International Vaccine Design Center

Division of Adjuvant Innovation (New Dimensional Vaccine Design Team)

新次元ワクチンデザイン系・アジュバント開発分野

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The laboratory is consisted of two groups working on vaccine and adjuvant lead by Ken Ishii and Jun Kunisawa, respectively to conduct novel research on vaccine immunology towards rational vaccine design. In FY 2022, we reported various papers related to immunology on vaccine and adjuvant R&D.

1. *Making innate sense of mRNA vaccine adjuvanticity.*

Successful vaccines contain two essential immunological components: a protective antigen and an adjuvant. Adjuvants are essential for optimal antigen-specific immune responses, the so-called 'immunogenicity', but are often a cause of reactogenicity (even toxicity) that results in local and systemic inflammation. Therefore, to ensure vaccine efficacy and safety, it is critical to understand the molecular and cellular mechanism(s) by which adjuvants provoke the immune system. By introducing papers, we describe that there seems to be more room to improve the immunogenicity and reduce the reactogenicity of LNP-mRNA vaccine formulations by further study of immunization methods (including delivery systems and devices) and their built-in adjuvanticity.

2. *Anti-tumor immunity by transcriptional synergy between TLR9 and STING activation*

Agonists for TLR9 and stimulator of IFN genes (STING) offer therapeutic applications as both anti-tumor agents and vaccine adjuvants, though their clinical applications are limited; the clinically available TLR9 agonist is a weak IFN inducer and STING agonists induce undesired type 2 immunity. Yet, combining TLR9 and STING agonists overcame these limitations by synergistically inducing innate and adaptive IFNγ to become an advantageous type 1 adjuvant, suppressing type 2 immunity, in addition to exerting robust anti-tumor activities when used as a monotherapeutic agent for cancer immunotherapy. Here, we sought to decipher the immunological mechanisms behind the synergism mediated by TLR9 and STING agonists and found that their potent anti-tumor immunity in a Pan02 peritoneal dissemination model of pancreatic cancer was achieved only when agonists for TLR9 and STING were administered locally, and was via mechanisms involving CD4 and CD8 T cells as well as the co-operative action of IL-12 and type I IFNs. Rechallenge studies of long-term cancer survivors suggested that the elicitation of Pan02-specific memory responses provides protection against the secondary tumor challenge. Mechanistically, we found that TLR9 and STING agonists synergistically induce IL-12 and type I IFN produc-

tion in murine APCs. The synergistic effect of the TLR9 and STING agonists on IL-12p40 was at protein, mRNA and promoter activation levels, and transcriptional regulation was mediated by a 200 bp region situated 983 bp upstream of the IL-12p40 transcription initiation site. Such intracellular transcriptional synergy may hold a key in successful cancer immunotherapy and provide further insights into dual agonism of innate immune sensors during host homeostasis and diseases.

3. *Machine Learning-Assisted Screening of Herbal Medicine Extracts as Vaccine Adjuvants*

Adjuvants are important vaccine components, composed of a variety of chemical and biological materials that enhance the vaccine antigen-specific immune responses by stimulating the innate immune cells in both direct and indirect manners to produce a variety cytokines, chemokines, and growth factors. It has been developed by empirical methods for decades and considered difficult to choose a single screening method for an ideal vaccine adjuvant, due to their diverse biochemical characteristics, complex mechanisms of, and species specificity for their adjuvanticity. We therefore established a robust adjuvant screening strategy by combining multiparametric analysis of adjuvanticity in vivo and immunological profiles in vitro (such as cytokines, chemokines, and growth factor secretion) of various library compounds derived from hot-water extracts of herbal medicines, together with their diverse distribution of nano-sized physical particle properties with a machine learning algorithm. By combining multiparametric analysis with a machine learning algorithm such as rCCA, sparse-PLS, and DIABLO, we identified that human G-CSF and mouse RANTES, produced upon adjuvant stimulation in vitro, are the most robust biological parameters that can predict the adjuvanticity of various library compounds. Notably, we revealed a certain nano-sized particle population that functioned as an independent negative parameter to adjuvanticity. Finally, we proved that the two-step strategy pairing the negative and positive parameters significantly improved the efficacy of screening and a screening strategy applying principal component analysis using the identified parameters. These novel parameters we identified for adjuvant screening by machine learning with multiple biological and physical parameters may provide new insights into the future development of effective and safe adjuvants for human use.

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