

Tumor-specific T cells induced in melanoma patients either naturally or by vaccination with peptides, IFA ± CpG oligodeoxynucleotides

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Quantitative and qualitative T-cell measurements *ex vivo* allow to distinguish between spontaneous and vaccine-induced antigen-specific T-cell responses, and a combination thereof. The majority of currently applied tumor vaccines induce T-cell responses in only a fraction of patients, suggesting that patient and disease related factors are co-responsible for T-cell activation. In contrast, the use of CpG oligodeoxynucleotide 7909 as immune adjuvant leads to reproducible *ex vivo* detectable T-cell responses in all vaccinated patients, enabling for the first time to study immune responses that are dominantly elicited by the vaccine.

Spontaneous tumor antigen specific T-cell responses

The HLA-A2/Melan-A(MART-1) antigenic system constitutes a well-defined model for studies of CD8+ T-cell responses in humans (1, 2, 3). For all studies, we use a standardized approach for *ex vivo* T-cell monitoring (4). In untreated melanoma patients, Melan-A multimer+ CD8+ T cells can regularly be identified at relatively high frequencies in metastatic lymph nodes (1–15% of CD8+ T cells) and subcutaneous/soft tissue metastases. In peripheral blood of untreated patients, Melan-A specific T cells are rarely increased in frequency, but a significant fraction is antigen-experienced, in contrast to the exclusively naive cells found in healthy controls.

A dominantly monoclonal T-cell response

CD8+ T-cell responses against differentiation antigens such as Melan-A are frequently polyclonal. We have developed and applied a new technology based on flow cytometry guided cell sorting (5), whereby different subsets of multimer+ T cells are distinguished according to cell surface expression of CD45RA, CCR7 and CD28. In one patient studied in depth, a dominant monoclonal response was found in the Melan-A specific CD8+ T cells that were negative for the three surface markers. In contrast, naive (CD45RA+/CCR7+) and CD28+/CD45RA-/CCR7- Melan-A specific T cells from the same patient were highly polyclonal. Longitudinal studies revealed that the dominant clone arose already 4 years ago before immunotherapy. Subsequently, the patient was vaccinated with Melan-A peptide in Incomplete Freund's Adjuvant (IFA), and later with the same vaccine supplemented with CpG 7909, which resulted in increased frequencies of *ex vivo* detectable Melan-A specific T cells (maximally 3.42% of circulating CD8+ T cells). Interestingly, the clone was also dominant in tumor infiltrating lymphocytes of this patient, remained dominant over 4 years, expressed IFN-gamma and granzyme B *ex vivo*, and to a lower extent perforin, TNF-alpha and CD94. We generated representative T-cell clones bearing the same T-cell receptor and found efficient and specific cytotoxicity against autologous tumor cells. The patient had several distant metastases in the

past, but is now without detectable disease. Subsequent immune monitoring will further elucidate the nature of this strong and long-lasting clonal CD8 T-cell response.

T-cell responses resulting from combined stimulation by endogenous tumor antigen and peptide vaccination

In a cohort of 38 patients vaccinated with Melan-A peptides and conventional adjuvants (IFA or QS21/MPL), we were able to demonstrate that stimulation by endogenous tumor antigen contributed significantly to vaccine-induced T-cell responses. Responder patients (12/38) had statistically significantly more CD28 negative Melan-A specific T cells before vaccination than non-responder patients. Thus, T-cell responses occurred primarily in patients with T cells that were pre-activated (6).

T-cell responses that are clearly induced by vaccination

In the majority of clinical studies assessing synthetic peptide vaccines, T-cell activation remained undetectable in PBMC analyzed *ex vivo*, or the frequencies of specific T cells reached only low levels (on average 0.05-0.2% tumor antigen-specific T cells) (2). Recently, we tested whether a CpG oligodeoxynucleotide, 7909, by virtue of its ability to trigger dendritic cell (DC) maturation, would lead to strong activation of antigen-specific CD8+ T cells upon co-injection with a peptide vaccine. Melanoma patients received CpG 7909 and Melan-A peptide, emulsified in IFA. After four vaccinations, 8/8 patients had increased frequencies of Melan-A specific T cells (mean of 1.15% of circulating CD8+ T cells). 6/8 patients had enhanced frequencies already after 2 vaccinations. Thus, for the first time a synthetic peptide vaccine was able to rapidly and strongly increase T-cell frequencies in all patients studied, demonstrating that cancer vaccines can be optimized when combined with appropriate means to activate DC. It was previously suggested that CpG ODN would not be strong adjuvants, because in humans, only the plasmacytoid DC (pDC) but not the myeloid DC are activated by CpG ODN, and pDC had not been found to induce CD8+ T cells efficiently in the mouse. Therefore, the strong adjuvant effect of CpG in humans is not only encouraging but also somewhat unexpected. This finding is a typical example demonstrating the importance of clinical investigation associated to vaccine trials. Our technologies to assess T-cell repertoire and T-cell function *ex vivo* are now being applied to precisely characterize T-cell responses to vaccination. These are crucial steps to identify which vaccine components result in what type of immune response, and their relation with disease regression, recurrence and progression.

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