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Mobilizing the low-avidity T cell repertoire to kill tumors

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1. Introduction

Immune recognition of tumors in an antigen-specific manner was first illustrated by experiments involving transplantation of chemically induced tumors into laboratory mice [1, 2]. Specifically, the growth of a transplanted tumor could be prevented by prior exposure to the same tumor, but not a different tumor. Many investigators have since observed naturally developing tumor-specific T cell responses (reviewed in [3]) which, in patients treated with standard therapies, correlate with improved prognosis [4-10]. Despite this positive correlation, the tumor infiltrating lymphocytes (TIL) do not always control tumor growth. Tumor-specific T cells are ineffective in part due to active regulation and suppression by tumors. For example, tumors produce the tryptophan degrading enzyme indoleamine 2,3-dioxygenase that inhibits T cell proliferation [11]. In addition, tumors produce immune suppressive cytokines such as TGFβ [12,13] and IL-10 [14]. The mechanism of immune suppression by these cytokines includes the inhibition of proliferation and inflammatory cytokine production by immune cells. For detailed reviews of tumor-induced immune suppression see the other reviews in this issue and [15].

In this review, we focus on another mechanism responsible for the poor reactivity of the tumor-specific T cell repertoire, the low functional avidity of the responding T cells. Functional avidity, or the sensitivity of T cell to antigen, is an important factor influencing the efficacy of a T cell response. Virus-specific cytotoxic T lymphocytes (CTL) with high functional avidity clear viral infections better than T cells with low functional avidity because these CTL are more sensitive to small viral loads [16,17]. Analysis of the functional avidity of tumor-specific T cells has provided insight into why tumors develop despite the presence of TIL and how these T cells may be harnessed for cancer therapies. In this review we will discuss the factors influencing the functional avidity of CTL and how this affects the T cell response to tumors. Furthermore, we will discuss different approaches aimed at improving the functional avidity of the tumor-specific T cells with the goal of augmenting conventional treatments and T cell therapies against cancer.

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2. Affinity, functional avidity, and recognition efficiency of T cells

As mentioned above, T cell functional avidity is defined as the sensitivity of a T cell to activation by an antigenic peptide bound by an MHC molecule. The sensitivity of a T cell to antigen is influenced by multiple factors: the affinity of the TCR-peptide-MHC interaction, the engagement of multiple other receptors on T cells, and the density of these receptors on the T cell surface. The combination of these binding interactions with an APC determines the functional avidity of a T cell. Since avidity is often used to describe the multivalent binding between two molecules rather than the interaction between two cells, the term functional avidity may be misleading and is therefore also referred to as recognition efficiency [18]. We use "functional avidity" since it is used in most of the literature described in this review.

The readout for T cell functional avidity also varies within the field. Functional avidity is frequently determined by the relative capacity of T cells to produce effector cytokines or lyse target cells in an antigen-specific manner. However, as we discuss below, staining intensity of T cells with peptide-loaded MHC tetramers is also a common readout for T cell avidity. Therefore, a comprehensive understanding of what influences T cell function is crucial for understanding and measuring functional avidity of tumor-specific T cells. In the following sections we will dissect the molecular mechanisms that contribute to the activation of T cells and functional avidity.

2.1 TCR-*peptide-MHC affinity, kinetics, and T cell functional avidity*

Activation of a T cell is initiated by the ligation of the TCR by peptide-MHC complexes on an APC. However, the TCR is not a simple on/off switch, but can be activated to different degrees depending on the binding kinetics. The serial-triggering model of T cell activation explains how a T cell is activated by the low levels of peptide presented on the surface of an APC. This model proposes that one peptide-MHC complex binds multiple TCRs on the surface of the T cell, providing the sustained signal required for activation [19]. One important prediction of this model is that there is an upper limit to the TCR-peptide-MHC binding half-life ($t_{1/2}$), or dwell time, that results in activation of the T cell. Prolonged binding would prevent the limited numbers of peptide-MHC complexes from binding enough TCRs to transduce a positive signal and, as a result, would inhibit T cell activation [20]. The complementary kinetic-proofreading model proposes that full T cell activation will not occur unless the TCR-peptide-MHC interaction has a long enough half-life for the completion of a series of biochemical intracellular signaling events [21]. If the off-rate is too rapid the T cell will not be fully activated [22,23].

The consequences of both the kinetic-proofreading and serial-triggering models are that TCR-peptide-MHC interactions with too high or low affinity will not activate the T cell; only those with mid-range affinity will result in activation and differentiation of the cell (Figure 1). This conclusion has been verified by experiments showing that the strength of the initial signal received through the TCR, due to antigen concentration [24,25] or the affinity of the stimulating antigen [20,26-31], affects the activation of T cell clones. Interactions with exceptionally long half-lives result in impaired T cell activation [20,25,32-34]. The K_D of a productive TCR-peptide-MHC interaction is on average between 1-10 μM and the $t_{1/2}$ is 5-20 seconds (reviewed in [35]). The low affinity of TAA-specific TCRs for peptide-MHC complexes has been proposed as a mechanism for preventing efficient recognition of tumors. Interestingly, we have determined that the K_D of a TAA-MHC complex for a specific TCR is between 5-7 μM and the $t_{1/2}$ is 1.5-2 seconds [30,31] confirming that at least some tumor antigens are weak TCR agonists.

The functional avidity of a T cell is often estimated by the relative staining intensity of MHC tetramers loaded with peptide since TCR-peptide-MHC dwell time correlates with T cell

activation. While our group and others have shown that multimer binding correlates with T cell sensitivity to antigen [31,36-38], this correlation is not always strict [20,39-41]. In some experiments higher expression levels of TCR on the cell surface increased the multimer-binding intensity [36,39]. Therefore, MHC tetramer off-rates may better correlate with functional avidity since this controls for TCR expression levels [42]. In addition, as discussed above, peptide-MHC complexes that bind TCRs with a long enough half-life inhibit T cell activation and MHC tetramers of these complexes bind T cells with relatively high intensity [20]. Finally, as we will discuss in the next section the intrinsic affinity of the TCR-peptide-MHC complex is not the only binding interaction that influences the functional avidity of a T cell.

2.2 Other factors that influence the functional avidity of a T cell

The functional avidity of a T cell is also influenced by the overall binding strength between a T cell and APC that results from the additive effects of multiple receptor/ligand interactions (Figure 2). The CD8 co-receptor on CTL binds the alpha 3 domain of the MHC class I molecule, enhancing the binding of the TCR-peptide-MHC complex [43-47]. Since the binding of CD8 by MHC enhances the activation of T cells expressing low affinity TCRs, staining with mutated MHC class I-multimers that inhibit CD8 binding identifies T cells with high-affinity TCRs [48]. The co-stimulatory receptors CD80 (B7-1) and CD86 (B7-2) on APC bind to CD28 on the surface of T cells and enhance the magnitude of TCR signaling, decreasing the level of TCR ligation required for activation. Improved binding between the T cell and APC is also facilitated by the adhesion molecule pairs ICAM-1/LFA-1 [49] and LFA-3/CD2 [50]. Interestingly, TIL have decreased cell-surface expression of LFA-1, CD2, and CD8 suggesting that defects in these T cell binding interactions contribute to the poor functional avidity of TAA-specific T cells [51].

The proximity of TCRs to each other and other membrane-associated molecules on the surface of T cells also affects the functional avidity. Fahmy *et al*. demonstrated that the increased sensitivity of activated T cells, relative to naive T cells, correlates with increased avidity resulting from TCR reorganization within the cell surface membrane [52]. Increased association of CD8 with TCR on the surface of T cells also enhances T cell activation, likely due to increased co-localization of the CD8-associated Lck kinase [53]. Similarly, increased expression of intracellular Lck following T cell activation increases the sensitivity of T cells to antigen stimulation [54]. Defects in proximal TCR signaling, including Lck activation, are observed in TIL [55]. This signaling blockade may explain the decreased expression of adhesion molecules on TIL since Lck activation is necessary for the expression and activation of these receptors on the cell surface [51]. In summary, numerous molecular interactions between T cells and APC contribute to functional avidity and can be impaired in TIL.

3. Tolerance and the tumor-specific T cell repertoire

The immune system maintains a diverse repertoire of T cells with high avidity for foreign antigen while limiting the activity of T cells that recognize self antigen. Since most tumor antigens are self antigens, tolerance mechanisms greatly influence the quality of the antitumor T cell response. The degree to which tolerance affects tumor-specific T cells differs depending on the TAA, but in many cases both central and peripheral tolerance mechanisms directly influence the functional avidity of T cells for TAA. We discuss both features of TAA and mechanisms of tolerance governing the T cells that recognize these TAA below.

3.1 Tumor antigens recognized by T cells

Tumor antigens can be divided into two basic categories, tumor-specific antigens (TSA) and TAA (see Table I). TSA are often immunogenic since they are derived from viral antigens or

neoantigens created by mutations during the transformation process. Many of these mutations contribute to the malignant phenotype of the tumor cells (e.g. RAS, CDK4). While some mutations are found in multiple tumors, often they are unique to the tumor in which they were identified, limiting their clinical value as targets of general tumor immunotherapies.

As stated above, viral antigens expressed by oncogenic viruses are another source of TSA. Viruses that are associated with human cancers include human papilloma virus (HPV) [56], Epstein-Barr virus [57], Kaposi's sarcoma-associated herpes virus [58] and hepatitis B and C (HBV and HCV) [59,60]. The increased occurrence of virally associated cancers in immunocompromised patients relative to healthy individuals suggests that the expression of viral antigens by the transformed cells promote antitumor immunity. Vaccines against cancers that result from infections with oncogenic viruses have shown promise. A recently approved vaccine against HPV prevents both infection and the associated cervical neoplasia [61]. Similarly, a decrease in the incidence of hepatocellular carcinoma is observed following vaccination against HBV [62].

Although TSA are attractive targets for immunotherapy against cancers with viral etiology, the majority of tumor antigens from other cancers are TAA. TAA are non-mutated self antigens from proteins expressed in tumors as well as normal tissues (Table I). TAA can be characterized by their expression pattern as tissue-specific or ubiquitously expressed antigens (reviewed in [63]). For example, cancer-testis (CT) antigens are expressed in the testes and sometimes in the placenta, and are reactivated in tumor cells. The low levels of MHC expression in healthy testes and placenta prevent recognition of CT antigens by the immune system making them targets for T cell therapies. Differentiation antigens are present on both the tumor and the tissue from which the tumor arose and not in other tissues. The most studied of these antigens are the melanoma-differentiation antigens (reviewed in [64]). These include the gp100, Mart-1/Melan-A, pMel-17 and tyrosinase antigens that are products in the melanin-production pathway. These TAA are expressed both in melanomas and in normal melanocytes.

While tissue-specific antigens are expressed in a limited number of tissues, ubiquitously expressed antigens are found on most normal tissues but are often overexpressed in transformed cells. For example, overexpression of telomerase occurs in many cancers, see Table I for more examples. The increased expression of these antigens increases the amount of peptide presented by MHC molecules on the cell surface and augmenting T cell recognition.

TAA that are shared between tumors are practical targets for immunotherapies. However, because they are expressed in normal tissues, T cell responses to TAA may promote autoimmunity. Autoimmune destruction of normal melanocytes, or vitiligo, has been observed in both mice and humans following induction of an immune response against melanoma antigens [65-67]. Furthermore, because TAA are self antigens, the immune system is at least partially tolerant of these proteins, affecting the quality of the tumor-specific T cell repertoire.

3.2 T cell tolerance to tumor-associated antigens

The T cell repertoire must include TCRs that recognize foreign antigens and protect the host from autoimmunity. This balance is achieved through central and peripheral tolerance mechanisms. Central tolerance removes autoreactive T cells from the developing repertoire by negative selection in the thymus [68-70]. If a T cell expresses a TCR with avidity for MHC and self-peptide above a certain threshold, the cell is deleted before reaching the periphery.

Given that TAA are self-proteins, the majority of high-avidity TAA-specific T cells are eliminated in the thymus. Endogenous expression of the p53 tumor antigen results in the deletion of high-avidity p53-reactive T cells that are not deleted in a p53-null mouse [71]. Similarly, mice with a deleted tyrosinase gene have CTL with increased functional avidity for

TAA after vaccination with tyrosinase compared to mice sufficient for tyrosinase expression [67]. We have also observed similar results in studies of the gp70 TAA. T cells from mice lacking the gp70 gene bind MHC tetramers loaded with $gp70_{423\text{-}431}$ with increased intensity following vaccination with irradiated tumor compared to those from wild type mice (unpublished data). In each of these models antitumor immunity correlates with the detection of high-avidity TAA-specific T cells. Therefore, elimination of these T cells during negative selection in the thymus likely contributes to the low tumor-reactivity of the peripheral T cell repertoire.

3.3 T cell escape from central tolerance

Although negative selection eliminates a significant portion of the self-reactive repertoire, self-reactive T cells do escape deletion and are found in the periphery. Some T cells escape due to a lack of exposure to antigen in the thymus. In a model of experimental autoimmune encephalomyelitis, T cells specific for the proteolipid protein evade deletion because a shorter splice-variant of the protein is preferentially expressed in the thymus [72,73]. Self-reactive T cells may also escape negative selection because of poor antigen presentation by MHC [74, 75]. For example, the human melanoma antigen $gp100_{280\text{-}288}$ has a fast dissociation rate from HLA-A*0201 [74]. This poor MHC-binding prevents efficient presentation of antigen in the thymus and non-tolerized T cells enter the periphery. However, the low affinity of $gp100_{280\text{-}288}$ for MHC also results in poor presentation on peripheral tissues and tumors, preventing recognition by peripheral T cells.

T cells also escape deletion in the thymus if they have low avidity for self antigens. For example, influenza nucleoprotein (NP)-specific T cells from transgenic mice that express NP bind less MHC tetramer relative to T cells from wild type mice, suggesting that they escaped deletion as a result of low avidity for antigen [76]. Similar low-avidity T cells are detected in OT-I transgenic mice that express a TCR specific for $ovalbumin_{257\text{-}264}$ and express ovalbumin driven by the insulin promoter. These T cells not only bind MHC tetramers with lower intensity, but also have decreased functional avidity since they require ~ 10-fold more peptide to produce IFNγ [77]. Theobold *et al*. showed that, although expression of the p53 results in complete loss of reactivity towards the dominant epitope, CTL specific for a cryptic epitope are detected [71]. The T cells specific for the cryptic epitope are low avidity, i.e. they require more peptide antigen for CTL-mediated lysis than T cells specific for the dominant epitope from mice that are not tolerized to p53. These experiments demonstrate that although T cells with high avidity are deleted, some T cells with low avidity evade deletion in the thymus.

The presence of self-reactive T cells in the periphery does not guarantee destruction of tissues expressing the antigen as illustrated in the following examples. In a transgenic mouse expressing a TCR Vβ chain specific for self antigen, tissue destruction does not occur, despite approximately 4% of the natural repertoire of $CD8^+$ T cells being self-reactive [77]. Furthermore, in healthy individuals up to 2% of the $CD8^+$ T cells are Melan-A specific without any evidence of autoimmune destruction of melanocytes [78]. These observations highlight the role of peripheral tolerance mechanisms in preventing autoimmunity and tumor immunity by the low avidity T cells. However, these mechanisms can be overcome. For example, in cancer patients receiving immunotherapy, tumor regression was observed when 80-90% of the $CD8^+$ T cells were tumor-specific [79].

3.4 Peripheral tolerance and the tumor-specific T cell repertoire

Peripheral tolerance is achieved through a variety of mechanisms. In some cases the avidity of the T cells is too low to respond to the endogenous antigen found in peripheral tissues. This passive tolerance is demonstrated in tumor models in which growth of a spontaneous tumor is not sufficient to activate the small numbers of TAA-specific T cells [80,81]. Similarly, analysis

of melanoma-infiltrating lymphocytes shows that the tumor-specific T cells are activated by target cells loaded with high concentrations of peptide but are too low avidity to be fully activated by melanoma cells that express much lower concentrations of antigen [82].

Although passive tolerance explains the lack of autoimmunity in some cases, in many studies autoreactive T cells have proliferated and display activation markers suggesting that they respond to antigen in the periphery [83-85]. However, rather than becoming fully activated, these T cells become anergic upon encountering self antigen. In one study, TAA-specific T cells isolated from melanoma patients were non-cytolytic and did not produce cytokines in response to antigen [83]. The induction of anergy is in part due to activation of T cells in a non-inflammatory environment where they encounter immature dendritic cells ([86], reviewed in [87]). Therefore, TAA-specific T cells may also be rendered anergic because the tumor may not provide appropriate inflammatory stimuli. Induction of anergy in TAA-specific T cells in a number of animal models suggests that chronic inflammation of the tumor environment does not activate TIL [88,89]. Other studies show that tumor growth can induce the activation of tumor-specific T cells, although the T cells do not prevent tumor growth [90]. Why T cell activation occurs in some tumor systems is not clear, but it may be influenced by the frequency of tumor-reactive T cells at the time of tumor growth. Transfer of large numbers of TAA-specific T cells leads to improved T cell activation by the tumor due to a reduced requirement for CD4-T cell help [80].

Autoreactive T cells, specifically those of higher avidity, may be deleted following antigen stimulation in the periphery [91-93]. Molldrem *et al.* demonstrated that T cells with both high and low avidity for the PR1 leukemia antigen can be cultured from healthy individuals, but high-avidity PR1-specific T cells are deleted in leukemia patients due to the high expression levels of PR1 by tumors [94]. Interestingly, patients in remission retained a population of T cells with high avidity for the tumor antigen. Since deletion of PR1 cells was only observed at high antigen doses *in vitro* it would be interesting to determine if the lack of T cell deletion in the patients in remission correlates with low expression of PR1 by their tumors.

Finally, the tumor-reactive T cell repertoire can also be actively inhibited by regulatory T cells (Tregs) (reviewed in [12]). This inhibition occurs as a result of both direct contact and the production of soluble factors such as TGFβ. Interestingly, Tregs may specifically inhibit T cells with high avidity for tumor antigen. In the HER-2/*neu* transgenic (*neu*-N) mouse model of breast cancer, vaccination against the HER-2/*neu* tumor antigen following removal of Tregs elicited T cells that bind MHC tetramers with higher intensity [95].

In summary, both central and peripheral tolerance mechanisms lead to deletion and inactivation of T cells with the highest avidity for TAA. Low-avidity populations persist in the periphery although it is unlikely that they recognize the low levels of TAA expressed by tumors *in vivo*. These explanations are consistent with why, despite large numbers of tumor-specific T cells in some studies, tumor growth is uninhibited [3]. These results provide a rationale for the development of immunotherapies aimed at improving the avidity and activation of the tumor-specific T cell repertoire.

4. Enhancing the T cell response to tumors

Can the low-avidity tumor-specific T cell repertoire be manipulated to enhance the immune response to tumors? Typically, the low avidity of TAA-specific T cells for antigen prevents activation of these T cells in response to endogenous levels of tumor antigens. Therapies that enhance antigenic priming of tumor-specific T cells will likely elicit a more productive antitumor response. A number of strategies are being developed to improve the function of these T cells so that they may be used prophylactically or therapeutically against cancer. These

strategies exploit the binding properties of T cells for tumors both antigen-specifically and non-specifically.

4.1 Vaccination with peptide mimotopes

Vaccination with TAA to elicit functionally avid tumor-specific T cells is one strategy for augmenting antitumor responses. In fact, in one study vaccination of a melanoma patient with the Melan-A peptide elicited a population of T cells with increased avidity for antigen compared to the preimmmune T cells, but these cells did not prevent disease progression [96]. Unfortunately, in the majority of studies vaccination with the TAA increases the frequency of T cells that recognize tumor, but does not improve functional avidity and is insufficient to control tumor growth [97-99]. One possibility is that the affinity of the TCR for TAA-MHC is too low to sufficiently activate the tumor-specific T cells in these cases. Alternatively, the chemical structure of synthetically produced peptides may not adequately mimic naturally presented TAA, as the T cells elicited by peptide vaccines do not always recognize the tumor [100-102].

These studies with TAA peptides provide a rationale for the design of peptide-mimotope vaccines. Mimotopes are mimics of peptide epitopes also known as peptide analogues, agonists, heteroclitic peptides, or altered peptide ligands. Peptide mimotopes contain amino acid substitutions in the TAA that either enhance binding to the MHC [103-108] and/or improve the affinity of the TCR-peptide-MHC complex [30,38,109]. Mimotopes are hypothesized to enhance activation of tumor-specific T cells by providing optimal antigen presentation and stimulation of T cells, allowing for the acquisition of full effector function. In addition, mimotope vaccines target T cells that are not activated by the endogenous antigen, but cross-react with it once activated.

Both animal models (see Table II) and clinical trials (see Table III) of mimotope vaccines show increased numbers of tumor-specific T cells and, in some cases, improved antitumor immunity. In some studies, mimotope vaccines also elicit T cells with increased functional avidity for tumor antigen compared to those elicited by vaccination with the endogenous antigen [67, 106,110,111]. However, in other studies T cells with decreased functional avidity are detected following vaccination. Comparison of T cell clones from mimotope-vaccinated or unvaccinated melanoma patients show that the vaccine-elicited T cells do not lyse melanoma targets whereas T cells from the endogenous response do [112]. In these patients, mimotope vaccines preferentially expanded T cells with low functional avidity that could only lyse target cells coated with high concentrations of TAA peptide. It is proposed that some high-affinity mimotopes induce deletion of the highest-avidity T cells leaving only low-avidity T cells in the periphery. This hypothesis is consistent with experiments that show deletion of autoreactive T cells after vaccination with high-affinity peptide mimotopes in autoimmunity models [113].

Using the CT26 tumor model we showed that vaccination with mimotopes that increase the binding of the TCR-peptide-MHC interaction above a certain threshold results in a loss of tumor protection [31]. Direct *ex vivo* analyses of TIL showed that these mimotopes elicit T cells that do not produce IFNγ upon stimulation with either the TAA or the mimotope used for priming, at any concentration of peptide. We also identified mimotopes that elicited functional CTL that produced IFNγ and successfully elicited antitumor immunity. The affinity of the TCR-peptide-MHC interaction with these mimotopes is between that of the TAA and the mimotopes that anergized the T cells. Interestingly, TAA-loaded MHC tetramer binds T cells responding to both the high- and intermediate-affinity mimotopes with the same intensity, further indicating that the T cells elicited by these mimotope vaccines are anergic rather than low avidity.

Thus, vaccination with peptide mimotopes shows significant promise for improving antitumor responses, especially when used in combination with other therapies [65]. However, recent research suggests that there is an upper boundary for the affinity of peptide vaccines, above which the expansion of functionally unresponsive T cells occurs. Improved efficacy of these vaccines requires optimization to elicit T cells that recognize tumor with high functional avidity.

4.2 Adoptive cellular therapy

Another approach aimed at improving the T cell sensitivity to tumor is adoptive cellular therapy (ACT). In ACT tumor-specific T cells are removed from the patient, cultured *in vitro*, and transferred back into the patient. The T cells may be sorted, expanded, and manipulated to enhance their antitumor activity. Selective expansion of high-avidity CTL is achieved by *in vitro* stimulation with low concentrations of peptide [16,114]. These CTL have superior antitumor activity as shown by improved protection against challenge with the B16 murine melanoma [115]. Furthermore, antigen-specific *ex vivo* expansion of T cells in combination with IL-2 can reverse the non-functional state of TIL [116]. In a clinical trial, adoptive transfer of melanoma-specific T cells in combination with IL-2 therapy and lymphodepletion resulted in clinical responses in 50% of the patients treated, demonstrating the potential of this therapy [79].

With the goal of increasing the functional avidity of T cells or providing TAA-specific T cells for patients without endogenous T cell responses, T cells are being genetically engineered to express high affinity TAA-specific TCRs. Human PBMC transduced with TAA-specific TCRs recognize and kill tumors expressing the antigen *in vitro* [117-119] and were recently tested *in vivo* for the treatment of metastatic melanoma [120]. PBMC were retrovirally transduced with a high-affinity MART-1-specific TCR and transferred into patients. Although the frequency of circulating MART-1-specific T cells sometimes remained high for up to a year, clinical responses were only observed in 2 of 15 patients. Combining gene therapy with other immunotherapies such as vaccination or chemotherapy may improve the antitumor efficacy.

4.4 Other therapies for improving T cell function

Enhancing co-stimulation during activation may improve priming of TAA-specific T cells. Vaccination with viruses encoding the co-stimulatory molecules CD80, ICAM-1, and LFA-3 (also known as TRICOM) in combination with TAA improves antitumor activity [121,122]. Enhancing co-stimulation not only increases the number of antigen-specific T cells, but also elicits higher avidity T cells as determined by dissociation of MHC tetramers and tumor lysis assays. Furthermore, the TRICOM vaccine elicits more memory T cells which also have increased peptide sensitivity [123]. However, enhanced co-stimulation also leads to the deletion of T cells. CD28-signaling pathways lead to either activation-induced cell death or activation of both immature and peripheral T cells depending on the strength of TCR triggering [32,124]. CD28 ligation and weak TCR binding results in the enhancement of T cell activation while CD28 ligation combined with strong TCR binding results in antigen-induced apoptosis. Further understanding of these opposing roles of co-stimulation may explain why some tumor immunotherapies induce ineffective T cell responses.

Some therapies that incorporate chemotherapy or irradiation also improve the functional avidity of the antitumor T cell response [65,120]. One mechanism for this improved immunity is the depletion of regulatory T cells. As mentioned above, vaccination with cells expressing HER-2/*neu* in combination with chemotherapy prevents tumor growth in *neu*-N transgenic mice [95]. In this study the chemotherapy led to selective depletion of Tregs resulting in activation of a subset of high-affinity *neu*-specific $CD8^+$ T cells. Irradiation and chemotherapy can also enhance the activation of tumor-specific T cells by improving the expression and

cross-presentation of tumor antigens on both tumors and the surrounding stroma [125,126]. T cell therapies, such as ACT, may benefit from this increased antigen presentation.

5. Discussion

Analyses of the T cell response to tumor antigens have demonstrated that tumor growth still occurs despite large numbers of tumor-reactive T cells. T cells must overcome a number of obstacles including tumor-induced immune suppression, cellular heterogeneity, and antigen loss from the tumor. This poor reactivity of T cells for TAA also results from central and peripheral tolerance mechanisms that delete or inactivate T cells with high avidity for tumor antigens. The remaining low-avidity T cells do not recognize the endogenous levels of tumor antigen. As a result the focus of research has turned towards developing therapies that enhance the activation and functional avidity of tumor-specific T cells.

The selective expansion of high-avidity T cells *ex vivo* is well established and ACT with these cells has shown potential. However, this therapy is labor intensive and the costs may be too great for general clinical use. Nevertheless, these studies have demonstrated that there is a subset of T cells within the TAA-specific T cell repertoire with sufficient avidity to recognize the levels of antigen expressed on the surface of tumors. Enhancing the survival and expansion of high-avidity T cells *in vivo* will improve the design of future T cell therapies. In some cases selecting for tumor reactive T cells requires enhancing the strength of the stimulating antigen. However, studies to date suggest that selective expansion of high-avidity T cells from the full repertoire may be more complicated. For example, vaccination with peptide mimotopes elicits T cells with both strong and weak reactivity to the endogenous tumor antigen. Understanding why, in some cases, vaccines elicit T cells with low functional avidity for TAA is crucial. Further insight into the mechanism behind peripheral deletion and functional inactivation of tumor-specific T cells will likely contribute to the answer. Other therapies such as lymphodepletion and optimization of prime-boost schedules that select for high-avidity memory T cells are also promising. Overall, the shift towards strategies aimed at improving the frequency and the functional avidity of tumor-specific T cells will likely be crucial for improving clinical efficacy of current immunotherapies.

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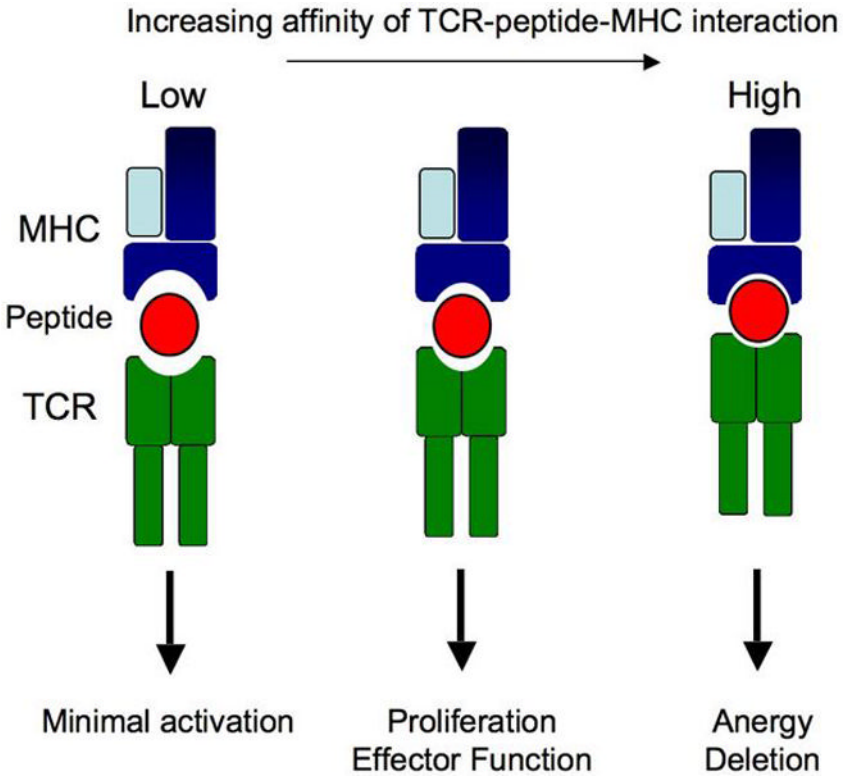

Figure 1. Goldilocks model for the affinity of TCR-peptide-MHC interactions and T cell activation

Peptide-MHC complexes that bind TCR with low affinity fail to induce complete intracellular signaling resulting in a lack of T cell activation (left). TCR-peptide-MHC binding interactions with long half-lives prevent serial triggering of the TCR and lead to impaired T cell activation (e.g. anergy or deletion, right). Optimal T cell activation requires an affinity in-between for complete induction of proliferation and acquisition of effector function (middle).

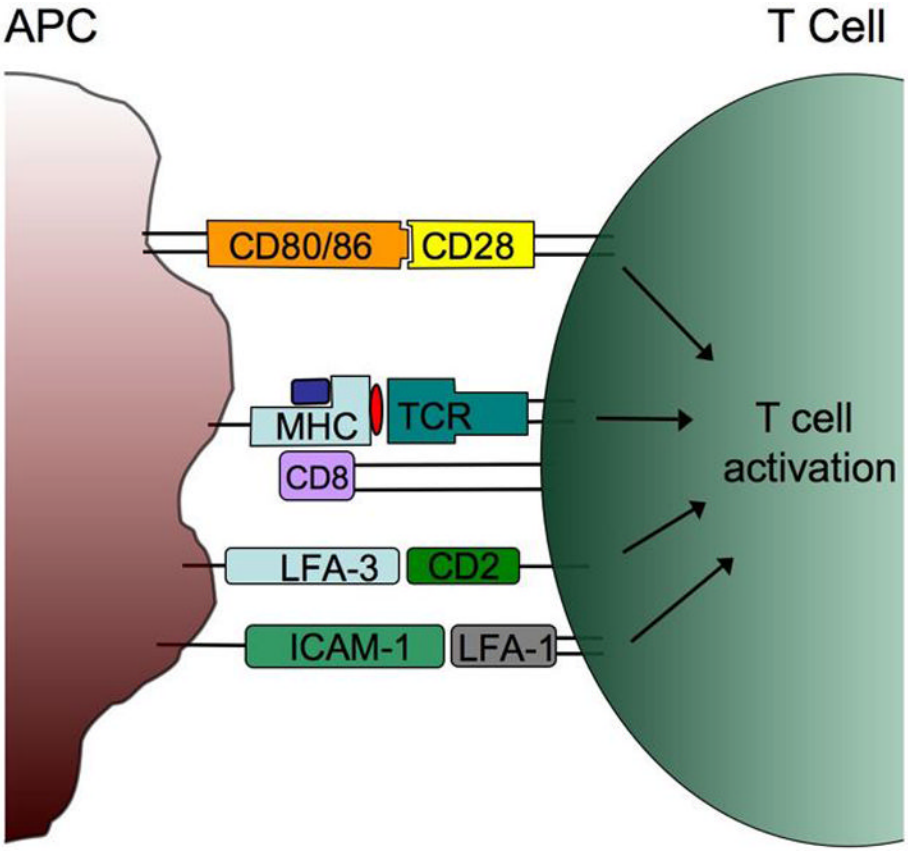

Figure 2. Molecular interactions influencing T cell functional avidity

Multiple receptor/ligand interactions between the T cell and APC enhance functional avidity. The affinity and kinetics of the TCR-peptide-MHC interaction, and binding of the CD8 co-receptor, contribute to TCR signaling. Sensitivity to antigen is also affected by ligation of the CD28 co-stimulatory molecule by CD80 (B7-1) and CD86 (B7-2). The adhesion molecule pairs (CD2/LFA-3 and ICAM-1/ LFA-1) enhance functional avidity by increasing the binding strength and intracellular signaling events.

Table I

Common tumor antigens expressed by murine and human tumors.

	Type of Antigen	Gene	Expression pattern	Ref
Tumor-specific antigens (TSA)	Mutated antigens	b-catentin	Melanoma	[127]
		CDK4	Melanoma	[128]
		Myosin	Melanoma	[129]
		RAS	Melanoma	[130]
	Chimeric proteins	Abl-bcr	CML	[131]
		ETV6/AML	ALL	[132]
		NPM/ALK	Large cell lymphomas	[133]
	Viral antigens	E6/E7	Cervical neoplasia	[134]
		EBNA-3	Immunoblastic lymphoma	[135]
Tumor-associated antigens (TAA)	Cancer-testis antigens	MAGE	Spermatocytes and placenta	[136]
		BAGE	Spermatocytes	[137]
		GAGE	Spermatocytes and placenta	[138]
		NY-ESO-1	Spermatocytes and ovary cells	[139]
	Differentiation Antigens	CEA	Embryonic tissue, epithelial cells	[140]
		gp100	Melanocytes	[141]
		Melan-A/ MART-1	Melanocytes	[142]
		NY-BR-1	Mammary tissue	[143]
		PSA	Prostate gland	[144]
		TRP-1/2	Melanocytes	[145]
		Tyrosinase	Melanocytes	[146]
	Ubiquitously expressed antigens	AFP	Expressed in multiple tissues	[147]
		HER-2/neu		[148]
		hTERT		[149]
		MUC1		[150]
		p53		[151]
		p15		[152]
		SART-1/2/3		[153-155]
		WT1		[156]

CML = Chronic myelogenous leukemia, ALL = Acute lymphoblastic leukemia

Table II

Antitumor activity elicited by mimotope vaccines in mice.

Tumor Antigen	MHC haplotype/epitope	Mimotope	Improved binding to MHC or TCR	Tumor assay	Response	Ref
$gp70_{423-431}$	H-2L[d] SPSYVYHQF	SPSY**A**YHQF	TCR	Prophylactic	50% protection	[30]
		MNKY**A**YH**ML**	TCR	Prophylactic	50% protection	[31]
$mTERT_{988-997}$	HLA-A*0201[†] DLQVNSLQTV	**Y**LQVNSLQTV	MHC	Prophylactic	33% protection	[75]
$Her2/Neu_{773-782}$	HLA-A*0201[††] VMAGVGSPYV	**F**MA**N**V**AI**P**HL** **F**M**HN**V**PI**PY**L** **FY**A**N**V**P**SP**HL**	n.d.	Therapeutic	40-50% delayed tumor growth	[157]
$Her2/Neu_{435-443}$	HLA-A*0201[†] ILHDGAYSL	ILH**N**GAYSL	TCR	Therapeutic	30% protection	[158]
$TRP1_{222-229}$	H-2K[b] TWHRYHLL	T**AY**RYHLL	MHC	Prophylactic	90-100% protection	[103]
$TRP2_{180-188}$	H-2K[b] SVYDFFVWL	**SI**YDFFVWL	TCR	Therapeutic	No protection	[159]
$gp100_{25-33}$	H-2D[b] EGSRNQDWL	**KVP**RNQDWL	MHC	Therapeutic (ACT and IL-2)	80-100% tumor inhibition	[65]
				Therapeutic (ACT)	100% delayed tumor growth	[104]

n.d.= not determined

[†]Antitumor activity evaluated in HLA-A*0201-transgenic HHD mice.

[††]Antitumor activity evaluated in double transgenic mice: HLA-A*0201 and *neu*-N.

Table III

Clinical trials of therapeutic cancer vaccines using modified HLA-A*0201-restricted tumor antigens

Cancer	Antigen	Adjuvant	Positive *in vitro* responses	Clinical response	Ref
Extensive metastatic melanoma	$gp100_{209-217}$ $gp100_{209-217}$(210M) $gp100_{209-217}$(210M)	IFA IFA IFA+IL-2	2/8 IFNγ* 10/11 IFNγ* 3/19 IFNγ*	1/9 CR 0/11 CR, 3/11 MR 8/19 CR, 3/19 MR, 3/19 SD	[97]
Stage I-III melanoma	$gp100_{209-217}$(210M)	IFA +/- IFNα**	28/29 tetramer 9/9 IFNγ*	Not Reported	[160]
Melanoma	$gp100_{209-217}$(210M)	IFA IFA+IL-12 IFA+IL-2	7/7 tetramer* 4/5 tetramer* 2/11 tetramer*	Not Reported	[161]
Stage III/IV melanoma	$gp100_{209-217}$(210M) $tyrosinase_{368-376}$ (370D)	IFA+/- IL-12	34/40 Skin test*≠ 33/38 IFNγ* 37/42 tetramer*	24/48 relapsed, 10 died	[162]
Stage IV melanoma	$gp100_{209-217}$(210M) $tyrosinase_{368-376}$ (370D)	DCs	2/16 Skin test* 11/16 IFNγ* 0/16 tetramer*	1/16 CR 2/16 MR, 2/16 SD 10/16 died	[163]
Stage IIA/IIB melanoma	$gp100_{209-217}$(210M) $tyrosinase_{368-376}$(370D)	IFA+/- GM-CSF	34/39 ELISA 37/42 tetramer	After 24 months: "favorable:" 7 relapsed, 2 died	[164]
Recurrent small cell lung or colon cancer	$CEA_{605-613}$(610D)	Flt3	7/12 CTL 10/12 tetramer*	2 CR, 7 PD 2 SD, 1 MR	[165]

CR=Complete response, PD=progressive disease, SD=Stable disease, MR=Mixed response, IFA=Incomplete Freund's adjuvant, DCs=dendritic cells

* specific for native peptide

≠ response measured to gp100, not tyrosinase

** IFNα does not interfere with the antigen-specific response.