REVIEW

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Tumor resistance to CD8$^+$ T cell-based therapeutic vaccination

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Abstract

CD8$^+$ cytotoxic T lymphocytes (CTLs) play an important role in antitumor immunity. Induction of tumor-specific CTLs is one major strategy for tumor immunotherapy. However, therapeutic vaccinations used to treat firmly established tumors are generally ineffective. A thorough understanding of the mechanisms underlying tumor resistance to CTL-based therapeutic vaccination is very important in the tumor immunology field. There are two main mechanisms by which tumors develop resistance to CTL-based therapeutic vaccinations. One is that tumors induce peripheral tolerance of tumor-specific CD8$^+$ T cells. The other is that tumor cells themselves develop immune evasion mechanisms to prevent recognition and killing by CTLs. This review focuses on recently reported cellular and molecular mechanisms of CD8$^+$ T cell tolerance and immune evasion in tumors and discusses about the possibilities to improve tumor immunotherapy.

Key words: CTL, therapeutic vaccination, tumor immunotherapy.

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INTRODUCTION

CD8$^+$ cytotoxic T lymphocytes (CTLs) can recognize tumor-associated antigens (TAAs) [56, 139, 152] and adoptive transfer of tumor-specific CD8$^+$ T cells can eradicate the tumors in certain situations [45]. This suggests that CD8$^+$ T cells play an important role in antitumor immunity. Therefore, induction of tumor-specific CTLs is one major strategy for the development of tumor vaccines. Preventive (prophylactic) vaccinations are used to provide protection against subsequent tumor challenges, whereas therapeutic vaccinations are used to induce immune responses to treat preexisting tumors [111]. Preventive vaccinations or vaccinations early after tumor cell inoculation can be effective in the elimination of inoculated tumor cells; in contrast, therapeutic vaccinations used to treat firmly established tumors are generally ineffective [45, 99, 105, 129]. Therefore, a thorough understanding of the mechanisms underlying tumor resistance to CTL-based therapeutic vaccination is crucial for the design of effective approaches to eliminate tumors.

ACTIVATION OF CD8$^+$ T CELLS AND CTL-BASED TUMOR IMMUNOTHERAPY

Dendritic cells (DCs) capture antigens in peripheral tissues, migrate to local lymphoid organs, and present antigens to naïve CD8$^+$ T cells in the secondary lymphoid organs [163]. Most DCs in peripheral tissues are immature. They can efficiently take up antigen, but cannot productively present antigens to naïve T cells. After stimulation by products of bacterial or viral pathogens through Toll-like receptors (TLRs) [57], proinflammatory cytokines, or ligation of surface CD40 by CD40 ligand (CD40L) on CD4$^+$ T helper cells [117], DCs become mature and the expression of costimulatory molecules such as CD80 (B7-1) and CD86 (B7-2) is upregulated [8]. Mature DCs provide naïve CD8$^+$ T cells with two signals for efficient activation. With these proper stimulations, CD8$^+$ T cells undergo proliferation and differentiate into CTLs [70, 73]. Activated CTLs can migrate to peripheral tissues and exert effector function by releasing cytokines, such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α to mediate

local inflammation [123], and killing target cells through apoptosis induction [33, 136]. CTLs can recognize virus--infected cells and tumor cells which display MHC class I peptide complexes through "direct presentation" of endogenous antigens. Two major mechanisms underlie CTL cytotoxicity: the perforin-dependent granule exocytosis pathway [59] and the FasL-Fas pathway [60].

A variety of TAAs have been identified to discriminate between normal and malignant tissue [83]. Some TAAs are self proteins overexpressed by tumor cells [87]; other TAAs are encoded by viral genes or by mutated cellular genes [83]. TAAs can be processed and presented as peptides through the MHC class I pathway in tumor cells. Antigen-specific $CD8^+$ CTLs can recognize these peptide/MHC class I complexes and kill tumor cells. By a variety of approaches, such as vaccinations, cytokine administration, and adoptive cell transfer, the immune system has been manipulated to induce tumor-specific CTLs that can effectively recognize and kill tumor cells [110].

DNA tumor vaccines are bacterial plasmid vehicles encoding TAA genes, which are used for *in vivo* transfection and antigen expression [130]. Following DNA vaccinations, $CD8^+$ T cells are activated by direct priming by transfection of DCs and/or by cross-presentation by DCs through acquiring antigens from other cells, such as myocytes or keratinocytes [44]. Furthermore, similarly to DNA vaccines, recombinant viruses or bacteria were used as vectors to deliver TAA genes to induce tumor-specific CTLs. For instance, in human colon cancer vaccines the recombinant poxvirus encoding carcinoembryonic antigen (*CEA*) [1, 40, 50, 62, 63], papillomavirus pseudoviruses encoding *CEA* [53], and attenuated bacteria encoding *CEA* [90, 155, 156] were used to induce CEA-specific CTL responses and anti--tumor immunity.

Since DCs play a pivotal role in the activation of T cells, DCs have also been manipulated to induce effective antigen-specific CTL responses for tumor immunotherapy. *Ex vivo* antigen-pulsed DCs were used to activate tumor-specific T cells [8, 135]. RNA-transfected DCs [84, 85], heat shock protein-peptide complex [12], or DC-derived exosomes [164] were also used to induce tumor-specific CTLs. In a similar strategy, irradiated tumor cells engineered to express granulocyte-macrophage colony-stimulating factor were used for immunization to activate DCs and enhance tumor-specific CTL responses [31]. Because ligation of CD40 on DCs by CD40L provides DC maturation signals, an agonistic anti-CD40 antibody was combined with intravenous administration of tumor-derived peptide to elicit a potent CTL-mediated antitumor response [89]. Altered peptide ligands with higher affinities have been used to improve the reactivity of T cells specific for self/tumor antigens [99, 112, 128]. It has been demonstrated that a combination of adoptive transfer of tumor--specific $CD8^+$ T cells, T cells stimulation through antigen-specific vaccinations using an altered peptide ligand, and co-administration of interleukin (IL)-2 could induce the regression of large established tumors [99].

PERIPHERAL TOLERANCE OF $CD8^+$ T CELLS

Central tolerance of $CD8^+$ T cells occurs in the thymus, where self-reactive $CD8^+$ T cells with very high affinities are deleted by negative selection [13, 36, 92]. Some self-reactive $CD8^+$ T cells can escape negative selection. Thus, peripheral tolerance is required for mature self-reactive T cells to avoid autoimmunity [140]. In addition, non-self-reactive T cells can also undergo peripheral tolerance. Peripheral tolerance of $CD8^+$ T cells may occur through immunological ignorance [94], clonal deletion [2], or peripheral anergy [124, 140]. In certain situation, antigen-specific $CD8^+$ T cells can maintain a naïve phenotype even in the presence of antigens. This phenomenon in which the antigen neither activates nor anergizes these $CD8^+$ T cells is called immunological ignorance [48, 93, 147, 148]. In some tumor models, the immunogical ignorance was explained by none or too few tumor cells reaching draining lymph nodes during early tumor development [93], expression levels of the ignored antigen, or the anatomic site in which the antigen is expressed [68, 80]. Mature $CD8^+$ T cells can also be deleted in the periphery, which is called clonal deletion. Repetitive, systemic administration of antigenic peptides can result in peripheral deletion of naive $CD8^+$ T cells *in vivo* [2, 69]. Clonal deletion by multiple peptide injections can prevent a virus-induced T cell-mediated autoimmune diabetes in a transgenic mouse model [3]. Peripheral anergy of T cells is defined as a complete unresponsiveness on re-encounter with antigen. T cell anergy can be induced when T cells encounter antigens on immature or resting antigen-presenting cells (APCs), while effective T cell activation is induced through antigen-specific interactions with activated, mature APCs [8, 27, 37, 46, 47, 118].

In T cell anergy models, tolerant T cells often become only partially anergized [74]. Otten and Germain demonstrated that a CTL clone was unable to secrete IL-2, but retained cytolytic activity in the absence of costimulation, which was described as "split anergy" [98]. Vezys et al. showed that chronic encounter of antigen by intestinal $CD8^+$ T cells resulted in a loss of IFN-γ secretion function, but cytolytic activity was retained [141]. In our tumor model, we also found "split tolerance" of tumor-specific $CD8^+$ T cells at a late stage of tumor growth. Those tumor-specific $CD8^+$ T cells lost cytolytic activity, but retained IFN-γ production function [54]. Hernandez et al. [49] showed that proliferation potential and gain of effector function were separable events in the differentiation program of $CD8^+$ T cells. It was reported that during chronic lymphocytic choriomeningitis virus (LCMV) infection, LCMV-specific $CD8^+$ T cells lose cytotoxic activity or the ability to secret cytokines, but those tolerant cells are capable of proliferating *in vivo*, as shown by uptake of bromodeoxyuridine [161]. The "split tolerance" of $CD8^+$ T cells suggests that cell proliferation, cytokine production, and cytolytic activity can be triggered through independent

mechanisms. Therefore, tolerized CD8+ T cells might become partially dysfunctional in different situations.

Overall, mature CD8+ T cells can undergo peripheral tolerance through different mechanisms. Therefore, in tumor-bearing hosts, peripheral tolerance of tumor-specific CD8+ T cells may cause the failure of CTL--based therapeutic vaccinations.

ACTIVATION AND TOLERANCE INDUCTION OF TUMOR-SPECIFIC CTLs DURING PROGRESSIVE TUMOR GROWTH

In tumor-bearing hosts, tumor-specific CD8+ T cells have different fates in different tumor models. Tumor--specific CD8+ T cell tolerance has been frequently reported in tumor-bearing hosts [149]. Most TAAs are self proteins overexpressed in tumor cells. Therefore, these self-reactive tumor-specific CD8+ T cells are maintained in a tolerant state in the periphery [95]. In melanoma patients, systemic tumor-specific CD8+ T cells developed in the periphery could not directly lyse melanoma target cells or produce cytokines in response to a mitogen [72]. Using a non-self tumor-antigen model, Shrikant et al. [126, 127] showed that tumor expressing ovalbumin (OVA) tolerized adoptively transferred OVA-specific CD8+ T cells by inhibiting CD8+ T cell proliferation. In a sporadic tumor model, spontaneous tumors avoided destruction by inducing tumor--specific CD8+ T cell tolerance [150]. In contrast, some reports showed that tumor-specific CD8+ T cells were not tolerized in the presence of tumors. In the non-self tumor-antigen models, LCMV-specific CD8+ T cells in the spleen and tumor-infiltrating lymphocytes (TILs) were not tolerized by tumor expressing the LCMV antigen [93, 105]. In a self-antigen model, the LCMV antigen is expressed as a self tumor antigen in endogenous tumors. The tumor growth enhanced cross-presentation and led to CD8+ T cell activation without tolerance, and these CD8+ T cells have effector functions [89].

Most tumor cells are not professional APCs. Therefore, it was proposed that tumor cells could induce the tolerance of tumor-specific CD8+ T cells due to the lack of immunologically costimulatory molecules [20]. However, it was also reported that tumors could activate tumor-specific CD8+ T cells through cross-presentation by professional APCs that acquire tumor antigens from tumor cells [91, 129, 153]. Uncontrolled cell growth is a key feature of a tumor which disrupts the local tissue architecture and consequently provides proinflammatory signals [101]. These "danger" signals will induce inflammation, activate innate immune cells with antitumor activity, and stimulate professional APCs to uptake tumor-derived antigens and migrate to draining lymph nodes to trigger an adaptive T cell immunity [14]. The different functional states of tumor-specific CD8+ T cells in different tumor models might be due to different growth behaviors of tumor cells, different host immunity in different animal strains, or different time points during tumor growth.

As shown in Fig. 1, we investigated the status of HPV-16 E7-specific CD8+ T cells at different time points in response to E7+ tumors and/or vaccinations [54]. In tumor-bearing mice, a weak CTL response is normally induced at the early stage, most likely through cross-presentation. Subsequent therapeutic vaccination amplifies CTL response and efficiently eliminates tumor cells, which delays tumor growth, whereas without therapeutic vaccination, the low level of CTL response in tumor-bearing mice cannot efficiently delay tumor growth. When the tumor grows progressively, the increased tumor antigen load induces more tumor-specific CTLs, probably through cross-presentation, and the CTL response reaches a very high level, even in the absence of therapeutic vaccinations. At the late stage, tumor-specific CD8+ T cells are tolerized and lose cytolytic activity, and further vaccination cannot induce effective CTL response. In general, tumor-specific CD8+ T cells enter the activation phase at the early stage, tumor-specific CTL response reaches a maximal level in the middle stage, and then tumor-specific CD8+ T cells lose cytolytic function at the late stage.

PERSISTENT ANTIGENS INDUCE PERIPHERAL TOLERANCE OF TUMOR-SPECIFIC CD8+ T CELLS

The mechanisms underlying peripheral tolerance of tumor-specific CD8+ T cells remain unclear. We demonstrate that tumor-specific CD8+ T cells are partially dysfunctional at the late stage in tumor-bearing mice: they lose cytolytic activity while keeping IFN-γ production function [54]. In chronic viral infection, the fate of virus-specific CD8+ T cells is similar to our findings in tumor-specific CD8+ T cells. In HIV-infected patients, HIV-specific CD8+ T cells produce IFN-γ, but are impaired in cytolytic function [5]. In murine chronic LCMV infection, viral persistence results in functional impairment of CD8+ T cells. Production of IL-2 and *in vitro* cytolytic capacity are the first functions compromised, followed by the ability to make TNF-α, whereas IFN-γ production is most resistant to functional exhaustion [146]. "Functional exhaustion" was used to describe antigen-specific CD8+ T cells during chronic HIV infection [39, 67, 74, 122], simian immunodeficiency virus infection [143, 157], and hepatitis C virus infection [42].

Tumor growth is comparable to chronic viral infections because antigens are persistently presented to CD8+ T cells and T cells have to respond to continuous antigenic stimulus. It is speculated that persistent tumor-antigen stimulation causes tolerance of tumor-specific CD8+ T cells during tumor growth [54, 107]. Using adoptive transfer of transgenic CD8+ T cells for the male antigen and different amounts of male bone marrow cells into female mice, Tanchot et al. [134] showed that a lower amount of male bone marrow cells

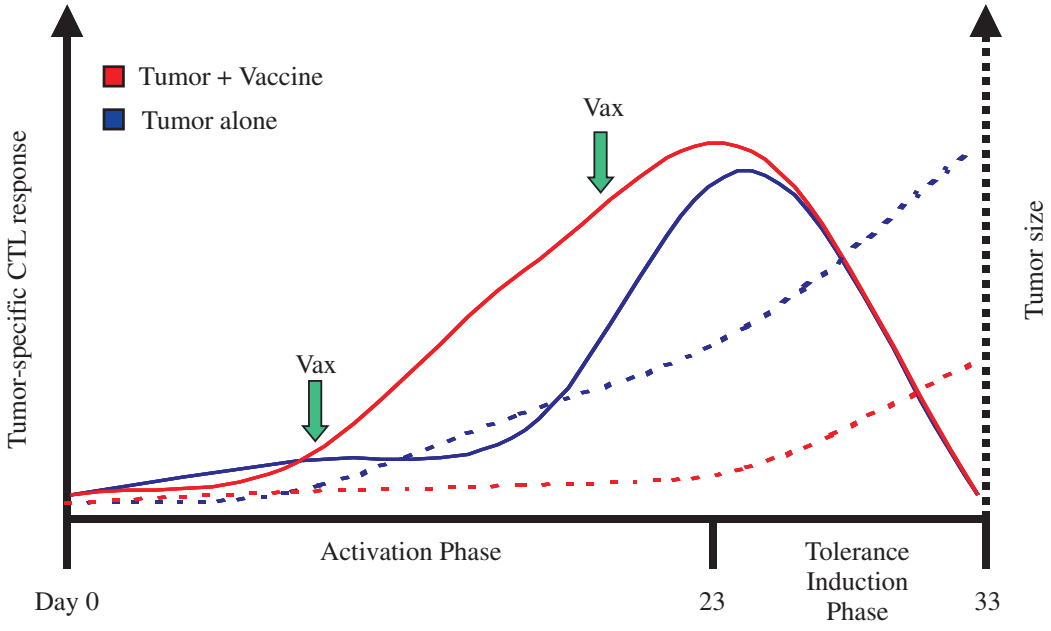

Fig. 1. A model of the effects that a progressively growing tumor has on functional states of tumor-specific CD8+ T cells and therapeutic vaccinations. The solid blue line indicates tumor-specific cytolytic activity of CD8+ T cells and the dashed blue line indicates tumor growth in tumor-bearing mice. The solid red line indicates tumor-specific cytolytic activity of CD8+ T cells and the dashed red line indicates the tumor growth in tumor-bearing mice with therapeutic vaccinations. The green arrows indicate the time points for vaccinations. In tumor-bearing mice, a weak CTL response is normally induced at the early stage, which is not sufficient to lead to tumor rejection. Subsequent therapeutic vaccination induces a more rapid and greater CTL response and eliminates more tumor cells, which slows down tumor growth, whereas in the absence of therapeutic vaccination, the low level of CTL response by tumor-primed CD8+ T cells cannot efficiently delay tumor growth. During progressive tumor growth, the increase in tumor antigen load induces more tumor-specific CTLs through cross-presentation, and tumor-specific CTL response reaches a maximal level even without therapeutic vaccinations. At the late stage, tumor-specific CD8+ T cells are tolerized and further vaccination cannot induce tumor-specific CTL response. Therefore, tumor-induced tolerance of CD8+ T cells at the late stage contributes to a gradual invalidation of therapeutic vaccinations during progressive tumor growth. Tumor-specific CD8+ T cells enter the activation phase at the early stage, tumor-specific CTL response reaches a maximal level in the middle stage, and then tumor-specific CD8+ T cells enter the tolerance-induction phase at the late stage, which depends on both the persistent tumor burden and the time course.

was eliminated by activation of CD8+ T cells; a higher amount of male bone marrow cells induced CD8+ T cell tolerance, and both CD8+ T cells and male cells persisted in the periphery. It was proposed that tolerance is a consequence of antigen persistence and an excessive antigen load [134]. It has been speculated that antigen-induced unresponsiveness [11, 26, 58] might be a generic consequence for T cells after prolonged antigenic stimulation in different experimental systems. During HIV infection, HIV-specific CD8+ T cells in the periphery can produce antiviral cytokines, but are impaired in cytolytic function. Since the perforin level is impaired in these CD8+ T cells, chronic stimulation of HIV-specific CD8+ T cells in the infected patient might cause the low perforin level by repeated contact with virus-infected cells and subsequent degranulation [5].

THE ROLE OF CD4+ T REGULATORY CELLS IN PERIPHERAL TOLERANCE OF TUMOR-SPECIFIC CD8+ T CELLS

Naturally occurring CD4+CD25+ T regulatory (T_{reg}) cells are selected in the thymus and comprise about 5–10% of peripheral CD4+ T cells in mice [114]. The development of effective antiviral or tumor immunity can be compromised by T_{reg} cells [15, 77, 113, 124]. These CD4+ T_{reg} cells express high levels of cytotoxic T lymphocyte-associated antigen (CTLA)-4, the glucocorticoid-induced TNF-related receptor (GITR), and the forkhead transcription factor Foxp3 [52]. In the Friend leukemia virus chronic infection model, effector functions of CD8+ T cells are impaired due to CD4+CD25+ T_{reg} cells [29]. Tumor-specific CD4+ T_{reg} cells frequently localize within the tumor stroma [24, 145] and tumor-specific CD8+ T cells often become tolerant in the presence of tumors. By adoptive transfer of tumor-specific CD8+ T cells and CD4+ T_{reg} cells into tumor-bearing mice, Chen et al. [21] showed that HA-specific CD4+ T_{reg} did not inhibit the proliferation of HA-specific CD8+ T cells but suppressed their cytolytic activity in the presence of HA+ tumor *in vivo.* Further, the suppression of cytolytic activity depends on signaling through the transforming growth factor β receptor on CD8+ T cells [21]. It has been reported that the proportions of CD4+CD25+ T_{reg} cells were increased in tumor sites [154], metastatic melanoma lymph nodes [142], and even in the peripheral blood [55, 75, 97, 116, 151]. In

tumor immunotherapy, prior to vaccinations, treatment of tumor-bearing mice with anti-CD25 significantly enhanced the antitumor effect, which might be due to depletion of T_{reg} cells [131].

MOLECULAR MECHANISMS UNDERLYING PERIPHERAL TOLERANCE OF CD8+ T CELLS

The T cell receptor (TCR) is composed of the αβ dimer for antigen binding and the signal-transducing CD3 δ, ε, γ, and ζ chains. These chains contain one (δ, ε, γ) to three (ζ) immunoreceptor tyrosine-based activation motifs that transduce the activation signal into the cell [106]. Upon $CD8^+$ T cell binding to target cells, CD8 is recruited to the TCR complex to stabilize the antigen-specific binding [162]. CD8 association with the TCR recruits Src-related kinase $p56^{lck}$ into close proximity with CD3ζ [6]. Following TCR stimulation, phosphorylated CD3ζ recruits ZAP70, which in turn phosphorylates linker for activation of T cells (LAT), followed by the recruitment of additional proteins and the activation of phospholipase Cγ-1. Then, calcium flux is initiated, resulting in the coordinated activation of additional downstream signaling pathways [34, 88, 106]. Alterations in the signal-transduction pathway may cause peripheral tolerance of $CD8^+$ T cells [81, 86].

In the male antigen model mentioned above, at relatively low antigen doses, efficient memory T cells can be generated, while high antigen doses lead to peripheral tolerance of $CD8^+$ T cells [108, 134]. By comparing functional memory and dysfunctional tolerant antigen-specific $CD8^+$ T cells, Tanchot et al. characterized the signaling modifications that were associated with tolerant $CD8^+$ T cells. They found tolerant $CD8^+$ T cells did not mobilize Ca^{2+} after anti-CD3 stimulation, but constitutively produced large amounts of IL-10. The defect was overcome by stimulation with phorbol myristate acetate (PMA)/ionomycin. This indicated a proximal defect in the TCR signaling pathway that blocks TCR-mediated stimulatory events in tolerant T cells [134]. They further compared the proximal TCR signal transduction and found that *in vivo* TCR stimulation led to constitutive phosphorylation of CD3ε, recruiting Zap70, in both memory and tolerant cells. However, in tolerant cells the phosphorylation was much more significant, the CD3ε and ζ chains were disassociated, the Src kinases p56Lck and p59Fyn were inactive, and the phosphorylation of ζ chain was defective [43]. This suggests that when the antigen load is too high, signal transduction might be altered by mechanisms such as the recruitment of Zap70 to CD3ε becoming excessive, leading to TCR complex destabilization, Src kinase dysfunction, and signal arrest [43].

CD3ζ chain is essential in TCR assembly and expression [61, 81]. The immunological dysfunction of T cells isolated from tumor-bearing hosts or in infectious diseases often correlates with CD3ζ deficiency [16]. It was hypothesized that sustained exposure to antigen and chronic inflammation may be responsible for the downregulation of CD3ζ and impairment of T cell function. In a bacterial antigen-stimulation model, sustained exposure to bacterial antigen *P. gingivalis* resulted in IFN-γ-dependent CD3ζ downregulation and impaired T cell function in both $CD4^+$ and $CD8^+$ T cells. CD3ζ downregulation was caused mainly by enhanced lysosomal degradation [16]. During chronic HIV infection, HIV-specific CD8 T cells down-modulated not only CD3, but also the costimulatory receptor CD28 [137]. Downregulation of CD3ζ and CD28 on tolerant $CD8^+$ T cells might increase the activation threshold for full effector functions.

$CD8^+$ T cells in the TILs can lose *ex vivo* cytolytic function, which can be recovered after short-term culture *in vitro* [66]. By comparing the signaling molecules in nonlytic and lytic $CD8^+$ TILs, a defective TCR signaling was identified in nonlytic (tolerant) $CD8^+$ TILs. Upon cognate antigen recognition, nonlytic $CD8^+$ TILs were defective in F-actin localization and unable to recruit molecules required for the release of cytolytic granules. These TILs were blocked at a proximal step because LAT was not phosphorylated and ZAP70 was only weakly phosphorylated. Upon conjugation with cognate tumor cells, immunological synapses were formed, but CD2, the CD3 complex, and CD8 disassociated from the TCR and were excluded from the synapse in TILs. These nonlytic $CD8^+$ TILs did not flux calcium and ultimately prevented exocytosis of cytolytic granules. These results suggest that defective proximal TCR signaling inhibits $CD8^+$ TIL cytolytic function [66]. Signaling events further downstream can also be impaired in tolerant $CD8^+$ T cells. In certain $CD8^+$ T cell tolerance models, PMA/ionomycin stimulation of dysfunctional LCMV-specific or tumor-specific $CD8^+$ T cells does not fully restore cytokine production [72, 161].

CAN PERIPHERAL TOLERANCE OF TUMOR-SPECIFIC CD8+ T CELLS BE REVERSED TO EFFICIENTLY ELIMINATE TUMOR CELLS?

Curtsinger et al. [25] showed $CD8^+$ T cells could be rendered "permanently tolerant" during an initial priming and unresponsive to even a potent secondary stimulation with antigen and adjuvant. Therefore, a key question for therapeutic vaccinations is whether tolerant tumor-specific T cells can regain functional properties by specific interventions. If so, we can develop some strategies to reverse peripheral tolerance of tumor-specific $CD8^+$ T cells and elicit effective antitumor immunity.

CTLA-4 is expressed by activated $CD8^+$ T cells [144] and is a potent negative regulator of T cell activation [19, 104]. Attenuation of T cell activation by CTLA-4 limits the potency of tumor immunity. Administration of blocking antibodies to CTLA-4 has had marked effects on enhancing antitumor immunity in murine models and recent clinical trials [14]. The administration of

antibodies that block CTLA-4 function inhibits the growth of moderately immunogenic tumors and, in combination with tumor vaccines, increases the rejection of poorly immunogenic tumors [51].

Some vaccination strategies should have more advantages in breaking $CD8^+$ T cell tolerance than others, although the efficacies may be comparable in activating non-tolerant $CD8^+$ T cells. Yang et al. [158] compared the efficacy of two different vaccination strategies: virus- and DC-based vaccines encoding HA antigen. In a non-tolerant environment, both vaccines were comparable in activating naïve HA-specific $CD8^+$ T cells; however, in a tolerant environment, in mice expressing "self" HA antigen, virus-based vaccine reversed tolerant HA-specific $CD8^+$ T cells, whereas DC-based vaccine reversed the tolerance only after removal of T_{reg} cells or the co-administration of TLR ligand or an irrelevant virus [158]. It was speculated that TLR signals might help reverse the T_{reg} cell-mediated $CD8^+$ T cell tolerance [158] because ligation of TLRs on DCs can block T_{reg} cell-mediated suppression [102, 103].

GITR is constitutively expressed on the cell surface of $CD4^+$ T_{reg} cells. Anti-GITR monoclonal antibody treatment can diminish the suppressive function of $CD4^+$ T_{reg} cells *in vivo*. This antibody treatment *in vivo* does not deplete T_{reg} cells, but provides an agonistic signal to GITR on T_{reg} cells [125]. In a tumor model, Sakaguchi and his colleagues showed that a single administration of agonistic anti-GITR monoclonal antibody to tumor-bearing mice intravenously or directly into tumors provoked potent tumor-specific immunity and eradicated established tumors without eliciting overt autoimmune disease [65]. Yu et al. [160] showed that $CD4^+CD25^+$ T_{reg} cells accumulated inside tumors and suppressed $CD8^+$ T cells at the local tumor site. After intratumor depletion of $CD4^+$ suppressive cells, the well-established tumors were rejected [160]. Therefore, removal of T_{reg} cells or blockade of suppression by T_{reg} cells can be a major contribution to the reversal of $CD8^+$ T cell tolerance. However, Dittmer et al. [29] showed that during persistent retroviral infection, effector functions of $CD8^+$ T cells could be suppressed by $CD4^+$ T_{reg} cells. Blocking the suppression with anti-GITR antibody from T_{reg} cells was ineffective in overcoming the tolerance of endogenous virus-specific $CD8^+$ T cells. This suggests that functional impairment of $CD8^+$ T cell by T_{reg} cells could be long-lived or even irreversible [29].

Cytokines are often used to enhance the efficacy of tumor immunotherapy. It was reported that established tumors were eradicated by adoptive transfer of tumor-specific $CD8^+$ T cells in the combination of vaccinations and in exogenous administration of IL-2 or IL-15 [64, 99]. Shrikant and Mescher [127] showed that systemic injection of IL-2 reversed tumor-induced anergy of $CD8^+$ T cells by stimulating their proliferation. Overwijk et al. [99] showed that strong vaccination induced the proliferation and tumor localization of antigen-specific T cells, but that these T cells did not effectively destroy the tumor unless provided with exogenous IL-2. Thus, IL-2 can act as a costimulatory/activation factor to T cells for full effector functions.

TUMOR IMMUNE EVASION OF RECOGNITION AND KILLING BY CTLs

In addition to tumor-induced peripheral tolerance of $CD8^+$ T cells, tumor cells themselves develop many immune evasive strategies to escape recognition and killing by tumor-specific CTLs. Genetic alteration is often associated with tumor development, which provides tumor cells with mechanisms for immune evasion [71].

$CD8^+$ CTLs recognize peptides presented by MHC class I molecules on tumor cell surfaces. The antigen processing in the MHC class I pathway starts with endogenous proteins being ubiquitinated and subsequently degraded by the proteasome into antigenic peptide fragments. The peptides are translocated across the endoplasmic reticulum (ER) membrane via the transporter associated with antigen processing (TAP) subunits TAP-1 and TAP-2. Within the ER, MHC class I heavy chain is synthesized and associates with β_2-microglobulin (β_2m) with the help of chaperone proteins (BiP, calnexin, calreticulin, and ERp57). The MHC class I/β_2m complex then associates with tapasin, which allows the dimeric complex to interact with TAP and ensures peptide loading into the complex. Then, the trimeric MHC class I/β_2m/peptide complex is transported to the plasma membrane [22, 38, 100, 109]. A variety of mechanisms underlying the down-regulation of the antigen-processing machinery (APM) contributes to the immune evasion by tumor cells [120].

The proteasome is composed of a proteolytic core (20S) and regulatory caps (19S) which assemble into a 26S complex. The core 20S proteasome consists of 4 rings. The two outer rings are identical and each is composed of 7 different α subunits. The two inner rings are also identical and each contains 7 different β subunits which surround a central chamber where proteolysis occurs [132, 133]. Two proteasome-inducible β subunits, low-molecular protein (LMP)2 and LMP7, are induced by IFN-γ and replace two constitutive β subunits in the standard proteasome to form an "immunoproteasome" [41]. This replacement can alter the cleavage specificity of the proteasome [17, 32, 35]. The immunoproteasome expressing inducible LMP2 and LMP7 decreases the presentation of an RU1-derived tumor peptide epitope in renal cell carcinoma cells [82]. The melanoma-associated antigen Melan-A/MART-1 also cannot be cleaved by the immunoproteasome, despite being efficiently cleaved by the standard proteasome. Consequently, MART-1-specific CTLs cannot recognize the LMP2- and LMP7-expressing melanoma cells [82]. Therefore, expression of the immunoproteasome subunits may alter the cleavage specificity of tumor cells as an immune-escape mechanism [28].

Deficiencies in TAP, and the chaperone proteins, can also downmodulate antigen processing and contribute to the immune escape of tumor cells from killing by CTLs

[120]. TAP expression is downregulated in many tumor cells [4, 23, 119], which may be due to a decrease in the activity/expression of trans-acting factors regulating TAP-1 promoter activity and/or a decrease in TAP-1 mRNA stability [121]. Restoration of TAP expression in lung carcinoma increases tumor-specific immune responses [76].

Downregulation of MHC class I molecules has been found in a variety of tumors [78]. It can be caused by impairments in the expression and/or function of components of the class I APM, as mentioned above [120]. β_2-Microglobulin gene mutations in tumor cells can also result in a lack of MHC I molecule expression [9, 10]. Reduced transcription or translation of MHC class I genes has also been reported in tumor cells [30].

The loss of TAA expression is also a common immune-escape mechanism [138]. There is a loss of cognate melanoma antigens in the tumor cells of melanoma patients with peptide vaccinations or treated with adoptive transfer of antigen-specific $CD8^+$ T cells, [96, 159]. In a murine melanoma model, tumor vaccinations also led to the immunoselection of tumor cell variants that have lost the expression of these target antigens [115]. It was demonstrated that "antigenic drift", mutations in CTL epitopes, was one of the mechanisms for tumor evasion of destruction by CTLs [7].

Another mechanism of tumor escape from immune surveillance is through the expression of antiapoptotic signals and the suppression of apoptosis in tumor cells. Human U266 myeloma cells are inherently resistant to Fas-mediated apoptosis and express high levels of the antiapoptotic protein Bcl-xL [18]. It was reported that a metastatic variant of a human prostate carcinoma line was more apoptosis-resistant than a nonmetastatic variant. The apoptosis resistance was associated with higher levels of expression of the antiapoptotic BCL-2 and lower levels of the proapoptotic BAX and BAK [79].

CONCLUDING REMARKS

Tumors can induce peripheral tolerance of tumor-specific $CD8^+$ T cells through a variety of mechanisms. Furthermore, even when functional tumor-specific CTLs exist, tumor cells themselves develop immune-evasion mechanisms to prevent recognition and killing by those CTLs. Uncovering the mechanisms underlying tumor resistance to therapeutic vaccinations will help develop reasonable treatment regimes for tumors: when to give therapy and how to enhance efficacy. Surgical removal of the tumor mass will drastically reduce the tumor cell load. It should be promising to eliminate the remaining tumor cells if an effective tumor-specific CTL response can be induced timely and strongly.

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