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Artificially synthesized helper/killer-hybrid epitope long peptide (H/K-HELP): Preparation and immunological analysis of vaccine efficacy

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ABSTRACT

To elucidate the immunologic mechanisms of artificially synthesized helper/killer-hybrid epitope long peptide (H/K-HELP), which indicated a great vaccine efficacy in human cancers, we prepared ovalbumin (OVA)-H/K-HELP by conjugating killer and helper epitopes of OVA-model tumor antigen *via* a glycine-linker. Vaccination of C57BL/6 mice with OVA-H/K-HELP (30 amino acids) but not with short peptides mixture of class I-binding peptide (8 amino-acids) and class II-binding peptide (17 amino-acids) combined with adjuvant CpG-ODN (cytosine-phosphorothioate-guanine oligodeoxynucleotides), induced higher numbers of OVA-tetramer-positive CTL with concomitant activation of IFN-γ-producing $CD4^+$ Th1 cells. However, replacement of glycine-linker of OVA-H/K-HELP with other peptide-linker caused a significant decrease of vaccine efficacy of OVA-H/K-HELP. In combination with adjuvant CpG-ODN, OVA-H/KHELP exhibited greater vaccine efficacy compared with short peptides vaccine, in both preventive and therapeutic vaccine models against OVA-expressing EG-7 tumor. The elevated vaccine efficacy of OVAH/K-HELP might be derived from the following mechanisms: (i) selective presentation by only professional dendritic cells (DC) in vaccinated draining lymph node (dLN); (ii) a long-term sustained antigen presentation exerted by DC to stimulate both CTL and Th1 cells; (iii) formation of three cells interaction among DC, Th and CTL. In comparative study, H/K-HELP indicated stronger therapeutic vaccine efficacy compared with that of extended class I synthetic long peptide, indicating that both the length of peptide and the presence of Th epitope peptide were crucial aspects for preparing artificially synthesized H/K-HELP vaccine.

1. Introduction

The development of preventive and therapeutic vaccines is an important issue to treat cancer and infectious diseases. Since Aichele et al. [1] reported that vaccination of mice with MHC class I-binding short peptide protected the mice from subsequent challenge of a relevant live virus, many investigators have focused on the development of peptide vaccines against infectious diseases and cancer using class I-binding short peptides [2,3]. However, it has been hard to induce complete cure of mice with established tumor tissue by the administration of class I-binding short peptide vaccine even though the vaccine could protect mice from live tumor challenge [4,5]. Short peptide vaccine has also shown a limited therapeutic efficacy in clinical trials [6,7]. Thus, short peptide vaccine appeared to be suboptimal for curing cancer because short peptide sometimes induced immunological tolerance rather than

Abbreviations: CTL, cytotoxic T lymphocytes; DC, dendritic cells; HPV, human papilloma virus; SLP, synthetic long peptide; dLN, draining lymph node; ndLN, non-draining lymph node; CpG-ODN, cytosine-phosphorothioate-guanine oligodeoxynucleotides; OVA, ovalbumin; H/K-HELP, helper/killer-hybrid epitope long peptide; Short peptides-Mix, class I and class II short peptides mixture; long peptides-Mix, extended class I and class II long peptides mixture; APC, antigen presenting cells; IFA, incomplete Freund adjuvant; LCMV, lymphocytic choriomening it is virus; APC, allophcocyanin; PE, phycoerythrin; FITC, fluorescein isothiocyanate; f.p., footpad; ELISA, enzyme-linked immunosorbent assay; CFSE, carboxyfluorescein diacetate sucinimidylester.

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preventive immunity when used in combination with incomplete Freund adjuvant (IFA) [8,9]. Moreover, the presence of helper epitope peptide appeared to be necessary for overcoming strong immunosuppressions and inducing Th1-dependent fully activation of CTL and CTL memory [10–12]. Indeed, the first successful experiment of cytotoxic T lymphocyte (CTL) short peptide vaccine against lymphocytic choriomeningitis virus (LCMV) reported by Aichele et al. [1] was due to the fact that their LCMV short peptide (15 amino acids sequence) included a helper epitope recognized by $CD4^+$ T cells and it was longer than the general minimal CTL epitope peptide (8–10 amino acids) though it was considered as a short peptide at that time [13]. Thus, the presence of helper epitope in peptide vaccine appeared to be requested for developing an efficient peptide vaccine.

To improve the problems of class I-binding short peptide vaccine, Melief's group [14] has developed a novel peptide vaccine designated as synthetic long peptide (SLP). They indicated that human papillomavirus (HPV) 16 SLP, which was a HPV16-derived 35 amino-acid-long peptide containing naturally occurring Th epitope and CTL epitope peptides, induced strong HPV-specific $CD4^+$ and $CD8^+$ T cell immunity to eradicate established HPV-positive tumor cells in mice [5]. The HPV16 SLP also exhibited a great therapeutic efficacy in human clinical trial of vulvar intraepithelial neoplasia [15]. The elevated vaccine efficacy of SLP vaccine was due to the selective processing and presentation by professional DC, which was preferable for inducing a strong activation of CTL in inflamed draining lymph node (dLN) [5,16]. Although they initially investigated SLP containing Th and CTL epitopes derived from naturally occurring peptide [5], they also found that the extended class I long peptide was selectively presented by professional DC and if used in combination with CpG-ODN caused downmodulation of tolerance induction and increased duration of epitope presentation to induce CTL [8]. However, the therapeutic vaccine efficacy of the extended class I peptide has not been demonstrated yet, in contrast to naturally occurring SLP vaccine containing Th and CTL epitope peptides [5,16]. Thus, it was indicated that the presence of Th epitope peptide in addition to the length of SLP appeared to be key factors for designing therapeutic long peptide vaccine. However, it might be hard to design proper length of SLP comprising highly immunogenic Th and CTL epitopes from naturally occurring peptide of all tumor-associated antigen.

To extend their great findings and develop an easier method to design long peptide vaccine containing Th and CTL epitopes, we prepared artificially synthesized 30–40 amino acids long peptide, termed as helper/killer-hybrid epitope long peptide (H/K-HELP) by conjugating Th1-epitope and CTL epitope peptide with glycine-linker. Compared with SLP using natural occurring antigen sequence, the hybrid peptide has a merit to easily constitute artificial long peptide vaccine using newly identified Th epitope, CTL epitope and linker peptide.

Indeed, we previously reported an elevated vaccine efficacy of artificially synthesized H/K-HELPs, which were prepared by conjugating both helper and killer epitopes of MAGE-A4 or Survivin human cancer-associated antigen with glycine-linker [17,18]. The innovative long peptide vaccines, MAGE-A4– and Survivin–H/K-HELP successfully induced cancer peptide-specific cellular immunity (Th1 and Tc1 cells) and humoral immunity (IgG1 and IgG3 complement fixing Abs) in cancer patients. Moreover, MAGE-A4-H/K-HELP cancer vaccine exhibited a great inhibition of metastatic colon cancer and Survivin–H/K-HELP successfully induced a complete response in triple-negative breast cancer patient [17,18]. However, the detailed mechanisms concerning artificially synthesized H/K-HELP vaccine have not been resolved yet.

To evaluate the precise mechanisms of H/K-HELP cancer vaccine in animal model, we prepared H/K-HELP vaccine of OVA model tumor antigen (OVA-H/K-HELP; 30 amino-acids) by conjugating both helper (17 amino-acids) and killer (8 amino-acids) epitopes with glycine-linker. Then, we investigated the cellular mechanisms of OVA-H/K-HELP vaccine using normal C57BL/6 mice or mice bearing with tumors derived from OVA-expressing EG7 tumor cells. In this paper, we examined whether artificially synthesized OVA-H/K-HELP conjugated with glycine-linker could induce preferable antitumor immunity likely as SLP and discussed about its mechanisms for inducing long-term sustained antitumor immunity.

2. Materials and methods

2.1. Mice

C57BL/6 mice were obtained from Charles River Japan (Yokohama, Japan). OT-I and OT-II TCR transgenic mice were provided by F.R. Carbone (University of Melbourne, Victoria, Australia). C57BL/6-background Ly5.1 mice were purchased from RIKEN Bioresource Center (Tsukuba, Takanodai, 305-0074, Japan). CD45.1 OT-I and OT-II mice were bred in our facility. All mice (5–8 weeks old) were maintained in specific pathogen-free conditions according to the guidelines for animal care at Institute for Genetic Medicine Hokkaido University.

2.2. Tumor cells

We used EG-7 tumor cells, which express OVA protein as a model tumor antigen by transducing full-length OVA gene into EL-4 tumor cells. In some experiments, EL-4 tumor cells were used as control target cells of EG-7 tumor cells. Both EG-7 and EL-4 tumor cells were cultured in RPMI-1640 medium (Wako Pure Industries, Ltd., Osaka, Japan) containing 10% heat-inactivated fetal carf serum (Invitrogen, Carlsbad, CA, USA) plus penicillin G (200 U/ml) and 0.1% streptomycin (complete medium) supplemented with 100 μg/ml G418 (Wako Pure Industries, Ltd., Osaka, Japan).

2.3. Antibodies and reagents

The following monoclonal antibodies (mAbs) purchased from BD Bioscience (California, USA) were used for all the experiment: APC-conjugated anti-CD11c mAb (HL3), APC-conjugated anti-IFN-γ mAb (XMG1.2), APC (or FITC)-conjugated anti-CD8 mAb (53–6.7), PE-conjugated anti-CD3 mAb (145-2C11), PE-conjugated anti-CD19 mAb (1D3), FITC-conjugated anti-CD44 (IM7), PE-Cy7-conjugated anti-CD4 mAb (RM4–5), PE-Cy7-conjugated-anti-CD45.1 mAb (A20). 7-Amino-actinomycin D (7AAD) was purchased from Beckman Coulter (Miami, FL, USA). H-2 K^b OVA tetramer-SIINFEKL-PE (OVA-tetramer) was purchased from Medical and Biological Laboratories (MBL) Co., Ltd. (Nagoya, Japan). Microbeads conjugated with anti-CD4 mAb, FITC-CD8 mAb or anti-APC mAb were purchased from Miltenyi Biotec K.K. (Tokyo, Japan).

2.4. Peptides

As illustrated in Supplementary Fig. S1, we have synthesized long peptide termed as OVA-H/K-HELP by conjugating CTL ($OVA_{257–264}$) and Th ($OVA_{323–339}$) peptides using glycine-linker. Also, we have used the following peptides: $OVA_{257–264}$, $OVA_{323–339}$, $OVA_{241–270}$ and $OVA_{317–346}$ (Fig. 8A and Fig. S1). Moreover, we synthesized modified OVA-H/K-HELP by substitution of glysine-linker with other peptide-linkers (Fig. S1). The synthesis of all