Adaptive Resistance to Cancer Immunotherapy

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14.1 Introduction

Adoptive transfer of tumor-specific T cells or T cells engineered to express tumor-specific receptors (TCRs, CARs) has provided proof that T cells mediate tumor destruction in humans [1–4]. Pre-clinical studies [5–7], as well as the adoptive transfer of enriched CD8+ T cells into patients [8] firmly establish that CD8+ T cells can provide robust anti-tumor immunity. CD4+ T cells have been shown to support CD8+ T cell responses [9, 10], possess cytotoxic function [11], and can promote tumor destruction through multiple mechanisms, including cytokine production and recruitment/activation of innate immune cells [12–15].

These data confirming the anti-tumor properties of T cells notwithstanding, tumors emerge when they acquire the capacity to evade T cell attack. The immunoediting hypothesis argues that tumors must develop mechanisms to evade host immunity. Indeed, there is considerable evidence from pre-clinical models and clinical observations that tumors emerge more readily in the absence of an immune response [16]. Further, tumors that emerge in immune competent hosts often display a wide range of mechanisms to bypass the host immune response. The local immunosuppression within tumors is typically considered in static terms. However, emerging data argue that the immunosuppressive tumor environment is actually a direct response to ongoing immune attack and, thus, reflects a dynamic response that adapts to the nature and magnitude of the T cell attack. This ability of the tumor microenvironment to adapt to immune attack represents a significant barrier to the development of effective and durable immunotherapies. The following review will discuss the current knowledge in this emerging area and potential implications for the design of future immunotherapeutic strategies.

14.2 Immune Surveillance and the Immunoediting Hypothesis

The idea that the immune system can effectively control the growth of cancer dates back to the early 1900s, when Paul Ehrlich proposed the idea

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of host immune protection from cancer [17, 18]. The 'immune surveillance' hypothesis, however was not officially proposed until 1957 by Macfarlane Burnet [19] who proposed that an accumulation of tumor cells possessing novel target antigens could elicit an effective immune response, leading to tumor clearance in the absence of clinical detection [19]. Studies designed to investigate whether immunocompromised mice displayed greater tumor incidence were largely inconclusive. The immune surveillance hypothesis was eventually abandoned when it was observed that immune deficient athymic nude mice developed similar frequencies of chemically induced tumors as wild type mice [20]. However, subsequent investigation revealed that the nude mice used for that research, although immune compromised, were not completely immune deficient and did have detectable populations of functional T cells [21].

The concept of immune surveillance returned in the early 1990s, when more sophisticated mouse models allowed for the direct assessment of immune-mediated cancer control. Indeed, the increased frequency of chemically induced tumors observed in the absence of IFN-γ signaling [22–24], perforin [25], or RAG-2, which is required for T and B cell maturation [24], strongly supported a role for the immune system in preventing tumor growth. Pivotal studies revealed that the immune system cannot only act to eliminate tumors but can also shape their immunogenicity [24], leading to the evolution of the tumor immune surveillance hypothesis towards the concept of cancer immunoediting.

The cancer immunoediting theory comprises three distinct phases: Elimination, Equilibrium, and Escape [18]. In the elimination phase, developing tumors are destroyed by combined innate and adaptive immune responses. Given the genomic instability of tumor cells, daughter cells may emerge that acquire resistance to the antitumor immune attack. When such resistant subclones arise, the tumor enters a state of equilibrium where the overall size may stabilize as the immunogenic tumor cells are eradicated while the resistant subclones continue to proliferate. As such, the tumor is "edited" by the immune system to be comprised of cells with resistance to immune attack. Ultimately, immunoediting will select for tumor cells that are resistant to eradication by host immune cells, allowing the tumor to escapes immune control and grow out uncontrollably [26, 27].

While many of the observations regarding cancer immunoediting have come from studies using laboratory mice, increasing evidence suggests that the same principles apply to human cancers. The elimination phase is exemplified by the increased risk of developing both virally and non-virally induced malignancies among those with immune deficiencies [16, 26], as in the case of individuals with AIDS or those receiving immunosuppressants following organ transplant [16, 28]. Tumor equilibrium may help to explain the improved prognosis for patients exhibiting strong T cell infiltrate and local production of cytokines, such as IFN-γ and TNF-α [16, 26]. Tumor equilibrium is also consistent with reports of cancer patients entering phases of progression free survival or stable disease following treatment with cancer vaccines, [29–32], checkpoint blockade antibodies [33–36], or adoptive T cell transfer-based therapies [4]. Additionally, the report of two kidney transplant recipients developing malignant melanoma after both receiving organ transplants from a woman who had been successfully treated for melanoma 16 years previously [37], suggests that the melanoma metastases had been held in equilibrium within the kidneys for a prolonged period prior to transplant and only emerged in the transplant recipients because they received immunosuppressive drugs. Lastly, clinically detectable tumors are poorly immunogenic and possess intrinsic mechanisms of circumventing or suppressing host immune responses (as will be discussed), suggesting these tumors have effectively escaped immune control.

14.3 Mechanisms of Immune Escape by Tumors

In accordance with the immune editing theory, growing tumors develop an immune refractory microenvironment that limits attack by infiltrat-

ing immune cells, presenting a major hurdle to successful cancer immunotherapy. This section will focus on underlying mechanisms of immune evasion and immune suppression within the local tumor environment, which contribute to limited anti-tumor immunity against growing tumors.

14.3.1 Defects in Tumor Antigen Presentation

Abnormalities in MHC I antigen presentation have been documented in a diverse set of solid and hematological tumors [38], representing an important mechanism through which tumors can escape recognition by CD8+ T cells. Loss of MHC Class I expression has been reported in both murine and human tumors [39, 40], ranging from down regulation to complete absence of protein expression [41–43] and has been associated with poor survival prognosis and disease progression [43, 44]. Indeed, MHC class I expression has been observed to correlate with tumor regression or progression within individual metastatic lesions [45], suggesting that restoring antigen presentation is likely an important determinant in the success of immunotherapy. Defective antigen presentation by tumor cells often results from impaired expression of proteins associated with antigen processing. In some cases, the defect in antigen presentation is irreversible due to genetic alterations. As examples, reports have described mutations in β2-microglobulin [46] or components of the antigen processing machinery [42], as well as loss of heterozygosity at MHC I loci [42, 47–49]. In other cases, the impairment in antigen presentation is reversible as epigenetic alterations have been shown to result in diminished gene transcription and MHC Class I presentation. Such reversible impairments may be therapeutic targets as MHC expression and immunogenicity may be restored in tumor cells through the use of DNA de-methylating agents [50], HDAC inhibitors [51], or through treatment with immunostimulatory cytokines, such as IFN-γ [40, 50, 52].

14.3.2 Immunosuppression Within the Tumor Environment

Growing tumors secrete chemokines that promote tumor infiltration by cell populations that suppress T cell immunity, including regulatory T cells (Tregs; recruited by CCL22) and tumor associated macrophages (recruited by CCL2, CCL5, CCL7, CCL8, CXCL12) [53, 54]. Both of these immune cell subsets play important roles in promoting tumor growth and suppressing anti-tumor immune responses *in situ.* In this regard, both tumor cells and tumor infiltrating immune cells can secrete a range of factors that suppress the anti-tumor activity of infiltrating immune effector cells including T cells [55]. Specifically, IL-10 and TGF-β are often found within the tumor environment [56] and act to suppress T cell immunity by preventing T cell proliferation, cytotoxicity, and cytokine release, promoting T_{regs} function, and inhibiting the pro-inflammatory function of APCs [57–59]. PGE_2 is also often present at high levels in tumor tissue [60] and acts to inhibit DC maturation, limit T cell proliferation and function, increase immunosupression by myeloid cells, and enhance the suppressive effects of T_{regs} [56, 61]. VEGF, an important angiogenic factor required for tumor growth, has been reported to promote recruitment of myeloid derived suppressor cells (MDSCs) and M2 (healer) macrophages to the tumor [56] and prevents immunostimulatory function of APCs [62]. Adenosine, a purine nucleoside derived through the catabolism of adenine nucleotides by the enzymatic activity of CD39 and CD73, is often present at high levels within the tumor [63]. Produced through the activity of both the tumor [64] and T_{regs} [65], adenosine has both pro-angiogenic as well as immunosuppressive functions and limits the function of T cells [63, 65]. Lastly, local production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) within the tumor can contribute to T cell suppression and tolerance by preventing TCR/MHC interactions, impairing T cell responsiveness [66–68], and limiting tumor infiltration by T cells [69].

The availability of essential amino acids are often reduced by local catabolism within the tumor, consequently reducing the proliferative and functional capacity of the infiltrating immune cells. L-arginine is catabolized by arginase and NOS, enzymes often implicated in tumor-induced immune suppression and expressed at high levels within the tumor [56, 67]. Lack of L-arginine results in downregulation of the TCR ζ chain and inhibits the activity of T cells [70, 71]. Similarly, the enzyme IDO, which is also expressed at high levels by tumor cells, stromal cells, and infiltrating immune cells [56], acts to degrade the essential amino acid tryptophan, thereby limiting local immune activity. Additionally, kynurenine and other metabolites resulting from tryptophan breakdown have been shown to have suppressive/toxic effects on T cells, as well as additional immune cell populations including B cells and NK cells [72].

Together, the localized activity mediated by these factors acts to impair local anti-tumor immune responses. While effective inhibitors for many of these factors have been identified and observed to correlate with improved treatment outcome in pre-clinical cancer studies [56], the breadth of immunosuppressive processes within the tumor represents a major hurdle to the production of effective and prolonged anti-tumor immunity.

14.3.3 Immunosuppressive Ligands and Receptors in the Tumor Environment

In addition to locally produced immunosuppressive factors within the tumor, numerous receptor/ligand interactions can also act to promote immune evasion and tumor progression. The finding that apoptosis-inducing FasL and TRAIL are expressed in tumors [73–75] suggests a mechanism by which the tumor can eliminate infiltrating immune cells expressing cognate receptors and underscores active measures by the tumor to evade host immune attack. Similarly, while ICOS-L is expressed on tumor cells [76] and could provide a source of co-stimulation for activated tumor-specific T cells, stimulation of ICOS-expressing regulatory T cells results in increased expansion of IL-10-producing T_{regs} [76], which may suppress local immune activity within the tumor. While CD80 and CD86 are often considered to promote T cell function, binding of CD80 or CD86 to CTLA-4, a T cell suppressive receptor (commonly known as a *checkpoint receptor*) that is upregulated following T cell activation [77], leads to suppression of T cell function. CTLA-4 has a higher affinity for CD80 and CD86 than CD28. As a result, the negative signal from the CTLA-4 receptor on activated T cells will supercede any positive signals from CD28 [78]. Moreover, ligation of CTLA-4 by T_{regs} can promote upregulation of IDO by APCs [79, 80], which produces indirect T cell inhibition by Tregs [81]. PD-1, another member of the CD28 receptor family, is upregulated following T cell activation. Similar to CTLA-4, ligation of the PD-1 checkpoint receptor via the ligands PD-L1 and PD-L2, impairs TCR signaling, cytokine production, and cell survival [82]. Unlike the ligands for CTLA-4, PD-L1 and PD-L2 are often expressed on tumor cells providing a direct mechanism by which tumors can suppress T cell function [83–86]. PD-1 expression has been shown to correlate with reduced functionality of TIL [87]. Additional inhibitory or checkpoint receptors, have been identified that contribute to immune evasion within the tumor. TIM3, a receptor expressed on CD4+ and CD8+ T cells, as well as DCs, monocytes, and other lymphocyte populations [88], has been shown to negatively impact T cell responses through interaction with its ligand galectin-9 [89]. LAG-3 interacts with MHC II [90, 91] and negatively regulates TCR signaling, leading to functional impairment [92]. Recent evidence suggests that LAG-3 can also initiate reverse signaling via MHC II that can protect MHC II-positive tumor cells from apoptosis [93], while LAG-3 expressed on T_{regs} can also interact with MHC II and suppress DC function [94]. Inhibition of TIM3 activity has been shown to improve T cell proliferation and cytokine production [95] and antibody-mediated TIM-3 blockade can enhance T cell-dependent anti-tumor immunity [96–98]. Similarly, LAG-3 blockade enhances cytokine

production by T cells and shows a synergistic improvement in anti-tumor immunity when combined with PD-1 blockade [99], suggesting that blocking multiple checkpoint pathways simultaneously may further improve anti-tumor immunity.

14.4 Immune Suppression in the Tumor Microenvironment Occurs in Response to Immune Attack

As stated previously, the immunosuppressive tumor microenvironment is a critical barrier to successful cancer immunotherapy. While the suppressive mechanisms employed by individual tumors can be varied, these processes are often thought to be intrinsic properties of the tumor. However, emerging evidence suggests that the immunosuppressive microenvironment of the tumor may actually reflect an adaptive response by the tumor to immune attack that has been termed *adaptive resistance* [100].

A study by Taube *et al.* [83] reported that B7-H1 (PD-L1) expression in human melanoma lesions was strongly associated with T cell infiltration and the expression of IFN-γ, indicating that the PD-L1 expression was elevated in direct response to immune attack. A follow up study by the same group further emphasized the intricacies of the inflammatory tumor microenvironment in PD-L1+ tumors of human melanoma patients compared to PD-L1- tumors [101]. In this study, the authors identified a number of markers consistent with T cell activation (CD8A, PRF1, IL-18, IL-21), as well as increased expression of IFN-γ, PD-1, LAG-3, IL-10, and IL-32-γ in PD-L1+ tumors, further emphasizing the concept that PD-L1 is elevated as a consequence of T cell attack. Using *in vitro* studies, the authors further identified IL-10 and IL-32-γ as factors that could enhance expression of PD-1 ligands on monocytes but not tumor cells, suggesting a complex mechanism by which both tumor cells and infiltrating immune cells may act to regulate inflammatory attack within the tumor. An independent study by Kluger *et al.* [102] adds further support to these findings, where the authors reported strong association between PD-L1 expression and the presence of TILs in biopsies collected from different anatomical sites from patients with advanced metastatic melanoma. High expression of PD-L1 was associated with increased TIL density (Total CD3+ and CD8+) and improved patient survival, presenting an apparent paradox where the levels of PD-L1, an immunosuppressive ligand, actually correlate with improved outcome. In truth, there is no paradox as PD-L1 expression is simply a direct measure of local immune activity in melanoma.

Similarly, analysis of tumors from HPV-associated head and neck cancer patients revealed that TILs co-localized with PD-L1-expressing tumor cells [103]. Expression of both CD8 and IFN-γ was higher in PD-L1-positive tumors than PD-L1-negative tumors, reinforcing the concept that TIL activity directly contributes to induction of PD-L1, and, in turn, immune suppression, within the tumor microenvironment. In line with this, the authors noted that PD-1+ CD8+ TILs were functionally impaired compared to PD-1+ peripheral CD8+ T cells. Furthermore, the presence of PD-L1+CD68+ macrophages within the analyzed tumors suggests that infiltrating hematopoietic cells can also contribute to the adaptive resistance within the tumor.

Using an *in vitro* co-culture system to model T cell/tumor cell interactions, Dolen and Esendagli [104] observed that myeloid leukemia cells could provide effective co-stimulation to CD4+ T cells, enhancing T cell activation and proliferation. In turn, the activated CD4+ T cells triggered up regulation of PD-L1 and B7-DC (PD-L2) on the leukemia cells. When these "T cell-conditioned" leukemia cells were used in subsequent co-culture assays with naive CD4+ T cells, the T cells displayed poor proliferative capacity, diminished expression of activation markers (CD25, CD154), and reduced capacity for cytokine production, providing direct evidence that the tumor cells adapt to T cell attack and augment their immunosuppressive properties. Importantly, the blockade of PD-1 in this co-culture system led to reversal of the immunosuppressed CD4+ T

cell phenotype, restoring T cell proliferative and functional capacities confirming a key role for PD-1 ligands in adaptive resistance.

Additional support for adaptive resistance was reported by Spranger *et al.* [105], who observed a strong correlation between CD8+ T cell infiltration in human melanoma and the expression of PD-L1 and IDO, and the infiltration of FoxP3+ T_{regs}. Using mouse models, the authors observed that the induction of IDO and PD-L1 was mediated by CD8+ T cells and IFN-γ. Further, CD8+ T cells reacting to the tumor triggered both *in situ* proliferation and increased tumor infiltration of T_{regs} through a CCL22/CCR4 dependent chemokine axis. Similarly, Hosoi *et al.* [106] observed that tumor infiltration of suppressive myeloid populations, in particular $CD11b^{+}Gr\text{-}1^{int}Ly6C^{+}$ monocytic MDSCs, was driven by tumor-specific CD8+ T cells and the production of IFN-γ. The tumor infiltrating MDSCs were observed to suppress T cells through a variety of mechanisms including iNOS and Arginase I activity, as well as the production of reactive oxygen species (ROS) [106]. In line with this, by analyzing immune cell populations isolated from the ascites of ovarian cancer patients, Wong *et al.* reported that activated type-1 immune cells (NK cells and CD8+ T cells), via secretion of IFN-γ and TNF-α, could promote the production of suppressive factors by MDSC's including IDO1, NOS2, IL-10, and COX2 [107]. Importantly, this "counter-regulatory" immune suppression could be largely reversed through treatment with the COX2 inhibitor celecoxib and resulted in reduced production of these immunosuppressive factors by MDSC's, leading to increased production of IFN-γ and TNF-α by NK cells and restored proliferation of Granzyme B+ CD8+ T cells following MDSC co-culture, suggesting that the COX2/PGE_2 axis functions in multiple ways to promote MDSC-mediated immune suppression in response to local inflammatory events. These results demonstrate that adaptive resistance is more complex than simple up regulation of PD-1 ligands and the adaptation includes both tumor cell intrinsic effects (ex. up regulation of PD-L1) and tumor extrinsic effects (ex. infiltration of Tregs and MDSCs) [105, 106, 108].

We observed that the adaptive response is a key hurdle that limits the therapeutic effect of cancer vaccines. Using the B16F10 murine melanoma model, we noted that as soon as the vaccine-induced T cells infiltrated the tumor, a broad adaptation occurred with up regulation of a multitude of suppressive pathways, including checkpoint receptors/ligands (PD-1, LAG-3, TIM-3 and their corresponding ligands), Arginase and iNOS [7] [and unpublished data]. Expression of these suppressive factors was driven by CD8+ T cells and, to a large extent, the production of IFN-γ. Strikingly, these pathways were upregulated as soon as the vaccine-induced T cells infiltrated the tumor demonstrating the rapidity of the adaptive resistance. Unlike previous reports, we noted a temporal relationship between the adaptive response and T cell immunity within the tumor. Whereas the vaccine-induced T cells were initially highly functional within the tumor, over time the functionality of the intratumoral, vaccine-induced T cells waned while the adaptive response gained in magnitude, resulting in a very transient growth suppression. These findings are of particular clinical interest, as immunotherapies, including vaccines, often require long treatment intervals or multiple immunizations to generate high numbers anti-tumor T cells. In turn, such therapies may instigate suppressive events in the tumor early in the course of treatment and long before maximal immune reactivity against the tumor is achieved.

Using the same model, we determined that tumor regression could be achieved by combining vaccination with either administration of immunomodulatory antibodies (anti-CD137 + anti-PD-1) [109] or adoptive transfer of tumor-specific CD8+ T cells [7]. Tumor regression did not result from the absence of an adaptive response following those therapeutic strategies. In contrast, we observed that the effective therapies produced a heightened anti-tumor T cell response, which actually resulted in an elevated magnitude of the adaptive immunosuppressive response. In fact, throughout our studies, we observed that the magnitude of the adaptive response was directly related to the magnitude of the therapy-induced immune attack. Thus, adap-

tive resistance may be a barrier, but it is not absolute and can be overcome when sufficient numbers of T cells are present in the tumor, despite impairments in T cell function that arise from the adaptive immune suppression.

Since adaptive immune suppression is evidence of immune attack within the tumor, measures of the adaptation may provide prognostic value. Tumeh *et al.* [108] and Taube et al. [83] observed co-localization of tumor infiltrating CD8+ T cells with expression of immune-inhibitory PD-1/PD-L1 markers, consistent with the theory that immune attack is responsible for adaptive immune resistance within the tumor microenvironment. Taube *et al.* observed that metastatic melanoma patients with elevated PD-L1 expression survived longer than those with low PD-L1 expression. Tumeh *et al.* went further and determined that patients with a high density of TILs and markers of adaptive resistance (PD-1/PD-L1) at both the invasive margin and in the tumor were more likely to respond to PD-1 blocking therapy (pembrolizumab) when compared to patients with poor TIL infiltration or PD-1/PD-L1 expression. The authors employed this information to develop a model that predicted which patients would respond or progress on PD-1 blocking therapy. Furthermore, Gajewski has emphasized that patients showing favorable clinical outcome often had pre-treatment tumor transcriptional profiles consistent with T cell infiltration and an inflamed tumor microenvironment, but that these same tumors also had the highest expression of genes associated with inhibitory mechanisms, including IDO, PD-L1, and a profile consistent with FoxP3+ T_{regs} infiltration [110–115].

Conceptually, the existing experimental data support induction of multiple adaptive resistance mechanisms in tumors in response to immune attack and it is likely that additional pathways/mechanisms will be identified. Currently, two possible scenarios have emerged whereby the adaptive response has the potential to be overcome therapeutically to promote anti-tumor immunity with curative potential. As depicted in Fig. 14.1a, inflamed tumors, which can arise either spontaneously or in response to immunotherapy, induce only low level immune attack on the tumor, resulting in adaptive resistance, localized immune suppression, and tumor growth. In this case, adaptive resistance mechanisms can be partially overcome through interventions aimed at disrupting these immunosuppressive pathways (including co-stimulatory agents, checkpoint blockade, as well as chemical inhibitors) to promote re-invigoration of local immune attack and tumor regressions, whether complete or transient. In contrast, delivery of more robust immunotherapies, such as ACT, can initiate rapid immune attack and result in tumor regressions despite the presence of the same adaptive resistance mechanisms [7], suggesting that increasing the magnitude and/or rate of immune attack on the tumor may also improve the likelihood of overcoming adaptive immunosuppressive mechanisms and achieving therapeutic benefit (Fig. 14.1b).

14.5 Adaptive Immune Suppression in the Tumor: Does the Tumor Benefit from Conventional Homeostatic Mechanisms of Immune Tolerance?

Chronic inflammation had been implicated in driving immunosuppressive mechanisms within the tumor, thereby limiting anti-tumor immune responses [116–120]. However, as described above, the emerging concept of an adaptive immune resistance argues that the broad network of suppressive factors within the tumor microenvironment may actually be instigated as a consequence of immune attack on the tumor. Adaptive immune resistance does not appear to be unique to tumor tissue, as many of the same suppressive mechanisms have also been implicated in the maintenance of immune tolerance under normal homeostatic conditions and in the control of autoimmune pathology under chronic inflammatory conditions.

PD-L1 expression has been observed to increase with pancreatic inflammation in a mouse model of diabetes [121]. In the womb, PD-L1 is expressed in the placenta [122] and by T_{regs} [123]

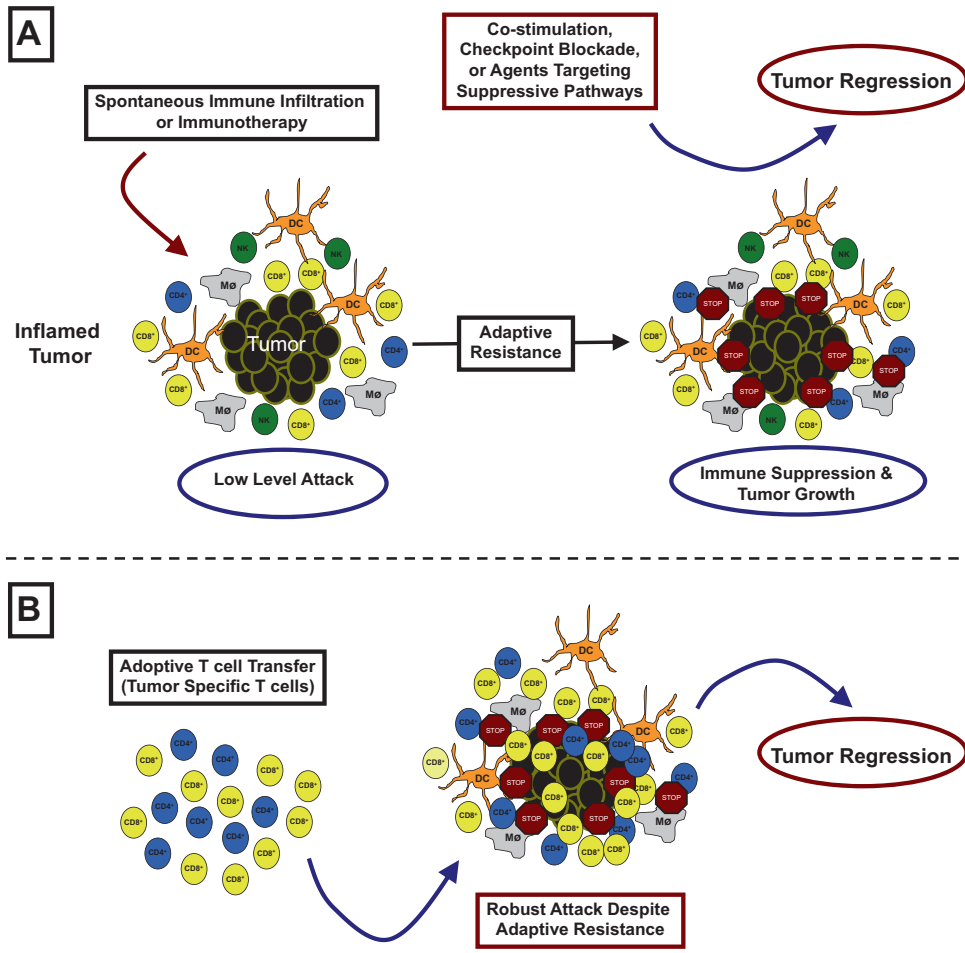

Fig. 14.1 Adaptive resistance occurs in direct response to immune attack on tumors but can be overcome therapeutically. (**a**) Spontaneous immune infiltration or delivery of immunotherapy results in an inflamed tumor microenvironment but only low level immune attack, resulting in adaptive resistance, immune suppression, and continued tumor growth. Intervention(s) with agents that act to enhance or restore local immune attack (co-stimulatory molecules, checkpoint inhibitors, or agents targeting immunosuppressive pathways) can partially overcome adaptive resistance mechanisms, leading to tumor regression. (**b**) Delivery of more robust immunotherapies (such as ACT) results in vigorous immune attack on tumors despite induction of adaptive resistance mechanisms and leads to tumor regression

to prevent immune attack on the semi-allogeneic fetus. PD-L2 has been implicated in the maintenance of oral tolerance to ingested antigens [124] and has been shown to aid in controlling airway asthmatic responses [125]. Not surprisingly, the checkpoint receptors PD-1, LAG-3, and TIM-3 play a role in the suppression of inflammatory processes to control autoimmune pathologies or tissue homeostasis [126–128], as do arginase [71], iNOS [129], TGF-β1 [130, 131], IDO [132, 133], and Galectin-9 [89]. These observations argue that tumor tissue is not unique in its ability to evade inflammatory attack, but instead exploits natural homeostatic mechanisms that limit unwanted auto-immune destruction of healthy tissues. IFN-γ is particularly interesting in this regard as it has been implicated as critical effector molecule promoting anti-tumor immunity, while also playing a key role in the induction of the many immune suppressive pathways within the tumor. The contribution of IFN-γ to tissue homeostasis is underscored by the emergence of severe autoimmune pathology in mice deficient in IFN-γ or the IFN-γ receptor [134–138]. IFN-γ,

therefore, acts as a double-edged sword, regulating both inflammation and immune destruction [139]. Developing strategies to mitigate the regulatory properties of IFN-γ while maximizing tumor destruction will greatly enhance the effectiveness of cancer immunotherapy.

14.6 Adaptive Resistance: Knowledge to Practice

Blockade of checkpoint receptors (i.e. CTLA-4, PD-1) and their ligands (i.e. PD-L1) has confirmed therapeutic benefit in multiple clinical trials across a broad range of tumors [34–36, 140–149]. These important successes notwithstanding, single-agent checkpoint blockade only achieved therapeutic effects in a fraction of patients and data emerging from both mouse and human studies has demonstrated additional therapeutic benefit through blockade of multiple immunosuppressive pathways simultaneously [7, 34, 99, 149–152]. As expected, given the complexity of adaptive resistance, the combination of anti-CTLA-4 and anti-PD-1 produced more objective responses in melanoma than either agent alone [153]. The requirement to overcome multiple resistance mechanisms is further supported by analysis of tumor-specific T cells isolated from tumor-positive lymph nodes of patients, which revealed that these T cells upregulate a host of suppressive receptors including LAG-3, TIM-3, PD-1, BTLA, 2B4, and CTLA-4 [154], further demonstrating that multiple pathways contribute to T cell impairment. Indeed, the effects of these pathways are additive as T cells receiving multiple suppressive signals possess greater functional impairments [96, 155–157]. Based on these observations, it is clear that a combinatorial approach will be required to effectively block local immunosuppressive processes and achieve more consistent objective responses in a larger number of patients. Given the high cost associated with each of the checkpoint blockade antibodies, however, it is unlikely that payers will accept using all blockade strategies with all patients. In this regard, it is imperative that we develop effective predictive tools that can determine which checkpoint blockade strategies will be most effective with individual patients. Adding further complexity to this challenge, a recent report investigating combination radiotherapy and CTLA-4 therapy revealed that PD-L1 was upregulated in therapy-resistant tumors [158], suggesting that re-invigoration of tumor attack by overcoming a single immunosuppressive pathway may, in fact, lead to induction of additional non-redundant mechanisms of adaptive resistance. Interestingly, adoptive T cell therapy can produce tumor regression despite clear evidence of adaptive resistance by the tumor [7]. Thus, it is possible to overcome the adaptive immune resistance when the level of immune attack is high enough. Of course, adoptive T cell therapy is imperfect and will likely require some aspect of checkpoint blockade to maximize clinical activity. Nevertheless, the impressive clinical outcomes with checkpoint blockade and adoptive T cell therapy support further research to identify not only mechanisms leading to the induction of adaptive resistance in tumors, but also to understand potential cross talk, interplay, as well as differences in the expression kinetics/upregulation of well-characterized and emerging immunoregulatory mechanisms that function to limit immune attack on tumors.

Collectively, these studies demonstrate that immune attack on the tumor triggers a complex and dynamic feedback mechanism through which cells present within the tumor actively respond to the attack by upregulating immunosuppressive pathways that limit the durability of the therapeutic anti-tumor effects. Understanding the triggers of these responses will be key in the development of strategies to suppress the adaptive resistance and enhance clinical outcomes with immunotherapy.

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