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Title	Ninjurin-1 Negatively Regulates Humoral and Cellular Immune Responses Induced by the Saponin-Based Adjuvant Quil-A in Mice
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論　文　内　容　の　要　旨

氏　名　（　邵 玫瑰　）	
論文題名	Ninjurin-1 Negatively Regulates Humoral and Cellular Immune Responses Induced by the Saponin-Based Adjuvant Quil-A in Mice (Ninjurin-1はマウスにおいてサポニン系アジュバントQuil-Aによって誘導される液性免疫応答と細胞性免疫応答を負に制御する)

論文内容の要旨

Vaccination remains one of the most effective strategies for reducing morbidity and mortality associated with infectious diseases. To improve safety, many modern vaccines employ purified protein, polysaccharide, or recombinant antigens rather than whole pathogens. However, such antigens often exhibit limited intrinsic immunogenicity and therefore require adjuvants to elicit sufficient protective immune responses. Despite their clinical use, the molecular mechanisms of many approved adjuvants remain incompletely understood, limiting their rational design and optimized use in vaccination. A better understanding of their mechanisms could guide evidence-based vaccine development strategies.

Adjuvants enhance vaccine efficacy primarily through activation of antigen-presenting cells (APCs), such as dendritic cells(DCs) and macrophages, via multiple mechanisms. Some adjuvants directly engage pattern recognition receptors by mimicking pathogen-associated molecular patterns, thereby activating APCs. Others act through distinct pathways that involve the induction of cellular stress or perturbations, leading to the release of intracellular molecules that modulate the activity of surrounding cells, including APCs. The release of intracellular molecules induced by such adjuvants is an active process mediated by molecular mechanisms. Elucidating both the molecules responsible for intracellular molecule release and the effector molecules released during this process is crucial for enabling rational control of adjuvants that act through pathways promoting the release of intracellular mediators.

Saponins are plant-derived compounds with potent adjuvant activity, and saponin-based adjuvant systems such as Adjuvant system 01 and Matrix-M have been incorporated into multiple licensed vaccines. Compared with traditional aluminum-based adjuvants, which primarily induce humoral immunity, saponin-based adjuvants can elicit robust cellular as well as humoral immune responses and have therefore attracted increasing attention. Several saponin-based adjuvants have been reported to activate the NLRP3 inflammasome and induce plasma membrane rupture (PMR). Activation of the NLRP3 inflammasome is generally associated with pore formation in the plasma membrane and the subsequent release of low-molecular-weight intracellular molecules, such as interleukin-1β (IL-1β). Notably, NLRP3 can restrict the adjuvant activity of the saponin-based adjuvant QS-21, suggesting that NLRP3-dependent mediators negatively regulate saponin-induced immune responses. In contrast, PMR leads to the release of high-molecular-weight intracellular molecules that can mediate inflammation. Elucidating the molecular mechanisms of PMR induced by saponin-based adjuvants would help to better understand and modulate their adjuvant effects.

Ninjurin-1 (NINJ1) has recently been identified as a key executor of PMR under various stimulations. NINJ1 is a transmembrane protein that oligomerizes at the plasma membrane in response to membrane-disruptive stimuli, resulting in membrane rupture and release of intracellular contents. Given that saponin-based adjuvants also induce PMR, this study aimed to determine whether NINJ1 mediates PMR

induced by the saponin mixture Quil-A and its immunological consequences.

Lactate dehydrogenase (LDH), a high-molecular-weight intracellular molecule released during PMR, is commonly used as a marker of PMR. To adequately induce NLRP3 inflammasome activation and subsequent IL-1β release, cells were primed with the Toll-like receptor 4 (TLR4) agonist MPLA. In vitro experiments demonstrated that Quil-A stimulation induced LDH release in wild-type peritoneal exudate cells (PECs) and bone marrow-derived dendritic cells (BMDCs), regardless of MPLA priming, indicating that PMR occurs independently of TLR4 activation. In contrast, IL-1β release was observed predominantly in MPLA-primed cells following Quil-A stimulation. Quil-A induced minimal IL-1β release in *Nlrp3*$^{-/-}$ cells, whereas LDH release was comparable to that observed in wild-type cells. Importantly, *Ninj1*$^{-/-}$ cells exhibited significantly reduced LDH release upon Quil-A stimulation, while IL-1β release remained unaffected. These results indicate that, under Quil-A stimulation, the release of the low-molecular-weight intracellular molecule IL-1β is NLRP3-dependent but NINJ1-independent. In contrast, PMR-associated release of the high-molecular-weight intracellular molecule LDH is NINJ1-dependent but NLRP3-independent. In vivo, immunization with Quil-A and ovalbumin (OVA) resulted in significantly higher levels of OVA-specific IgG, IgG2b, and IgG2c in *Ninj1*$^{-/-}$ mice compared with wild-type mice. Splenocytes from *Ninj1*$^{-/-}$ mice produced increased amounts of interferon-γ in response to stimulation with both MHC class I- and class II-restricted OVA peptides. Moreover, *Ninj1*$^{-/-}$ mice exhibited a higher frequency of OVA-bearing cells in the draining lymph nodes, particularly among monocyte-derived dendritic cells, compared with wild-type mice.

To explore the mechanism underlying enhanced antigen delivery in *Ninj1*$^{-/-}$ mice, intracellular ATP levels were assessed in PECs and BMDCs following Quil-A stimulation. ATP availability influences numerous cellular processes, including migration, antigen processing, and metabolic activity, all of which could affect the accumulation of antigen-bearing APCs in draining lymph nodes. Quil-A reduced intracellular ATP levels to a similar extent in both wild-type and *Ninj1*$^{-/-}$ cells, indicating that the enhanced immune responses observed in *Ninj1*$^{-/-}$ mice are unlikely to result from differences in ATP availability. Based on these findings, we suggest that Quil-A-induced PMR in wild-type cells promotes the release of intracellular molecules that exert paracrine inhibitory effects on the trafficking or accumulation of antigen-bearing APCs in draining lymph nodes.

In conclusion, this study identifies a previously unrecognized negative regulatory mechanism in vaccine adjuvant responses, whereby NINJ1-mediated PMR limits immune activation by suppressing antigen delivery to draining lymph nodes. These findings highlight NINJ1 as a potential target for enhancing the efficacy of saponin-based vaccines. Furthermore, because multiple clinically approved adjuvants, including aluminum-based and emulsion-based formulations, also induce the release of intracellular molecules, NINJ1-dependent negative regulation may represent a broader mechanism constraining adjuvant activity. A detailed understanding of NINJ1-mediated PMR and its downstream inhibitory mediators may facilitate the rational design of next-generation adjuvants that maximize immune activation while minimizing unwanted negative regulatory pathways.

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

ワクチンは感染症の罹患率および死亡率の低減に最も有効な手段であり、その効果を高めるために抗原とともにアジュバントが投与される。サポニンは植物由来のアジュバント活性を有する化合物であり、複数の臨床承認ワクチンに組み込まれている。サポニン系アジュバントは、従来のアルミニウム系アジュバントでは達成困難な液性免疫と細胞性免疫の両方を誘導できる点で優れているが、その作用機序には未解明の点が多い。サポニン系アジュバントに曝露されたマクロファージや樹状細胞では、NOD-like receptor family pyrin domain containing 3（NLRP3）インフラマソーム依存的なinterleukin-1β（IL-1β）の放出に加え、形質膜破裂（plasma membrane rupture; PMR）が誘導され、高分子量の細胞内分子が細胞外へ放出されることが報告されている。しかし、PMRを媒介する分子機構およびPMRがアジュバント誘導性免疫応答に及ぼす影響は不明であった。近年、Ninjurin-1（NINJ1）がリポ多糖刺激などに応答したPMRの主要な実行因子として同定されたことから、サポニン系アジュバントによるPMRにおけるNINJ1の関与およびアジュバント効果への影響の解明が求められていた。

Shao氏は、博士後期課程において、サポニン系アジュバントQuil-Aにより誘導されるPMRにおけるNINJ1の役割を解析し、NINJ1がアジュバント誘導性免疫応答に及ぼす影響を検討した。Shao氏の行った研究の成果は、下記（１）～（５）の通りである。

（１）Quil-A刺激により、マウス腹腔マクロファージおよび骨髄由来樹状細胞において、PMRの指標であるlactate dehydrogenase（LDH）の放出がNLRP3非依存的に誘導されること、一方でIL-1βの放出はNLRP3依存的であることを明らかにした。さらに、Ninj1欠損細胞においてQuil-A誘導性のLDH放出がほぼ完全に抑制される一方、IL-1β放出はNinj1欠損の影響を受けないことを示した。

（２）Quil-Aとovalbumin（OVA）で免疫したNinj1欠損マウスにおいて、野生型マウスと比較してOVA特異的総immunoglobulin G（IgG）、IgG2bおよびIgG2cの血清中レベルが有意に上昇することを明らかにし、NINJ1がサポニン系アジュバント誘導性の液性免疫応答を負に制御することを示した。

（３）Quil-Aで免疫したNinj1欠損マウスの脾細胞が、主要組織適合遺伝子複合体クラスII拘束性ペプチド（OVA323-339）およびクラスI拘束性ペプチド（OVA257-264）刺激により、野生型マウスと比較して有意に高いinterferon-gamma（IFN-γ）を産生することを明らかにし、NINJ1がヘルパーT細胞1型応答および細胞傷害性T細胞応答の両方を負に制御することを示した。

（４）Quil-Aとfluorescein isothiocyanate（FITC）標識OVAで免疫したNinj1欠損マウスの所属リンパ節において、OVA保持細胞の頻度が野生型マウスと比較して有意に高く、特に単球由来樹状細胞においてその差が顕著であることを明らかにした。このことから、NINJ1依存的なPMRが所属リンパ節における抗原保持細胞の出現を抑制し得る可能性を示唆した。

（５）Quil-A刺激後の細胞内adenosine triphosphate（ATP）レベルが野生型とNinj1欠損細胞の間で同程度に低下することを確認し、Ninj1欠損マウスにおける免疫応答の増強が細胞内ATPの利用可能性の差では説明できないことを示した。

Shao氏の研究は、サポニン系アジュバントQuil-Aが誘導するPMRの実行因子としてNINJ1を同定し、NINJ1依存的なPMRがアジュバント誘導性の液性免疫応答および細胞性免疫応答を負に制御するという、これまで知られていなかった制御機構を明らかにしたものである。本研究は、NINJ1依存的なPMRがアジュバント効果を制限する制御点であることを提示し、サポニン系アジュバントの作用機序の理解とアジュバント作用の最適化に向けた分子基盤を与えるものである。今後、NINJ1依存的なPMRを介して放出される免疫抑制性分子の同定が進むことで、より効果的なワクチン製剤の設計に貢献することが期待される。

以上より、博士（薬科学）の学位論文に値するものと認める。