International Vaccine Design Center

Division of Human Immunology (Human Immune-Profiling Team)

ヒト免疫プロファイリング系・ヒト免疫学分野

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The laboratory is consisted of two groups working on vaccine and immunometabolism lead by Ken Ishii and Noriko Sorimachi, respectively to conduct novel research on vaccine immunology and immunometabolism towards human immune-profiling to understand why and how our immune system respond to infection and other immunological disorders. In FY 2022, we reported two independent phase-I clinical trials for seasonal influenza vaccine with a novel adjuvant HP-bCD, and phase-Ib study for mono-therapeutic application of humanized CpG ODN K3, for cancer patients.

1. *Safety and immunogenicity of a quadrivalent seasonal influenza vaccine adjuvanted with hydroxypropyl-β-cyclodextrin: A phase 1 clinical trial*

Objectives: Hydroxypropyl-β-cyclodextrin (HP-β-CyD), an oligosaccharide used as an excipient in pharmaceutical preparation, was recently reported to function as a vaccine adjuvant to co-administered antigens. In this study, we investigated the safety and immunogenicity of a seasonal influenza vaccine adjuvanted with HP-β-CyD (FluCyD-vac) in healthy adults compared with those of a standard seasonal influenza vaccine (Flu-vac).
Methods: We conducted a single-blinded randomized phase 1 clinical trial study, and used two quadrivalent split seasonal influenza vaccines: FluCyD-vac containing 9 μg of HA/strain and 20% w/v of HP-β-CyD, and Flu-vac containing 15 μg of hemagglutinin (HA)/strain only. All participants were randomly assigned to receive a single dose of Flu/CyD-vac or Flu-vac at a ratio of 2:1. We assessed solicited and unsolicited adverse events (AEs) and immune responses using hemagglutination inhibition (HI) titers. In addition, we assessed T-cell function in peripheral blood mononuclear cells (PBMCs), after stimulation with HA vaccine strains, using flow cytometry.
Results: Among 36 healthy volunteers enrolled in the study (FluCyD-vac, n = 24; Flu-vac, n = 12), FluCyD-vac was well tolerated. Most of the solicited AEs were mild local skin reactions at the injection site. No serious AEs were reported in either group. HI titers 21 days after vaccination with FluCyD-vac were comparable with those of Flu-vac and sufficient to meet international criteria, despite reduced HA antigen doses. When PBMCs were stimulated with the four HA antigens in the vaccine, tumor necrosis factor (TNF)-α-producing CD4+ T cells were enhanced in the FluCyD-vac group.
Conclusion: FluCyD-vac was well-tolerated and immunogenic, despite containing 40% less HA antigens than Flu-vac. This study showed that HP-β-CyD is a potentially safe, novel adjuvant for human influenza vaccine.
Clinical trial registry: UMIN000028530.

2. *CpG ODN (K3)-toll-like receptor 9 agonist-induces Th1-type immune response and enhances cytotoxic activity in advanced lung cancer patients: a phase I study*

Background: Cytosine-phosphate-guanine oligodeoxynucleotide (CpG ODN) (K3)-a novel synthetic single-stranded DNA immune adjuvant for cancer immunotherapy-induces a potential Th1-type immune response against cancer cells. We conducted a phase I study of CpG ODN (K3) in patients with lung cancer to assess its safety and patients' immune responses.
Methods: The primary endpoint was the proportion of dose-limiting toxicities (DLTs) at each dose level. Secondary endpoints included safety profile, an immune response, including dynamic changes in immune cell and cytokine production, and progression-free survival (PFS). In a 3 + 3 dose-escalation design, the dosage levels for CpG ODN (K3) were 5 or 10 mg/body via subcutaneous injection and 0.2 mg/kg via intravenous administration on days 1, 8, 15, and 29.
Results: Nine patients (eight non-small-cell lung cancer; one small-cell lung cancer) were enrolled. We found no DLTs at any dose level and observed no serious treatment-related adverse events. The median observation period after registration was 55 days (range: 46-181 days). Serum IFN-α2 levels, but not inflammatory cytokines, increased in six patients after the third administration of CpG ODN (K3) (mean value: from 2.67 pg/mL to 3.61 pg/mL after 24 hours). Serum IFN-γ (mean value, from 9.07 pg/mL to 12.7 pg/m after 24 hours) and CXCL10 levels (mean value, from 351 pg/mL to 676 pg/mL after 24 hours) also increased in eight patients after the third administration. During the treatment course, the percentage of T-bet-expressing CD8+ T cells gradually increased (mean, 49.8% at baseline and 59.1% at day 29, $p = 0.0273$). Interestingly, both T-bet-expressing effector memory (mean, 52.7% at baseline and 63.7% at day 29, $p = 0.0195$) and terminally differentiated effector memory (mean, 82.3% at baseline and 90.0% at day 29, $p = 0.0039$) CD8+ T cells significantly increased. The median PFS was 398 days.
Conclusions: This is the first clinical study showing that CpG ODN (K3) activated innate immunity and elicited Th1-type adaptive immune response and cytotoxic activity in cancer patients. CpG ODN (K3) was well tolerated at the dose settings tested, although the maximum tolerated dose was not determined.
Trial registration: UMIN-CTR number 000023276.

3. *Metabolic regulation of macrophage's inflammatory functions by a lysosome-resident amino acid transporter, SLC15A4; a therapeutic target for controlling macrophage-mediated inflammatory diseases*.

Background: Controlling inflammation is critical for alleviation of immune-mediated diseases including infection-related subsequent complications. The endolysosome system plays critical roles in inflammatory responses since endolysosomes function as signal transduction hubs to convert various environmental danger signals into gene expression. Such endolysosome-dependent signals cause metabolic adaptation of immune cells which required for efficient orchestration of inflammatory responses including the termination of inflammation and the progression of tissue repair. However, the precise mechanisms of endolysoosme-dependent metabolic regulation are still largely unclear.

SLC15A4 is an endolysosome-resident amino acid transporter that regulates innate immune responses, and is genetically associated with inflammatory diseases such as systemic lupus erythematosus (SLE) and colitis. SLC15A4-deficient mice showed the amelioration of symptoms of these model diseases, and thus SLC15A4 is a promising therapeutic target of SLE and colitis. In this study, we investigated SLC15A4's functions in endolysosome-dependent metabolic regulation of macrophages and their impact on inflammatory responses.
Methods: We used BioID to identify molecules that are in close-proximity to SLC15A4. Based on the results of BioID, we compared metabolic properties between *Sls15a4*$^{+/+}$ and *SLC15A4*$^{-/-}$ mouse-derived macrophages using Seahorse flux analyses and mass-spectrometry-based metabolomics analyses.
Results: We identified 9 proteins involved in the mTOR signaling pathway, including RagA, RagB, RagC, and Lamtor1/2 by BioID, and further investigated the functional linkage between SLC15A4 and mTORC1. We revealed that SLC15A4 loss disturbed the coupling of glycolysis and the TCA cycle, and SLC15A4-deficient macrophages preferred to use glutamine rather than glucose as a carbon source for the TCA cycle. SLC15A4-deficient macrophages produced low levels of itaconate and pro-inflammatory IL-12 cytokine members.
Conclusions: Our findings reveal a novel mechanism of metabolic regulation in which an amino acid transporter acts as a gatekeeper that protects immune cells' ability to acquire an M1-prone metabolic phenotype in inflammatory tissues by mitigating metabolic stress. SLC15A4 pivotally and widely affects inflammatory and metabolic signaling at the endolysosome, and loss of SLC15A4 inhibits multiple inflammatory signals in macrophages.

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