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Mimotope vaccine efficacy gets a "boost" from native tumor antigens

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Abbreviations: IL-7Rα, interleukin-7 receptor α; KLRG1, killer cell lectin-like receptor subfamily G member 1; poly(I:C) polyinosinic:polycytidylic acid; TAA, tumor-associated antigen; TERT, telomerase reverse transcriptase

Tumor-associated antigen (TAA)-targeting mimotope peptides exert more prominent immunostimulatory functions than unmodified TAAs, with the caveat that some T-cell clones exhibit a relatively low affinity for TAAs. Combining mimotope-based vaccines with native TAAs in a prime-boost setting significantly improves antitumor immunity.

Vaccination with mimotopes, that is, peptides engineered to mimic tumor-associated antigen (TAA) epitopes, has been shown to increase the frequency of tumor-specific cells in clinical trials, yet such immune responses are often insufficient to mediate tumor regression.[1] Indeed, many mimotopes elicit T cells that exhibit low functional avidity for TAAs and hence do not efficiently kill tumor cells, nor they eradicate tumors. Conversely, unmodified peptide antigens administered in combination with optimized adjuvants result in comparatively fewer T-cells, but those cells exhibit increased functional recognition as compared with T cells elicited by mimotopes.[2] Our recent work demonstrates the utility of appropriate adjuvants and peptides to improve antitumor immunity.[3] Specifically, priming T-cells with a mimotope and then boosting mimotope-elicited responses with native TAAs combines the advantages conferred by each vaccine component alone and improves antitumor immunity.

Using murine CT26 cells as model for immunogenic colon carcinoma, we have previously identified multiple mimotopes that stimulate responses to the immunodominant MHC Class I-restricted TAA AH1. The suboptimal mimotope 15 elicits many non-crossreactive, low avidity AH1-specific $CD8^+$ T cells that fail to protect most mice from a challenge with tumor cells. Similar to the responses elicited by peptide vaccines in humans, immunization with the native AH1 peptide expands few AH1-specific T cells, yet those T cells exhibit increased functional avidity relative to those expanding upon the administration of mimotopes. However, priming the immune system with mimotope 15 followed by a boost with the native AH1 antigen increases the quantity of functional T cells as compared with an AH1-prime AH1-boost setting, and their quality as compared with a 15-prime 15-boost scenario. AH1-specific T cells elicited by a 15-prime AH1-boost approach exhibit increased avidity for AH1, secrete elevated levels of pro-inflammatory cytokines, and are primarily composed of killer cell lectin-like receptor subfamily G member 1 $(KLRG\text{-}1)^+$ interleukin-7 receptor α $(IL\text{-}7R\alpha)^-$ effector cells, which mediate efficient cytotoxic responses.[4] Consequently, mice immunized with the mimotope 15 and then boosted with the native AH1 peptide are largely protected from challenges with living tumor cells. T-cell receptor (TCR) sequencing analyses of AH1-specific cells revealed that the boost with AH1 results in an enrichment of T cells with TCRs primed by the mimotope, which have previously been shown to correlate with tumor protection by optimal mimotopes.[5] The simple adjustment of incorporating unmodified native TAAs following the administration of mimotopes may significantly improve T-cell responses,[3] especially when combined with other immunomodulatory agents such as IL-2 or antibodies blocking T-cell inhibitory receptors.

Recently, a similar approach has been undertaken for the vaccination of non-small cell lung cancer patients. In particular, the cryptic telomerase reverse transcriptase $(TERT)_{572}$ peptide and the mimotope $TERT_{572Y}$ were employed.[6,7] TERT is commonly expressed by human tumors and the relatively low affinity of the $TERT_{572}$ peptide for MHC Class I molecules can be improved using a general anchor residue modification.[8] The vaccine known as Vx-001 entails two initial immunizations with the modified $TERT_{572Y}$ mimotope emulsified in Montanide ISA51, performed 3 weeks apart. Three weeks after the second vaccine, the patients are boosted 4 more times, against with 3 weeks intervals, with the unmodified $TERT_{572}$ peptide. In setting, the detection of an early specific immune response correlated with improved progression-free (5.2 vs. 2.2 mo) and overall

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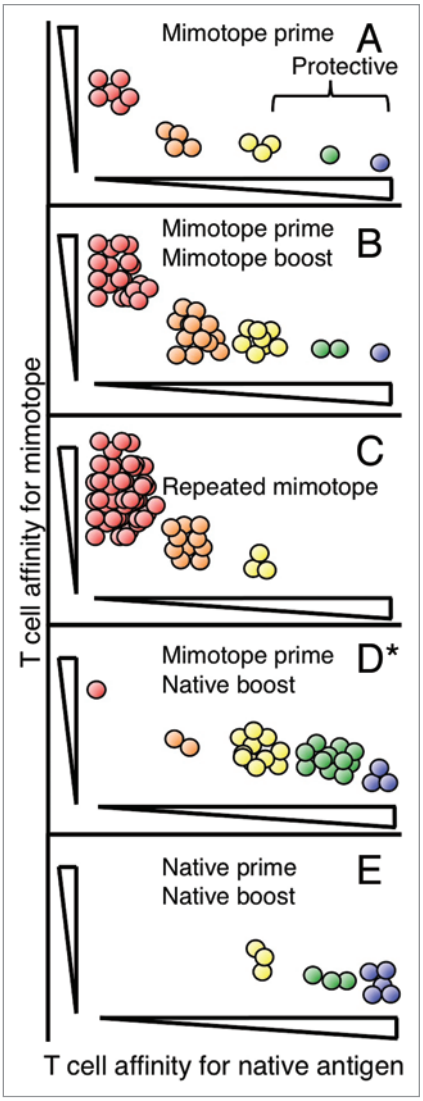

Figure 1. Affinity of antigen-specific T cells elicited in a prime-boost model for peptide vaccination. (**A**) Peptide mimotope vaccination elicits a broad repertoire of tumor-specific T cells exhibiting variable affinity for both the mimotope and tumor-associated antigen (TAA). (**B**) High affinity mimotope-specific T cells that do not cross-react with tumor cells (red) expand more effectively than cross-reactive cells (other colors) in response to a mimotope boost. (**C**) Repeated mimotope booster vaccines may preferentially select for high affinity mimotope-specific T cells at the expense of their cross-reactive counterparts. (**D***) Boosting the mimotope response with native TAAs expands high affinity tumor-specific T cells resulting in improved antitumor immunity. (**E**) The small population of tumor-specific T cells expanded by the native TAAs used during the prime and boost overlaps with that of mimotope-elicited T cells.

survival (20 vs. 10 mo).[7] Importantly, this vaccine strategy was demonstrated to elicit more consistent T-cell responses, with increased avidity for $TERT_{572}$, than a continuous boosting with $TERT_{572Y}$.[6] Our data are consistent with these results and provide further evidence that a boost with native TAAs preferentially expands a subset of high-avidity tumor-specific T cells that exhibit increased effector functions.

Mimotope vaccines elicit antitumor T cells with a wide range of affinities and functions (**Fig. 1**), as well as mimotope-specific T cells that do not cross-react with malignant cells. T cells with shared antigen specificity compete for several different signals during the initial phases of priming, including the access to antigen-presenting cells and cytokines.[9] The competition among T cells should be taken into particular account for the development of mimotope vaccines. Ideally, mimotope vaccines should elicit T cells exhibiting significant cross-reactivity for native TAAs. However, if the mimotope elicits non-cross-reactive T cells, then sequential immunizations with the mimotope may promote the selection of T cells exhibiting high affinity for the mimotope rather than for the native TAA. It is likely that the non-cross-reactive mimotope-specific T cells exhibit high affinity for the mimotope, providing them an advantage from subsequent mimotope boosts. This process may be amplified if non-cross-reactive T cells outnumber their cross-reactive counterparts, further limiting the expansion of the latter.

Future improvements to the use of native TAAs to boost mimotope vaccines may involve the modification of the pro-inflammatory environment administered together with the peptide. A prime combined with local pro-inflammatory signals, such as peptide-loaded dendritic cells, followed shortly by a boost with systemic pro-inflammatory signals, such as polyinosinic:polycytidylic acid [poly(I:C)] or pathogen-based vaccines expressing TAAs, generates robust T-cell responses in a two-injection murine model.[10] We have observed that the mimotope-prime and native TAA-boost regimen using the same adjuvant elicits weaker responses as compared with a prime-boost regimen consisting of different adjuvants, supporting the use of heterologous strategies to optimize T-cell responses. As new adjuvants become available for use in humans, vaccine protocols will be modified and optimized to elicit the rapid T-cell expansion that is required for therapeutic anticancer immunotherapy. Combining antigen-specific vaccines with other antigen-non-specific immunotherapeutic regimens will generate ever more effective immune responses against tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Buhrman JD, Slansky JE. Improving T cell responses to modified peptides in tumor vaccines. Immunol Res 2013; 55:34-47; PMID:22936035; http://dx.doi.org/10.1007/s12026-012-8348-9
2. Speiser DE, Baumgaertner P, Voelter V, Devevre E, Barbey C, Rufer N, et al. Unmodified self antigen triggers human CD8 T cells with stronger tumor reactivity than altered antigen. Proc Natl Acad Sci U S A 2008; 105:3849-54; PMID:18319339; http://dx.doi.org/10.1073/pnas.0800080105
3. Buhrman JD, Jordan KR, U'ren L, Sprague J, Kemmler CB, Slansky JE. Augmenting antitumor T-cell responses to mimotope vaccination by boosting with native tumor antigens. Cancer Res 2013; 73:74-85; PMID:23161490; http://dx.doi.org/10.1158/0008-5472.CAN-12-1005
4. van Duikeren S, Fransen MF, Redeker A, Wieles B, Platenburg G, Krebber WJ, et al. Vaccine-induced effector-memory CD8+ T cell responses predict therapeutic efficacy against tumors. J Immunol 2012; 189:3397-403; PMID:22914049; http://dx.doi.org/10.4049/jimmunol.1201540
5. Jordan KR, Buhrman JD, Sprague J, Moore BL, Gao D, Kappler JW, et al. TCR hypervariable regions expressed by T cells that respond to effective tumor vaccines. Cancer Immunol Immunother 2012; 61:1627-38; PMID:22350070; http://dx.doi.org/10.1007/s00262-012-1217-5
6. Vetsika EK, Papadimitraki E, Aggouraki D, Konsolakis G, Mela ME, Kotsakis A, et al. Sequential administration of the native TERT572 cryptic peptide enhances the immune response initiated by its optimized variant TERT(572Y) in cancer patients. J Immunother 2011; 34:641-50; PMID:21989412; http://dx.doi.org/10.1097/CJI.0b013e31823284a6
7. Kotsakis A, Vetsika EK, Christou S, Hatzidaki D, Vardakis N, Aggouraki D, et al. Clinical outcome of patients with various advanced cancer types vaccinated with an optimized cryptic human telomerase reverse transcriptase (TERT) peptide: results of an expanded phase II study. Ann Oncol 2012; 23:442-9; PMID:21873272; http://dx.doi.org/10.1093/annonc/mdr396
8. Tourdot S, Scardino A, Saloustrou E, Gross DA, Pascolo S, Cordopatis P, et al. A general strategy to enhance immunogenicity of low-affinity HLA-A2. 1-associated peptides: implication in the identification of cryptic tumor epitopes. Eur J Immunol 2000; 30:3411-21; PMID:11093159; http://dx.doi.org/10.1002/1521-4141(2000012)30:12<3411::AID-IMMU3411>3.0.CO;2-R
9. Kedl RM, Kappler JW, Marrack P. Epitope dominance, competition and T cell affinity maturation. Curr Opin Immunol 2003; 15:120-7; PMID:12495743; http://dx.doi.org/10.1016/S0952-7915(02)00009-2
10. Pham NL, Pewe LL, Fleenor CJ, Langlois RA, Legge KL, Badovinac VP, et al. Exploiting cross-priming to generate protective CD8 T-cell immunity rapidly. Proc Natl Acad Sci U S A 2010; 107:12198-203; PMID:20616089; http://dx.doi.org/10.1073/pnas.1004661107