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The Function of Bee Venom PLA2 and Its Impact on Immune Responses

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Abstract

Bee venom (BV) is the secretion that is produced by a needle device for protecting the bee from an enemy. However, BV has been applied in folk medicine for various diseases because it has many enzymes which contain anti-inflammatory or anti-cancer action. Above all, phospholipase A2 (PLA2) is a hydrolytic enzyme that cleaves membrane phospholipids and occupies up to 12% of bee venom. PLA2 has been analyzed in great detail. This mini-review sets out the latest scientific evidence concerning the therapeutic effects of PLA2 in the context of diseases and provides a detailed description of the mechanisms.

Keywords

Bee venom
PLA2 (phospholipase A2)
Enzyme activity
Microglial cells
Phospholipids

1. Introduction

Bee venom is a secretion produced by bees from their sting apparatus to protect themselves from enemies. It has been applied in folk medicine to treat various diseases because it contains many enzymes, and has anti-inflammatory and anti-cancer effects. Recently developed analytical methods have shown that bee venom contains a total of 102 proteins and peptides. Among these, PLA2 (phospholipase A2), an enzyme in bee venom that accounts for up to 12%, has been analyzed in great detail and is known to induce inflammation via arachidonic acid metabolism. This paper outlines the characteristics of bee venom PLA2 and its action on the main immune responses and diseases.

PLA2 is the generic name for a group of enzymes that hydrolyze glycerophospholipids, the major component of biological membranes, to produce free fatty acids and lysophospholipids. PLA2 is not only produced by the aforementioned bee venom but also by mammals, and more than 30 molecular species have been identified, which are broadly classified into several groups based on their structure, localization, and evolution, including cytoplasmic and extracellular secretory types. Initially, it was shown that cytosolic PLA2 releases arachidonic acid from plasma membrane phospholipids, leading to the metabolism of lipid mediators such as prostaglandins and leukotrienes. Recent improvements in lipid metabolome analysis

techniques and other factors are beginning to elucidate the immunoregulatory mechanisms of PLA2 other than cytosolic PLA2. The largest PLA2 subgroup, secretory PLA2 (sPLA2), has 10 active isozymes with different cellular distribution and selectivity for substrate phospholipids. Among these, type III (sPLA2-III) is the only isozyme homologous to bee venom PLA2 and is the only one that induces allergic reactions in humans. sPLA2-III transgenic mice with ApoE deletion (Tg), spontaneously developed atherosclerosis when fed a high-cholesterol diet, there was an increase in blood lysophosphatidylcholine (LPC) and accelerated atherosclerosis, as well as a marked increase in endogenous sPLA2-III expression in human atherosclerotic lesions, indicating that sPLA2-III is involved in atherosclerosis by promoting lipoprotein degradation and denaturation in the vascular wall [1]. In addition, spontaneous onset of dermatitis is frequently observed when sPLA2-III Tg mice are kept for long periods of time.

2. Structure of bee venom PLA2 and its effects on immune responses and diseases

Bee venom PLA2 is an enzyme consisting of a protein with a molecular weight of approximately 14–18 KD and with a structure of three major α-helices and a two-stranded, inversely parallel β-sheet. It has a conserved enzymatic activity due to four non-hydrophobic amino acid residues (H34, D35, Y87, and D64) and an active center consisting of a histidine-aspartic acid pair [2]. It also has a calcium-binding motif and requires millimolar levels of calcium to function as an enzyme. There are reports on the effects of bee venom PLA2 on a variety of immune responses and diseases [3].

2.1. Suppressive effects on neurodegeneration

Microglial cells expressing the human leukemic antigen HLA-DR and their responses have been implicated in neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis.

In Parkinson's disease, microglial cells are activated in response to neuropathy, causing widespread secondary damage to dopamine neurons in the substantia nigra. Activated microglial cells produce pro-inflammatory cytokines such as TNF-α, IL-1β, and IFN-γ, with inducible nitric oxide synthase (i-NOS) and caspase 3 expression, and leads to neuropathy exacerbating factor production such as activation of caspase 8. Free radicals such as NO and superoxide cause lipid peroxidation and neuronal DNA damage, leading to mitochondrial respiratory arrest and cell death. In addition, HLA-DR-expressing microglial cells induce positive signaling, which in turn induces CD4-positive helper T cells to differentiate into Th1 and Th17 cells, which produce inflammatory cytokines. While insulin HLA-DR-expressing microglial cells also induce a positive feedback loop towards neuropathy by stimulating glial cells to inhibit the secretion of insulin-like growth factor (IGF-1), which acts as a neural survival signal. HLA-DR-expressing microglial cells also cause neuropathy by activating the Fas/Fas-ligand pathway, which is an apoptosis-inducing signal. However, bee venom PLA2 acts as a negative regulator of these pathological signs. Bee venom PLA2 binds to lectin-type CD206 receptors on dendritic cells and induces the expression of prostaglandin E2 (PGE2). PGE2 then binds to the EP2 receptor on naive CD4T cells, which induces CD4T cells to differentiate into Foxp3-positive regulatory T cells. Regulatory T cells end inflammation by activating microglia and attenuating T-cell infiltration, as shown in **Figure 1** [4].

Alzheimer's disease is also caused by the formation of amyloid-β peptide in the CNS, which leads to increased extracellular senile plaques in the hippocampus and cortex, intracellular neurofibrillary tangles and neuronal shrinkage, and symptoms such as dementia, and behavioral and cognitive dysfunction. Bee venom PLA2 plays a role in attenuating Alzheimer's disease. In a group of Alzheimer's

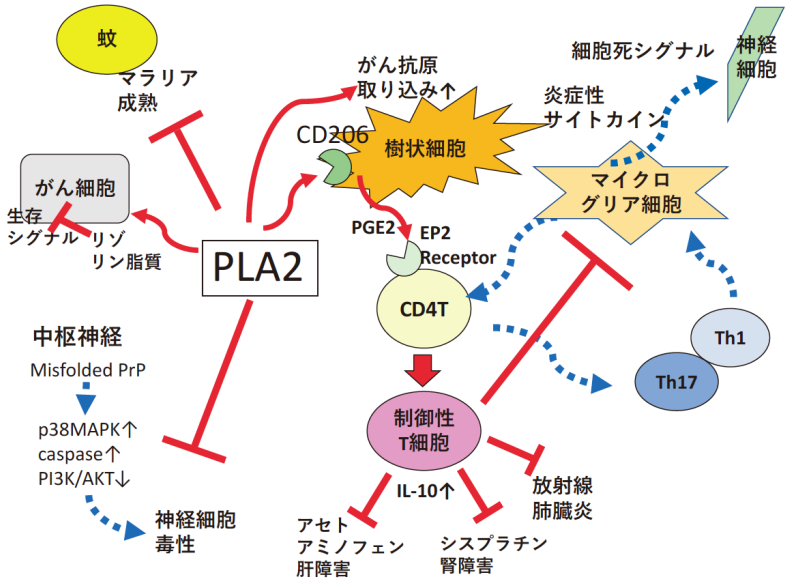

Figure 1. Summary of the effects of bee venom PLA2 on the suppression of neurodegenerative diseases, anti-inflammatory, anti-cancer, and control of parasitic infections. The solid line shows the action of PLA2 and the dotted line shows the signal towards cytotoxicity.

disease model mice expressing three dementia-related genes and treated with bee venom PLA2, cognitive function was improved compared to a control group, brain glucose metabolism was increased, amyloid-β deposition in the hippocampus and T-cell infiltration in the hippocampus was also reduced. In addition, bee venom PLA2 had an enhancing effect on the regulation of microglial activation by regulatory T cells.

Furthermore, bee venom PLA2 attenuates neuronal death caused by prion disease, a neurodegenerative disorder caused by a proteinase K-resistant prion protein called PrP fragment. In prion diseases, normal intracellular prion protein mutates into scrapie prion protein, which blocks the PI3K/AKT pathway, and activates the caspase and p38 mitogen-activated protein kinase (MAPK) pathways, causing neuronal cell death. The addition of bee venom PLA2 to human neuroblastoma cells prior to the addition of prion protein blocked the activation of the p38 MAPK pathway by the prion protein and attenuated caspase, restoring the PI3K/AKT pathway and sparing prion protein-induced cell death, which can be seen in **Figure 1**.

2.2. Anti-inflammatory effects

Bee venom PLA2 also has an inhibitory effect on acetaminophen-induced acute liver injury in antipyretic analgesics. In a mouse model of liver injury induced by acetaminophen, 5-day pre-administration of bee venom PLA2 reduced the levels of enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST), which are indicators of liver injury. Inflammatory cytokines such as IL-6, TNF, and NO have also been reported to be reduced compared to controls. Bee venom PLA2 also exerts a protective function against cisplatin-induced nephrotoxicity by increasing the production of regulatory T cells and the inhibitory cytokine IL-10. Furthermore, bee venom PLA2 has been reported to regulate allergic airway inflammation. Administration of bee venom PLA2 attenuates the inflammatory cell infiltration in bronchoalveolar lavage fluid and goblet cell proliferation in the airway epithelium induced by egg white albumin administration. Bee venom PLA2 also has protective effects against radiotherapy-induced acute lung inflammation via regulatory T cells. A total of six intraperitoneal doses of bee venom given to mice three weeks after irradiation resulted in a clear attenuation of the inflammatory response compared to the control group, which was not observed in mice in which the regulatory T cells had been removed.

2.3. Anti-cancer effects

In various cancer cell lines, bee venom PLA2 has cytolytic and antiproliferative effects. The tumor lysates produced promote differentiation and maturation into monocyte-derived dendritic cells and provide adjuvant action in immunotherapy against tumor cells. The bee venom PLA2 acts on phospholipids in the plasma membrane to produce lysophospholipids, which form micelles and affect the structure of the plasma membrane, impairing the correct function and expression of cell surface macromolecules and receptors, thus disrupting cell survival signaling pathways from the start. These effects also disrupt the PI3/AKT and extracellular signal-regulated kinase (ERK1/2) signaling pathways, which are necessary for cell survival. LPC, a lysophospholipid produced by bee venom PLA2, also activates intracytoplasmic signaling pathways that produce calcium channels and free radicals, inducing plasma membrane damage. These cytotoxic effects have a significant impact on rapidly growing tumor cells by killing them.

Furthermore, dendritic cells play a major role in this anti-cancer effect by presenting antigens to T cells. The bee venom PLA2 can irreversibly bind to hydrophobic groups on the plasma membrane of any cell type and can electrostatically bind to the anions of plasma membrane phospholipids so that the binding of a candidate cancer antigen peptide to the C-terminal region of PLA2 acts as an anchor from which the PLA2 + antigen peptide complex protein is incorporated. The C-terminal region of PLA2 can act as an anchor for the uptake of PLA2 + antigen-peptide complex proteins. In preparation for cancer antigen vaccines in dendritic cells, mutation of the 34th histidine of the bee venom PLA2 to glutamine to enhance cross-presentation by MHC class I peptides and presentation of MHC class II peptide antigens abolishes the enzymatic action of PLA2 itself and prevents the uptake of cancer antigens by dendritic cells. PLA2 is an effective cell membrane-binding vector in dendritic cell-based cancer vaccines [5].

2.4. Regulatory action against bacterial and parasitic infections

It has also been reported that bee venom PLA2 has antibacterial and antiparasitic effects. Bee venom PLA2 has also been shown to have antiparasitic activity against trypanosomes and antimicrobial activity against *Enterobacter*, *Escherichia coli*, and others. Mosquitoes expressing the bee venom PLA2 gene sequence specifically in the gut epithelium have been reported to prevent malaria infection in mice by affecting the maturation of *Plasmodium falciparum* in the gut [6]. This antimalarial effect of bee venom PLA2 is thought to prevent the binding of *Plasmodium falciparum* to mosquito midgut cells.

3. Conclusion

The clinical application of PLA2 still has great potential. We found that bee venom PLA2 enhanced activation by pathogen components in human skin cells and speculated that the action of bee venom PLA2 on human skin cell membranes was responsible for its enhancing effect (article under revision). In the future, we are considering the possibility of clinical applications, such as using PLA2 in combination with pathogen components to enhance anti-cancer activity, or in combination with burns or bedsores to increase the rate of skin proliferation.

Disclosure statement

The author declares no conflicts of interest.

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