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Adoptive immunotherapy
New assay for the identification of T cells with optimal avidity

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T cells expressing high avidity T-cell receptors (TCRs) have been shown to mediate superior therapeutic effects. A novel k_{off}-rate assay allows for the quantitative and reproducible assessment of the avidity of TCRs for their ligands directly on living T cells, ex vivo. This assay might facilitate the selection of T cells with an optimal avidity for their target, hence favoring the development of adoptive immunotherapeutic regimens.

CD8$^+$ T lymphocytes are potent cytotoxic immune effectors that can eliminate infected or malignant cells. The immune system comprises a highly diverse repertoire of T cells expressing unique T cell receptors (TCRs). TCRs generally bind to MHC molecules loaded with antigenic peptides of viral or neoplastic origin. However, T cell clones that would mediate therapeutic effects are often absent or inhibited in patients suffering from chronic infections or cancer. Thus, there is increasing interest in the therapeutic restoration of protective antigen-specific CD8$^+$ T lymphocytes by adoptive cell transfer. The technologies that allow for the detection and isolation of T cells with defined specificities have been substantially improved over the past few decades. Importantly, the adoptive transfer of minimal numbers of antigen-specific T cells can be sufficient to establish a diverse and long-lasting immune response.[1,2] The therapeutic efficacy of adoptively transferred T-cell populations depends not only on their proliferative potential, effector functions and in vivo persistence, but also on the (structural) binding avidity of both the TCR and CD8 (which operates as a co-receptor) for their ligand (peptides complexed with MHC class I molecules).[3,4] As mentioned above, T lymphocytes bearing high-avidity TCRs specific for tumor-associated antigens (TAAs, including mutant polypeptides as well as overexpressed self proteins) are scarce in cancer patients, owing to central and peripheral tolerance mechanisms. This implies that the identification and isolation of high-avidity T cells could significantly improve the clinical profile of adoptive cell transfer-based immunotherapy.

Until recently, the methods to precisely assess the binding avidity of TCRs for their targets were very laborious and not compatible with the screening of large numbers of T cell clones. To circumvent this issue, we have recently developed a new assay based on reversible MHC *Strep*tamers, allowing for the assessment of the dynamic dissociation (k_{off}-rate) of fluorescently labeled, peptide-loaded MHC class I monomers from TCRs expressed on the surface of living T cells (**Fig. 1**). In this setting, an elevated structural binding avidity is reflected by a low dissociation (k_{off}) rate, de facto indicating that the molecular complex made up by the antigenic peptide presented in the context of MHC class I molecules, the corresponding TCR and CD8 has a long half-life. This new assay enables a simple, quantitative and reproducible measurement of the k_{off}-rate as a reliable indicator of the TCR binding avidity.[5] It is indeed possible to analyze T cells and process them for additional in vitro manipulations, including the cloning of the TCR-coding sequence by single-cell PCR[6,7] approaches, or for adoptive cell transfer.Importantly, we were able to demonstrate in two distinct murine models that T cells bearing TCRs with a low k_{off}-rate (indicative of high binding avidity) mediate superior therapeutic effects upon adoptive transfer. In addition, we could show that the k_{off}-rate of a native TCR is maintained upon transgene-driven re-expression in CD8$^+$ Jurkat T cells, indicating that high-avidity TCR-coding genes identified via our method can be employed in the genetic engineering of T cells for therapeutic applications.[5]

In the future, it will be important to characterize numerous TCRs for their k_{off}-rate and correlate these values with the therapeutic efficacy of adoptively

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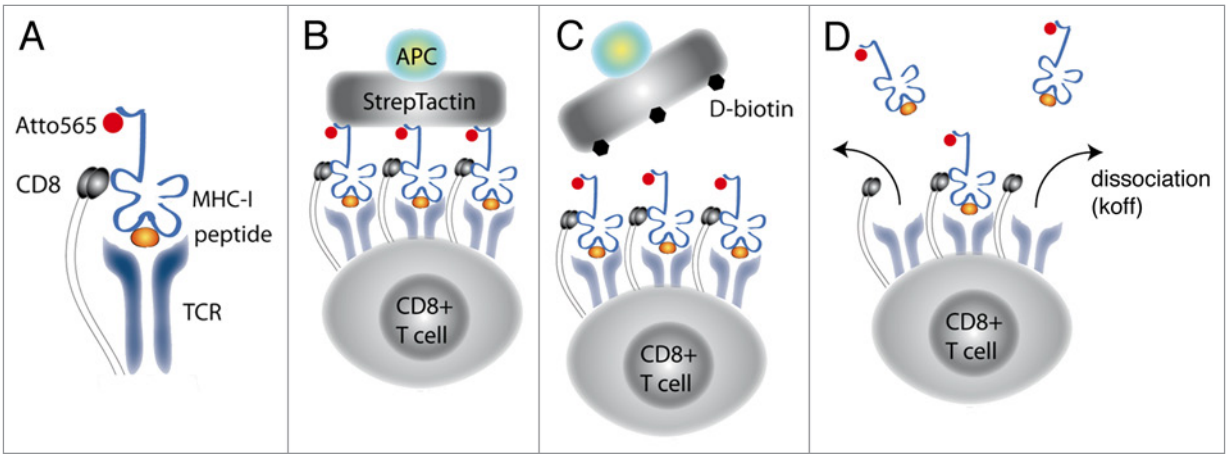

Figure 1. Principle of the k_{off}-rate assay. (**A**) The structural avidity of T cell receptors (TCRs) is defined as the molecular interaction between the TCR, CD8 (which operates as co-receptor) and peptide-loaded MHC class I molecules. (**B**) Specific $CD8^+$ T cells are stably labeled with dichromatic *Strep*tamers (pMHC-Atto565 in red; *Strep*Tactin-APC in blue). (**C**) The addition of *D*-biotin results in the disruption of the *Strep*tamer, leaving Atto565-labeled MHC monomers on the T cell surface. (**D**) The dissociation (k_{off}) rate of peptide-loaded MHC molecules from TCRs can hence be monitored as a decay of the Atto565-dependent fluorescence by real-time microscopy.

transferred T cells. This approach might allow for the identification of threshold k_{off}-rate values below which the therapeutic potential of adoptive cell transfer is compromised. So far, we have compared TCRs exhibiting relatively large differences in their k_{off}-rate values, observing that half-lives longer than 80–100 s consistently correlate with an effective recognition of target cells.[5] However, a broader and more detailed characterization is needed to define the actual threshold for optimal avidity. This value might be implemented into screening strategies aimed at the identification of therapeutically relevant TCRs.

A diverse and rich source for therapeutic TCRs is the naïve T-cell repertoire. Although T-cell clones specific for some antigens (notably TAAs) can be rather rare, sophisticated multimer staining protocols allow for the visualization of extremely small cell subsets. We have recently tested a number of HLA-A*0101-restricted T cell clones specific for the cytomegalovirus-encoded protein pp65 that had been obtained upon multimer-guided cell sorting from the naïve T-cell repertoire.[8] These clones exhibited positive multimer staining with a variable intensity; yet, we observed no correlation between staining intensity and functionality. In fact, T cell clones exhibiting the highest staining intensity were generally weak responders in functional assays. Conversely, the k_{off}-rate of these clones completely correlated with their functionality. In particular, only the T cell clones bearing TCRs with a half-life higher than 100 s demonstrated a high functional avidity, while all other clones (which manifested a poor functional profile) were characterized by extremely short half-lives (20–40 s). These data are very promising, as they lend support to the interpretation that measuring k_{off}-rates is a valuable approach for identifying therapeutically relevant TCRs.

One possible strategy to generate high-avidity T cells for immunotherapeutic applications is the genetic alteration of genes coding for low-avidity TCRs, followed by their re-introduction in T cells. Also in this scenario, measuring k_{off}-rates might constitute a valuable tool for TCR screening and characterization. Preliminary clinical trials have already demonstrated that genetically enhanced TCRs can indeed confer improved on-target effector functions to T cells.[9] However, 2 patients receiving affinity-enhanced melanoma antigen family A3 (MAGEA3)-specific T cells have recently died owing to the recognition of another protein expressed in the pulsating cardiac tissue by adoptively transferred lymphocytes.[10] Of note, such a fatal cross-recognition could not observed with the corresponding unmodified, wild-type TCR. Thus, methods need to be refined to allow not only for the prediction of on-target therapeutic activity, but also for the identification of putative safety issues.

In summary, the k_{off}-rate assay that we have recently developed allows for a quantitative and reproducible measurement of TCR binding avidity on living T cells. Importantly, k_{off}-rate values correlate with the functionality of T cells, both in vitro and in vivo. Besides constituting a tool to address several questions of basic immunology, this assay offers a convenient means to select T cells that exhibit optimal avidity for adoptive transfer based-immunotherapy as well as to characterize primary or genetically-engineered T-cell transplants. Furthermore, the k_{off}-rate assay might be employed to estimate the quality of therapy-induced or pre-existing T cell responses as a diagnostic marker, especially in neoplasms in which the tumor microenvironment significantly alters the functional profile of infiltrating T cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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