

Relevance of the T cell Receptor-Ligand Avidity for Immunity to Infection

Magdalena Nauerth¹, Kristof Wing^{1,2}, Heinrich Körner² and Dirk H Busch^{1,3,4*}

¹Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich, Germany

²Menzies Institute for Medical Research, Medical Science Precinct, Hobart, Tasmania, Australia

³DZIF - National Centre for Infection Research, Munich, Germany

⁴Focus Group "Clinical Cell Processing and Purification", Institute for Advanced Study, Technische Universität München, Munich, Germany

Abstract

Cytotoxic lymphocytes are critical for fighting viral and certain bacterial infections. Therefore, assessing the quality of cytotoxic T cell responses might have important clinical implications. TCR-pMHC binding (avidity) is a key determinant of T cell quality. Here we review currently available technologies for the measurement of TCR-pMHC avidity and their potential relevance for translational applications.

Keywords: T cell; T cell receptor; Avidity; Intracellular infections; Immunotherapies

Introduction

Chronic infections with viruses and intracellular bacteria remain a leading cause of morbidity and mortality worldwide. The Human Immunodeficiency Viruses (HIV) account for over 4% of the Years of Life Lost globally [1], continue to be largely incurable, and have increasing rates of therapeutic resistance [2]. *Mycobacterium tuberculosis* is estimated to latently infect between two and three billion people worldwide [3], with mortality in active, extensively drug resistant (XDR-TB) cases approaching 50% in spite of rigorous treatment [4]. Similarly, cytomegalovirus (CMV) and Epstein-Barr virus (EBV) cause significant morbidity and mortality in immunosuppressed patients, and are regularly refractory to current therapies [5]. The treatment of each of these conditions is associated with, at times, severe toxicities that limit management and prevent curative treatment [6-8]. These inadequacies have led to the investigation of novel immunotherapies, including vaccination and adoptive cell therapy (ACT).

The control and clearance of intracellular pathogens usually requires robust cytolytic immunity, pivotally mediated by antigen-specific CD8⁺ T cells [9]. Highly diverse repertoires of CD8⁺ T cells in the pool of lymphocytes, with each cell expressing a unique T cell receptor (TCR) on its surface, are provided by thymic development and selection. These TCRs bind, together with the CD8 co-receptor, to major histocompatibility complex I molecules loaded with cognate peptides (pMHC), which are expressed on the surface of target cells. Infected cells presenting peptides from the intracellular pathogen can thereby be specifically recognised by T cells, and are killed or modulated due to the consequent effector functions of the T cell. In this context, the term 'T cell avidity' describes the efficiency of these effector functions after antigen encounter and is principally affected by the binding strength between the TCR, its cognate pMHC ligand, and associated co-receptors, the so called 'structural avidity'. The structural avidity of TCR-pMHC binding can be expressed by the equilibrium constant K_D , a ratio between the association (k_{on}) rate and the dissociation (k_{off}) rate [10,11] with low equilibrium constants indicative of high avidity TCR-pMHC complexes. Interactions characterised by high structural avidities are correlated with increased T cell functional avidities [10,12], with a major determinant of the structural TCR avidity being the k_{off} -rate [10,13]. There is growing interest in identifying T cells with high avidity TCRs, as they have been shown to possess superior efficacy towards target cells *in vivo* and *in vitro* [14-16]. Given that high

avidity T cells recognize targets earlier and mediate superior effector functions [14], their generation and precise detection becomes an important aspect for the development of more effective anti-infective immunotherapies.

Measuring TCR-pMHC Interaction

Functional assays, such as interferon- γ synthesis, interleukin-2 release or *in vitro* cell-mediated lysis, can be strongly affected by the expression level of TCRs and co-receptors, and alterations in the signaling cascade [17], and are cumbersome to undertake. Several different methods have been developed, which aim to assess the structural avidity of TCR-pMHC binding in order to overcome the limitations of functional assays, as proxies for functionality. Surface Plasmon Resonance (SPR) (Figure 1a), initially developed by Liedberg et al. [18], has been adapted to assess the binding of TCRs or MHCs devoid of co-receptors, with one member of the pair being soluble and one substrate-bound, by detecting changes in the mass of the bound complexes. Typically, this technique is complicated by the technically challenging and labour intensive production of high-purity soluble TCRs and MHC molecules. Furthermore, the contribution of the CD8 co-receptor and other potentially involved surface molecules to binding is unable to be interrogated, thus preventing assessment of the physiological conditions present at the T cell surface.

Conversely, assays based on multimer binding (Figure 1b) and dissociation (Figure 1c) attempt to measure the structural avidity of TCR-pMHC binding on the surface of living T cells [13,19]. These assays monitor the dissociation of multimeric complexes, can thus have half-lives of dissociation in the range of hours to days, are strongly influenced by the quality of multimer reagents, as well as the concentration and affinity of MHC-blocking antibodies used, a

*Corresponding author: Dirk H Busch, Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich, Germany, Tel: +49 89 28901; E-mail: dirk.busch@tum.de

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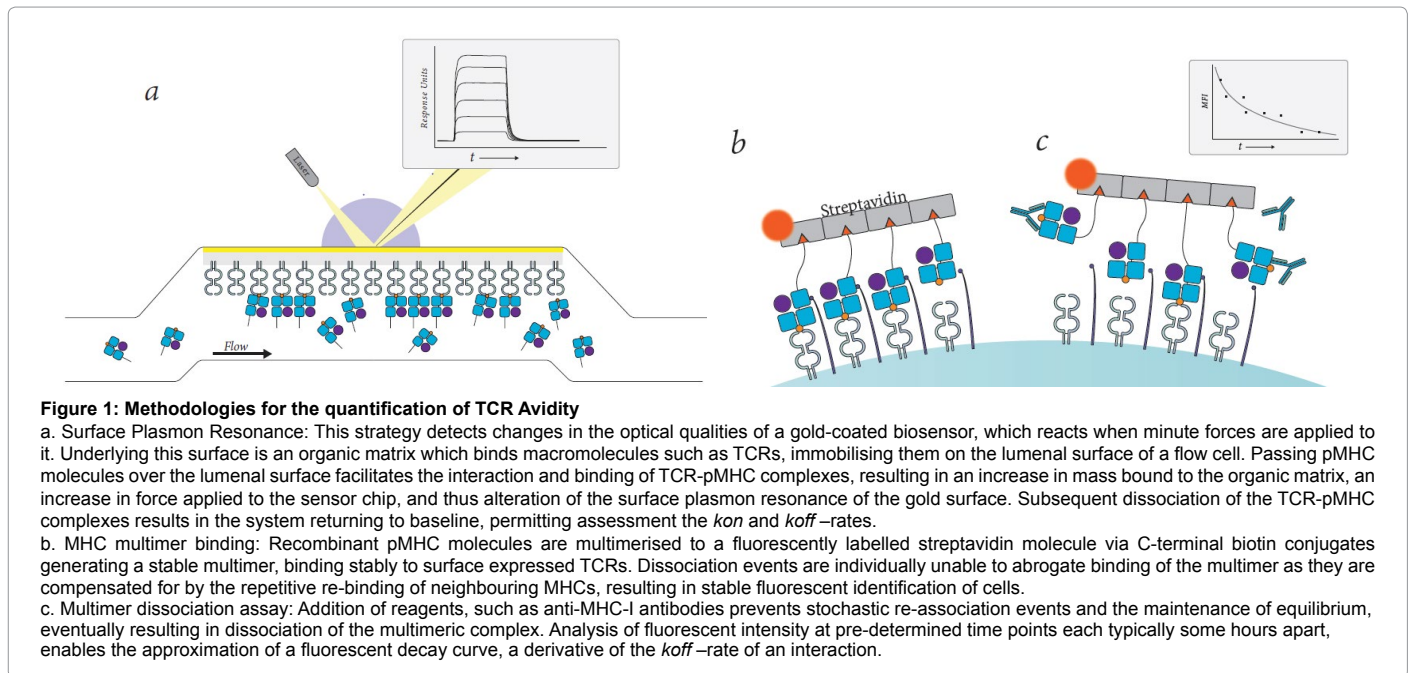


Figure 1: Methodologies for the quantification of TCR Avidity

a. Surface Plasmon Resonance: This strategy detects changes in the optical qualities of a gold-coated biosensor, which reacts when minute forces are applied to it. Underlying this surface is an organic matrix which binds macromolecules such as TCRs, immobilising them on the luminal surface of a flow cell. Passing pMHC molecules over the luminal surface facilitates the interaction and binding of TCR-pMHC complexes, resulting in an increase in mass bound to the organic matrix, an increase in force applied to the sensor chip, and thus alteration of the surface plasmon resonance of the gold surface. Subsequent dissociation of the TCR-pMHC complexes results in the system returning to baseline, permitting assessment the k_{on} and k_{off} -rates.

b. MHC multimer binding: Recombinant pMHC molecules are multimerised to a fluorescently labelled streptavidin molecule via C-terminal biotin conjugates generating a stable multimer, binding stably to surface expressed TCRs. Dissociation events are individually unable to abrogate binding of the multimer as they are compensated for by the repetitive re-binding of neighbouring MHCs, resulting in stable fluorescent identification of cells.

c. Multimer dissociation assay: Addition of reagents, such as anti-MHC-I antibodies prevents stochastic re-association events and the maintenance of equilibrium, eventually resulting in dissociation of the multimeric complex. Analysis of fluorescent intensity at pre-determined time points each typically some hours apart, enables the approximation of a fluorescent decay curve, a derivative of the k_{off} -rate of an interaction.

pre-requisite for the assay as it permits dissociation of the multimeric complex [20]. These factors result in significant inter-assay and inter-investigator variability, negatively effecting the reliability of this assay.

In an effort to overcome these inadequacies, Huang et al. [21] and Huppa et al. [22] have developed two-dimensional TCR-pMHC binding assays to better approximate the physiological situation, where both binding partners are embedded in 'two-dimensional' membranes. These assays provide valuable insights into TCR-pMHC physiology, but have thus far been unable to lend themselves to high-throughput translational applications.

Determining T cell Avidity: The TCR-Ligand k_{off} -Rate Assay

To enable the rapid and accurate measurement of truly monomeric TCR-pMHC interactions on living T cells, we developed the TCR-Ligand k_{off} -rate assay (Figure 2) based on reversible MHC multimers, so-called MHC *StrepTamers* [23]. Fluorescently-labelled MHC-I molecules, which associate with their cognate TCRs are multimerized onto *StrepTactin* molecules via C-terminal *StrepTag* sequences, permitting the stable identification of living CD8⁺ T cells via cooperative binding (Figure 2a). In the presence of D-biotin, the *StrepTag* is very rapidly displaced from the *StrepTactin* molecule, causing disintegration of the multimer complex, whilst leaving monomeric MHC-I molecules bound to their cognate TCRs (Figure 2b). Dissociation of these MHC molecules from the surface of the T cell is visible as a decay in fluorescent intensity over time, readily permitting the assessment of the k_{off} -rate (Figure 2c) [24].

We analysed human T cells specific for CMV from healthy donors' PBMCs via the fluorescence microscopy k_{off} -rate assay, and demonstrated high reproducibility between experiments, dissociations, and even unknown identical clones [24]. Correlation of the k_{off} -rate and *ex vivo* cytokine and cytolytic assays demonstrated a clear association between the k_{off} -rate and functionality. Specifically, we have not yet found any endogenous epitope-specific T cell which exhibits fast k_{off} -rates and still high functionality [17], a constellation

that could be achieved by a predominantly fast k_{on} -rate. For CMV-specific CD8⁺ T cells, differences in k_{off} -rate again correlated with the functional competence of the respective populations. Furthermore, we have demonstrated a clear correlation between protectivity and the k_{off} -rate of T cells used in adoptive T cell transfer, in preclinical murine models of *Listeria monocytogenes* and murine CMV infection. In the meantime, others have also developed TCR-pMHC k_{off} -rate assays based on the same principles as our *Streptamer* based assay [25]. Their methodologies have found a clear correlation between the TCR-pMHC k_{off} -rate and the functionality of the analysed cells [26], supporting our previous findings.

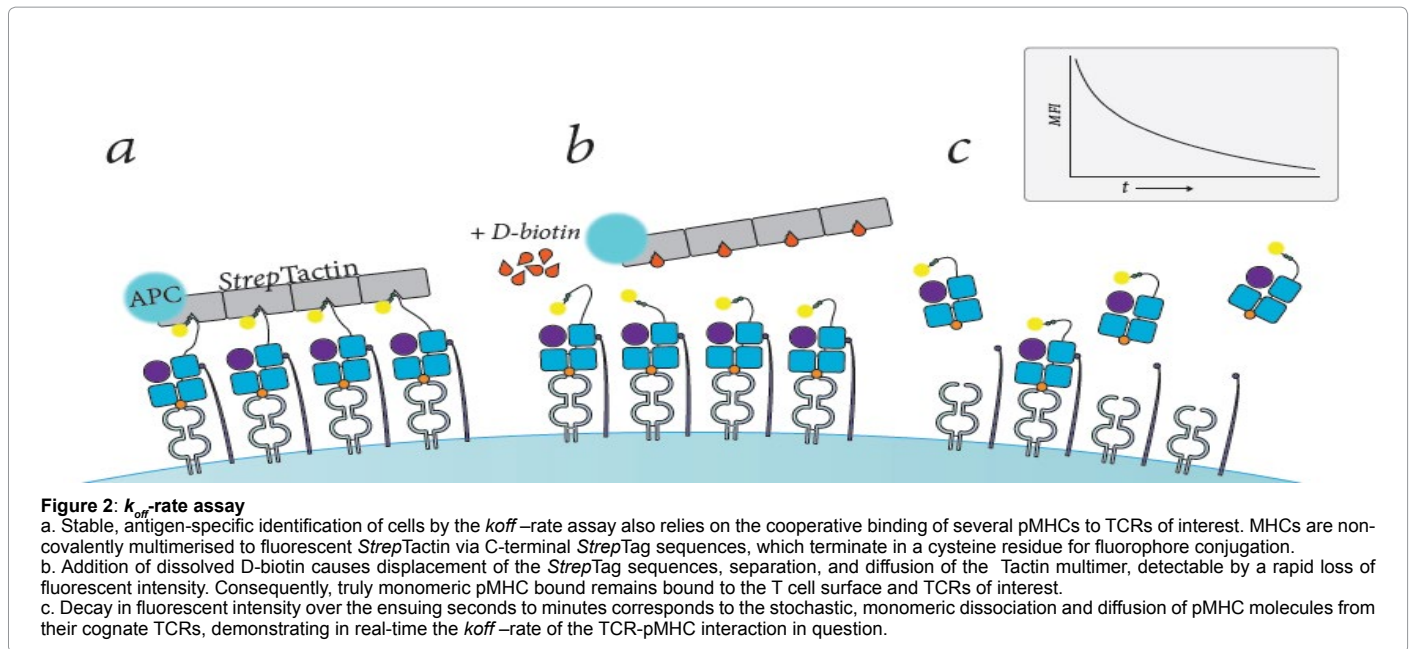
Altogether, our data and the published work of other groups indicates that the k_{off} -rate is an important parameter for the identification and selection of highly functional T cells for different therapeutic and diagnostic applications [17,27].

Applications of the TCR-Ligand k_{off} -Rate Assay

Adoptive T cell transfer experiments in preclinical mouse models have clearly demonstrated the superior protective capacity of T cells with long TCR-pMHC dissociation half-lives and thus low k_{off} -rates [24]. In addition, Stemberger et al. [28] found that the transfer of very high-quality antigen-specific T cells, which is additionally determined by the subset differentiation, can provide exceptionally robust protection against pathogens even at very low cell numbers. Together, these data suggest that the application of the TCR-pMHC k_{off} -rate assay for the identification of highly functional T cell populations could lead to a reduction of the number of T cells needed for the adoptive transfer, reducing costs and possible side effects.

Clinical relevance

The selection of T cell receptors most suitable for genetic engineering of autologous T cell populations could be guided by the k_{off} -rate assay, a technology which has the capacity to broaden the applicability of adoptive T cell therapy. Putatively, individual high-avidity T cells could be identified in a high-throughput manner via the k_{off} -rate assay,



with TCR extraction and subsequent recombinant TCR expression [24,29,30]. This process has the capacity to permit the identification of ideal, high-avidity, pathogen-specific TCRs, with opportunities for the characterisation of TCRs bearing the greatest clinical relevance, and the development of novel cellular therapeutics. In patients who have successfully cleared or controlled infections, the k_{off} -rate assay has the potential to identify highly protective populations that have undergone clonal selection and evolution, enabling the directed extraction of TCRs for investigation and clinical application. Several investigators have demonstrated that the transfer of TCRs, in addition to the transfer of antigen specificity [31,32], endows the recipient cells with functional avidities comparable to that of the original population [30,33]. Thus, the development of novel, k_{off} -rate-identified cellular therapeutics for infectious diseases is a tangible application of this technology to the clinical setting.

For example, Appay et al. [9] identify that CD8⁺-specific responses are crucial in the immune control of HIV, and that high avidity T cells result in superior control of viral replication by exerting a greater selective pressure on variable viruses, which as a result exhibit reduced replicative fitness, elegantly demonstrated in the work of Varela-Rohena et al. [34] Similarly, Sud et al. [35] identify the importance of cytotoxic T cells in controlling infections of *M. tuberculosis*, in cooperation with other members of the adaptive immune system. Aside from the application of the k_{off} -rate assay to adoptive cell therapies, the assay has the potential to be of great advantage in diagnostic settings. Conceivably, the k_{off} -rate of antigen-specific T cell populations could demonstrate the quality of existing immunity and permit a degree of prognostication about response to illness to occur. Indeed, the work of Hadrup et al. [36] and Wikby et al. [37] have identified several features of an individual's immune system that are predictive of premature mortality in the aged. Given the short timeframe in which a k_{off} -rate assay can be performed, and the lack of unique equipment required to perform the assay, it would be easily integrated into the clinical environment. Furthermore, the quality of induced CD8⁺ T cell immune responses after vaccination are of critical importance and are able to be analysed with this assay. Identifying so-called non-responders early in vaccine development and monitoring cohort responses throughout

the developmental course of new vaccines are two key applications of this assay. In the context of HIV, it has been shown that the variability of existing assays and the resultant inability to confidently correlate structural TCR-pMHC data with functional data has hampered the development of effective vaccines [9]. Additionally, Parida et al. [4] exemplify the importance of the development of both prophylactic and therapeutic vaccines for *M. tuberculosis*, as they lament that even with the availability and use of new anti-tuberculous medications, *M. tuberculosis* will continue to develop therapeutic resistance. Foreseeably, the k_{off} -rate assay could overcome practical difficulties by permitting the inference of functionality from structural avidity with regard to vaccine-induced T cell responses in both the pre-clinical and clinical phases of vaccine design and testing.

Relevance to research

The applications of the k_{off} -rate assay in basic research and clinics are manifold. Comparing k_{off} -rates of T cell populations after primary and secondary infection with *Listeria monocytogenes*, we found an unexpectedly large variance in the k_{off} -rates of T cells induced after primary infection, with a clear focussing to higher k_{off} -rates for some epitope specificities after secondary infection (unpublished data). This observation is in line with previous findings that narrowing of the repertoire towards higher avidity T cells [38] is largely due to failed recruitment of T cells with low structural avidity into secondary response [39].

The k_{off} -rate assay has also permitted the detailed investigation of chronic immune responses, further demonstrating its application to basic infection research. Infections in which sterilising immunity isn't achieved, including the pathogens HIV, CMV, EBV, and *M. tuberculosis*, are characterised by several mechanisms to control pathogen replication, including memory inflation [40]. A well studied example is the course of CMV infection during ageing [36,37], the studies of which have led to the theory of 'The Clonal Changing of the Guard' [41]. According to this theory, high avidity T cells become exhausted and eventually deleted during ongoing infection. Due to reduced thymic output of novel, potentially high-avidity T cells, they are gradually replaced by sub-dominant clones [42], each of which has a lower affinity

for MHC-presented viral antigens. This requires larger populations to prevent fulminant viral infection, such that global functional avidity is maintained, and so gradually occupy a larger proportion of the peripheral T cell pool. Pertinently, in a mouse model of chronic murine CMV infection and in accordance with this theory, we found a clear tendency towards inflating populations with decreasing k_{off} -rate during chronic infection (unpublished data). Further investigation of immune phenomena using the k_{off} -rate assay will indubitably yield new insights into the physiology of the adaptive immune system.

In summary, the TCR-pMHC k_{off} -rate assay allows for the accurate determination of the TCR-ligand avidity of cytotoxic T cells. This can be used for the identification and isolation of highly functional T cells to improve adoptive T cell therapy of severe infections, to monitor the quality of existing and induced immunity, and to gain insights into the course of infections in basic research.

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