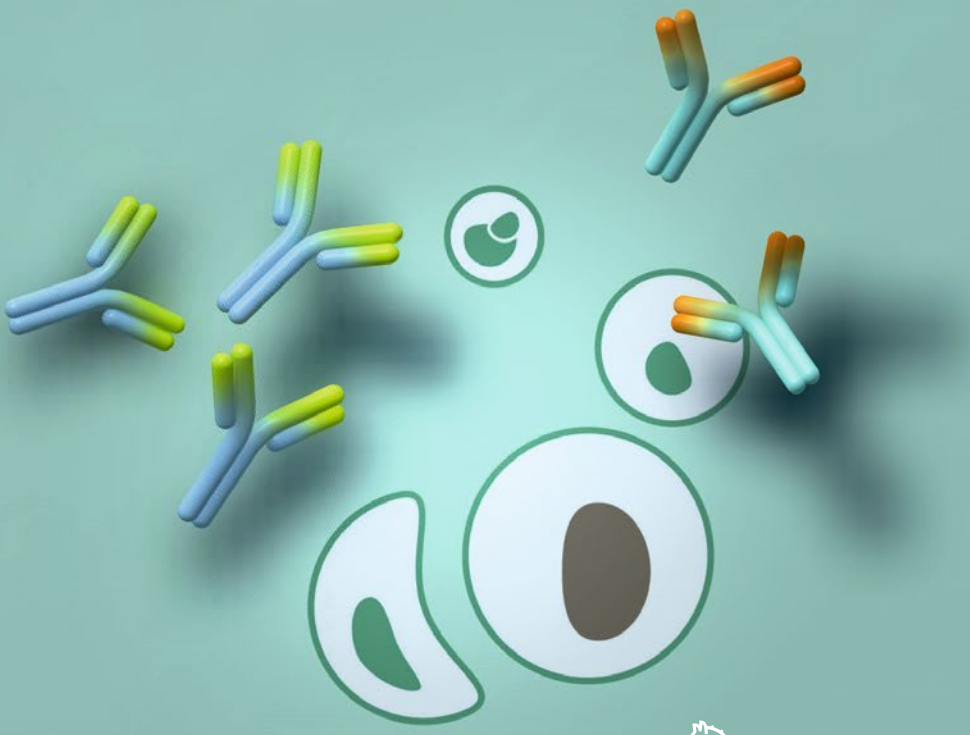
Haidong Dong
Svetomir N. Markovic *Editors*

The Basics of Cancer Immunotherapy



Springer

The Basics of Cancer Immunotherapy

Haidong Dong • Svetomir N. Markovic
Editors

The Basics of Cancer Immunotherapy

Springer

Editors
Haidong Dong
Department of Urology,
Department of Immunology
College of Medicine, Mayo Clinic
Rochester, MN, USA

Svetomir N. Markovic
Department of Oncology, Department
of Medicine/Division of Hematology,
Department of Immunology
College of Medicine, Mayo Clinic
Rochester, MN, USA

ISBN 978-3-319-70621-4 ISBN 978-3-319-70622-1 (eBook)
https://doi.org/10.1007/978-3-319-70622-1

Library of Congress Control Number: 2017961878

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Acknowledgements

We are grateful to the pioneers, our mentors, and colleagues in the field of cancer immunology and immunotherapy. We dedicate our book to our patients and their families for fighting cancer with us. Our research has been supported by the National Institute of Health, the National Cancer Institute, and the Mayo Foundation. We appreciate Dr. Khalid Jazieh, MBBS, for helpful review of the drafts of each chapter and in designing several graphs and suggesting the idea for the cover design. We apologize for not being able to cite all of the excellent studies and clinical trials in this field due to space limitations.

Haidong Dong
Svetomir N. Markovic

Contents

Chapter 1
The Basic Concepts in Cancer Immunology and Immunotherapy

Haidong Dong

Contents

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

H. Dong (✉)
Department of Urology, Department of Immunology, College of Medicine, Mayo Clinic, Rochester, MN, USA

H. Dong, S.N. Markovic (eds.), *The Basics of Cancer Immunotherapy*,
https://doi.org/10.1007/978-3-319-70622-1_1

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.

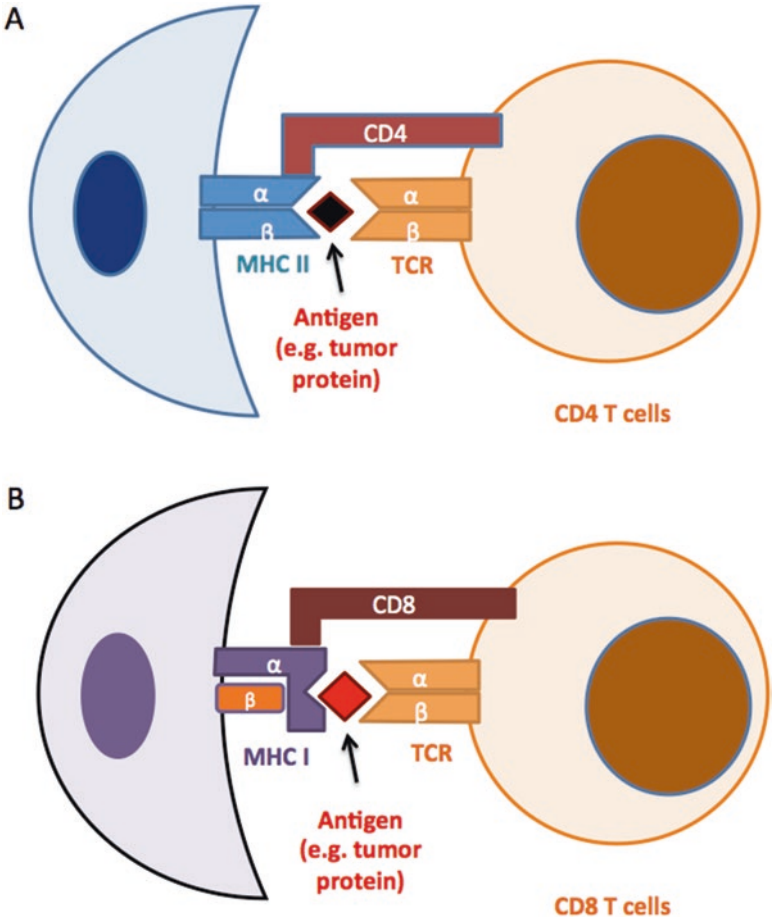


Fig. 1.1 Tumor antigen can be presented to CD4 T cells through the major histocompatibility complex II (MHC II) or to CD8 T cells through MHC I in order to activate T-cell receptors (TCRs)

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.