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Abstract	In this chapter we present an historic overview of the relationship between cancer and the immune system. We will see how it was not always clear that the immune system was able to recognize and fight cancer and how different theories have evolved, from immunosurveillance to the recent immunoediting. We will see how the tumor microenvironment is extremely rich in different immune cell populations. Then we will broadly revise, the main components of the immune system and how it roughly works (immunology in a very small nutshell) to be able to understand how cancer cells not only evade the immune system to remain undetectable and sustain within the host but how they can also hijack immune cells to help cancer progression. The molecules and cells that are major players in these processes will also be addressed. We then conclude this chapter by describing the several new revolutionary approaches to fight cancer using the patient's own immune system.	

Cancer Immunoediting and Hijacking of the Immune System

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What Will You Learn in This Chapter?

In this chapter we present an historic overview of the relationship between cancer and the immune system. We will see how it was not always clear that the immune system was able to recognize and fight cancer and how different theories have evolved, from immunosurveillance to the recent immunoediting. We will see how the tumor microenvironment is extremely rich in different immune cell populations. Then we will broadly revise, the main components of the immune system and how it roughly works (immunology in a very small nutshell) to be able to understand how cancer cells not only evade the immune system to remain undetectable and sustain within the host but how they can also hijack immune cells to help cancer progression. The molecules and cells that are major players in these processes will also be addressed. We then conclude this chapter by describing the several new revolutionary approaches to fight cancer using the patient's own immune system.

Learning Objectives

After reading this chapter, students should be able to:

1. Describe the immunosurveillance concept.
2. Describe the immunoediting process.
3. Explain the differences between innate and adaptive immunity.
4. Discuss how the immune system controls cancer cells.
5. Describe the cell players that constitute both arms of immunity and their general function.
6. Describe the major cellular components of TME.
7. Discuss the immune evasion mechanism, providing some examples.
8. Describe the use of immunotherapy for cancer-treatment and discuss why some treatments work so well in some patients while not at all in others.

Important Concepts Discussed in This Chapter

- Cancer Immunosurveillance – cells and tissues are constantly "watched" by the immune system that in early stages is able to eliminate the first cancer cells but then it becomes entangled in a cross-interaction with tumor cells that corrupts their initial surveillance role.
- Cancer Immunoediting – is the result of the cross-interactions between the anti-tumor response of the immune system and the tumor cells, leading to the selection of immune-resistant clones/variants.
- Cancer Immune evasion – is a strategy used by cancer cells to escape the host immune response, increasing its probability to thrive in the immune competent host.
- Cancer Immune suppression – is a reduction of activation of the immune system functions.
- Immunotherapy is a type of cancer treatment that enhances the patients' natural defenses to fight cancer.
- Immune checkpoint blockers – are the "brakes" of the immune system, that normally act to avoid auto-immune responses.

7.1 Cancer Immunoediting and Hijacking of the Immune System

All living organisms are hosts for other species, establishing different types of symbiotic interactions. However, all organisms and all cells in a multicellular organism need to defend themselves from dangerous invaders like bacteria, viruses, fungi or larger

parasites which have not evolved a positive and/or neutral relationship. In parallel, dead cells resulting from normal tissue homoeostasis also need to be cleared. Vertebrates have two major armies of defense: the innate and adaptive immune system, which perform these functions.

7.1.1 Cancer Immunosurveillance Hypothesis

A hundred years ago Paul Ehrlich (1909) put forward an hypothesis in which the immune system could recognize and destroy tumor cells, a concept that was further developed by Burnet and Thomas (1957) as the **cancer immunosurveillance hypothesis** [1]. This theory proposed that cells and tissues were constantly monitored by a vigilant immune system, and such immunosurveillance was responsible for recognizing and eliminating the majority of incipient cancer cells [2].

This immunosurveillance hypothesis was supported by the observations of the "father" of immunotherapy – William Coley (1891). Coley was a surgeon that noticed that some cancer patients who got infections after surgery had their tumors regressed more efficiently than patients who didn't get infections. He hypothesized that infection had stimulated the body's "resisting powers" [3]. At first Coley injected live bacteria into tumors, but later he used heat-killed bacteria to induce a high temperature (fever) without causing a real infection. However, not all patients responded well to this immune activation and also the advent/introduction of radiotherapy lead to Coley's approach to be mostly forgotten until the recent advent of immunotherapy [3].

The immunosurveillance theory was supported by other later observations such as:

- Increase of certain cancers in immunodeficient individuals, like immunosuppressed organ transplant recipients and HIV/AIDS patients [4].
- In mice, tumors were rejected when transplanted into syngeneic hosts (genetically identical) whereas normal tissues grow with no constrains, suggesting existence of tumor-specific antigens [5].
- Patients with high number of intratumoral lymphocytes and Natural Killer (NK) cells in different types of cancers show increasing survival rates [6].
- Effectiveness of the bacterium *Baccillus Calmette-Guérin* (BCG) vaccine in the treatment of superficial bladder cancer [7].

However, there were also several other contradicting observations that suggested a positive role for the immune system in cancer development. For example:

- Thymus excision after birth correlated to a reduction of breast cancer prevalence [8] and immune reconstitution restored susceptibility to cancer [9].
- Immunosuppressed patients present less chance to develop breast carcinomas compared with immunocompetent individuals [10].
- Low incidence of human cancers in leprosy and sarcoidosis diseases, which are characterized by immunosuppression [11].

Therefore, the concept of cancer immunosurveillance has been shaped with debate until the early '90s, where finally the development of new immunodeficient mouse models made it possible to address these questions in a more reliable manner [12].

7.1.2 The Cancer Immunoediting Concept

Using a chemical carcinogen, Schreiber and colleagues, found that 58% of RAG2$^{-/-}$ mice (which lack adaptive immunity, i.e., NK, T and B cells) developed tumors, contrasting to only 19% in the wild-type (WT) strain. A striking finding was that, when these tumors were transplanted into WT immunocompetent recipients (from the RAG2$^{-/-}$ or WT donors), the tumors derived from RAG2$^{-/-}$ were more immunogenic as a group i.e. had a higher rate of rejection in comparison to the tumors derived from the WT strain (Fig. 7.1). These results showed that tumors that had developed in the WT immunocompetent host were subjected to a selective immune-related pressure (**editing process**) whereas the others did not [2].

In summary, these and other experiments led to the idea that highly immunogenic cancer cell clones are routinely eliminated in immunocompetent hosts, leaving behind only weakly immunogenic variants (poorly recognized by the immune system) to grow and generate "immunoedited" tumors. These weakly immunogenic cells can then colonize very efficiently in immunocompetent recipients. In contrast, when arising in immunodeficient hosts (RAG2$^{-/-}$), the immunogenic cancer cells are not selectively depleted and can prosper in an immunodeficient recipient (unedited). However, when transplanted into an immunocompetent host they are no longer able to thrive because they were not previously "edited" (negatively selected), they are now recognized and eliminated. These results show that the immune system not only protects the host against tumor formation, but also edits/selects tumor immunogenicity.

These and others studies led to the current view of **cancer immunoediting**, which integrates the **paradoxical anti- and pro-tumoral roles** of the immune system [13].

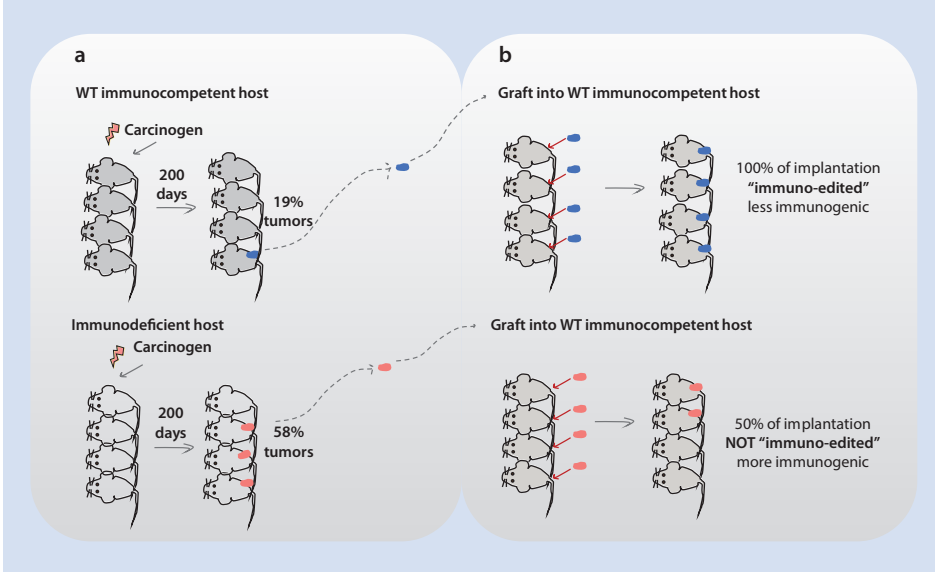

Fig. 7.1 Tumor immunoediting. **a.** Immunodeficient mice (Rag2$^{-/-}$) were more susceptible to carcinogen-induced tumors formation (58%) when compared with WT immunocompetent host (19%). **b.** WT-derived tumors when transplanted into WT recipients implant with 100% efficiency (blue). In contrast, tumors derived from mice lacking adaptive immunity (red) are more immunogenic (where not previously eliminated/edited) and only 50% were able to implant in WT recipients. (Adapted from Schreiber et al. [2])

Schreiber and colleagues developed the concept of "cancer immunoediting", a dynamic process composed by three phases [12]:

1. Elimination – in which transformed cells are killed by the action of innate and adaptive immunity
2. Equilibrium – a state of equilibrium between immune and tumor cells.
3. Evasion/escape – which concludes with the appearance of clinically detectable tumors.

During cancer immunoediting, the host innate, and adaptive immune system interact dynamically with the tumor, determining its progression. In this dynamic process, some tumor cells variants have the capacity to evade and/or suppress the immune system and in this way, are immunologically sculped by Darwinian selection, expanding in an uncontrolled manner in the immunocompetent host [12].

In this chapter we will discuss some of the known molecular mechanisms involved in this process of **immune evasion** but also how cancer cells can **hijack the immune system** to help tumor development. However, before we enter into the molecular details, we need to take a detour and look at the components of the tumor microenvironment and also into the basics of immunology.

7.1.3 The Tumor Microenvironment (TME)

Tumors have been recognized as complex entities, where during the course of tumorigenesis they build their own microenvironment, which can contribute to tumorigenesis *per se* (Fig. 7.2).

More than just cancer cells, the cellular TME can be composed by (Fig. 7.2) [14, 15]:

- Cancer-associated fibroblasts (CAFs)
- Endothelial cells (ECs)
- Pericytes (PCs), that surround ECs

and a variety of immune-related cells types, all derived from hematopoiesis:

- Myeloid lineage
  - Macrophages – tumor associated macrophages (TAMs)
  - Neutrophils – tumor associated neutrophils (TANs)
  - Mast cells (Mcs)-granulocytes rich in histamine and heparin
  - Dendritic cells (DCs)
  - Myeloid-derived suppressor cells (MDSCs)
- Lymphoid lineage:
  - T cells, including $CD8^+$ cytotoxic T lymphocytes (CTLs), $CD4^+$ T helper cells (Th), regulatory T cells (T-Regs) and γδ T cells
  - B cells
  - Natural killer cells (NK)

7.1.4 Immunology in a Very Small Nutshell

As evident by this list of possible TME cellular components, the immune system is highly represented in the tumor ecosystem. As referred before, the immune system can be divided

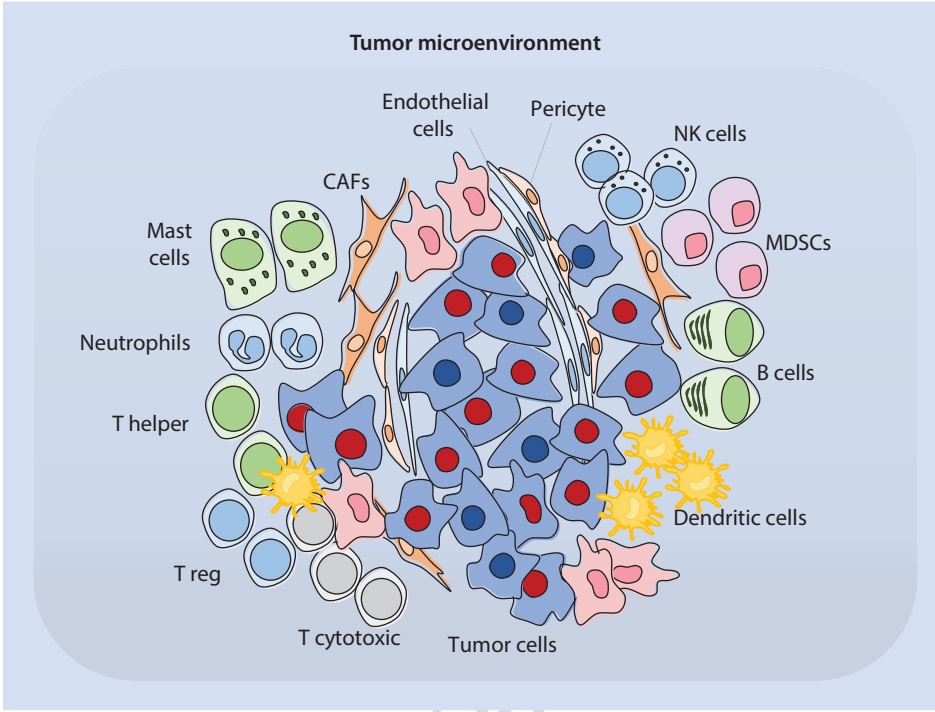

Fig. 7.2 Some cellular components of the tumor microenvironment. TME mainly include CAFs, endothelial cells and pericytes as well as many bone marrow-derived cells as immune cells from myeloid and lymphoid origin, which can be present in different stages of their differentiation state

in two categories: the innate and the adaptive immunity which dynamically interact to defend the host (Fig. 7.3).

7.1.4.1 Innate Immunity

Innate immunity is considered the basic defense mechanism, as a first-line of response to infection and disease and is not specific for a given pathogen or antigen (Ag). However, innate immunity is essential to call in the highly specific adaptive response allowing both armies to work together to eliminate the threats [16, 17].

The innate arm includes:

- The **complement system** – consists of ~30 interacting soluble inactive-proteins produced in the liver that go into circulation and can get activated by three types of pathways: classical pathway (antibodies), lectin pathway (lectins) and alternative pathways. Once activated, all pathways converge in the activation of the potent anaphylatoxins C3a and C5a and in the formation of the membrane attack complex – MAC (composed by C5b, C6, C7, C8 plus several C9) – a pore complex responsible for cell and pathogen lysis. During this process several small peptides are generated by cleavage, recruiting immune cells to assist the fight.
- **Macrophages** and **granulocytes** (ex. neutrophils) – are phagocyte cells which are able to engulf and kill invading pathogens by a combination of strategies involving degrading enzymes (lysozymes), antimicrobial peptides and oxygen-derived toxic molecules (superoxide, oxygen peroxide and hydroxyl radicals). In addition, they express pattern recognition receptors (PRRs) that bind specific pathogen-associated

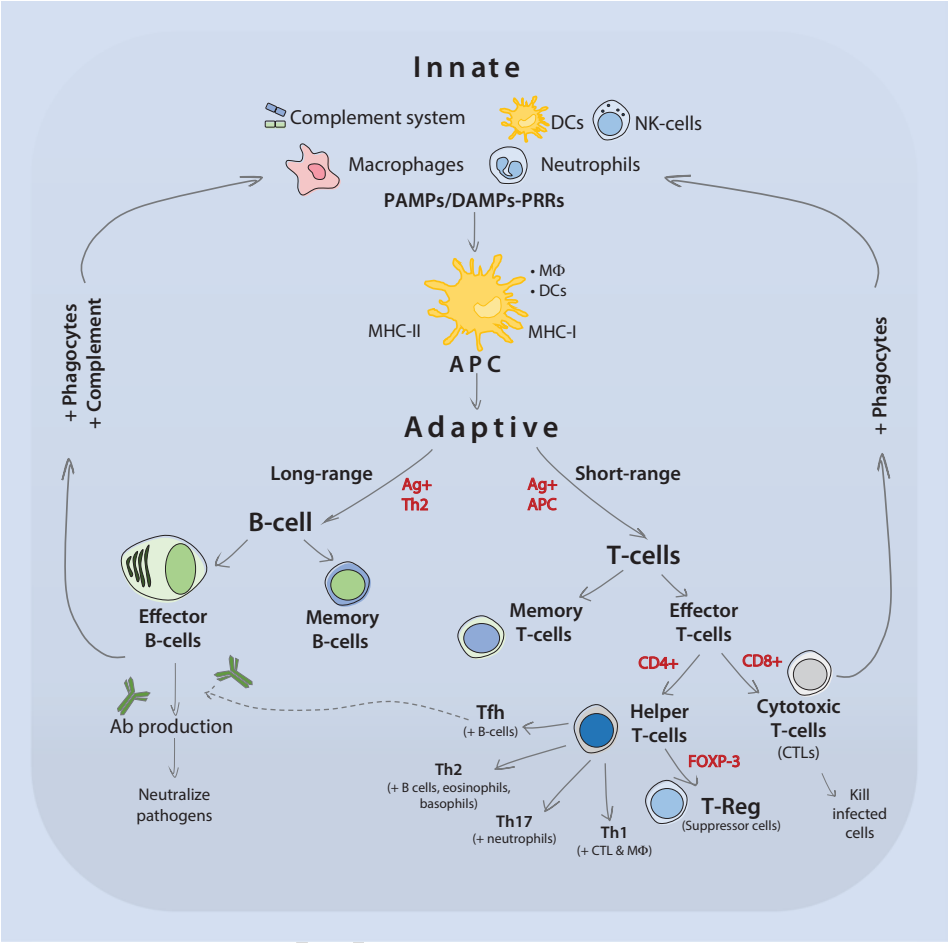

Fig. 7.3 Immunology in a very small nutshell. The immune system comprises the innate and adaptive arms. Innate immune mechanisms are the first line of defense and is not specific. Innate responses include the action of soluble factors (complement, chemokines and cytokines) as well as activities mediated by cellular components, mainly myeloid cells (neutrophils, macrophages etc.) as well as NK and γδ T cells (lymphoid lineage). These innate cells express Pattern recognition receptors (PRRs), which constitutes an alarm system that recognizes pathogen-associated molecular patterns (PAMPs – present in microbial pathogens) and damage-associated molecular patterns (DAMPs – molecules that are released/expressed by damaged or dying cells). Activation of PRRs leads to release of inflammatory cytokines, activation of the complement system. Antigen presenting cells (DC and macrophages mainly), after phagocytosing the pathogens/debris present antigens through MHC molecules to the adaptive T-cells, constituting a direct link between innate and adaptive immunity. The cells of adaptive immunity, B and T lymphocytes express specific receptors. T cells recognize the antigens through the TCRs and MHC bound peptides at the surface of APCs. MHC class I is presented to CTLs ($CD8^+$) that kill infected cells, and MHC class II to T helper cells ($CD4^+$). $CD4^+$ helper cells differentiate in secondary lymphoid tissues into T-Reg, Th1, Th2, Th17 and Tfh (follicular T helper cells). Th1 assist activation of CTLs and macrophages while Tfh help in the differentiation of B cells. In fact, B cells proliferate and may differentiate into effector cells, termed plasma cells (upon release of cytokines by Tfh cells), which are short-lived cells that secrete specific antibodies (Ab) against pathogens. Others may differentiate into memory cells that help to mount an effective response in a second exposure to the antigen. The net result of activation of antibodies and effector T cells ends with a positive feedback of activation of the innate immune cells (phagocytosis). T cells secreted chemokines and cytokines and recognition of the antigen bound to the antibodies lead to activation of the complement, or the direct activation of macrophages through recognition of the Fc portion of the Ab by the Fc receptor present in macrophages

molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). Activation of PPRs triggers the inflammatory response (secretion of cytokines, chemokines, prostaglandins, NF-β signaling, interferon response).

- **Dendritic cells (DCs)** – are the most important antigen presenting cells (APC) and the main link between innate and adaptive immunity. DCs are specialized at presenting antigens, small peptides and proteins, to activate naïve T cells (adaptive immunity), and therefore are also known as **professional antigen presenting cells** (**APCs**). The activated dendritic cells cleave proteins of the "pathogen" into small peptides, that then bind to newly synthesized MHC proteins, which carry the fragments to the cell surface. Activated/matured DCs express co-stimulatory markers (CD40, CD80, CD83, CD86) and MHC class I and II molecules. Then they migrate to lymph nodes where they present the peptide-MHC complexes to T-cells of the adaptive arm, starting the adaptive response.
- **Natural killer cells (NKs)** – as mentioned before, belong to the lymphoid lineage but mediate innate immune responses. NK cells patrol the body and are able to kill tumor cells and virus infected cells by inducing apoptosis. This apoptosis can be mediated by granzymes and perforin or via expression of Fas ligand and TRAIL (TNF-related apoptosis-inducing ligand). However, their killing activity is dependent on the **balance between activating and inhibitory receptors** on the NK cell surface. These inhibitory receptors bind MHC class I molecules explaining why **NK cells preferentially kill cells that express low levels of MHC class I** and do not kill normal healthy cells (that express MHC-I). Downregulation of MHC-class I is a strategy employed by virus to avoid being detected by T cells. Nevertheless, we will see that cancer cells also use this strategy. NK cells seem to have evolved as a response to this adaptation – so virus infected cells and tumor cells cannot hide from the NK cells! Moreover, NK cells secrete cytokines such as IFNγ and TNFα, which act on other immune cells like macrophage and dendritic cells to enhance the immune response.

7.1.4.2 Adaptive Immunity

Adaptive immunity is a defense mechanism that requires a sophisticated gene recombination strategy to generate antigen specific receptors (TCRs and BCRs) and antibodies that identify **specific targets** and **remember them** (immunological memory), generating a **very precise** way to **recognize** and **kill foreign threats**, even if they come back later in time. This recombination strategy allows adaptive immunity to respond to millions of different foreign antigens in a highly specific manner [17]. It is amazing!

Adaptive immunity can be subdivided in two classes of immune responses:

- **Antibody responses** (humoral immunity) – mediated by **B cells** that are activated to secrete antibodies which circulate in the blood stream and therefore act over **long distances.** Antibodies neutralize pathogens (blocking their binding to specific cell receptors) or by marking pathogens to be dwelt by innate immunity through phagocytes (that recognize these Ab through Fc receptors), NK cells or the complement system.
- **T-cell mediated responses** (cellular immunity) – in general may require cell-cell contact and therefore act over **short distances** at the lymphoid organ (activating B cells) or at the site of infection. **T cells** recognize foreign Ag bound to MHC molecules on the surface of the APC, such as dendritic cells, macrophages or B cells. T cells act either by directly killing the infected cells (CTLs) or by stimulating phagocytes or B cells to help fight infection (Th).

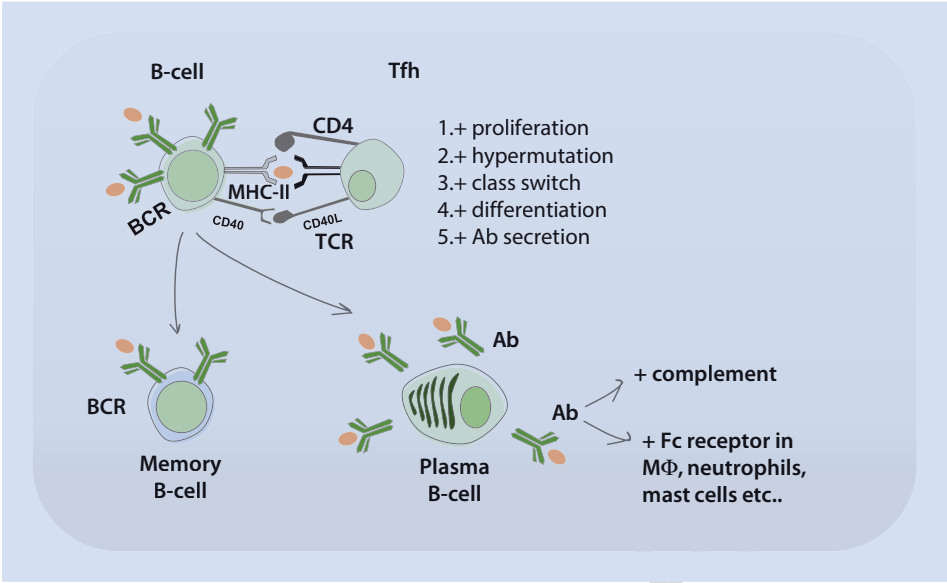

Fig. 7.4 B cell differentiation in the lymphoid follicles. B cells are produced and undergo maturation in the bone marrow (BM) expressing BCR on their surface. After they leave the BM, they circulate through blood and peripheral lymphoid organs. If they recognize an antigen, they will endocytose both BCR and the antigen. Then the antigen will be processed and presented as a small peptide through MHC-II to follicular helper T-cells (Thf) (co-stimulated by CD40/CD40L). Tfh in turn, promote B-cell proliferation, somatic hypermutation, class switch recombination, differentiation into memory B cells and differentiation into plasma cells that abundantly secrete antibodies. The order of these processes does not occur necessary in sequence and differentiation/maturation of B-cells in germinal centers is still under intense investigation

B Cells

B cells are the cells that produce and secrete antibodies. However, B cells first synthesize the antibodies which are immunoglobulins (Igs) in a membrane bound form – the B cell receptors (BCRs), that are produced in billions of arrangements, each with a different amino-acid sequence, with a unique binding site by a process of somatic recombination called V(D)J recombination [18]. Only after antigen recognition do B cells start secreting the antibodies. Antigen binding to the BCR together with co-stimulatory factors, provided by follicular helper T cells (Tfh), activate B cells to proliferate and differentiate into either memory B cells (long-lived) or antibody-secreting effector cells, which are called plasma cells (short-lived cells) [17, 19] (Fig. 7.4).

Box 7.1

V(D)J recombination – somatic mechanism of DNA recombination to generate diversity of antigen receptor genes during B and T lymphocyte development. B cells generate the innumerable antibodies and T cells generate the TCRs. It is directed by two enzymes: the recombination activating gene 1 (RAG1) and RAG2 that bind and cleave genomic DNA at specific recombination signal sequences next to antigen receptor gene segments [20].

Somatic hypermutation (SHM) – recombination process that generates diversity of the variable regions of the immunoglobulin genes during B cell differentiation/maturation being fundamental for the development of high-affinity antibodies [21].

Class-switch recombination (CSR). Another recombinational process that replaces the immunoglobulin heavy chain constant region Cμ (which encodes the Fc portion of IgM) for that of the constant region of IgG, IgA or IgE, (Cγ, Cα or Cε respectively) [22].

T Cells

All T cells express T-cell receptors (TCRs), which are cell surface antigen receptors, encoded by genes that are assembled by multiple gene segments during T cell development and also generated by a V(D)J genetic mechanism of recombination.

Besides memory T cells, there are four main classes of effector T cells:

- **Cytotoxic T-cells** (**CTLs**) are characterized by expressing αβTCRs and CD8 co-receptors and kill infected cells by inducing cells to undergo apoptosis. CTLs activate apoptosis either by activating the Fas pathway or through cytotoxic proteins (granzymes and perforin) that lead to the activation of the caspases cascade.
- **Helper T-cells** (**Th** cells) are characterized by expressing αβTCRs and CD4 co-receptors and are responsible for:
  1. Secrete cytokines
  2. Activate $CD8^+$ T cells
  3. Activating B cells to proliferate and differentiate to become Ab secretory, start hypermutation and class switch
  4. Activate macrophages, granulocytes and effector cells
- **Regulatory T-cells** (**T-Regs**) are characterized by expressing CD4 co-receptors and the master transcription factor FOXP3. T-Regs suppress the activation, development or function of most other types of immune cells by secretion of immune suppressive cytokines like TGFß and IL10 and inhibitory proteins like CTLA-4 and PD-1.
- **γδ T-cells** are characterized by expressing γδTCRs but with reduced diversity, do not express CD4 and CD8 co-receptors and are activated in an MHC-independent manner. Upon activation produce cytokines, chemokines, induce cytolysis (due to secretion of perforin, granzymes and TRAIL) and interact with other immune cells. Similar to NK, exhibit features of innate and adaptive immune system and are abundant in epithelial barriers like in the gut mucosa, skin and uterus. Therefore, are referred as innate lymphoid cells [23].

T and B lymphocytes continuously circulate between the different peripheral lymphoid organs via the lymph and blood stream and only when lymphocytes expressing their unique cell-surface antigen receptors (BCR and TCR) encounter their matching antigen (presented in the peripheral lymphoid organs) in the peripheral lymphoid tissues where they engage proliferation and differentiation into **effector** and **memory** cells.

As referred before TCRs recognize peptide fragments displayed in MHC proteins on the surface of APCs. APCs present Ag to cytotoxic T cells through MHC-class I molecules and CD8 co-receptors, whereas helper T cells receive Ag by MHC-class II molecules and activation is mediated by CD4 co-receptors. Besides the Ag binding, lymphocytes need co-stimulation by other molecules: B cells depend on Tfh cells to provide co-stimulatory molecules such as CD40L and T cells depend on co-stimulatory reactions between the CD28 receptor and the B7 molecules expressed at surface of APC (Fig. 7.5) [24].

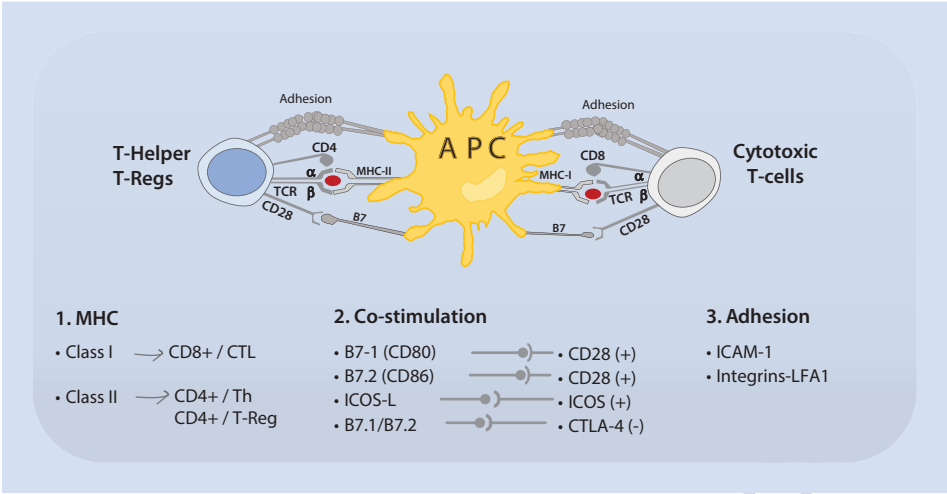

Fig. 7.5 Interactions between APCs and T cells. APCs present Ag through MHC molecules that will bind to the TCR of T lymphocytes. CTLs ($CD8^+$ cells) recognize an Ag bound to MHC class I whereas Th cells (CD4+) associates with MHC class II molecules. Co-stimulatory molecules such as B7.1 (CD80) and B7.2 (CD86) are present on APC's which interact with CD28 on T-cells to mount an immunological response. Also for a full functional immunological synapse APCs must bind T-lymphocytes through adhesion molecules such as intracellular adhesion molecule (ICAM) and integrins

During maturation of B and T cells, mechanisms to ensure that B and T cells do not react against the host's own cells and molecules had to evolve – a process called **immunological self-tolerance**. In other words, during the maturation process, cells with BCRs and TCRs that recognize self, are eliminated or diverted to regulatory pathways by several mechanisms. Over-activation of the immune response also has to be regulated and several checkpoints and negative feedback loops ensure that the massive cellular expansion and cytokine storm that accompany the immune response do not overwhelm the host and do not incorrectly destroy healthy cells (autoimmune reaction)!

We will see straightaway how cancer cells exploit exactly these defense mechanisms, that dampen the immune response (to avoid autoimmunity) to their own benefit. Tumor cells can evade/escape the immunity through several mechanisms, they can become invisible, kill and suppress the immune system or even sabotage and hijack the immune cells to work for them to fuel tumorigenesis instead of fighting it! This is one of the reasons why it is so difficult to fight this devastating disease – the police is corrupted.

7.2 Immune Evasion Mechanisms

Evasion – "the act of physically escaping from something (an opponent or a pursuer or an unpleasant situation". During tumor immunoediting, high immunogenic (highly reactive) clones get eliminated, while low immunogenic clones remain (i.e., get selected because they are the ones that are not eradicated), allowing the survival of these tumor cell variants in an immunologically unrestricted manner [25]. Many mechanisms have been reported that enable cells to pass undetected and evade the immune system (Fig. 7.6). Keep in mind that the examples given below are not an exhaustive review.

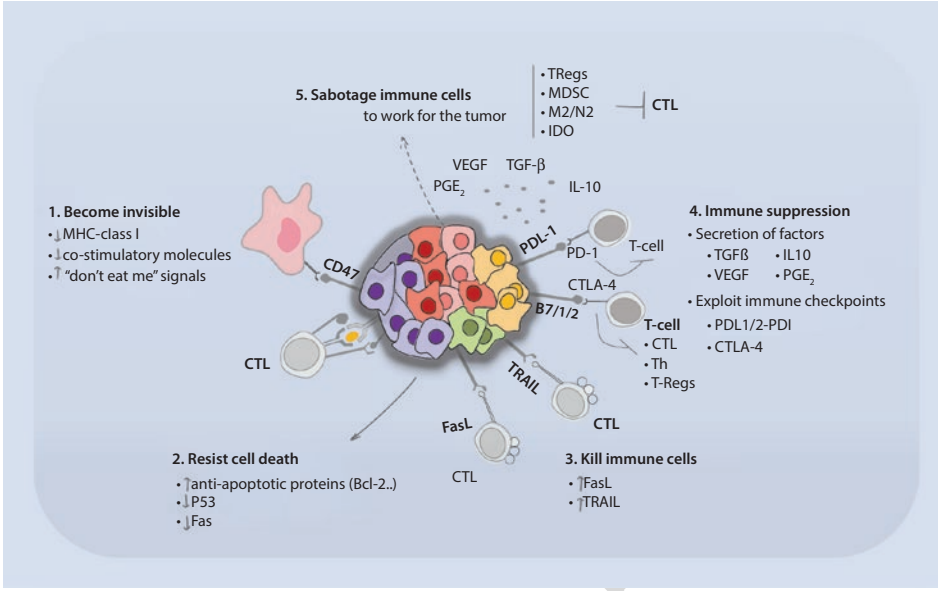

Fig. 7.6 Tumor escape mechanisms. An illustration of different key factors governing tumor immune evasion. (1) Tumor escape can occur through cell-contact-dependent mechanisms, in which tumor cells have acquired mechanisms to become undetectable by the immune system. (2) Tumor cells developed apoptotic resistance. (3) Capacity to kill immune cells via TRAIL or FasL upregulation that will lead to activation of caspase pathway. (4) Tumor cells may present aberrant expression of cell-surface ligands that downregulate T-cell activity, such as PD-L1/CTLA-4. In addition, tumor cells also employ cell contact-independent mechanisms like secretion of tumor-derived factors like VEGF, IL10, ROS, IDO, PGE2 and TGFβ. (5) Finally, tumor cells can also manipulate various myeloid and lymphoid cells to contribute to tumor growth

7.2.1 Mechanisms to Become "Invisible" – The "Harry Potter" Invisibility Cloak

- Downregulation of the MHC class I, through alterations in the expression of MHC molecules or in the processing or presentation of tumor-associated antigens (TAA), lead tumor cells to become invisible for CTLs and can only be recognized by NK cells [26].
- Expression of "**don't eat me signals**" like CD47, a cell surface molecule that inhibits the phagocytic activity of macrophages and DCs. CD47 molecules seem to function as a negative innate immune checkpoint and a marker of self to ensure that healthy cells are not inappropriately phagocytosed during inflammatory conditions. Once more, tumors exploit this mechanism for their own benefit avoiding being phagocytosed by macrophages and DCs [26].
- Lack/reduction of expression of co-stimulatory molecules necessary for proficient T-cell activation (PD-L1/B7 family) [26].

7.2.2 Mechanisms to Resist Cell Death (See Chap. 5)

- Upregulation of anti-apoptotic proteins such as **Bcl2** and **Bcl-xl**
- Downregulation or loss of pro-apoptotic factors such as **P53** and **Fas receptor**. Fas-mediated killing is an important defense mechanism during the effector phase

of the immune reaction. Thus tumor cells that express high levels of Fas receptor will get killed, remaining the Fas^{low} resistant variants – this is an example of the sculpting action of the immune system [27].

7.2.3 Mechanisms to Suppress the Immune System

7.2.3.1 Tumor Cells Secrete Immunosupressive Factors

Tumor cells **secrete factors** that have multiple repressive effects on the immune system affecting all sort of cells of the immune system:

- **Transforming growth factor-β (TGFβ) has been shown to** [28]:
  - Inhibit $CD8^+$ CTL clonal expansion and inhibit transcription of key genes such as perforin, granzymes, blocking the "cytotoxic program"
  - Induce FOXP3-T-Reg cell differentiation
  - Inhibits B-cell proliferation and Ab secretion
  - Inhibit proliferation and function of NK cells
  - Promote pro-tumoral macrophages and neutrophils and mediate the immune suppression function of MDSCs (see ahead).
- **Prostaglandins (PGE2)** have multiple and paradoxical effects. It is critical to start the inflammatory response, promoting local vessel dilatation and attraction/activation of neutrophils, macrophages, and mast cells. However, it also shuts down this early response by directly suppressing the production of several pro-inflammatory cytokines (IL2, IL12, IL15 for example) and promoting the production of suppressive IL10, leading to a general immune suppression affecting both innate and adaptive immunity at multiple molecular and cellular levels. PGE2 suppresses the innate cells (NK, macrophages, neutrophils to perform their function, i.e., call in CTLs) and suppresses adaptive immunity (inhibit activation and expansion of CTL and inhibit Th1 favoring Th2), but also promotes the development and activity of suppressive cells (T-Regs, MDSC and pro-tumoral macrophages-M2-like) [29].
- **Interleukin-10 (IL10)** is an anti-inflammatory cytokine with also paradoxical roles in cancer. Some studies point to an immune suppressive pro-tumoral role whereas others show exactly the opposite. IL10 can directly modulate innate and adaptive immunity. IL10 is thought to inhibit MHC class I on tumor cells and MHC class II and co-stimulatory molecules B7-1/B7-2 expression on APC (monocytes/macrophages) reducing production of pro-inflammatory cytokines (IL1α and β, IL6, IL12, IL16, and TNF-α) and chemokines. IL10 can also act directly on helper T-cells $CD4^+$, inhibiting proliferation and production of pro-inflammatory cytokines (IL2, IFN-γ, IL4, IL5 and TNF-α). These direct effects on monocytes/macrophages and Th cells are thought to lead to an overall reduction of T cell activation and differentiation in the lymph nodes and decrease of pro-inflammatory responses in tissues. However, IL10-deficient mice and humans develop inflammatory bowel disease (IBD) and are more susceptible to cancer. Moreover, tumor cells overexpressing IL10 are rejected! Also, treatment with IL10 can induce tumor rejection. Therefore, although in many reviews you will find that IL10 is an immunosuppressive molecule that helps tumors escape the immune system, you need to be cautious because there are conflicting reports showing that IL10 can actually activate T-cells [30–32]. Definitely we need to learn more!
- **VEGF** inhibits pro-inflammatory reactions within the tumor microenvironment by promoting the expansion of MDSC and impairing DC maturation and activation [25].

7.2.3.2 Exploit Immune Checkpoints of Self-Tolerance

As referred before the immune systems has evolved mechanisms to control overactivation, preventing for instance auto-immune diseases – these are called mechanisms of self-tolerance i.e. mechanisms that dampen the immune response – the immune-checkpoint pathways. It is now clear that tumors co-opt these pathways as a major mechanism of immune resistance, particularly against T cells.

- **Programmed death receptor-1 (PD-1)** is found expressed on T, B and myeloid cells. PD-1 receptor interacts with its ligands PD-L1 (also termed B7-H1) or PD-L2 and leads to blockage of T cell proliferation and cytokine production. The inhibitory effects of PD-1 were initially observed when PD-1-deficient mice developed autoimmune diseases. PD-L1 is strongly expressed on a variety of tumors cells as well as DC's and macrophages present on the TME and inversely correlates with patient prognosis [33].
- **Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)** is constitutively expressed in T-Regs. CTLA-4 is induced after T cell activation as a negative feedback mechanism that competes with CD28 for B7 ligands inhibiting T cell proliferation and IL2 secretion [34].
- **Indoleamine 2,3-dioxygenase (IDO)** is a heme-containing enzyme that catalyzes the first and rate-limiting step in the kynurenine pathway (NAD^+ production from tryptophan). IDO has been shown to be expressed in tumor cells, endothelial or innate immune cells such as DC, MDSCs and macrophages of the TME [35], generating **2 major effects:**
  - Production of soluble factors (kynurenine and downstream metabolites) that bind and activate the aryl hydrocarbon receptor (AhR), which in turn activate T-Reg differentiation and push dendritic cells (DCs) and macrophages to an immunosuppressive phenotype [7]. Thus, when IDO is active, APCs which in normal conditions would produce inflammatory cytokines such as IL12, instead express IL10 and TGFβ inhibitory cytokines [35].
  - IDO can also create a local suppression of effector T cells by metabolic depletion of tryptophan and production of the catabolite kynurenine [7]. Thus, IDO up-regulation can alter the phenotype of the APC itself, activate T-Regs, and induce the production of suppressive cytokines, changing the whole local ecosystem from immunogenic to tolerogenic. Overall the main mechanism of IDO pathway mediated immune suppression is to reduce T cell infiltration in TME.
- **Depletion of intratumoral T cells - tumor cells kill immune cells!** Tumors can induce T cell death by:
  - Upregulation of ***Trail* expression** which binds TRAIL-receptor (TRAIL-R1) positive CTLs cells, leading to their apoptosis [36].
  - Upregulation of **Fas ligand** (FasL) that expressed or released by tumor cells in tumor-derived exossomes, activating Fas in T cells inducing also their apoptosis [37].

7.2.4 Immune "Sabotage" and "Hijacking" Mechanisms

Tumor cells can manipulate and hijack the cells themselves (lymphoid or myeloid immune cell populations) to work for them by sabotaging normal defense mechanisms of immune tolerance i.e., inducing immune suppressive cell phenotypes that then contribute to tumor escape and progression [15]. In both mice and humans, a number of immune suppressive

cell sub-types have been identified, including T-Regs, myeloid-derived suppressor cells (MDSCs), pro-tumoral macrophage (M2-like) and neutrophils (N2-like).

7.2.4.1 Regulatory T Cells (T-Regs)

T-Reg cells occur naturally and act to inhibit autoimmune responses but can also suppress the generation of tumor-specific T-cell responses, possibly through similar mechanisms [26]. Increased numbers of T-Reg cells have been found in the peripheral blood of different cancer types [38]. T-Regs can suppress effector T cells and thus prevent the development of anti-tumor immunity by four basic "modes of action" [26]:

- Expression of inhibitory cytokines, like TGFβ, IL10 and IL35
- Directly kill CTLs by expression and release of granzymes and perforin
- Indirectly kill CTLs by cytokine deprivation: by expressing high affinity IL2-Receptorα (CD25), T-Regs scavenge IL2, decreasing its levels in the TME leads to CTLs death
- Blocking DCs maturation or function

7.2.4.2 Myeloid Derived Suppressor Cells (MDSC)

Tumor progression evolves with the accumulation of inhibitory myeloid cells, designated as Myeloid Derived Suppressor Cells (MDSC). MDSCs are expanded in several pathological conditions, not only in cancer. MDSCs are not a defined subset of myeloid cells, but a heterogeneous population of myeloid progenitor cells and immature myeloid cells (IMCs) that have been blocked from fully differentiating into mature cells. In the steady healthy state, IMCs lack suppressive activity and are present exclusively in the bone marrow and found in secondary lymphoid organs only in pathological conditions. When activated, in these pathological conditions, MDSC can suppress anti-tumoral immune functions [39].

It has been shown that several tumor-derived factors can induce expansion and activation of these MDSC, which migrate from the bone marrow to the lymphoid organs and to the tumor site.

These expansion factors include: PGE2, granulocyte macrophage CSF factor (GM-CSF), M-CSF, stem-cell factor (SCF), vascular endothelial growth factor (VEGF), IL10 and IL6 [40]. Most of these molecules activate signaling pathways that activate Janus kinase (JAK) family members and STAT3 (see Chap. ► 3) that promote the expansion of the MDSCs. For activation of the immune suppressive activity it has been shown the involvement of other signaling molecules such as: IFNγ, ligands for Toll-like receptors (TLRs), IL4, IL13 and TGFβ that ultimately lead to activation of STAT1 and NF-Kβ [41].

A number of studies have implicated these MDSCs in immunosuppression, mainly through [25, 39, 41]:

- Secretion of immune suppressive cytokines **TGFβ and IL10**
- As a major source of PGE2 (they highly express cyclooxygenase 2 – COX-2)
- Production of **arginase 1**, which leads to arginine depletion, inhibiting T-cell proliferation
- Reduction of local **tryptophan** levels due to the activity of IDO
- Production of inducible nitric oxide synthase (**iNOS**) that results in the generation of reactive oxygen species (**ROS**) including nitric oxide (NO) and peroxynitrite ($ONOO^-$), which ultimately alter T-cell signaling, activation and survival (TCR nitrosylation)
- Expression of inhibitory **PDL-1** in MDSC in the TME
- **T-Reg cells** induction (lymphoid organ) and attraction to the TME

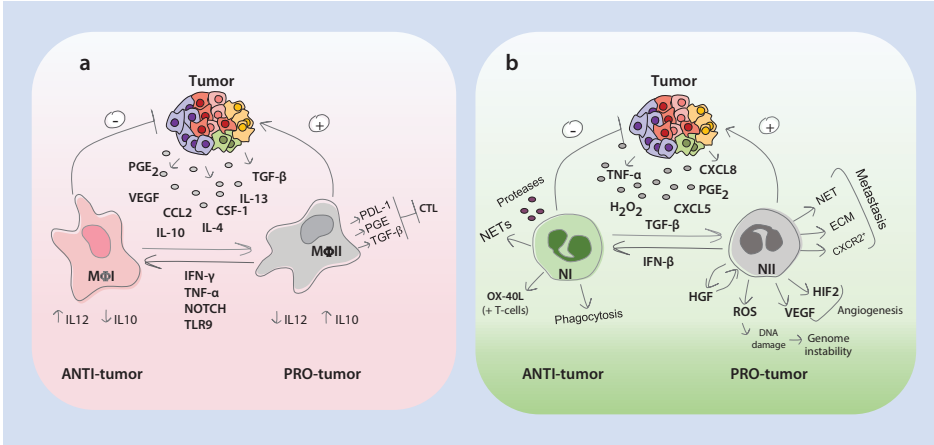

Fig. 7.7 Dynamic states of anti- and pro- tumoral macrophages and neutrophils. **a**. Anti-tumor macrophages (M1-like) and pro-tumoral macrophages (M2-like) phenotypes and signaling involved. IFN-γ and TNF-α have been reported to induce activation of the M1 phenotype. M1-like macrophages produce high levels of IL12 and low levels of IL10 cytokines and can contribute to tumor control. In contrast, the M2-like phenotype has an $IL12^{low}$, $IL10^{high}$ cytokine profile and a pro-tumorigenic role. IL4, IL13, TGFβ, PGE2, VEGF, CCL2, and CSF1 can induce M2-like macrophages. **b** Anti-tumor (N1-like) and pro-tumoral (N2-like) phenotypes and signaling molecules involved in neutrophil behavior. Besides being "killing" machines (N1), neutrophils can revert to a pro-tumorigenic role and impact on angiogenesis (activation of VEGF) and metastasis by remodeling the extracellular matrix via matrix metalloproteinases (MMPs) for example [43, 45, 46]

MDSCs can be found in the lymphoid organs and in the TME, where they engage on different mechanisms of immunosuppression. In peripheral lymphoid organs, immunosuppression by MDSC is contact dependent, mainly antigen-specific whereas in TME suppression is more potent and non-antigen-specific, nevertheless both rely on activation of the two key enzymes: **arginase1** and **iNOS** [39].

7.2.4.3 M2-Like Macrophages and N2-Like Neutrophils

Within the myeloid-derived cell compartment, the tumor associated macrophages (TAMs) and neutrophils (TAN) can either adopt an anti-(M1/N1-like) or pro-tumoral (M2/N2-like) phenotype (Fig. 7.7), which can be reverted and modulated by tumor-derived signals. We would like to highlight that the terms M1/N1 M2/N2 is for the sake of simplicity, since nowadays researchers are realizing that there are many different subtypes and "states" of these cells and that these are highly dynamic. **IL10**, **IL4**, **IL13**, **CCL2**, and **CSF-1** secreted by tumor cells can drive TAMs into pro-tumoral M2-like macrophages (Fig. 7.7a). M2 exert a pro-tumoral role through several mechanisms of immunosuppression (PDL-1, PGE2, TGFβ, IL10, CCL2, etc), that can block anti-tumor T cell activity and interferon type I responses (IFN) [42–45]. In contrast, N2-like neutrophils are more involved in promoting angiogenesis and metastasis than immune suppression *per se* but nevertheless also contributes to tumorigenesis [43, 46].

In summary, it appears evident that tumors develop a parasitic relationship with its host to take control of both myeloid and lymphoid compartments to further prolong tumor growth and progression.

7.3 Immunotherapy

The great advances in understanding this dynamic cross-talk between tumor cells and immunity has led to the emergence of immunotherapy as a transformative approach to cancer treatment. Immunotherapy aims at unleashing the patient's own defense mechanisms to fight cancer and is giving hope to the most mortal types of cancer like melanoma and renal cell carcinoma.

To date, cancer therapies such as conventional chemo- and radiotherapy fail to obtain long term responses, probably due to the escape of resistant sub-clones. So, if we are able to block the immunosuppressive mechanisms and turn tumor cells visible for the immune system, the innate and adaptive armies will be able to find these small hidden clones, no matter where they are and eradicate the disease before it reaches vital organs – or even after dissemination – this is hope for cure…. Nevertheless, unleashing the immune system can also have adverse effects similar to auto-immunity – there is a delicate balance between activation and inhibition of immunity to fight cancer but at the same time do no harm to the normal cells….

There are several approaches to boost the immune system to fight cancer described below.

7.3.1 Administration of Cytokines

Administration of cytokines like Interleukin-2 (IL2) and IFN- α, boost the activity of the anti-tumor immune response. **IL2** administration was the first method to show that immunotherapy – exploiting the body's own immune system to kill cancer – could actually work (if we don't take in account Coley's early work). After 66 failed attempts, Dr. Steven Rosenberg and colleagues were finally able to induce a complete remission of a metastatic melanoma patient (1984) [47]. This was the first cancer patient to respond to IL2 infusion and to demonstrate that modulation of the immune system, by stimulation of T cells, could mediate complete destruction of cancer. From then on many melanoma and renal-cell carcinoma patients were treated with IL2 with an overall response rate of ~15% [47]. Although, other types of cancer do not respond to IL2 treatment, IL2 had a profound impact on the development of cancer immunotherapy. IL2 allowed the *in vitro* expansion of T-cells, permitting the development of another type of immunotherapy: adoptive cell therapy also pioneered by Rosenberg and colleagues [47, 48].

7.3.2 Adoptive Cell Therapy

Adoptive cell therapy (ACT) involves isolation of tumor-specific T cells from patients and their expansion *ex vivo* to increase the number of these cells in order to infuse them back into patients to fight cancer [48]. IL2 is used not only to grow T cells *in vitro* but also is administered together with the infused cells to support their growth and survival in patients [47].

Exome sequencing of tumor mutations has showed that tumor-infiltrating lymphocytes (TILs) are able to recognize unique tumor mutations – named neo-antigens or tumor-associated antigens (TAA). This explains why tumors with high mutational burden,

like melanoma, smoking-induced lung cancer or tumors with mismatch repair mutations may have a better chance of response to immunotherapy [47], i.e. these tumors are more immunogenic! The larger the number of mutations, the higher is the probability to generate neo-antigens that will exhibit a strong binding to a MHC molecule for tumor recognition! So, ACT can be coupled with tumor sequencing to identify the tumor neo-antigens to then engineer or select T-cells capable of targeting more specifically and efficiently the tumor cells of that particular patient.

There are numerous forms of adoptive T cell therapy used for cancer treatment:

- Expansion of tumor-infiltrating lymphocytes (**TILs**)
- **CAR-T** cells – T cells engineered to express chimeric antigen receptors (CARs) that recognize cancer-specific antigens, rendering them more efficient in recognizing and attacking specifically tumor cells. The process of generating CAR-T cells involves extracting patient's T cells, transfecting them with a gene for a **chimeric antigen receptor** and reinfuse them back into the patient. In 2017, CAR T-cell therapies targeted to CD19 were approved for children with acute lymphoblastic leukemia (ALL) and for adults with advanced B-cell lymphomas [49, 50].

7.3.3 Immune Checkpoint Therapies

This strategy aims at removing inhibitory pathways that block anti-tumor T cell responses, in the tumor microenvironment (Fig. 7.8). These therapies use monoclonal antibodies against specific molecules that modulate the immune repressive mechanisms, that in normal conditions refrain the immune system to avoid autoimmunity [7].

As mentioned earlier, T cell activation depends, not only on direct contact with APCs, which present Ags through MHC molecules to the corresponding TCR but also depends on the interaction of co-stimulatory molecules such as CD28 and B7 that are mandatory for full activation (Fig. 7.6). However, to avoid catastrophic over activation of the

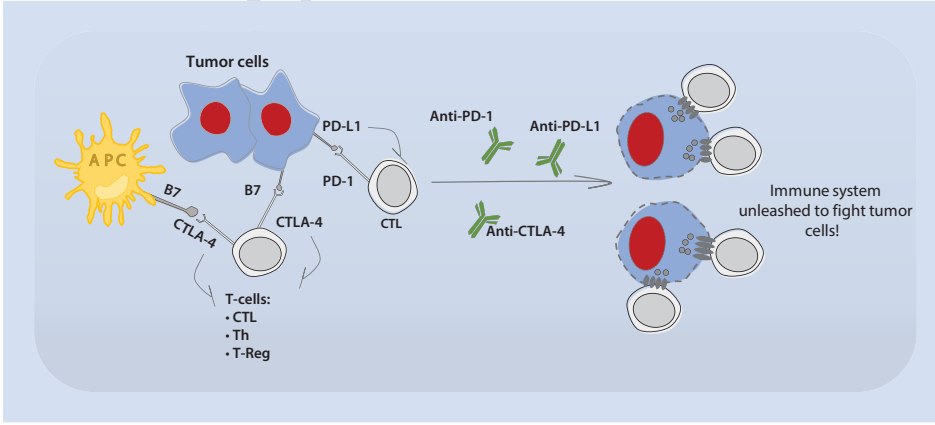

Fig. 7.8 Mechanism of action of immune checkpoint inhibitors. When activated T-cells encounter a PD-L1-expressing tumor cell, PD-1 receptor is activated in T-cells leading to T-cell exhaustion. CTLA-4 competes with CD28 for B7 ligands (CD80/CD86) decreasing T cell activity. Therefore, blocking PD-1/PD-L1 and CTLA-4 activity with immune checkpoint antibodies blocks immune suppression and stimulate effector T cells, boosting anti-tumor responses

immune system, T cell activation is highly regulated and subjected to feedback regulation by inhibitory checkpoints and T-Regs.

Seminal work by James Allison, Padmanee Sharma and colleagues showed that CTLA-4, which is constitutively expressed in T-Regs and induced after T cell activation, competes with CD28 for B7 ligands with much more affinity, inhibiting proliferation and IL2 secretion by T cells (abrogating its anti-tumor response). It was also shown that CTLA-4 blocking antibodies could treat tumors in immune competent animal models and later in clinical trials showed very promising results. In 2011, FDA (Food and Drug Administration) approved the first anti-CTLA-4 antibody – Ipilimumab – to treat metastatic melanoma [51, 52].

Another immune checkpoint molecule expressed by activated T cells to suppress activation is PD-1 (programed death receptor 1) and it has been shown that PD-L1 (ligand) expression can be exploited by many tumors to evade immune attack. Antibodies blocking the PD-1 and PD-L1 inhibitory axis can unleash activated tumor-reactive T cells and have very encouraging results [7, 51]. Anti-PD-1 (Nivolumab) and anti-PD-L1 (Pembrolizumab) were also recently FDA approved for metastatic melanoma and advanced/metastatic non-small cell lung cancer. The combination of anti-PD-1/PD-L1 with complementary checkpoint inhibitor CTLA-4 has also been shown to have promising results in many other types of cancer [51]. James Alison for CTLA-4, together with Tasuku Honjo for the PD-1/PDL-1 therapies, just got the *2018 Nobel Prize* in Physiology or Medicine!

In the clinic, the presence of TILs and PD-L1 expression correlates with patient survival/better prognosis. This hint, of a "hot" tumor microenvironment, indicates that the patient will benefit with either TILs or anti-PD-L1 therapy. In contrast, if the tumor microenvironment is "cold", anti-CTLA-4 should be administered to drive T cells into the tumor and induce PD-L1 expression, in order to be responsive to combinatorial therapy.

7.3.4 Combinatorial Immunotherapy

The efficiency of the immune checkpoint blockade with monoclonal antibodies in cancer treatment is remarkable and has durable effects. However, only a fraction of patients benefits from this therapy. Therapeutic intervention often fails because tumor cells are not immunogenic enough i.e. they do not express sufficient Ag to be recognized and presented to T cells or they may face other suppressive mechanisms present in the TME. To enhance and broaden the anti-tumor activity of immune checkpoint inhibition it is possible to combine other agents [7, 53]. For example:

- Chemotherapy or radiotherapy – have been shown to expose tumor antigens and therefore aid recognition of tumor cells by the activated T cells
- IDO inhibition – IDO when expressed in the TME either by tumor or host immune cells, leads to immunosuppression by increasing T-Regs and decreasing proliferation of effector T cells. Combination of IDO inhibition and immune checkpoint blockage are currently under clinical investigation.

7.3.5 Cancer Vaccines

Although most cancer vaccines are employed as therapeutic rather than preventive agents, there is one paradigm that revealed to be a huge achievement – the Human Papilloma

Virus (HPV) vaccine that protects women against cervical cancer (ovarian). All other cancer vaccines, in general have a therapeutic action and involve administration of TAAs in the form of either peptides, recombinant proteins, DNA or even whole cells to stimulate the immune system to attack cancer cells. The stimulation of immunity can be either direct, i.e. directly administrated to patients, or the tumor antigens can be presented to immune cells *ex vivo* (*in vitro*) to expand them and to then re-infuse the activated/selected cells into patients (Dendritic cell vaccines) [7, 26]. For now, in humans, the majority of vaccines are only being use in clinical trials.

Many more immunotherapies are being developed, some focusing also in the innate cell compartment. For example, the inhibition of CSF-1R (receptor of macrophage colony stimulating growth factor) reduces the frequencies of TAMs and increases IFN production, confining tumor progression. Additionally, this therapy can also synergize with anti-PD1 or anti-CTL4 antibodies [54]. Another strategy reported is the use of blocking "don't eat me" signals, to unleash the phagocyte activity of macrophages [55].

In conclusion, this new approach to fight cancer using the patient's own immune system, just like Coley originally proposed, is giving hope to finally manage or even cure this shattering disease. However, not all patients respond, so there is still a long way to go in research to understand all the strategies cancer cells employ to avoid and suppress the immune system to make immunotherapy a reality for all patients.

Check out these movies:

- ▶ https://youtu.be/3hlGq-3F1uQ
- ▶ https://youtu.be/K09xzlQ8zsg

Take Home Message

- Immune system evolved to protect the host against diseases – innate and adaptive immunity work together to eliminate possible threats
- Immunosurveillance is the first step in preventing and fighting cancer
- Tumors are edited and immunologically sculpted over time leading to detectable cancers
- There is an active dialogue between cancer and immune cells in the tumor microenvironment that influences immune anti- or pro-tumoral function
- Tumors are able to circumvent immune attack employing immunosuppressive mechanisms and mechanism of death resistance
- Inhibitors of mechanisms responsible for tumor escape could restore anti-tumor immune responses in cancer patients
- Cancer Immunotherapy as a pillar of cancer therapy – can be combined with other types of therapies to enhance its efficiency for long term

Questions

1. What are the differences between innate and adaptive immunity? Who are the players of each type of immune response?
2. Explain the concept of cancer immunoediting referring briefly the three processes underlying it.
3. Which types of immunity can be provided by the adaptive immune system? Describe the main cells involved in both responses.

4. Describe how innate interacts with adaptive system and how they work together to carry out the function of protect the body against cancer.
5. What are the major components of the tumor microenvironment?
6. What are the relevant immune evasion mechanisms that cancer cells employ to circumvent immune response? Which molecules act on those processes?
7. Provide some examples of adoptive cell therapy.
8. Explain the mechanism of immune checkpoint therapies.

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