Multilevel mechanism of immune checkpoint inhibitor action in solid tumors: History, present issues and future development (Review)

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Abstract. Immunotherapy with checkpoint inhibitors (antibodies that target and block immune checkpoints in the tumor microenvironment) is included in the standard of care for patients with different types of malignancy, such as melanoma, renal cell and urothelial carcinoma, lung cancer etc. The introduction of this new immunotherapy has altered the view on potential targets for treatment of solid tumors from tumor cells themselves to their immune microenvironment; this has led to a reconsideration of the mechanisms of tumor-associated immunity. However, only a subset of patients benefit from immunotherapy and patient response is often unpredictable, even with known initial levels of prognostic markers; the biomarkers for favorable response are still being investigated. Mechanisms of immune checkpoint inhibitors efficiency, as well as the origins of treatment failure, require further investigation. From a clinical standpoint, discrepancies between the theoretical explanation of inhibitors of immune checkpoint actions at the cellular level and their deployment at a tissue/organ level impede the effective clinical implementation of novel immune therapy. The present review assessed existing experimental and clinical data on functional activity of inhibitors of immune checkpoints to provide a more comprehensive picture of their mechanisms of action on a cellular and higher levels of biological organization.

Contents

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1. Introduction

Modern science has acquired understanding of the innate control of cell immunity; however, understanding of adaptive immune mechanisms in cancer is relatively limited. Despite a long history of cancer immunotherapy, strategies to restore antitumor-immunity have not delivered satisfactory results (1). A specific pro-tumor part of adaptive immunity following the escape phase of the immune surveillance process promotes tumor growth and cannot be reprogrammed (2). Novel immunotherapies targeting adaptive immunity by inhibiting certain immune checkpoints are a key breakthrough in oncology, providing therapeutic strategies that improve the outcome of various types of cancer, such as melanoma, renal cell and urothelial carcinoma and lung cancer (3,4). A new approach based on specific inhibition of checkpoints is different to the previous strategies aimed at boosting anti-tumor immunity.

The mechanisms underlying the effectiveness of immune checkpoint inhibitors (ICIs) may be associated with the role of T cells in tumor development. Tumor-infiltrating immune cells, namely regulatory T cells (Tregs), serve as a cellular basis for cancer immunotherapy (5). A better understanding of their role in the tumor microenvironment is key to determine mechanisms underlying immunotherapy and identify prognostic biomarkers. Treatment strategies aim to deplete or block Tregs, resulting in significant intratumoral Treg depletion, coinciding with long-term antitumor activity in solid tumor models; the success of aforementioned approach however has been limited to a subset of respondents (6). Immunotherapy targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein-1 (PD-1) has achieved long-term remission in patients with multiple types of solid tumors, continuously revolutionizing treatment strategies for many malignancies.

Response to immunotherapy is often unpredictable, even with known starting levels of predictive biomarkers (7). Despite clinical success, the response rate is 20-40% and biomarkers for favorable response are still being investigated (8,9).

Consequently, obstacles to clinical application of immunotherapeutic regimens include limited response rate, inability to predict clinical efficacy and potential side effects. A better understanding of the biological events following checkpoint blockade is necessary to identify reliable predictive biomarkers of successful PD-1/PD-L1 (programmed cell death ligand 1) blockade and tailor immune therapies for specific clinical conditions. The present review aimed to summarize the history of the development of ICIs and to analyze the underlying mechanism of this type of immunotherapy in the context of tumor-associated adaptive immunity. Understanding of the basic principles, advantages and limitations of novel immunotherapy-based techniques may improve development of novel strategies and clinical efficacy.

2. Immune surveillance: Understanding host-tumor interaction

The immune system serves a key role in the host response to tumors (10). However, immune surveillance is a controversial issue in tumor immunology. In the early 20th century, Paul Ehrlich proposed immune surveillance, according to which the immune system scans tissue for transformed cells and eradicates them using immune mechanisms (11). Sir MacFarlane Burnet proposed clonal selection to explain self-tolerance by deleting self-reactive clones in 1957 (12). The 1960 Nobel Prize was awarded to Burnet and Peter Medawar for immunological tolerance. According to the theory of clonal selection, the concept of immune control of a cell types heterogeneity is based on existing mechanisms of antitumor immunity acting under the condition of permanent appearance of altered cells in the body (13). Thomas (14) suggested that lymphocytes serve as sentinels in recognizing and eliminating continuously arising, nascent transformed cells. Cancer immune surveillance is a key host mechanism to prevent cancer via inhibition of carcinogenesis and regular monitoring of tissue homeostasis (15). Two problems with Burnet's theory were formulated by Hodgkin (16). The first is the cell type dilemma, which states that novel T cell subtypes may still be discovered due to technological advances, and understanding of the behavior of different types of T cell is not complete. The second issue is the complexity of cooperation between immune cells via modifying signals that elicit different responses. Thus, the immune surveillance hypothesis underlying cancer immunology is important for understanding how the immune system functions in this case. However, the theory has contributed little to attempts to treat cancer via immunological mechanisms (17). It has been suggested that immune surveillance primarily functions as a component of a more general dynamic process of 'cancer immunoediting' that has three phases: Elimination, equilibrium and escape (18,19). As long as the elimination and equilibrium phases continue, immunity serves to protect against tumors. Escape from immune surveillance leads to manifestation of tumorous tissue and a change in the direction of immune reactions from anti-tumor to pro-tumor by which the escaped cells survive in immunocompetent hosts. The escape phenomenon is an event that leads to tumor formation; therefore, it is an attractive but very challenging target for immunotherapy, since evasion is only a transient moment between the completed phases of elimination and equilibrium and manifestation of tumor tissue (Fig. 1). The elimination phase assumes the predominance of effector cytotoxic mechanisms for the eradication of malignant cells, while the equilibrium phase exists due to tolerance to the emerging pool of tumor cells. The manifestation of a tumor as tissue and its development in the presence of immune cells in the microenvironment indicates the failure of previous mechanisms and the formation of a new type of interaction between immune and tumor cells. The development of immunoediting theory determined that the primary site of action in tumor-associated immunity is interactions between immune and tumor cells. The expression of checkpoint molecules on immune cells suggests the possibility of interaction with other populations of intratumoral cells. These functional receptors serve an essential role in the control of cell fate and tissue homeostasis (20). Therefore, receptors that determine these antigen-driven interactions, on both immune and tumor cells, have become research target for effective antitumor strategies (21).

3. Immunity in maintaining tissue homeostasis

Tissue homeostasis is achieved when the behaviors of constituent cells, including proliferation, differentiation and apoptosis, are in balance (22). This dynamic equilibrium between cells and their environment requires constant control of regulatory systems at different levels (Fig. 2). Under physiological conditions, the function of the immune system, in addition to protecting against infections, is also morphogenetic-monitoring morphological and genetic tissue homeostasis to protect against malignant transformation (23). However, the innate immune reactions of host defense against pathogens cannot be applied to mechanisms that protect against cancer involving adaptive immunity (24). Immune surveillance is the ability of the immune system to detect cellular imbalances and respond by activating adaptive cellular immunity to restore tissue homeostasis (25). The immune system contributes to permanent tissue renewal and remodeling following damage (26,27). To maintain tissue equilibrium, key elements of the central part of the immune system, namely, thymus-derived lymphocytes, are functionally represented in each developing tissue; to ensure this representation, immune cells have unique ability to move between compartments and realize feedback mechanisms for inverse correlation with the thymus as a central organ (20,28). Thymus-derived regulatory T cells, Tregs, are considered to have a homeostatic function (23,29). The mechanism of transforming autoreactive thymocytes into Tregs, which do not induce inflammatory reactions in self body tissue, is involves 'education and differentiation' in response to autoantigens (30,31). Accordingly, self-antigen-recognition by a specific T cell receptor (TCR) is the predominant requirement for the induction of thymic Tregs (32). Due to their homing capacity that allows them to target tissue-specific migration, T lymphocytes monitor tissue homeostasis constantly. Migrating cells are continuously recycled between the core and peripheral compartments of the immune system, penetrating tissue through post-capillary vessel walls (33). This immunological process of tissue 'patrolling' has biological rationality in the monitoring and controlling of antigenic constancy. TCRs are not only
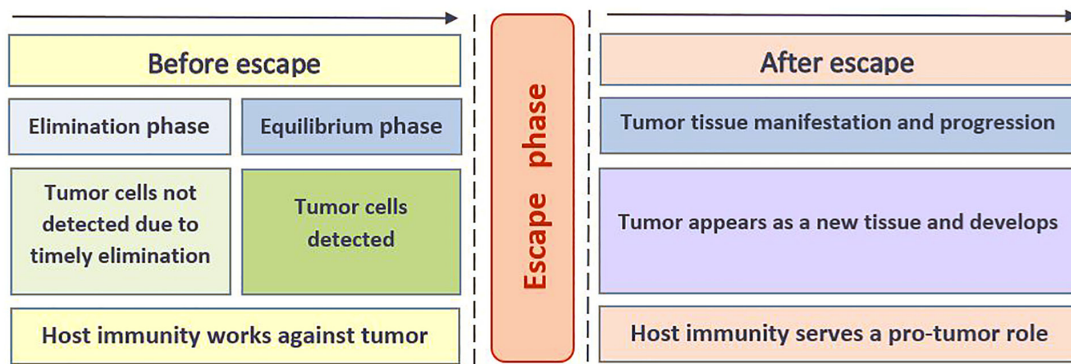
Figure 1. Host-tumor interaction in tumorigenesis. Schematic of the interaction between host immunity and tumor at different points of time during the known phases of immune editing process. The escape phase is a key event before the manifestation of tumor tissue and the moment when the direction of the immune response changes from anti-tumor to pro-tumor.

involved in specific T cell clone induction and maturation, but also determine the destination of migrating lymphocytes in a particular tissue. Interaction of TCRs with ligands in the endothelium of post-capillary venules allows entry into tissue in a controlled manner (26). TCRs recognize molecules of the major histocompatibility complex (MHC), which are tissue-specific and ubiquitously expressed on cells of different tissue (34). The binding specificity of the TCR is a key component in MHC recognition (35). Migration of T lymphocytes, directed by TCRs, occurs due to ligand-integrin interaction of T cells with adhesive molecules on the endothelium of blood vessels (34,36). Capillary endothelial cells select lymphocytes for active movement into tissue according to recognition of the 'homing' receptors (33).

Movement of lymphocytes through lymph and blood and their migration into tissue is targeted (20). The trafficking of T cells to peripheral tissue is performed in response to homeostatic chemokines, which are permanently expressed by cells of the microvascular endothelium to attract the corresponding clones of lymphoid cells to certain tissue sites (36). Lymphocytes leave the bloodstream, entering tissue between adjacent endothelial cells (33). Recirculating lymphocytes are a highly mobile population of cells able to enter through vessel walls into tissue and back into circulation. Thus, migrating lymphocytes control tissue homeostasis via bidirectional cell transfer, which is beneficial for tissue development (37-39). When immunocompetent T cells appear in the tissue, they become part of the local microenvironment. Complex interactions between lymphocytes, extracellular matrix proteins, sedentary immune cells and tissue cells determine the outcome of immune responses at the tissue level (28). Functioning tissue exhibits an immune regulatory compartment-zone located around the post-capillary venules where interactions between endothelial cells, lymphocytes and tissue structures occur (40,41). Tissue lymphocytes are represented primarily by T cells, which are defined as a regulatory population, originating from double-positive helper/suppressor cells (42,43). Lymphatic tissue constantly maintains a recirculating pool of T lymphocytes, which are generated in the thymus and undergo T lymphocytes during their circulation cycle reside in non-lymphoid tissues where they complete differentiation and acquire immunological specificity (44). The selectivity of the migration of T cell clones, termed 'homing', determines

formation of a functional complex, which consists of a specific T cells clone tissue, and regional lymph nodes (26,45). Targeted migration of regulatory T lymphocytes into the tissue compartment is necessary for tissue development and renewal (46). Consequently, renewing tissue under non-inflammatory conditions favors preferential recruitment of a highly restricted repertoire of specific Tregs for development (27,42).

Nevertheless, mechanisms underlying tissue homeostasis regulation by the immune system have not been completely elucidated and require further clarification. Multicellular organisms function as stable integrated systems due to physiological intercellular interactions (47). The substrate for storing and transmitting information in biological systems is nucleic acid sequences in the form of RNA and DNA. Previous studies have shown that cells communicate via direct exchange of genetic patterns in the form of extracellular vesicles, such as exosomes, microvesicles and apoptotic bodies that deliver functional RNA/DNA molecules (37,48). The majority of cells secrete exosomes into the extracellular environment and exosomes have been observed in the cytoplasm of primary T lymphocytes (49). Exosomes fuse via phagocytosis with recipient cells, including macrophages and dendritic cells, which internalize exosomes (50,51). Fusion of exosomes with membranes of recipient cells has also been described in cancer cells (52). Intercellular communication via exosomes is a potential driver of phenotypical changes and cell plasticity during tissue regeneration (53). Targeted transfer of genetic information is key in tissue development and this mechanism underlies the immune-editing function of the T cell arm of the immune system (47). Specific genetic messages are designed for incorporation into acceptor cells to promote tissue-specific differentiation. The exosomes necessary for induction of differentiation are provided by apoptosis of immune cells, which underlies their mechanism of action (54). microRNAs within exosomes regulate innate immune responses; exosomes from T cells directly fuse with host tissue cells, releasing miRNAs (55). The genetic information contained in exosomes affects target cells in various ways, inducing activation, differentiation or apoptosis (47).

The thymus undergoes notable decline in both size and function during the postnatal period but does not undergo atrophy and continues to perform a key role in T cell arrangement in adulthood by maintaining the development and homeostasis
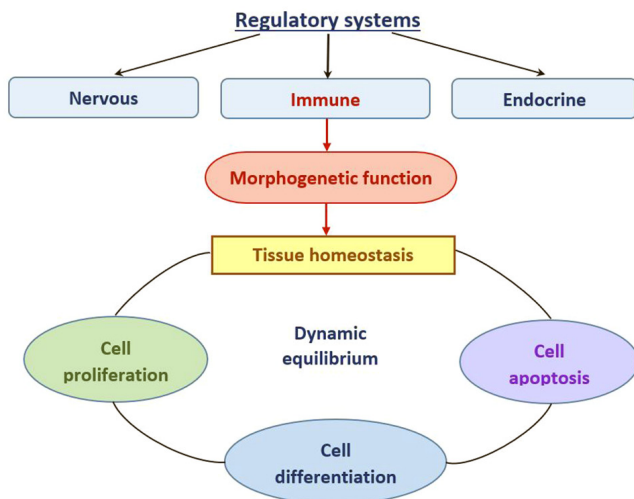
Figure 2. Control of tissue homeostasis by regulatory systems. The immune system as one of the regulatory systems is involved in maintaining tissue homeostasis. The morphogenetic function of immunity is realized by controlling the dynamic equilibrium between the processes of cell proliferation, differentiation and apoptosis. All arrows indicate the direction of the regulatory influence.

of the T cell arm of immunity (56). This is supported by the fact that thymus epithelium stem cells are constantly generated and their pool is dynamically regulated by signals from the periphery in response to tissue needs (57-59). Maturation of T cells in the thymus requires a constant supply of T cell progenitors from bone marrow (60). The role of lymphocytes in regulation of cell differentiation is confirmed by their presence in the microenvironment of differentiating hematopoietic stem cells in bone marrow (61). Skin and mucous membranes must be constantly monitored to ensure homeostasis as they serve as a barrier to the external environment. Thus, these tissues are most predisposed to developing cancer (62).

4. Tumor as a new tissue

Malignant tumors evolve by developing mechanisms to evade antitumor immune-based programs and conscripting them to promote carcinogenesis (2,62). Having passed through the escape phase of immune surveillance, the tumor appears as a new tissue and develops according to self-regulating mechanisms and is subject to the same central regulatory rules as a normal tissue. This new tissue evolves as what appears to be a unique tissue and the immune system continues to maintain homeostasis in the newly appeared anatomical area, allowing tumor development instead of targeting the new tissue (63,64). This is supported by the fact that developing and progressive tumor tissue can exist as a symbiont of an organ (65). The paradoxical role of adaptive and innate lymphocytes is that they serve as key regulators in cancer development and progression (66). Tumor progression is not a random process and it follows internal rules and mechanisms of central regulation in the host-tumor system that are still under investigation (67-69).

There is evidence to support the importance of a central mechanism in regulating tumor progression. The formation of metastatic niches in the form of stromal restructuring in tissue remote from the site of the primary tumor begins before dissemination of malignant cells (70). Tumor cells acquire metastatic properties before they migrate from the primary tumor site (71) and systemic circulation of tumor cells may remain latent or unproductive for an extended period (72). Additionally, increased formation of blood and lymphatic vessels in tumors contributes to metastasis (73,74). Stimulation of vascular growth in tumors occurs via the same mechanism as that in normal tissue (75). For blood vessels, a tumor lesion is an extra cell mass that requires nutrition and elimination of tissue metabolic products (65). The aforementioned processes support the hypothesis that the effect of host regulatory systems on formation of the tumor microenvironment is a factor initiating metastasis.

The tumor microenvironment includes heterogeneous immune cell populations (10). Advances in single-cell characterization have provided insight into involvement of T cells in the tumor microenvironment. Solid tumors typically contain functionally active antigen-specific tumor-infiltrating lymphocytes that paradoxically do not interfere with tumor growth and progression (76,77). During each phase of the metastatic process, tumor cells are targeted by immune cells, which recognize them as harmful and restrict their development (78). However, numerous studies have shown that tumor-infiltrating immune cells promote the metastatic cascade (2,64). Tregs may serve a role in increasing the number of surviving tumor cells in the circulation and at sites of metastasis (2). Tregs have been found in lymph nodes containing micrometastases (79); moreover, the detection of this population of lymphocytes precedes detection of metastatic lesions in regional lymph nodes (80).

Leukocytes infiltrating tumor tissue are predominantly Tregs with a $CD4^+CD25^+$ forkhead box P3 ($Foxp3^+$) phenotype that serve a key role in immune editing (81,82). Phenotype, differentiation status and function of regulatory immune cells differ depending on the anatomical compartment in which they reside; this shows that immune cells that originate from the same precursors but reside in different types of tissue are affected by organ-specific factors (20,45). Consequently, each tissue possesses its own antigens to activate the immune system and generate local immune responses that are associated with homeostasis of that tissue (83,84). The features of Treg behavior make it possible to understand the mechanisms underlying immune-mediated regulation of tissue homeostasis as well as to assess the role of the thymus as a central organ controlling the location and function of T cells populations in the periphery (58).

In solid tumors, the density of Tregs in tumor lesions and their imbalances in blood are associated with clinical outcomes (85-87). Experimental data are consistent with the hypothesis that tumor-specific Tregs originate in the thymus during T cell development and are preferentially recruited into tumor tissue compared with the diverse systemic Treg pool (88,89). Clinical data reporting an increase in the population of Tregs in peripheral blood of patients with solid tumors are consistent with experimental data on increased yield of mature thymocytes and migration into the peripheral circulation (85,90). The direction of target migration of T lymphocyte clones determines their destination and recruitment in the tumor tissue (5,81). According to clinical and experimental
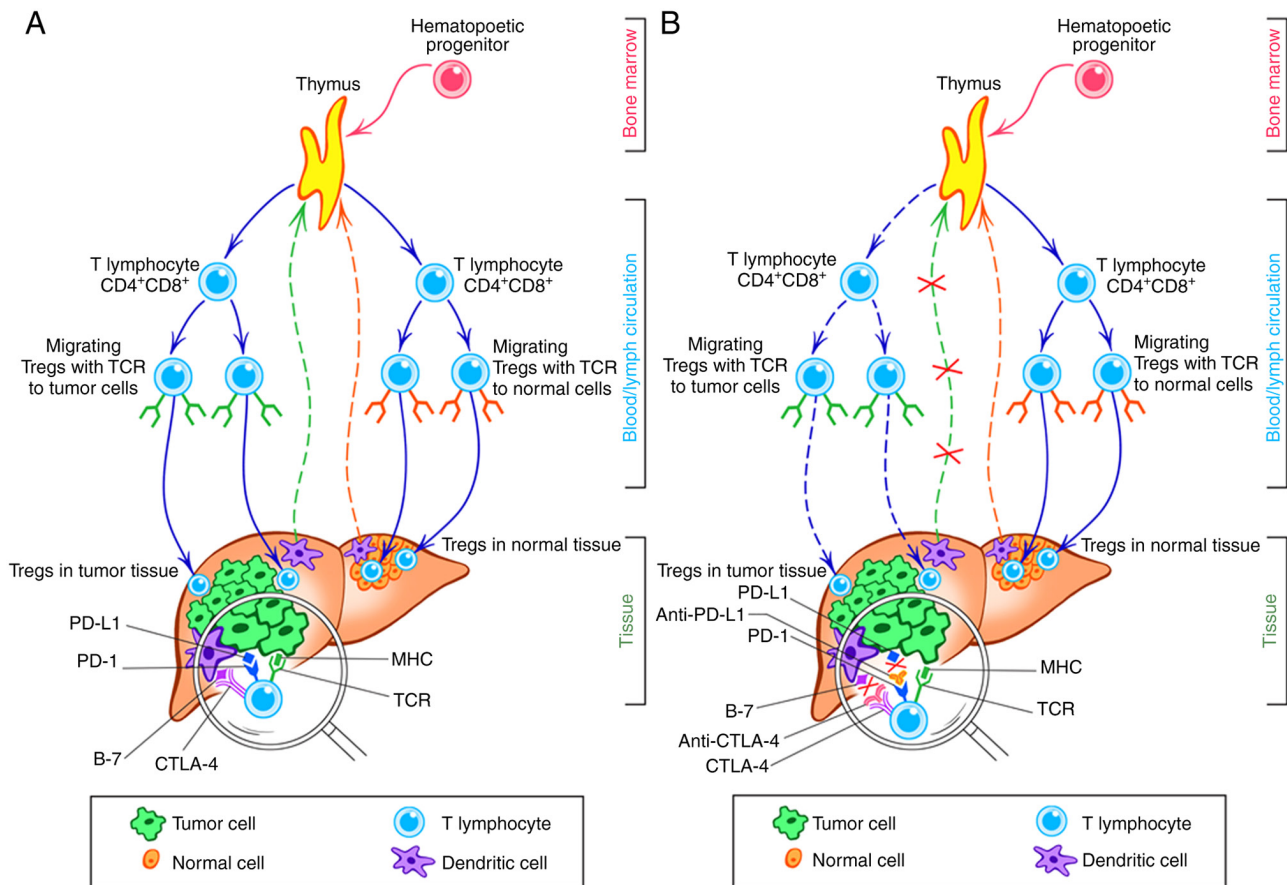
Figure 3. Immune regulation of tissue homeostasis in an organ affected by tumor before and after tumor exposure to ICIs. (A) Schematic of central immune system-mediated regulation of homeostasis in normal and tumor tissue in the case of a clinical tumor. An organ affected by cancer consists of tumorous and normal tissue containing Tregs intended for each tissue type. Tissue-specific Tregs dynamically emerge in the thymus in response to signals from the periphery according to the needs of the tissue. T cells begin to differentiate in the thymus and complete differentiation in the peripheral tissue compartment to which they migrate due to innate homing ability. Maturation of T cells in the thymus requires a constant supply of T cell progenitors from bone marrow. Recirculating Treg clones are similar in phenotype and differ only in the direction of migration to the specific tissue, dictated by unique MHC antigens that attract corresponding TCRs. CTLA-4 and PD-1 are functional receptors of Tregs with ligands B-7 and PD-L1, respectively. (B) Schematic of the multilevel mechanism of action of ICIs. Exposure of tumor to ICIs leads to disruption of contact between tumor tissue and immune cells by inhibiting functional receptors on Tregs. Further impairment of tumor tissue homeostasis occurs due to weakening of self-sustaining mechanisms of central immune regulation. The quantitative side (the number of immune checkpoints as targets) of interaction between receptors on immune and on tumor cells and the rate of generation of new clones of tumor-associated T lymphocytes determine the effect of ICIs at the tissue level. ICI, immune checkpoint inhibitor; Treg, T regulatory cell; MHC, major histocompatibility complex; TCR, T-cell receptor; CD, cluster of differentiation; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand-1.

studies, tumor infiltrating leukocytes are mainly represented by the same regulatory subpopulation of thymic lymphocytes (88,91,92).

Tumor tissue development is accompanied by formation of clones of T lymphocytes designed for that tissue. Tumor-infiltrating regulatory lymphocytes undergo early differentiation in the thymus and complete differentiation in the tumor tissue, which promotes in generation of immune signals to support tumor development (87). Therefore, tumor tissue can also be considered a peripheral compartment of the immune system, consisting of post-capillary vessels endothelial cells, a circulating and settled pool of Tregs, tumor cells and active components of the extracellular matrix; however, the function of this compartment is not conducive with physiological function of the immune system. Accordingly, in an organ affected by a tumor, each type of tissue (tumor and normal) has a regulatory zone infiltrated by T lymphocytes from the regulatory population; meanwhile, in the peripheral circulation of the host, T cell clones intended both for tumor and normal tissue. Both these sets of T cell possess the same phenotype (CD4+Foxp3+) but they differ in the direction of TCR-driven migration into corresponding tissue, either normal or tumor, due to each of them carrying unique histocompatible antigens (Fig. 3A) (91,93).

Numerous studies have confirmed the role of Tregs in regulating the pace of solid tumor progression: Increased quantities of Tregs in tumor tissue are associated with a higher degree of tumor differentiation and favorable prognosis (5,85,90). A decrease in Treg content among tumor-infiltrating lymphocytes following effective neoadjuvant chemotherapy in patients with breast cancer has been found in a comparative study of postoperative tumor samples (94). Clinical studies have demonstrated an association between tumor regression in response to chemotherapy and a decrease in Treg population, indicating that Treg levels may serve as a prognostic marker (5,95). The role of Tregs in cancer development provides a rationale for targeting Tregs for future therapeutic strategies.

5. Discovery of ICIs

The microenvironment of tumor cells has a complex, heterogeneous and dynamic nature (96). The region of interaction between cancer and immune cells is decisive for tumor tissue progression (10). Previous cancer immunotherapies (such as immunostimulatory cytokines, vaccination with tumor-specific antigens, stem cell therapy) aimed at stimulating the immune system has not yielded satisfactory results (1,97), although novel immune therapies (such as immune checkpoints inhibitors) that target precise blockades rather than non-specific stimulation have shown promising efficacy. This type of immunotherapy focuses on the tumor microenvironment rather than tumor cells. The effectiveness of ICIs has shown that tissue regulation by the immune system serves a key role in tumor progression and Tregs are important for tumor development (23,81,98). The ambiguous dual role of Tregs in cancer development has been shown as Treg inactivation and depletion may initiate an antitumor immune response (99). Thus, investigation of Treg behavior and their association with tumor cells may support methods to modulate host response to malignant tissue transformation. Targeted immunotherapies should aim to either enhance the antitumor properties and/or prohibit the pro-tumor properties of immune cells (100).

Research on the association between tumor cells and activated immune cells led to the discovery of immune checkpoint blockade, a novel form of immunotherapy. James Ellison and Tasuku Honjo discovered functional receptors, CTLA-4 and PD-1, on activated T lymphocytes, which are key to developing checkpoint-blockade immunotherapeutic agents, revolutionized cancer treatment and was awarded the 2018 Nobel Prize in Physiology or Medicine (101). The clinical efficacy of checkpoint blockade in various types of cancer, e.g. in melanomas, kidney cancers, urothelial carcinoma and non-small cell lung cancers (102-105), proves the universality of its mechanism of action (106,107).

6. Current status of immunotherapy with ICIs

Targeted immunotherapy against CTLA-4 and PD-1 lymphocyte receptors in clinical practice has demonstrated efficacy in a subgroup of patients with aggressive solid tumors, including disseminated melanoma, renal cell carcinoma and non-small cell lung cancer (3,8,21,108). The patients who respond to immunotherapy show a pronounced and enduring clinical response that persists following treatment discontinuation and may resume in response to a similar treatment in case of disease progression (102). Successful clinical application of ICIs has contributed to development of novel immunotherapeutics for treatment of metastatic and locally advanced disease to application in a neo- and adjuvant setting in earlier stages of high-risk disease (109,110). Currently, ICIs are the standard treatment for numerous types of solid tumor (111) and their application is expanding following study of their effects in combination with radiotherapy, chemotherapy and targeted therapy (4).

New immunotherapies have a notable effect on Treg subpopulations (107). Immunotherapeutic agents targeting lymphocytes alter the immunological tumor microenvironment, thereby decreasing the promoting effect and facilitating induction of immunological tolerance to tumor tissue (82,87). Immunological tolerance may be considered as a deprivation of tumor support from the host (112). Tumor-infiltrating Tregs are functional and their activity is mediated by apoptosis (93,113,114). A subset of regulatory T cells with high expression of functional receptors CTLA-4 and PD-1 realize effector functions, such as motility, migration and apoptosis (115). These Tregs express similar receptors (PD-1, lymphocyte-activating 3 and T cell immunoreceptor with Ig and ITIM domains) (116).

Based on the association between decreased number of Tregs and tumor regression, it is hypothesized that tumor undergoes involution, becoming deprived of Treg-mediated signaling due to successful blockade of the PD-1/PD-L1 axis (6). The functional failure of Tregs resulting from PD-1/PD-L1 checkpoint blocking is the initial step in a series of reactions in the host-tumor system that leads to tumor regression (21). Activated mononuclear phagocytes/macrophages are required in PD-1-based therapy in experimental models, because destroyed/damaged tumor cells trigger the process of phagocytosis (117).

The self-regulatory loop, provided by feedback mechanisms from the peripheral to the central immune system, ensures functional activity of Tregs in tumor tissue (116). The generation of adaptive immunity to cancer is a cyclical process that can be self-sustaining, leading to amplification and broadening of the T cell response (118). Regulatory feedback mechanisms of the immune cycle can promote or limit development of immune reactions (119). Experimental data have confirmed the participation of regulatory T cells in tumor progression and the presence of central immune-mediated mechanisms that regulate tumor spread (95,120). Moreover, according to experimental and clinical data, the central mechanisms of immune regulation determine the nature, direction and results of cell interactions at the tissue level (2,121). A novel therapeutic ICI strategy has shown that only functional blockade of lymphocytes regulating homeostasis in tumor tissue leads to significant tumor regression due to deprivation of immunological support from the host (21).

7. Discussion

The therapeutic effect of immune checkpoint inhibition can successfully cure certain patients with solid tumors. However, it is unclear why PD-LI blockade yields a complete response in only certain patients, especially given all ICIs have a common focus of action in targeting inhibition of the immune checkpoints of the PD-1/PD-L1 axis. Improving the effectiveness of ICI therapy and expanding the population of responders requires clarification of mechanisms underlying successful response to inhibition of immune checkpoints. However, theoretical explanation of the actions of ICIs at the cellular level does not allow clinicians to evaluate their integral implementation at the organism level (107).

Further research is required to determine the best predictors of response, how to distinguish between real progression and atypical patterns of response, such as pseudo- or hyper progression; and how to avoid dangerous side effects to achieve the feasibility of tailored treatment regimens and the

optimal duration of treatment (9). To address the aforementioned issues, comprehensive insight is needed concerning the events initiated by ICIs at cell, tissue and organ levels. The mechanisms underlying cross-interactions between tumor and immune cells in their environment have been studied (10); however, the role of central regulatory organs and circulatory systems remains to be investigated with regard to interactions in the tumor-host system. The lack of clear understanding of the mechanisms underlying ICIs complicates identification of clinically useful predictive biomarkers (122). Therefore, additional studies are required to uncover the immune mechanisms underlying tissue homeostasis.

Depletion of Treg pools is associated with successful antitumor therapy (90,94,95). Tregs have a similar phenotype irrespective of tumor location but are highly heterogeneous due to MHC-specific clonality (34). The cross-talk between Tregs and tumor cells is important because inhibition of this axis may lead to tumor shrinkage or progression (123). Due to Tregs being tissue-specific (antigen-experienced T cells), irrespective of them being a similar phenotype, the central mechanisms of tissue competency formation define interactions in the 'tumor-host' system (121). Despite the promoting role of tumor-infiltrating immune cells at each stage of the metastatic cascade (2,77,124), interactions between tumor and immune cells are traditionally considered in the context of tolerance or suppression only at the tissue level, without assessing the role of immune cell-mediated central thymic regulation. As Tregs are thymus-derived and operate in a tissue-specific manner (125), it is necessary to determine the centrally driven mechanisms underlying tumor regression following checkpoint inhibition and formation of self-regulatory loops that provide feedback mechanisms from central regulatory structures.

Tumor-infiltrating Tregs are functional and their activity is mediated by apoptosis via activation of PD-1 receptors (93,113). Apoptosis, essentially altruistic cell suicide that necessary for transmitting information to acceptor cells, underlies the effector mechanisms of immune cells (54). The aforementioned association between tumor regression and decreased numbers of Tregs supports the hypothesis that tumors deprived of essential signals from Tregs due to successful checkpoint blockage of the PD-1/PD-L1 axis undergo involution (112). Tregs may exert a regulatory effect and deliver key information to tumor tissue (126).

Tregs serve a key role in maintaining homeostasis in normal (127) and tumor tissue (29) and interactions between tumor cells and Tregs are targets for ICIs. Moreover, interactions between Tregs and tumor cells via the PD-1/PD-L1 axis are targeted by ICIs, which can act on both the lymphocyte receptor (PD-1) and the tumor cell ligand (PD-L1). When starting immunotherapy, quantitative assessment of tumor cells, active lymphocytes and the rate of generation of novel antigen-specific T cells are typically unknown. The conventional practice consists in initial measuring of only tumor markers such as PD-L1 expression and microsatellite instability, while it is also possible to quantify fluctuations of tumor-specific Tregs in the blood at varying time points during treatment to establish a host pattern.

The function of monoclonal antibody (mAb) is realized by Ab binding affinity with a specific antigen (128). Accordingly, a defined quantity of mAbs targeting PD-1 or PD-L1 decreases receptor/ligand ratio on the target cell by a specific amount. A number of specific Tregs serve a key role in treatment outcome (129), but it is difficult to quantitatively assess the interaction between Tregs and tumor cells, calculate a patient-specific mAb dose and determine the optimal duration of therapy, taking into consideration the distinct tumor mass, tumor burden and number of tumor-specific Tregs in patients of various ages and T cell response.

Through the in-depth dissection of existing data and inferences using Hermann Hesse's 'Glass Bead Game' principles (for example, intellectual synthesis of scientific facts and evidence of all ages to make them into organic whole), a multilevel mechanism of action of ICIs that better reflects real-life situations with various clinical outcomes is envisaged. The thymus generates T cell clones tailored to tumor tissue in response to tissue-specific signals. Lymphocytes infiltrating the tumor are predominately Treg with tumor-promoting activity. During effective therapy, a direct association between tumor regression and a decrease in Treg population is observed; blockade of immune checkpoints causes a functional failure of Tregs, resulting in tumor deprivation of co-stimulation signals. Depletion of Tregs as a result of checkpoint inhibition is the first step in a series of reactions in the host-tumor system leading to tumor decrease or progression. The dynamics of self-regulatory mechanisms preferentially maintain one of the processes at a given time: either growth or regression of tumor tissue.

The goal of effective inhibition of immune checkpoints is to reverse tumor progression, including by changing the direction of self-regulatory mechanisms (130,131). The interaction between receptors on immune and tumor cells and rate of generation of new clones of tumor-associated T lymphocytes determine the effects of ICIs at the tissue level (Fig. 3B).

8. Conclusion

Present antitumor immunotherapy-based strategies, namely immune checkpoints inhibitors, aimed at specific blockade of the tumor-associated part of the adaptive immunity show promising results. Neutralizing pro-tumor activity of immune cells in a case of effective treatment leads to deprivation of tumor tissue activity and provides a chance for preferential development of the essential normal tissue program. Novel immune therapies with ICIs demonstrate that regulation of the immune system serves a key role in tumor growth and metastatic spread (2,77,124). The aforementioned treatment options have highlighted potential mechanisms that affect solid tumors by targeting tumor cells through the microenvironment via immune-regulated mechanisms (21). Therefore, it is imperative to identify the population of tumor-associated immune cells that can be exploited for selective therapeutic intervention without affecting cells elsewhere in the body. Successful therapy aims to switch growth and development of tumor tissue to tumor shrinkage and involution. ICIs induce this change by blocking tumor-associated immune cells. Methodologies combining imaging-based biomarkers with tumor markers and host's tumor-specific immune characteristics are needed to improve patient selection and monitoring during immunotherapy. Enhanced imaging modalities and

laboratory-determined predictive markers may allow development of criteria to predict patient response to immunotherapy. Clarifying the underlying mechanisms of immunotherapy may identify the point at which pro-tumor activity of immune cells occurs and improve patient outcomes by inhibiting or reversing tumor growth.

Availability of data and materials

Not applicable.

Authors' contributions

NL has analyzed and systematized data and evidence related to the immune checkpoints to create a more comprehensive picture of their mechanism of action. The author has read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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