Long peptide-based cancer immunotherapy targeting tumor antigen-specific CD4$^+$ and CD8$^+$ T cells

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CD4$^+$ T cells promote cytotoxic T lymphocyte (CTL)-mediated anticancer immune responses. We have recently identified ideal tumor-associated antigen (TAA)-derived long peptides (LPs) that elicit not only TAA-specific T$_h$1 response, but also CTLs, through cross-presentation. The LP-specific T$_h$1 cell responses were augmented in cancer patients vaccinated with CTL epitopes. Our findings support the clinical application of LP-based immunotherapy.

The importance of CD4$^+$ T cells in orchestrating the activity of the immune system and inducing effective T cell-mediated anticancer immune responses is indisputable. CD4$^+$ helper T cells are necessary for the priming of tumor-specific CD8$^+$ T cells, influence the differentiation and expansion of tumor-associated antigen (TAA)-specific cytotoxic T lymphocytes (CTLs), and are essential for the generation and maintenance of long-lasting CD8$^+$ T-cell responses. Moreover helper T cells pave the way for the infiltration of neoplastic lesions by CTLs and T$_h$1 cells not only play a direct role in the elimination of malignant cells but also mediate anti-angiogenic effects, mostly via their ability to secrete interferon $\gamma$ (IFN$\gamma$).

Tumor-specific T$_h$1 cells also control neoplastic lesion by favoring the activation of dendritic cells (DCs), natural killer (NK) cells, and M1 macrophages. Therefore, the identification of peptides that activate both tumor-specific T$_h$1 cells and CTLs is an important goal for the induction of effective antitumor immune responses. Of note, DCs can process extracellular antigens and cross-present them on HLA class I molecules, leading to the activation of TAA-specific CD8$^+$ T-cell responses. Such T-cell responses are considered to be crucial for both tumor eradication and the generation of long-lasting immunological memory. Recent studies have shown that the administration of long peptides (LPs) encompassing T$_h$1 and CTL epitopes has the potential to elicit combined CD4$^+$ and CD8$^+$ T-cell responses through cross-presentation. Thus, anticancer vaccines based on a single polypeptide encompassing epitopes that elicit both T$_h$1 and CTL responses may exhibit superior efficacy as compared with peptides that activate either CD4$^+$ or CD8$^+$ T cells only.

Nakamura et al. analyzed the gene expression profile of various cancers and normal tissues using a genome-wide cDNA microarray encompassing 27,648 genes. By this technology, we have identified a number of TAAs that are not expressed by normal adult tissues. Most often, the expression level of these TAAs was correlated with poor disease outcome. In particular, we have identified several HLA-A24(A$^*$24:02)-restricted CTL epitopes that are able to elicit tumor-reactive CTLs but not autoimmune responses. In Phase I/II clinical trials involving lung, head and neck, pancreatic, gastric, and esophageal cancer patients, CTL epitopes derived from these novel TAAs were found to be safe and to induce not only robust TAA-specific CTL responses, but also a survival benefit, among individuals bearing advanced neoplasms.

Based on these results, in order to further improve the efficacy of peptide-based anticancer immunotherapies, we attempted to identify TAA-derived long peptides (LPs) that can induce both TAA-specific T$_h$1 cells and CTLs (Fig. 1). To select a candidate LP bearing both T$_h$1 and CTL epitopes, we combined information from a recently developed computer algorithm predicting HLA class II-binding peptides with known HLA-A2(A$^*$02:01) or HLA-A24(A$^*$24:02)-restricted CTL epitopes. It is well known that the incidence of HLA-A2 and HLA-A24 is relatively high in various ethnic groups, including Asians, Africans, Afro-Americans, and Caucasians. In terms of TAAs, we focused on a cancer testis antigen, NDC80 kinetochore complex component (NUF2, best known as cell division cycle associated 1, CDCA1), and kinesin family member 20A (KIF20A).
TH1 responses were boosted by the repeated administration of CTL epitopes. From these results, we speculate that tumor-specific TH1 responses in vaccinated patients may result from phenomena of intramolecular epitope spreading or antigen spreading initiated by CTL epitopes. Recent clinical studies have demonstrated that targeting helper T cells and CTLs by the concomitant administration of HLA class I- and II-restricted epitopes enhance vaccine-dependent immune responses and improve clinical responses. We investigated the synergistic effects of CDCA1- and KIF20A-derived LPs on the induction of antigen-specific CTLs by CTL epitopes. Both these LPs boosted the ability of CTL epitopes to elicit tetramer+ and CD107a+ CTLs. These findings indicate that LPs may be able to synergize with CTL epitopes in the elicitation of antigen-specific CD8+ T-cell responses, hence exacerbating the clinical potential of this immunotherapeutic approach.

Thus, we succeeded in identifying highly immunogenic CDCA1- and KIF20A-derived LPs encompassing both T\textsubscript{H1} and CTL epitopes. Such LPs (24–26mers) encompassed naturally processed T\textsubscript{H1} epitopes that are presented by various HLA class II molecules including HLA-DR53, DR4, DR8, DR9, DR15, and DP2. Based on the findings, CDCA1- and KIF20A-derived LPs should be useful in at least 86% and 93% of the Japanese population, respectively.

CDCA1- and KIF20A-derived LPs elicited T\textsubscript{H1} cells producing significant levels of IFN\textgamma, interleukin (IL)-2, tumor necrosis factor \alpha (TNF\textalpha), granulocyte macrophage colony-stimulating factor (GM-CSF), and chemokine (C-C motif) ligand 4 (CCL4, also known as macrophage inflammatory protein-1\beta, MIP-1\beta), but comparatively lower amounts of IL-4, IL-6 and IL-17. The cytotoxicity marker CD107a was also detected on CDCA1- and KIF20A-specific T\textsubscript{H1} cells elicited by LPs. Moreover, we demonstrated that the cross-presentation of LPs efficiently primes tumor-specific CD8+ CTLs, in vitro (with human cells) as well as in vivo (in HLA-A2 (HHD) or A24 (HHH) transgenic mice). Furthermore, the CDCA1-derived LP was superior to the corresponding (embedded) CTL epitope in the induction of CTL responses in vivo. We also assessed T\textsubscript{H1} responses specific for CDCA1- and KIF20A-derived LPs in the peripheral blood of cancer patients who were enrolled in phase I/II clinical trials based on three HLA-A24-binding, cancer testis antigen-derived CTL epitopes, namely, CDCA1\textsubscript{56–64} (from CDCA1), IMP-3\textsubscript{177–186} (from the U3 small nucleolar ribonucleoprotein protein, IMP3), and LY6K\textsubscript{177–186} (from lymphocyte antigen 6 complex, locus K, LY6K). We indeed detected CDCA1- and KIF20A-specific T\textsubscript{H1} responses upon vaccination with CTL epitopes. Interestingly, the frequency of these responses was significantly higher in vaccinated cancer patients than in patients before vaccination and in healthy individuals. In addition, in some patients, antigen-specific T\textsubscript{H1} responses were boosted by the repeated administration of CTL epitopes. From these results, we speculate that tumor-specific T\textsubscript{H1} responses in vaccinated patients may result from phenomena of intramolecular epitope spreading or antigen spreading initiated by CTL epitopes.

Recent clinical studies have demonstrated that targeting helper T cells and CTLs by the concomitant administration of HLA class I- and II-restricted epitopes enhance vaccine-dependent immune responses and improve clinical responses. We investigated the synergistic effects of CDCA1- and KIF20A-derived LPs on the induction of antigen-specific CTLs by CTL epitopes. Both these LPs boosted the ability of CTL epitopes to elicit tetramer+ and CD107a+ CTLs. These findings indicate that LPs may be able to synergize with CTL epitopes in the elicitation of antigen-specific CD8+ T-cell responses, hence exacerbating the clinical potential of this immunotherapeutic approach. The synergistic effects of anticancer vaccines and strategies aimed at blocking...
immunological checkpoints, for instance antibodies targeting cytotoxic T lymphocyte-associated protein 4 (CTLA4), programmed cell death 1 (PD-1) or its ligand (CD274, also known as PD-L1), have previously been illustrated. Thus, immunological checkpoint-blocking agents might constitute valid candidates for combination therapies involving CDCA1 and KIF20A-derived LP-based anticancer vaccines.

In conclusion, CDCA1- and KIF20A-derived LPs provide a good tool for the propagation of tumor-specific T\textsubscript{H}1 cells and CTLs. Our studies support the clinical application of LP-based anticancer immunotherapy.

Disclosure of Potential Conflicts of Interest

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