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High-quality and high-avidity T cell clones specific for tumor-associated antigens and how to find them

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Abbreviations: CTL, cytotoxic T lymphocytes; TCR, T-cell receptor; Th1, Type 1 helper; tgTCR, transgenic TCR; pMHC, peptide-MHC

The adoptive transfer of lymphocytes expressing high-avidity T-cell receptors with antitumor specificity provides a promising therapy for cancer patients. Recently, we compared 12 HLA-A2-restricted, tyrosinase peptide-specific CD8+ cytotoxic T-lymphocyte (CTL) clones and demonstrated that polyfunctional Type 1 helper (Th1)-cytokine secretion serves to rapidly select high-quality, high-avidity CTLs.

The adoptive transfer of lymphocytes expressing transgenic T-cell receptors (tgTCR) specific for a tumor-associated antigen is providing a new treatment option for patients with advanced cancer. This therapy can be used to treat not only leukemia, lymphoma and solid tumors, but also life-threatening viral infections.[1] In these settings, successful immunotherapy requires that antigen-specific TCR-engineered T cells with appropriate functional characteristics are transferred to patients to eliminate infected or transformed cells. In previous clinical studies, the adoptive transfer of tumor-infiltrating lymphocytes specific for multiple tumor-associated antigens was shown to successfully control melanoma in numerous patients.[2] While overexpressed self-antigens, like p53 and telomerase, are potential targets for adoptive T-cell gene therapy against multiple types of tumors, negative selection in the thymus yields only low-avidity T cells in the peripheral repertoire.[3] Thus, to obtain high-avidity T cells one must seek within a non-negatively selected T-cell repertoire, for example using foreign MHC-molecules to present self-peptides. Allo-restricted TCRs of high-avidity with peptide-specificity can be expressed as transgenic receptors in patient lymphocytes, providing suitable therapeutic reagents when matched to the MHC allotype and tumor type of the patient.[4] The adoptive transfer of lymphocytes with high-affinity tgTCR improved clinical efficiency[5] but also increased the risk of on-target toxicity, so that a careful selection of target antigens is essential.[6] In addition, the transfer of tgTCRs of various specificities may be necessary to avoid tumor immunoescape.[5] Finding suitable high-affinity TCRs is an important challenge that can be approached in several ways: they can be isolated from (1) transgenic mice with a complete human TCR repertoire that recognizes human tumor-associated antigens as foreign sequences; (2) by performing genetic affinity maturation; or (3) by tapping allo-restricted TCR repertoires.[1] Regardless of the approach, finding high-affinity TCRs takes time and is often hindered by poor rates of proliferation and survival of individual T-cell clones. Therefore, the rapid selection of relevant T cells is a critical point. Previous studies have used either functional parameters[7] or structural MHC-multimer binding characteristics[8] for the characterization of T cells. However, these approaches have never been compared on a larger panel of clones with identical specificity to determine which one works best for the rapid selection of CTL clones with high-avidity. Recently, we have performed a systematic analysis of a set of 12 CTL clones with identical peptide-MHC (pMHC) specificity the for tyrosinase$_{369–377}$ peptide presented by HLA-A*02:01 molecules.[9] This analysis included the assessment of peptide sensitivity, tumor lysis, secretion of 10 cytokines and chemokines and structural MHC-multimer binding properties. Because the CTLs had known peptide sensitivities that varied from 1×10^{-8} to 6×10^{-10} M (**Fig. 1A**), our retrospective analysis was designed to identify CTLs with high peptide sensitivity but using parameters that required a few cells. In the end, we wanted to identify early selection criteria to yield clones that had a high-avidity, a good capacity to kill tumor cells and secreting high amounts of interferon γ (IFNγ). At the structural level, an

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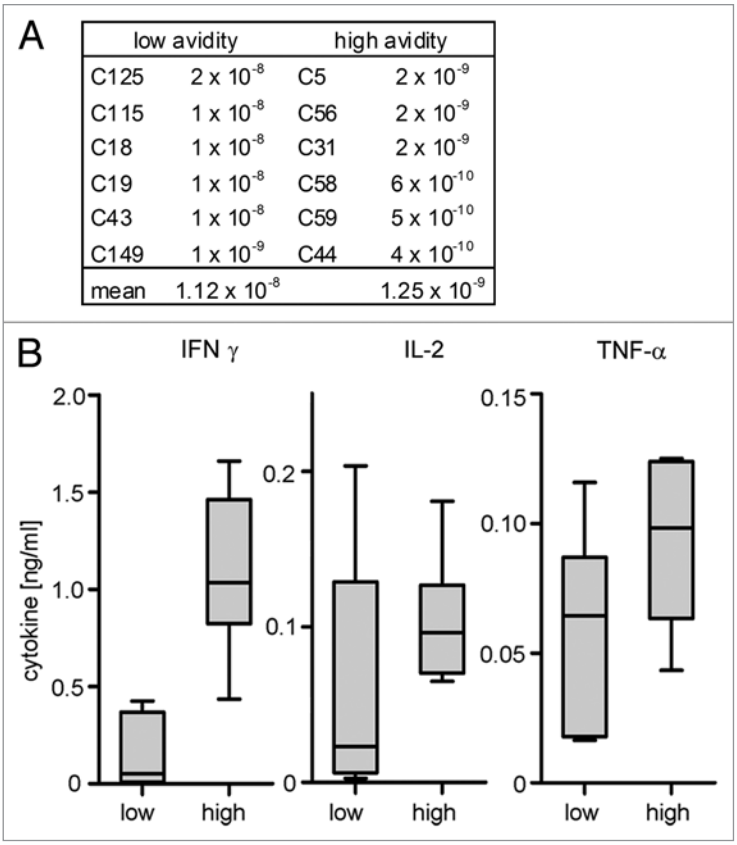

Figure 1. Functional characteristics of 12 cytotoxic T-lymphocyte (CTL) clones with the same specificity. (**A**) Peptide concentrations required for half-maximal cytotoxicity, as measured for each CTL clone measured in a standard ^{51}Cr-release assay of T2 target cells (HLA-A*02:01^{+}) loaded with varying concentrations of the tyrosinase$_{369-377}$ peptide. CTL fell in two groups, a low-avidity group (mean 1.12×10^{-8} M) and a high-avidity group (mean 1.25×10^{-10} M). (**B**) The capacity of CTL clones to secrete the the Type 1 (Th1) cytokines interferon γ (IFNγ), interleukin-2 (IL-2) and tumor necrosis factor α (TNFα) upon co-culture with tyrosinase$_{369-377}$ peptide-loaded T2 cells is shown as box and whiskers plots.

increased capacity to bind MHC multimers (on-rate) and a limited loss of bound multimers (off-rate) indicates stronger TCR-pMHC interactions. Thus, these characteristics appeared to be well suited to identify high-avidity CTL clones. Contrary to expectation, we failed to find a clear correlation between multimer binding and peptide sensitivity. For example, the two clones (C115 and C19) that best bound multimers (93% and 73% positive cells at 60 min) had relatively low peptide sensitivities (10^{-8} M) (**Fig. 1A**).

Since both human and murine virus-specific T cells with increased peptide sensitivity and virus control secrete multiple pro-inflammatory cytokines,[7,10] we applied a similar functional analysis to our CTL clones, comparing 10 cytokines and chemokines. Likewise, we found that CD8^{+} CTLs with enhanced peptide sensitivity and high capacity for tumor recognition secreted a triplet of CD4-associated Type 1 helper (Th1)-cytokines, including IFNγ, interleukin-2 (IL-2) and tumor necrosis factor α (TNFα), whereas lower avidity CTLs failed to produce these three cytokines simultaneously (**Fig. 1B**). These results extended the findings made with virus-specific CTLs to tumor-specific CD8^{+} T cells, by showing that high peptide sensitivity correlates with Th1-polycytokine production and a good control of tumor cells in vitro. Crucially, the TCR sequence of a high-avidity CTL clone transferred high antigen sensitivity, enhanced tumor recognition and capacity for Th1-polycytokine secretion to recipient lymphocytes, which represent the therapeutic form for clinical application.

Since CTL selection should allow for the early elimination of non-specific or low-avidity CTLs in order to focus on high-avidity clones with desirable properties, the requirements of different assays for cell numbers and culture times should not be neglected. Fortunately, the lowest cell requirements were needed for multiplex cytokine secretion assays while giving the best prediction for peptide sensitivity and tumor lysis. In conclusion, our recent study demonstrates that polyfunctional Th1-cytokine secretion, rather than multimer-binding parameters, is most suited for the early selection of CTL clones whose TCRs have functional properties that are compatible with the efficient development of adoptive cell therapy based on engineered lymphocytes.

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