

T cells: Functional and molecular analysis of vaccine elicited T-cell responses in melanoma

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Abstract

The aim of therapeutic vaccines for cancer or chronic viral infections is to elicit strong antigen-specific T-cell responses able to eliminate transformed or virus infected cells. We have recently identified a strong adjuvant formulation consisting of low doses of a tumor antigenic peptide and of a synthetic TLR-9 agonist, CpG 7909 (PF676), emulsified in Montanide ISA-51 (1). All metastatic melanoma patients ($n = 24$) immunized with the Melan-A₂₆₋₃₅ A27L peptide analogue, which binds strongly to the HLA-A2 molecule, had readily detectable A2/Melan-A tetramer+ CD8 T-cell responses after 2 to 4 subcutaneous injections of the vaccine. In contrast, only half of patients immunized with the same emulsion lacking the TLR-9 agonist had detectable tetramer+ CD8 T-cell responses after repeated vaccination (2). Moreover, the frequencies of specific T cells were approximately 10-fold higher in the group of patients receiving the TLR9 agonist containing vaccine.

Functional assessment of the postvaccination Melan-A tetramer+ CD8 T cells revealed robust differentiation to effector memory type, IFN- γ release and lytic activity comparable to viral antigen specific CD8 T cells present in the autologous peripheral blood lymphocyte pool from the same samples. In contrast, Melan-A tetramer+ CD8 T cells recovered from postvaccination tumor biopsies from two patients displayed reduced function. This was coincident with accumulation of relatively high numbers of regulatory T cells (3). Repeated vaccination led to a progressive increase in the fraction of Melan-A tetramer+ CD8 T cells that downregulated the expression of the CD28 coreceptor present in the circulating lymphocyte pool. These cells displayed an even more marked differentiation towards effector type cells and a reduction in the number of TCR clonotypes. Close to 60% of a series of independent CTL clones isolated from three vaccinated patients efficiently recognized and killed tumor cells in an antigen-specific fashion.

The efficacy of the vaccine formulation described here is additionally demonstrated by its ability to elicit strong CD8 T-cell responses to vaccination with the Melan-A₂₆₋₃₅ natural peptide, which forms unstable and low affinity complexes with the HLA-A2 molecule. The weak immunogenicity of such peptide had hindered thus far its use as a vaccine. Functionally, the specific CD8 T cells induced by the non-substituted peptide were also of the effector memory type. Surprisingly, however, they appeared to be superior in terms of both expressed effector molecules and tumor reactivity than those elicited by the substituted peptide analogue. Moreover, close to 100% of a series

of independent CTL clones isolated from three melanoma patients immunized with the natural Melan-A peptide were tumor reactive and had a high functional avidity of antigen recognition (4). These results suggest that weak self peptides can be superior to substituted analogues at inducing high avidity T cells provided that appropriately strong adjuvant formulations are used. These results may have wide ranging implications for therapeutic vaccine design.

It has become urgent to identify immune correlates of clinical efficacy of cancer vaccines. This is an important challenge at the present stage of vaccine development because of the low tumor response rates observed in consecutive non-randomized phase I clinical trials including small numbers of patients. In this regard, one approach we favor is to carry the analysis of vaccine-specific T-cell responses at the individual T cell level. We have optimized an experimental strategy based on the labeling of antigen-specific T cells with tetramers combined with cell surface markers for memory and effector subsets and sorting of large collections of single cells from each subset, from each patient and from sequential blood samples taken at different time points before and after vaccination (5). Gene expression analysis and identification of the TCRs is carried out cell by cell using conventional PCR on amplified cDNA. Work in progress shows a very dynamic picture of T-cell responses to peptide vaccines, with the establishment of few dominant clonotypes that are specific to each individual patient. Our results suggest that long term persistence of dominant T-cell clones might correlate with favorable clinical outcomes. In turn, persistence of dominant clones appears to be associated with long telomeres and slow transition to the highly differentiated effector phenotype characterized by high perforin content and loss of the CD28 and CD27 coreceptors.

It is widely acknowledged that poor clinical efficacy of current vaccine approaches may not only be explained by suboptimal vaccine formulation but also by multiple immune regulatory checkpoints active in the advanced metastatic cancer setting. In this regard, regulatory T cells may play a central role. In my talk, I will also discuss recent results on monitoring Melan-A antigen-specific regulatory T cells in vaccinated patients. In summary, functional and molecular monitoring of vaccine induced specific T cells at the single cell level provides invaluable lessons for future therapeutic vaccine development.

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