Chapter 1
The Basic Concepts in Cancer Immunology and Immunotherapy

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Why Do We Have Cancer?

Cancer is a cellular disease resulting from the uncontrolled growth of tumor cells. A massive amount of tumor cells accumulate in one or more parts of the body or spread throughout the blood. Documentation of human cancer can be found in literature dating as far back as 3,000 years ago in Egypt, and the search for the cause

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never comes to an end. While inheritance and environmental factors (ultraviolet radiation, pollution, etc.) are attributed as major causes of human cancer, a recent report pointed out that random errors in replication of genetic materials (genome) in our bodies seem to play a key role in cancer generation (Tomasetti et al. 2017). Once a genomic error happens, its consequence may or may not be harmful to our bodies and the cells harboring the error. The error or mutation (changes) in the genome can cause a normal cell to become a tumor cell. These errors in the genome are responsible for two-thirds of the mutations in human cancers (Greenman et al. 2007). In its early stages, a cancer cell does not look so different from other normal cells, yet they may behave differently, such as continuing to proliferate (divide) and consuming extra nutrients (higher metabolism). Contrary to normal cells, cancer cells have lost control over their proliferation; nothing can stop them until they take over the whole body. Since the errors that happen in our genome are random, most of our cancers may not be predictable or realistically preventable at the genetic level. However, not all mutations or errors in our cells will lead to cancer. We have both internal- and external-checking systems to monitor what happens to the cells in our bodies. If all these checking systems fail, tumor cells will proliferate and take control—the disease spreads throughout the body, eventually resulting in death if not treated.

The internal-checking system consists mainly of tumor suppressor genes that suppress the development and growth of mutant cells in a process called "programmed cell death." In this process, some enzymes will be activated to cut the genetic material of cells into small fragments that will stop the proliferation and survival of the cells. Basically, our cells are programmed to die if they detect any mutations within their genes that they cannot correct. If the tumor cells escape this internal check, they will face an external check that is mediated by the immune system. Our immune system has developed the ability to check for tiny changes in cells. Immune cells have very specific "eyes" to identify any subtle changes in nearby cells. The "eyes" of immune cells are receptors that function to detect very specific changes in our cells and will activate our immune cells accordingly. Usually, these changes are in the proteins (structure and function molecules) expressed by tumor cells. Cancer cells usually harbor many altered and abnormal proteins due to errors in the genome coding the proteins, or due to uncontrolled production of some proteins that should have been shut down when cells matured past their early stages. Certain environmental factors may also cause damage in the genome that results in the production of altered proteins. When our immune cells detect these altered proteins on the surfaces of the tumor cells, they will recognize them, become activated, and eventually destroy the tumor cells. As long as our immune system can recognize these changes inside any cancer cells, the cancer cells cannot accumulate and develop into a disease. Therefore, cancer is ultimately a disease caused by unlimited growth of tumor cells that escaped the attack of the immune system.

How Does the Immune System Protect Us from Cancer?

Many of us have tumor cells in our bodies, but most of us do not develop cancer as a disease. Our immune system prevents spontaneously generated tumor cells from developing into cancers. This phenomenon has been reproduced in animal models and has prompted a theory of "immune surveillance." There are four pieces of evidence that support this theory that the immune system indeed responds to cancer. First, humans with genetic defects in their immune systems tend to have a higher incidence of cancer than those whose immune systems are intact. Second, humans who have their immune systems suppressed by medicine in order to avoid rejection of transplants have higher cancer generation than people with normal immune function. Third, some cancer patients have "paraneoplastic syndromes" that are caused by the immune system's response to a cancer. For example, patients with lung cancer may develop disorders in the central nervous system (CNS) due to immune responses to certain proteins shared by CNS and lung cancers. It is further proof that internal immune responses to tumors are present as they can even begin to attack the normal tissues that share the same proteins with tumors. Last but not least, immunotherapy has been used to treat some human cancers in recent years. Immunotherapy regimens do not directly kill tumor cells but boost the immune system to find and destroy cancer cells. The success of this therapy provides direct evidence that we have pre-existing immune responses to cancer in our body, but at times they do not function as well as they should. However, once we give them a boost, they will do a great job in attacking cancer.

Two Types of Immune Responses

The two types of immune responses differ in their specificity of recognition and speed of response. One is called the innate immune response, in which innate immune cells lack precise specificity in recognition of their targets but have a rapid response to them. Macrophages (large eater cells) and natural killer (NK) cells are the main innate immune cells. They recognize their targets based on the general patterns of molecules expressed by target cells or pathogens.

The second response is called the adaptive immune response. Adaptive immune cells have a very restricted specificity in recognition of their targets, but usually have a delayed response to their targets because they need more time to divide and produce attacking molecules. There are two major sub-populations of adaptive immune cells: T cells and B cells, also called T lymphocytes and B lymphocytes (since they were originally identified in lymph nodes). The "T" in T cells means that these cells develop in the thymus, and the "B" in B cells means they develop in bone marrow. They recognize their target cells or pathogens using receptors (eyes) that are designed only for a very specific antigen. An antigen is a protein molecule or any substance capable of inducing an immune response that produces antibodies

(antigen-binding proteins) or attacking molecules. As this specificity is so detailed, T cells or B cells can recognize any tiny changes in a protein molecule. In order to recognize any potentially changed proteins or pathogens, our bodies have been bestowed with 300 billion T cells and 3 billion B cells. Normally we only have a few T cells for each single antigen in our bodies, but we can have thousands of them once they are activated by antigen stimulation and undergo expansion (proliferation). Although we cannot see the expansion of T cells, we can feel them. When you feel enlarged lymph nodes in your body after infection, these signs usually tell you that millions of immune cells proliferated.

Innate Immunity to Cancer

As we learned above, the innate immune response is fast, but not restricted to specific antigens. It is still unclear how innate immune cells recognize tumor cells, but they do have the ability to kill tumor cells once they are activated by environmental cues. Macrophages and NK cells are the two major types of innate immune cells that can attack tumors. There are other innate immune cells that do not directly kill tumor cells, but can present proteins expressed by tumor cells to other immune cells to instruct them to target these tumors. For example, dendritic cells (DCs) are innate immune cells that can present tumor proteins to adaptive immune cells (like T cells) and help activate T-cell responses; thus, the dendritic cells act as a "bridge" between the innate immune system and the adaptive immune.

Macrophages are big eater cells. Macrophages are present within most tissues of our body in order to clean up dead cells and pathogens. Once activated by environmental cues (like materials released from bacteria, viruses, or dead cells), macrophages infiltrate deep into tumor tissues and destroy cells via production of toxic oxygen derivatives (reactive O_2 intermediates) and tumor necrosis factor (TNF), or they directly eat the tumor cells (known as phagocytosis). In order to escape being eaten by macrophages, some tumor cells express "don't eat me" signal molecules to fool macrophages and escape them. Recently, reagents have been developed to block the "don't eat me" molecules on tumor cells. An example of a "don't eat me" molecule is CD47. CD47 on tumor cells interacts with signal-regulatory protein alpha (SIRP-α), an inhibitory receptor present on macrophages. Since engagement of CD47 with SIRP-α inhibits macrophage phagocytosis, blocking CD47 may enhance the "eating" of tumor cells by macrophages (Tseng et al. 2013). Thus, macrophages are "tumor" eater cells, but tumor cells can find a way to escape them. Recently, some drugs (e.g., CD47 antibody) that can help macrophages to eat tumor cells have been tested in clinical trials.

NK cells are circulating immune cells in our blood system and are believed to serve as the earliest defense against blood-borne metastatic tumor cells. In order for tumor cells to be recognized by NK cells, there must be something that distinguishes them from normal cells—such as expressing something abnormal and/or failing to

express something normal. NK cells are called natural killers because they do not need to be "coached" to see very specific antigens for their activation. They respond to their target cells by searching whether something is "missing" on the cell surface. In this regard, NK cells help us clean out many cancer cells in very early stages or those cancer cells circulating in our blood where we have plenty of NK cells. Patients with metastases have abnormal NK activity, and low NK levels are predictive of eventual metastasis. Recent studies suggest that some NK cells have a "memory" capability to recognize certain tumors or pathogens. However, there are limitations in NK cell-mediated antitumor immunity. First, only tumor cells with "missing" markers can be detected by NK cells. Second, there are a limited number of NK cells present in the bloodstream, as only 10% of lymphocytes are NK cells. In addition, tumor cells can avoid NK cell attacks by expressing immune suppressive molecules to inhibit NK cell function, in a manner similar to how they can avoid getting eaten by macrophages. To improve NK cell function, a cytokine called interleukine-2 has been used to activate NK cells for expansion.

Adaptive Immunity to Cancer

In contrast to innate immunity, the adaptive response is slower, specific to certain antigens, and has memory (can provide life-long protection). Since adaptive immune cells can remember antigens from their first encounter, they can respond to antigens much faster when they encounter the same antigens again. This process is called "immune memory" and is the foundation of protective immunization. The eyes of adaptive immune cells are "near sighted." They need a very close cell-to-cell contact to clearly and specifically "see" their antigen on the target cells. In order to remember their target antigens, adaptive immune cells need professional antigen-presenting cells (APCs) to "teach" them how to see and how to respond. Dendritic cells are professional APCs. Dendritic cells are called "dendritic" because they have dendrites (branches) that extend to surrounding tissues to catch proteins released from pathogens or tumors, but they cannot eat whole cells like macrophages. Once they catch proteins (antigens), they will "eat" (phagocytosis) and "digest" them using enzymes (degradation), and then "present" them in an antigen-presenting structure on their surfaces. The antigen-presenting structure is called the major histocompatibility complex (MHC) that is a set of cell surface protein complexes that contain a "pocket" in order to hold an antigen. The main function of MHC is to display antigens on the cell surface for recognition by the appropriate T cells (Fig. 1.1). Thus, the MHC is like a gauge indicating whether there are tumor antigens within a cell to which the immune response will be turned on to them.

There are two types of adaptive immunity: cellular (T-cell) and humoral (B-cell) immunity. T cells consist of CD8 and CD4 T cells. CD8 T cells are also called cytotoxic T lymphocytes (CTLs). They are the primary killers of tumor cells because they can distinguish cancer cells from normal cellsand directly destroy cancer cells.

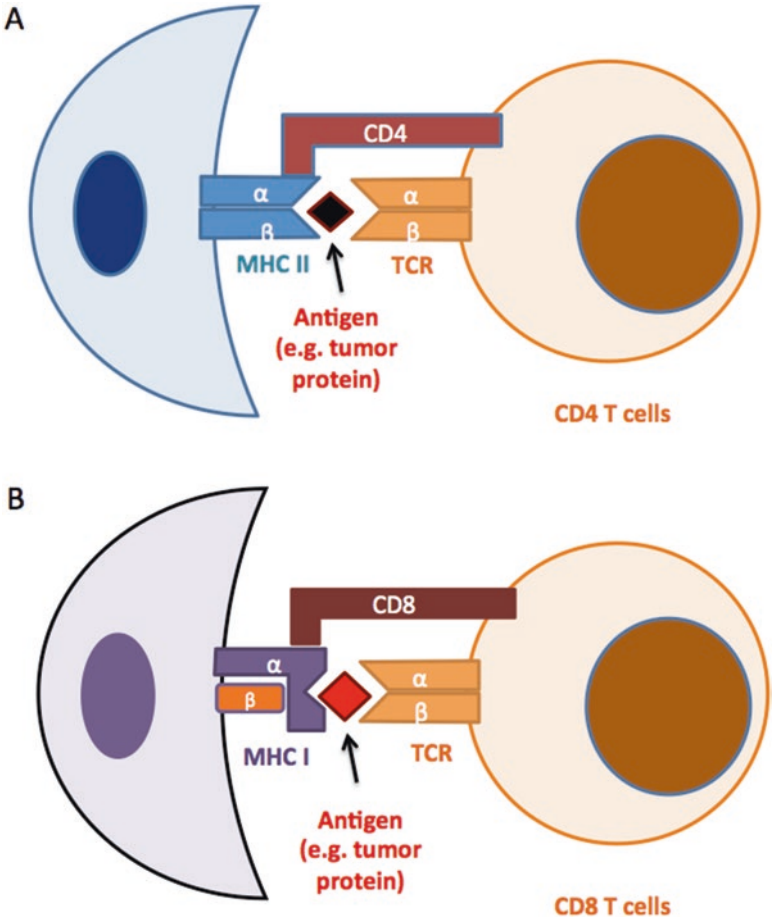

Fig. 1.1 Tumor antigen can be presented to CD4 T cells through the major histocompatibility complex II (MHC II) or to CD8 T cells through MHC I in order to activate T-cell receptors (TCRs)

CTLs kill cancer cells via a quick yet well controlled cell-to-cell contact process. They start by digging a hole in the cancer cells and inject enzymes that can dissolve their inner materials. Some injected enzymes can cut the genetic materials into very small pieces leaving the cancer cells to die in a manner known as apoptosis (a Greek word meaning falling apart). As for CD4 T cells, their major function is producing soluble proteins called cytokines. These cytokines are messages sent by CD4 T cells to regulate or help the function of other immune cells during an immune response. Some cytokines are called interleukins (ILs), because they deliver messages between leukocytes (white blood cells). CD4 T cells that use these messages to help other immune cells are called T helper cells (Th). We have several types of T helper cells according to their different production of cytokines (Th1, Th2, Th17, etc.). Among them, Th1 cells play a key role in suppressing tumor growth because they produce

a cytokine called interferon (IFN)-gamma that can inhibit the growth of tumor cells. Some CD4 T cells also have a cytotoxic function like CTLs in killing of tumor cells.

In order to kill tumor cells but not normal cells, T cells need to specifically distinguish tumor cells from normal cells. They can do this because tumor cells, unlike normal cells, express unique tumor antigens that can induce T-cell responses. It took a long time for people to discover tumor antigens because they are embedded in MHCs, rather than standing alone on the cell surface as people originally perceived. Dr. Boon and his colleagues discovered the first human cancer antigen in melanoma (van der Bruggen et al. 1991). They found a way to "flush out" the small protein fragments (called peptides) that are embedded in a pocket of MHC. There are two classes of MHCs: class I and class II. The class I MHCs present antigens to CD8 T cells, and the class II MHCs present antigens to CD4 T cells. Class I MHC molecules are expressed by almost every cell in humans, while class II MHC molecules are restricted to certain immune cells like macrophages and lymphocytes. The antigen peptides presented in MHC are recognized by T-cell receptors (TCRs) expressed by T cells. TCR is very specific for each tumor antigen peptide in a particular MHC. A T cell only expresses one type of TCR and can only recognize one type of antigen.

Unlike T cells, B cells do not kill tumor cells directly, but produce antibodies as their attack molecules. Their antibodies function like "catchers" that can grasp their target antigens. There are five classes of antibodies: IgG, IgM, IgA, IgD, and IgE, based on their different chemical structures and functions. IgG is the major antibody type that crosses the placenta to provide protection for the baby. IgM is the largest antibody in our bodies. IgA can be released to our intestines to control infections in our digestion system. IgE is the major antibody to control parasites but also causes allergies. IgD functions like a receptor for the activation of B cells. Each antibody can only bind to one antigen. Once antibodies bind to antigens, they either block the function of their target molecules or direct other immune cells (like macrophages and NK cells) to kill the target cells that express the antigens, a process called antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC plays a key role in the treatment of human cancers, especially cancer cells in the blood.

The Efficiency of the Immune System: The Power of Diversity

Since an immune (T or B) cell only can recognize a tiny part of an antigen and only a few cells have this specificity, the efficiency of the immune system in responses to any altered proteins or pathogens can be very low. To increase efficiency but not compromise specificity, diversity is granted to the immune system. This diversity is achieved at the genetic level in order to produce a battery of different kinds of receptors or antibodies for recognizing different antigens, and different MHC for presenting different antigens. Based on the gene arrangements that generate the diversity of MHC in a single cell, one cell can express at least 12 different MHCs

that present at least 12 kinds of different epitopes of an antigen. An epitope is the smallest portion of an antigen protein that can be recorgnized by T cells. For example, if a pathogen virus infected a host cell, this host cell can present 12 kinds of epitopes of a viral protein. Accordingly, there will be at least 12 kinds of T cells that express receptors for each antigen epitope and will be able to recognize the viral antigens of the infected cells and destroy them in order to stop infection. In the case of tumor cells, if a tumor cell has two tumor antigens, the tumor cell can present at least 24 kinds of epitopes and will stimulate 24 kinds of T cells. Since only one T cell is enough to kill one tumor cell, if we have 12 or 24 kinds of T cells in place, we will have more than enough T cells to kill the tumor cells. Therefore, the diversity of our immune system is a crucial mechanism in protection and elegantly balances the specificity of each immune cell.

Why Does the Immune System Fail to Control Cancer Cells?

If the immune system has the ability to protect us from cancer, why do some of us develop cancer as a disease? It has been observed that in many patients, cancer cells are surrounded by immune cells in tissues or co-exist with tumor-reactive immune cells in the peripheral blood. Despite this, their cancer continues to progress and spread all over the body. We have a name for this enigma: the Hellström paradox, after Ingegerd and Karl Erik Hellström, two immunologists who first described this paradox more than 50 years ago (Hellström et al. 1968). In the last few decades, many efforts have been made to tip the balance in favor of the immune system based on the assumption that there are not enough immune cells to keep the cancer cells in bay. Most recently, we realized that even if there are plenty of immune cells capable of killing cancer cells, these immune cells can be killed or suppressed by cancer cells at tumor sites. The fight back from cancer cells is so powerful that many tumor vaccine therapies and T-cell transfer therapies failed to control cancer due to the barriers built up in the tumor sites. The discovery of B7-H1 (also named PD-L1) expressed by human tumor cells opened a door for us in our understanding of how tumor cells escape immune surveillance (Dong et al. 2002). PD-L1/B7-H1 is used by cancer cells to disarm the immune system and blocking of PD-L1 restores the antitumor function of immune cells (Dong and Chen 2003; Iwai et al. 2002). PD-L1 and other immune regulatory molecules (CTLA-4, PD-1, B7-DC/PD-L2, etc.) are collectively called “immune checkpoint molecules” as they function as barriers for restraining immune responses. Accordingly, immune checkpoint blockade therapy is applied to restore the function of tumor-reactive immune cells by lifting the checkpoint barriers (Pardoll 2012; Korman et al. 2006). The success of immune checkpoint blockade therapy also tells us that the suppression put on the immune system is reversible as long as we have the right tool to do so. In the following sections, you will learn how the immune system is regulated, how cancer cells usurp the self-protection mechanism for their own safety from immune cells, and novel strategies in the treatment of cancer based on new discoveries.

The Checks and Balances on the Immune System

While the diversity of our immune system protects us from pathogen infections or cancer cells, it also mounts a great risk for us to be attacked by our own immune system if this system goes out of control and responds to any changes identified in our bodies. To avoid the overreaction of our immune system, a battery of checkpoint molecules is put in place to check and balance our immune responses.

Immune cell activation means a status of immune cells where they proliferate more and produce molecules that affect the fate of targeted cells. However, it is not an easy job to activate immune cells. Only specific antigens trigger the receptors of immune cells, and these specific antigens function like a specific key that is used to turn on the engine of a car. Antigen stimulation alone usually is not able to activate T cells, just like a car cannot move faster when only the engine is turned on. To have the car move faster, we need to press down the accelerator pedal in order to inject more gasoline into the engine. The accelerators of immune cells are called co-stimulatory signals. Immune cells need co-stimulatory signals to become fully activated. For safety, we also need a brake system to control the speed or movement of a car. The brake system on immune cells is called immune checkpoint molecules. When we are driving a car, we frequently use both the gas pedal and the foot brake to balance the speed according to surrounding situations in order to have a smooth and safe drive to our destination. During a process of immune responses, immune cells also need to consistently receive both co-stimulatory and checkpoint signals in order to work specifically and efficiently in places where they are eliminating infected cells or cancer cells.

Mechanisms Used by Tumor Cells to Escape Immune Attack

First of all, cancer cells do their best to hide from the detection of the immune system. Since tumor antigens presented by MHC can reveal their identity to the immune system, most of the time, tumor cells turn down or even turn off their presentation of tumor antigens by downregulation of MHC (antigen-presenting complex) expression or production of tumor antigens. Tumor cells accumulate many mutations in their MHC molecules that prevent them from being appropriately expressed, thus dampening the ability of MHC to present tumor antigens. In addition to MHC, tumor cells may also turn off the machines that can produce tumor antigens inside cells.

Tumor cells also take the advantage of the brakes in the immune system. Tumor cells express immune checkpoint molecules to actively turn down the immune responses against them. One important molecule is called B7-H1, which was discovered at Mayo Clinic in 1998 (Dong et al. 1999). B7-H1 was renamed PD-L1 in 2000 because it was found to be a ligand (binder) of PD-1 (Freeman et al. 2000). PD-1 is another important molecule of the immune system that was discovered in

1992 (Ishida et al. 1992). PD-L1 is expressed by most human solid cancer cells (Dong et al. 2002). When activated immune cells that express PD-1 come to tumor sites and get closer to tumor cells, their PD-1 will be engaged by the PD-L1 expressed by tumor cells. Once engaged, PD-1 transmits signals into those immune cells that will cause them to either die or lose their immune function (Dong et al. 2002; Iwai et al. 2002). That is where PD-1 gets its name from—programmed death-1 molecule.

Therefore, it is no surprise that high expression of PD-L1 predicts poor survivorship of patients with renal cell carcinoma, lung cancer, ovarian cancers, and some other cancers (Thompson et al. 2006). By extension, therapeutically targeting the interaction of PD-1 and PD-L1 using antibodies that block this contact will be able to restore the antitumor function of immune cells. Recently, the US Food and Drug Administration (FDA) approved two anti-PD-1 drugs and three anti-PD-L1 drugs in the treatment of several human cancers. Of note, all these drugs are antibodies specific for PD-1 or PD-L1. Once these antibodies are injected into the blood of cancer patients, they will find their target molecules PD-1 on T cells or PD-L1 on tumor cells and bind to them. Once these molecules are bound by appropriate antibodies, the interaction of PD-1 and PD-L1 will be blocked, and tumor cells cannot use PD-L1 to engage PD-1 and suppress T cells that are capable of killing them (Fig. 1.2).

Before immune cells travel to tumor sites, their function is also regulated at lymph nodes that are close to tumor sites. Another immune checkpoint molecule called CTLA-4 (cytotoxic T lymphocyte antigen-4) is expressed by activated T cells. CTLA-4 provides negative feedback signals for T-cell activation. To release the regulation of immune cells at lymph nodes and promote their potential antitumor function, CTLA-4 blocking antibodies became the first regimen in cancer immune checkpoint blockade therapy (Leach et al. 1996). CTLA-4 not only regulates tumor-reactive immune cells, but also self-reactive immune cells in the lymph nodes. Thus, a global blockade of CTLA-4 has the risk in increasing autoimmune responses, some of which could be fatal. Caution should be exercised in monitoring patient responses to immune checkpoint blockade therapy, either PD-1/PD-L1 or CTLA-4, to avoid and prevent potential risks of autoimmune responses, which are called self-toxic effects.

In addition to these immune checkpoint molecules, tumor cells invite their "friends" to help them escape immune attacks. They attract myeloid-derived suppressor cells (MDSCs) to help them turn down the activation of immune cells within tumor sites or lymph nodes near the tumor tissues. MDSCs are bone marrow cells in the process of becoming antigen-presenting cells, but their normal development process is disrupted by tumor cells that recruit them to suppress antitumor immunity (Bunt et al. 2006). Lymph nodes and tumor sites contain another type of immune cells called regulatory T cells (Treg), which also help tumors by dampening immune responses (Curiel et al. 2004; Casares et al. 2003). Treg cells have the ability to inhibit the antitumor function of tumor-reactive immune cells by competing for their nutrition or reducing T-cell activation.

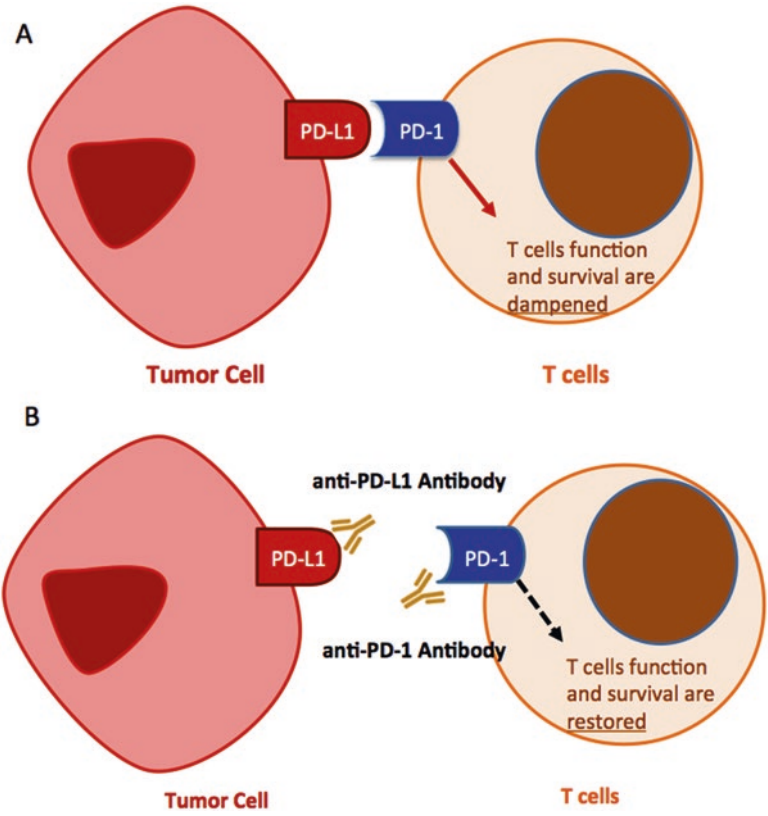

Fig. 1.2 (**a**) Tumor cells that express programmed death ligand 1 (PD-L1) activate the programmed death receptor 1 (PD-1) to inhibit T-cell function and cause T cells to die. (**b**) Antibodies that target PD-L1 or PD-1 can be used to stop tumor cells from suppressing T cells, allowing T cells to be reactivated to fight the cancer

Tumor cells can actively release soluble molecules to create an environment that is harsh to immune cells. They produce VEGF (vascular endothelial growth factor) not only to promote blood supply for themselves, but also to suppress the function of antigen-presenting cells (dendritic cells) and avoid having their identity exposed to the immune system. They also release cytokines (TGF-beta, IL-10, etc.) that directly inhibit the activation or function of immune cells. As we know, immune cells require a significant amount of energy to do their job, and to interfere with this an enzyme called indoleamine 2,3-dioxygenase (IDO) is produced in tumor tissues. This enzyme helps tumor cells use most of the essential amino acid tryptophan in the tumor environments, thus dramatically reducing the levels of tryptophan, which is a critical "food" for immune cells. When immune cells are starved of tryptophan, they lose their ability to fight cancer cells. To prevent that happening, drugs that block the function of IDO have been tested as cancer immunotherapy agents (Friberg et al. 2002).

Strategies to Harness the Immune System to Fight Cancer Cells

Cancer immunotherapy works through the immune system to control cancer; therefore, its direct target is the immune cells rather than the tumor cells. Cancer immunotherapy is aimed at restoring or enhancing the capability of immune cells to recognize and destroy cancer cells, and the therapeutic effects will be determined by the extent to which the immune cells eliminate tumor cells. While it is unreasonable to expect the immune system to deal with large tumor masses, reducing the tumor burden seems to increase the chance of success of immunotherapy. The ideal scenario is that sufficient numbers of tumor-reactive T cells (with "highly avid recognition") are generated by appropriate tumor-antigen stimulation, and T cells are able to move to tumor sites where they can destroy cancer cells with cytotoxic enzyme (granzyme B) or cytokines (TNF-alpha or IFN-gamma). Since PD-L1 (B7-H1) expressed by tumors suppresses this process (Dong and Chen 2003), PD-L1 blockade is required to improve this antitumor immunity. Successful immunity will lead to another round of immune response by releasing more tumor antigens through destruction of tumor cells. This process is called the cancer-immunity cycle (Chen and Mellman 2013), describing the sequence of events in priming, expansion, and effector phases of T-cell responses to tumors.

Improving Antigen-Presentation to Prime More Immune Cells

Activation of the immune system to bring therapeutic benefit to cancer patients has been the subject of more than 100 years of study. Dr. Coley is believed to be the first physician to perform clinical trials in the treatment of cancer patients using dead bacteria (Coley 1906). His idea was that a strong immune response triggered by pathogens that can cure an infection would be able to cure a cancer as well. Thus, the so-called Coley's toxin was originally used as a trigger of the immune system to treat human cancer. Most of his trials failed, but occasionally some of his cancer patients experienced tumor regression. From his pioneering work on cancer immunotherapy, immunologists have learned the immuno-stimulatory power of his "toxin" and dissected its effective components to discover new functions of immune adjuvants (enhancers). These adjuvants have been tested to help immune responses to tumor vaccination. For example, the dead bacteria bacille Calmette-Guérin (BCG) that causes tuberculosis has been successfully used in the treatment of human bladder cancer. BCG is used to induce inflammatory cytokine production, therefore increasing efficiency of tumor antigen presentation from dead tumor cells.

Some defined tumor antigens or irradiated tumor cells can be used as vaccines in combination with certain powerful adjuvants to prevent tumor growth. Most

human tumor antigens are normal, unmutated proteins that are aberrantly expressed on tumors. For example, gp100 and MART-1 tumor antigens are human melanoma tumor antigens, mainly expressed by melanoma cells rather than by normal cells. They are real tumor antigens because they can induce an immune response against the melanoma cells expressing them. However, these antigens need to be presented to immune cells by professional APCs. This requirement prompted the study of dendritic cell (DC) therapy using dendritic cells as APCs to present tumor antigen vaccines. The dendritic cells can be isolated from a patient's blood, loaded with the tumor antigen, and then re-injected into patients. Currently DC therapy is a complicated, costly procedure, but could be streamlined in combination with other immunotherapy. Besides tumor antigen-based vaccine therapy, viruses have been used as a new strategy to treat human cancers. Viruses that can attack tumor cells and cause them to die are called oncolytic viruses and have been selected for this treatment. Oncolytic viral therapy not only directly destroys the infected tumor cells, but also releases inflammatory mediators and tumor antigens that would further induce immune responses against the cancer.

Improving Immune Cell Expansion and Differentiation

Following activation by tumor antigens, T cells undergo proliferation either before or after they enter tumor sites. During this expansion period, they need additional signals to maintain their proliferation and to gain effector functions. To that purpose circulating proteins called cytokines provide them with the needed stimulation. Among them, interleukin 2 (IL-2) is an important factor for T cells to expand and become effector cells. The drug form of IL-2 (Proleukin®, aldesleukin) has been approved to treat human melanoma and kidney cancers based on their ability to help effector T cells to grow. However, the expansion of T cells is negatively controlled by the regulatory T cells (Treg). Before proliferation, T cells need to be activated. T-cell activation requires two signals—one is antigen stimulation through T-cell receptor (TCR), and the other is co-stimulation through CD28. CD28 receives signals from APCs by binding to B7 molecules expressed by APCs. Treg cells disrupt the interaction of CD28 and B7 by expressing CTLA-4 molecule because CTLA-4 has a higher affinity to binding B7 than CD28. Therefore, CTLA-4 can "out-compete" CD28 for binding and prevent co-stimulatory signals (Fig. 1.3). A current drug approved by the FDA in the treatment of cancer is ipilimumab (anti-CTLA-4). As an antibody to CTLA-4, it will block the function of CTLA-4 and prevent it from competing with CD28, resulting in more T-cell activation and expansion to fight cancers.

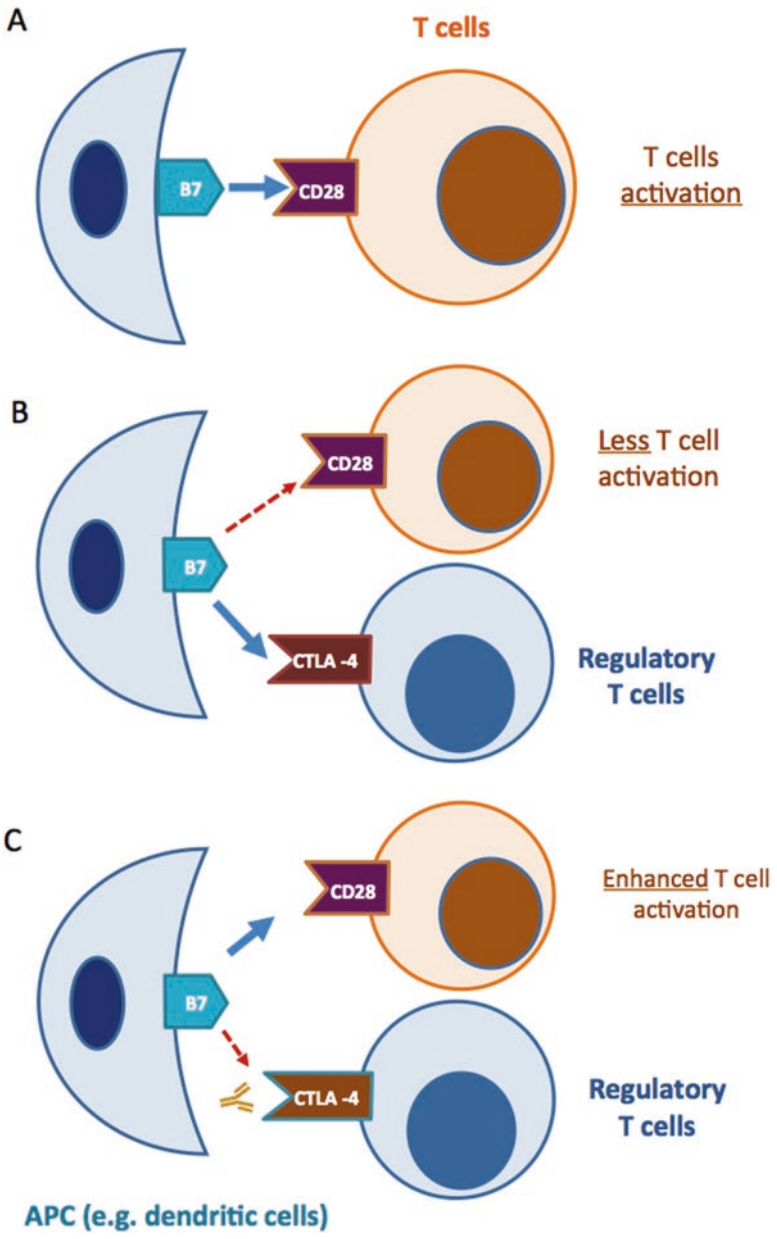

Fig. 1.3 (**a**) Antigen-presenting cells (APCs) use B7 to co-stimulate CD28 on T cells and activate them upon antigen stimulation. (**b**) Regulatory T cells have CTLA-4, which competes for B7 on the APCs, reducing the activation of T cells. (**c**) A blocking antibody to CTLA-4 can block CTLA-4 and let more B7 to interact with CD28, enhancing T-cell activation

Protecting Immune Cells from Suppressive Mechanisms Within Tumor Tissues

As mentioned above, once T cells are activated by antigen stimulation, they express high levels of PD-1, a receptor for transmitting signals that impede the function of T cells. While PD-1 expression could be good to prevent damage to healthy tissues or organs caused by over-activated T cells, PD-1 also restrains the antitumor activity of T cells. Tumor-reactive T cells tend to express high levels of PD-1 because they are continually exposed to tumor antigens in the tumor microenvironment. After such a long time of antigen stimulation, T cells become "exhausted." The hallmarks of exhausted T cells are weak function and poor survival. When PD-1 positive T cells come to tumor sites, their PD-1 will be engaged by PD-L1 expressed by most cancer cells. PD-L1 is a ligand of PD-1 and functions to induce T-cell death or reduce the T cell's ability to kill tumor cells. However, some exhausted T cells can be invigorated to restore their antitumor function and protected from cell death by blocking this PD-1/PD-L1 interaction (Gibbons Johnson and Dong 2017).

PD-L1 expression by tumor cells is an important mechanism of tumor immune evasion (a process tumor cells use to escape immune attacks). One effector molecule produced by T cells to suppress tumor growth is IFN (interferon) gamma that can directly inhibit the proliferation of tumor cells by impairing their DNA duplication. Interestingly, yet-to-be killed tumor cells take up a small amount of IFN-gamma as an inducer of their PD-L1 expression (Dong et al. 2002). This process is called adaptive resistance of the tumor to cancer immunity, as tumor cells become resistant to T-cell attacks when tumor cells increase their expression of PD-L1, which can disarm any T cells that approach (Taube et al. 2014). Not only do tumor cells express PD-L1, but some immune cells also express this ligand. APCs like dendritic cells and macrophages are known to express PD-L1. Since APCs play a key role in T-cell activation, they may use PD-L1 to restrain the degree of T-cell activation in order to prevent over-activation of T cells (Gibbons Johnson and Dong 2017). While it would be obvious that PD-L1 expression in human cancers may be useful in predicting response to PD-1/PD-L1 blockade therapy, patients with PD-L1 negative cancers have also responded to the same therapy. This suggests that PD-L1 expressed by host cells may be a therapeutic target similar to the PD-L1 expressed by tumor cells. PD-L2 (also called B7-DC) is another ligand of PD-1 (Tseng et al. 2001; Latchman et al. 2001). PD-L2 is not expressed by most human cancer cells, but is expressed by dendritic cells that infiltrate tumor tissues. Thus, PD-L2 expression could also be considered as a marker to evaluate the responsiveness to anti-PD-1 therapy. Besides PD-1 and PD-L1/2 pathways, many other immune checkpoint molecules (B7-H3, B7-H4, VISTA, PD-1H, Tim-3, LAG3, TIGIT, etc.) have been identified, and the therapeutic effects of blocking them have been tested in clinical trials (Yao et al. 2013). In the future, a rationalized and individualized combination of immune checkpoint blockade therapy could be formulated in one package that would fit individual cancer patients in order to improve the efficacy and reduce toxicity.

Adoptive T-Cell Transfer

As T cells are the final tumor killer cells, it has been speculated that injection of enough T cells into tumor sites would be able to reject tumors or suppress their growth. Since the early 1980s, T cells isolated from patients' tumors have been used to treat melanoma and kidney cancers (Rosenberg 2011). Recent technology allows us to expand T cells by thousands of times in culture dishes in a week or so. To increase the tumor-specificity of T cells, T cells are engineered to express receptors that are specific to tumor antigens present on the surface of tumor cells. These engineered T cells are called chimeric antigen receptor (CAR)-engineered T cells (CAR-T cells). Adoptive CAR-T cell transfer has achieved promising therapeutic effects in the control of some blood cancers. However, CAR-T cell therapy faces challenges in treatment of solid cancers as the transferred T cells may disappear rapidly if the tumor burden is large and may cause adverse effects in patients. More research is underway to improve its efficacy and safety.

Rational Combination in Cancer Therapy

Novel cancer immunotherapies targeting the immune checkpoint pathways have become a true paradigm shift in the treatment of patients with advanced cancers (Pardoll 2012), and are now the standard-of-care in seven different cancer types. However, only a small fraction of patients with solid cancers benefit from these immunotherapies with durable responses. One hurdle of cancer immunotherapy could be large tumor burdens. Strategies aimed to reduce tumor burden have the potential to promote efficacy or overcome resistance to cancer immunotherapy.

Radiation is known to trigger innate immune responses and impair immune suppressive cells. The potential of local radiation has been tested in many preclinical models to evaluate its ability to promote infiltration of tumor sites with effector T cells. To turn a local antitumor immune response to a systemic protection, additional methods are needed. Several clinical trials and preclinical studies have shown that administration of PD-1 or PD-L1 blockade can cause tumor regression in distant tumor sites that are not directly irradiated. This phenomenon is called the "abscopal effect" and suggests that immune cells primed at the irradiated sites would be able to circulate the whole body and find the same tumor cells and destroy them (Park et al. 2015). However, the optimal dose of irradiation and the timing of a combination of immune checkpoint inhibitors should be validated in future clinical trials.

The tumor cell death caused by certain chemotherapy agents provides a good source for releasing potential tumor-specific antigen proteins. This type of tumor cell death is called immunogenic cell death (ICD) (Obeid et al. 2007). In this regard, chemotherapy drugs that cause ICD could be used to "recharge" T cells by providing more tumor antigens that are released from dead tumor cells. This strategy could be

very helpful in cancer patients who otherwise do not have spontaneous release of tumor antigens. Releasing these tumor antigens to activate T cells provides an opportunity for the addition of immune checkpoint blockade that can further improve the expansion of activated immune cells. This can help achieve a good number of activated immune cells to overcome the resistance caused by the overwhelming numbers of cancer cells. Based on this potential synergy effect, the FDA recently approved the combined therapy of chemotherapy and immunotherapy to treat lung cancers.

Prospects of Cancer Immunotherapy

Cancer immunotherapy is a personalized medicine because each patient has his or her own unique immune responses to cancer. The uniqueness of immune responses to cancer is not only determined by the diversity and specificity of our immune system in presentation and recognition of tumor antigens, but also by the heterogeneity of cancer antigens within each patient. The future of cancer immunotherapy should be a rational combined therapy that will meet the specific needs of each patient. While this task is not easy and could be very costly, it is the goal we should to strive for. To achieve this objective, scientifically we need to address several fundamental questions in tumor antigen presentation, T-cell function, and regulatory mechanisms, and clinically we need to address the optimal dose and sequences of immunotherapy drugs and to define mechanisms of drug resistance. We also need biomarkers to identify and evaluate the tumor-reactive T-cell responses in cancer patients in order to predict and monitor patient responses to immunotherapy.

Finally, after reading through all these technical terms and explanations, you may have a question for me: what can I do to improve my own immunity to cancer? Although cancer immunologists can give you a list of ways in which to do that, I would suggest getting enough sleep. While we are waiting for scientific validation of my answer, I hope you did not doze off when you were reading this chapter. But if this chapter really helps you sleep, I would love to know that.

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