper or integrin βν died earlier than a wild-type counterpart after infection with DCV,

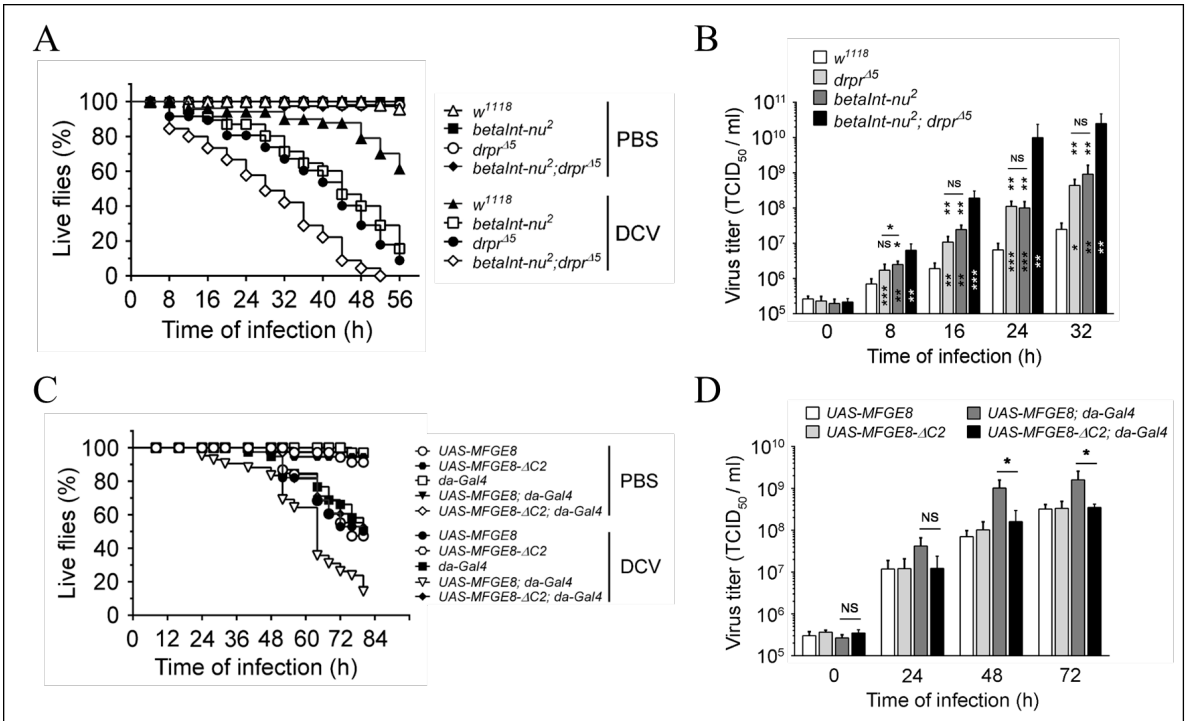

Fig. 2. PS-dependent, engulfment receptors-mediated protection of flies against DCV infection
(**A, C**) Adult flies of the indicated lines were abdominally injected with DCV or PBS as control, and the ratio of live flies was determined at the indicated time. Average values of the data from a 3-vial experiment are presented. (**B, D**) The indicated lines of flies were infected with DCV, collected at the indicated time points, and lyzed. The resulting whole fly lysates were analyzed for virus titer. Mean ± standard deviations of the data obtained from three independent experiments are presented. * $p < 0.05$ and *** $p < 0.001$.

and flies lacking both receptors succumbed to viral infection more severely than those lacking either receptor (Fig. 2A). The level of virus recovered from mutant flies was higher than that from control flies (Fig. 2B). In addition, the systemic expression of a phosphatidylserine-binding protein in flies led to early death of flies and a high level of viral production (Fig. 2C and 2D). Taking the data from *in vitro* experiments into consideration, it is most likely that the phagocytosis of DCV-infected cells by *Drosophila*

phagocytes is mediated by the two major engulfment receptors Draper and integrin αPS3βv as engulfment receptors, and phosphatidylserine as an eat-me signal.

Taken together, this study indicated that an antiviral mechanism involving the apoptosis-dependent phagocytic removal of virus-infected cells is an evolutionarily conserved innate immune response.

審査結果の要旨

ウイルス感染症は人類を含めた多くの生命体の生存を危ぶませる疾患のひとつである。タンパク質合成能を持たないウイルスは、侵入した細胞が持つ合成系を利用して自身を複製する。ウイルス感染防御の仕組みには、ウイルス自体が対象になる場合と感染細胞が標的になる場合とが知られる。後者に分類されるものに、ウイルス感染細胞がアポトーシスに依存して貪食除去される反応が哺乳類で報告されている。学位申請者は、遺伝学を利用する *in vivo* 実験と細胞生化学的な *in vitro* 実験を併用できるショウジョウバエを宿主とするウイルス感染実験を行い、この貪食を介する防御反応が昆虫にも存在するかどうかを調べた。その結果、ウイルス感染したショウジョウバエ細胞がアポトーシスを起こして被貪食能を獲得することがわかった。さらに、感染細胞の貪食でウイルス増殖が抑制され、感染後のショウジョウバエの生存期間が長くなることが示された。

アポトーシス依存的なウイルス感染細胞の貪食が、生物種を越えて共通の生体防御反応であることが明らかにされた。さらに、昆虫は獲得免疫系を持たないため、これがウイルス感染に応答した自然免疫反応であることが示された。本審査委員会は、研究成果の新奇性および口頭発表会における学位申請者の発表と討論の能力を考慮して、当該学位論文は博士（創薬科学）に値すると判定した。