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The antigen specific composition of melanoma tumor infiltrating lymphocytes?

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Large numbers of tumor associated antigens has been characterized, but only a minor fraction of these are recognized by tumor infiltrating lymphocytes of melanoma, although these have shown the ability to recognize tumor and provide tumor regression upon adoptive transfer. Thus the peptide recognition of the majority of the CD8 tumor infiltrating lymphocytes remains to be identified.

Adoptive therapy with tumor infiltrating lymphocytes (TILs) from melanoma, pioneered by the group of Steven A. Rosenberg,[1] and currently established in a small number of oncology-centers world-wide, has emerged as an important strategy to induce objective responses in metastatic melanoma patients. The TILs has shown both autologous and allogenic tumor cell recognition, but until recently very little was known about the antigen specific reactivity of these TIL preparations. Two publications by Sick Andersen et al. and Kvistborg et al. has recently demonstrated that TILs comprise T cells reactive against only a minor fraction of the previously described T cell epitopes of relevance for melanoma, and when peptide-specific responses were identified the frequency was often low (<1% of total $CD8^+$ T cells). To elucidate the recognition pattern of melanoma TILs both studies used a recently generated library of all published T cell epitopes of relevance for melanoma that includes 175 MHC-class I peptides restricted to HLA-A1, A2, A3, A11 and B7.[2] Screening of peptide-specific T cell responses was conducted by MHC-multimers, generated by peptide exchange from conditional ligand-HLA complexes and combinatorially encoded with different fluorescence molecules to generate unique two-color codes allowing parallel detection of large numbers of different antigen specific T cells.[4,5] Studies were conducted either for all mentioned alleles or for HLA-A2 only.[2,3]

Based on TILs from three different centers it was shown that T-cell populations recognizing described T cell epitopes are low-frequent and only a small fraction of the described melanoma-associated antigens are recognized. The most prominently recognized groups of antigens were differentiation antigens, with MART-1 and gp100 together accounting for more than half of the responses (**Fig. 1**). Strikingly few epitopes from the group of overexpressed antigens were recognized, and the majority of these were encoded in alternative open reading frames (ORFs). These observations induce a number of questions as to what else is recognized but yet not described, if these low-frequent tumor antigen specific populations are indeed sufficient for clinical responses, and if tolerance induction is prohibiting T cell responses in TILs against broadly expressed (cancer-overexpressed) antigens.

The first obvious gap in our knowledge relates to the HLA-restriction of the described epitopes. In the database generated for all described tumor associated T cell epitopes 57% of all epitopes (326 of 576) are restricted to HLA-A2. Although this allele is frequently expressed in many different populations,[6] even for an HLA-A2 positive individual the responses determined by the additional 5 HLA Class I loci may be of equal importance for the tumor recognition as the HLA-A2 restricted recognition. Thus, if we would extrapolate the results based on the HLA-A2 restricted recognition (average percent antigen-specific TIL: 3.5%, ranging from 0–39%) and assume similar recognition by all 6 loci this may add up to on average 21% of the TILs recognizing described antigens (but non-identified epitopes). This frequency of antigen specific T cells is well matching the autologous tumor cell recognition observed in our cohort of TILs with available autologous tumor cell-lines. Here, we found that on average 9% of the CD8 TILs recognize INFγ treated autologous tumor cells as measured by combined secretion of INFγ and TNFα (Donia M, manuscript submitted).

Another likely explanation for the detection of relatively few antigen specific T cells using this library of published T-cell epitopes of relevance for melanoma is the underrepresentation of mutated antigens. It was recently shown that mutated antigens can be a target for the immunoediting process and these "de-novo" antigens may drive the immunological recognition of tumors.[7,8] Only one response was detected against this group of antigens, but it is likely that the majority of these responses are patient specific. Recent technological advantages

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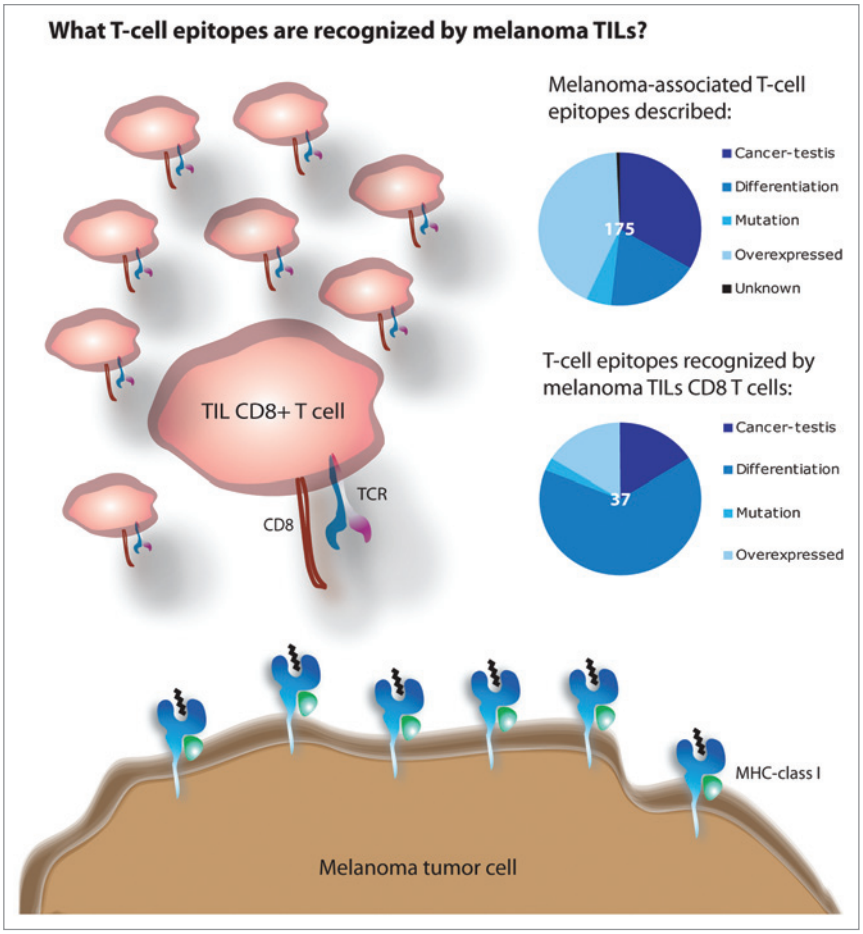

Figure 1. T-cell epitope specific reactivity in TILs. The illustration shows the distribution of the described T-cell epitopes of relevance for melanoma into four different antigen classes: Cancer-testis, Differentiation, Mutation and Overexpressed antigens; and the distribution of the $CD8^+$ T-cell responses found in TILs from 15 patients into the same four antigen-classes. An obvious caveat relates to the HLA-restriction of the different epitopes as this patient cohort where non-selected for HLA-expression.

in high-throughput sequencing and detection of T-cell responses will allow the identification of patient specific mutations and the recognition of these by patients TILs. The "de-novo" antigens represent an ideal source of targets for T-cell therapy, since they are exclusively expressed in the malignant cells, and no tolerance mechanisms has shaped the T-cell repertoire to abolish recognition of these.

Tolerance mechanisms may indeed be part of the explanation for the few T-cell responses found against the group of overexpressed antigens, and even three out of four responses found against this group of antigens were directed against epitopes expressed by an alternative ORF. We are currently elucidating how prominent this observation is in relation to the overall appearance of T-cell epitopes in alternative ORFs.

If we were able to identify the antigen specific T cells conveying most of the anti-tumor reactivity it would be ideal to selectively infuse these specific T cells instead of the whole T-cell culture. Antigen specific T cells can be selected by streptamers in a GMP controlled fashion,[9] but at this stage it is still unclear what fraction of the tumor-cell recognition resides in the described antigen-specific fraction. Thus currently, selection based on autologous tumor may seem most relevant, but due to the delay in tumor cell-line establishment this is possible only for patients where TILs have been frozen to await for certain clinical parameters to develop/normalize. Furthermore, the impact of these low-frequent antigen specific T cell populations may serve as an indicator to the level of responses needed to convey clinical efficacy, also for other immunotherapeutic strategies. To this end, the surprising low frequencies, both in TILs and blood after transfer holds promise to other means of inducing anti-tumor immune reactivity.

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