# INTRODUCTION TO TUMOR IMMUNOTHERAPY

## 肿瘤免疫治疗概论

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Introduction to Tumor Immunotherapy

**肿瘤免疫治疗概论**

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# PREFACE

Immunotherapy, which involves or uses components of the immune system, is the most promising therapy used to treat tumor patients. By re-awakening and enhancing the immune system to fight tumor, immunotherapy has achieved impressive clinical responses. However, many tumor types still do not respond to immunotherapy and many patients do not receive durable benefit, eventually developing resistance.

Broadening the clinical applicability of tumor immunotherapy requires a deep understanding of the molecular and genetic mechanisms that influence whether tumor cells resist or respond. With the goal of expanding the benefits of immunotherapy and finding actionable strategies to combat therapeutic resistance, an increasing number of studies have been conducting in pre-clinical and clinical models. Furthermore, significant efforts are underway to identify reliable predictive biomarkers of response and resistance to immunotherapy, such as checkpoint inhibitors.

This textbook describes the latest advancement of tumor immunotherapy: basic and clinical applications, and is specified for foreign language teaching of postgraduate students in colleges and universities who major in medical immunology. Meanwhile, this textbook describes the basic immunology principles that form the foundation of understanding how the immune system recognizes and rejects tumor cells. The role of the innate and adaptive immune responses is discussed and the implications of these responses for the design of clinical strategies to combat cancer are illustrated through both experimental clinical trials and review of current standard of tumor therapeutic agents. This information will be invaluable to both students of immunology and cancer research and practicing physicians who have patients with tumor. The textbook provides a comprehensive overview of the field, demonstrates how advances in basic immunology can and are being applied to cancer treatment, and describes the current status of approved immunotherapy regimens.

In the compiling course of the textbook, we collected and referred large numbers of the latest peer-reviewed research articles and papers in professional journals. We would very like to express our gratitude for the authors of the references.

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Figures & Papers

# CONTENTS

# Chapter 1

# Introduction

## 1.1 Basics of the Human Immune System

The human immune is extremely complex. It has evolved over hundreds of millions of years to respond to invasion by the pathogenic microbes that regularly attempt to infect our bodies, and invasion by the microbes that tried to infect our genetic ancestors. There are similarities between the immune system of humans and those of the most primitive of vertebrates, going back five hundred million years on the evolutionary ladder.

The immune system does not rely on one single mechanism to deter invaders, but instead uses many strategies, the most important of which are detailed below. The main division between the strategies is that between **innate immunity**, which does not require previous exposure to the invading microbe, and **acquired immunity**, whereby the immune system "remembers" how to deal with a microbe that it has dealt with before.

### *1.1.1 The Phagocytes*

Phagocytes are the soldiers of the immune system, and provide **innate immunity**. They are responsible for swallowing, killing and digesting invading microbes. The process of swallowing microbes is known as **phagocytosis**. There are two main types of phagocyte.

- **Microphages**. These cells are also known as Polymorphonuclear Leucocytes, PMNs and Polymorphs. These cells start life in the bone marrow. They are constantly circulating in the blood. They cannot replicate, and live for only a few days. The bone marrow contains large reserves of microphages.

- **Macrophages**. These cells start out life as **monocytes**, which originate in the stem cells in the bone marrow, but when they are first called into action, they turn into macrophages. Macrophages are not as numerous as microphages, and there are no large reserves of them, but they are longer

lived than microphages. Macrophages are stationed at strategic locations throughout the body, usually in places that are not otherwise well defended. These areas include the alveoli of the lungs, the abdominal (peritoneal) and chest (pleural) cavities, under the top layer of the skin and the intestines. Macrophages are the front line of defense against microbial invasion in these areas.

As mentioned above, the process of swallowing of microbes by the phagocytes is known as **phagocytosis**. After the invading microbe has been ingested, the next task for the phagocyte is to kill the microbe. This is achieved in two main ways.

- **Aerobically**, i.e. using oxygen. The phagocytes produce oxygen based chemicals that are highly disruptive to the swallowed microbe. Oxygen is highly chemically reactive, and these oxygen based chemicals "tear" the microbe apart. This process is known as the **oxidative burst**, or the **respiratory burst**.

- **Anaerobically**, i.e. without using oxygen. One way to kill the microbe without oxygen is by using a chemical that deprives the microbe of iron, thus preventing it from metabolising. Another way is to increase the acidity of the internal environment of the phagocyte.

When these tasks are complete, the **macrophages** have one further task to complete. They return to the lymph nodes, displaying the remnants of the destroyed invader on their surface. This has the effect of stimulating the cells of the **acquired immunity** system into action.

### *1.1.2 The Complement System*

The complement provides **innate immunity**. It is comprised of a collection of proteins that "recognise" corresponding proteins on the cell walls of invading microbes. When such invading microbes have been recognised, the following actions are taken.

- The "alarm" is sounded. Chemicals, known as **chemotaxins**, that attract phagocytes are emitted. This process is known as **chemotaxis**. The phagocytes follow the trail of **chemotaxins** to arrive at the site of invasion.

- The invading bacteria are "marked" with chemicals that make them stand out. These chemicals are known as **opsonins**, from the Latin word *opsonium*, meaning "sauce". This "marking" greatly increases the chances of the invading bacteria being phagocytosed.

- Chemicals are released which promote the **inflammatory response**. More on the inflammatory response later.

### *1.1.3 Acquired Immunity*

The acquired immunity system comprises B Cells and T Cells. Together, these cells which provide acquired immunity are known as **Lymphocytes**. The acquired immunity system further divides into two parts, humoral immunity and Cell Mediated Immunity (CMI).

**B Cells**

B Cells provide **Humoral Immunity**. Each B cell secretes a unique antibody, which acts against a particular **antigen**. An **antigen** is a chemical feature (a protein) which is unique to any given type of invading organism. When B cells meet an invading organism for which they have the antibody, they do one of two things.

- They may turn into **antibody factories** and start manufacturing as many copies of their antibody as they can.

- They may clone themselves, thus increasing the numbers of **antibody factories**, which results in an increased immune response to the target organism.

**T Cells**

T Cells provide **Cell Mediated Immunity**, often referred to as **"CMI"**. T cells have several functions. They can be:

- **Helper T cells**, which control other cells, such as B cells or Macrophages, directing them to carry out their task.

- **Suppressor T cells**, which dampen down the immune response when it is no longer needed.

- **Cytotoxic T cells**, which destroy host cells that have become infected with the invading organism.

**Cytokines**

Cytokines are the last element of the immune system which we shall discuss here. Cytokines (meaning "cell movers") are the messengers of the immune system. The above mentioned elements of the immune system (Complement, Phagocytes, Lymphocytes) do not work separately, but all

work together in co-operative fashion. If they are to work effectively, they need a good system for communicating messages. This system is provided by the cytokines. Among the cytokines are the **Interleukins**, of which there are known to be at least twelve, **Gamma-Interferon**, **Lymphotoxin**, and **Tumor Necrosis Factor**.

## 1.2 Conception of Tumor Immunotherapy

Tumor is a leading cause of mortality worldwide, accounting for one in seven deaths globally. Progress has been made in treating tumor, but a significant proportion of patients still die despite treatment, indicating that new, more effective targets for tumor therapy are needed. According to the definition of National Cancer Institute (USA), immunotherapy is any "treatment to boost or restore the ability of the immune system to fight cancer, infections, and other diseases". Although tumor progression involves a wide variety of methods to overcome host immunity, tumor immunotherapy can potentially revive the patient's suppressed immune system, ideally resulting in the eradication of the disease. By re-awakening and enhancing the immune system to fight tumor, such strategies have achieved impressive clinical responses. A range of tumor immunotherapy approaches have proven effective in many patients, including monoclonal antibodies, immune checkpoint blockers, cancer vaccines and cell-based therapies [1].

## 1.3 Progress of Tumor Immunotherapy

Currently, two major revolutions in tumor research and treatment are fulfilling the need for targeted treatments. One of these trends is based on significant advancement in cancer immunotherapy, allowing new treatments that enhance the body's antitumor immunity functions. Because this approach is so promising, "cancer immunotherapy" was named "Breakthrough of the Year" by *Science* in 2013. During the last decade, a rapid understanding of the mechanisms that most tumor cells use to hide from the immune system has led to the improvement of new immunotherapeutic approaches against tumor. Overall, there are four different immunotherapeutic strategies. These include immune checkpoint blockade, cytokine therapy, cellular therapy and therapeutic vaccines. To understand how immune checkpoint blockade works, it is critical to comprehend the constant interaction between tumor and immune cells in the continuous process of cancer development. Tumor cells exploit various immune-regulatory mechanisms to achieve immune escape, thus suppressing immune responses against them within the tumor microenvironment. This is more obvious as the disease progresses. Several immune-related cells work towards the establishment of an immunosuppressive microenvironment, including regulatory T cells (Tregs), dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs) and regulatory B cells. Therefore, within the

tumor microenvironment, cancer cells along with immune cells excrete inhibitory cytokines and express checkpoint inhibitors that dampen the anti-tumor activity of specific T cells. During the last decade, a rapid understanding of the mechanisms that most cancer cells use to hide from the immune system has led to the improvement of new, immunotherapeutic approaches against cancer. Especially, the use of anti-PD-1 or anti-PD-L1 monoclonal antibodies (mab) has yielded top-notch medical responses in several cancers [2].

### *1.3.1 Progress of Target Tumor Cells*

Chimeric antigen receptor (CAR) is the core component of CAR-T, which endows T cells with the ability to recognize tumor antigens in a HLA-independent manner and enables them to recognize more extensive target antigens than natural T cell surface receptor (TCR). A basic CAR includes a tumor-associated antigen (TAA) binding domain (usually from the scFv fragment of antigen-binding region of the monoclonal antibody), an extracellular hinge domain, a transmembrane domain and an intracellular signal domain [3].

The activation of T cells mediated by the first generation of CAR is accomplished by the tyrosine activation motif on CD3ζ chain or FcεRIγ. CD3ζ chain can provide "signal I" for T cell activation, cytolysis, regulation of IL-2 secretion and anti-tumor activity in vivo. However, the anti-tumor activity of the first generation of CAR modified T cells is limited in vivo, and the decreased proliferation of T cells ultimately leads to apoptosis. The second generation of CAR adds a new costimulatory signal in the intracellular region, which enlarges the original "signal Ⅰ" derived from TCR/CD3 complex. Many studies have shown that compared with the first generation of CAR, the second generation of CAR carrying "signal Ⅱ" has the same antigen specificity, increased T cell proliferation and cytokine secretion, enhanced secretion of anti-apoptotic proteins, and delayed cell death. The ubiquitously used costimulatory molecule is CD28, which have been gradually been replaced with CD137 (4-1BB). In addition, an idea of using NK cell receptor CD244 has also been proposed to promote sustained activation and proliferation of CAR-T cells [4].

In order to further improve the design of CAR, many studies began to focus on the development of the third generation of CAR, including not only "signal Ⅰ", "signal Ⅱ", but also additional costimulatory signals. Studies using different targets and costimulatory signals were conducted to compare the results of the second and third generations of CAR and obtained quite encouraging experimental results. Combination of CD28 and 4-1BB costimulatory signaling domains is to construct a CAR specific for prostate-specific membrane antigen (PSMA), and then induce the

strongest PI3K/Akt activation and Bcl-XL expression in vitro, and the least apoptosis in transduced peripheral blood $CD8^+$ T cells [5]. To target a different tumor marker, MUC1, a CAR is designed for containing a fused CD28/OX40/CD3ζ endo-domain, the engineered CAT-T cells and upon MUC1 stimulation can secrete proinflammatory cytokines indicative of both type-1 (IFN-γ) and Th17 (IL-17) differentiation in vitro [6]. It was noteworthy that IL-17 has been known as tissue destructive cytokine in autoimmune disease animal models, although its anti-tumor effect is still to be elucidated.

It is still uncertain which design is better between the second generation and the third generation of CAR. Additionally, the second and third generations of CAR have their own on-going clinical trials in the US, China and Europe, and the development and outcome of these clinical trials are being closely watched [4].

Immunotherapy with CAR-T cells has achieved tremendous successes in treatment of hematological malignancies. Two CD19-targeting CAR-T cell products, Kymriah from the Novartis (East Hanover, NJ USA) and Yescarta from the Kite Pharma (Santa Monica, CA USA), have been approved by the Unite States Food and Drug Administration (US FDA) for treating B cell acute lymphoblastic leukemia (B-ALL) and diffusing large B-cell lymphoma (DLBCL), respectively [7]. However, due to intricacies of solid tumors and their locations in the human body, treatment of solid tumors with CAR-T cells is facing multiple obstacles, such as the hostile tumor microenvironment, on-tumor/off-tumor toxicities, and undesired antigen specificity. Many strategies and approaches have been tried to overcome these obstacles, including arming CAR-T cells with knock-out of PD-1 expression or secretion of cytokines/chemokines and using CAR-T cells in combination with other treatments. Despite these efforts, there are still no CAR-T cells clinically approved for solid tumor treatment so far. Encouragingly and optimistically, in this landscape, more than forty clinical trials in treatment of solid tumors by CAR-T cells have been registered in China alone [4, 8, 9].

As innate immune cells, natural killer (NK) cells are unique and play pivotal functions in cancer immune surveillance. NK cells can eliminate a variety of abnormal or stressed cells without prior sensitization, and even preferentially kill stem-like cells or cancer stem cells. Upon forming immune synapses with target cells, NK cells release preformed cytolytic granules, including perforin, and granzymes, of which function is to induce cell lysis. Several studies have successfully exploited adoptive transfer of NK cells against various tumors, especially hematological malignancies [10].

Adoptive transfer of autologous NK cells expanded ex-vivo for treatment patients with lymphoma, colon cancer, breast cancer and lung cancer have been tested in a range of clinical trials. Only very limited antitumor effect was observed. The major reason was that the inhibitory receptors on autologous NK cells matched self MHC class I presented on tumor cells, and this self-recognition signals subsequently inhibited the activation of NK cells. Besides, autologous NK cells derived from cancer patients were actual in an immune suppression state with impaired functions, making these cells difficult to exhibit antitumor capability. The first piece of evidence showing that NK cells had a clinical benefit was reported in 2002. It has been confirmed that donor vs recipient NK cell alloreactivity, which was mainly resulted from KIR ligand incompatibility, could avoid relapse and graft rejection without GVHD in AML patients receiving HLA (Human leukocyte antigens) mismatch donor hematopoietic transplantation. Later, this strategy was used in adoptive cellular immunotherapy of ex vivo activated allogeneic KIR/KIR ligand mismatched NK cells derived from PBMC into AML patients. Subsequently, alloreactive PBMC derived NK cells have been widely investigated as an immunotherapy in clinical trials of hematologic malignancies, as well as in trials of solid tumors including melanoma, breast cancer, ovarian cancer, neuroblastoma, renal cell carcinoma, colorectal cancer, and hepatocellular cancer [11, 12].

Target-activated NK-92 (ta-NK) cells, another engineered variant of NK-92 cells modified with CARs, have been designed to target TAAs-expressing tumor cells. The stable CAR expression and activity of these cells have been demonstrated by HER2. taNK (HER2-specific target activated NK), a cell line that is now being tested in patients with recurrent HER2-positive Glioblastoma (NCT03383978). Unlike immunostimulatory strategies which involve the delivery of molecules to assist NK cells, genetic modification strategies induce changes in the genetics of NK cells directly, leading to far-reaching and sustained changes to the cells. Among them, genetic modification of NK cells with CAR constructs has drawn increasing attention. CAR-NK cells can be produced from different sources of NK cells including primary NK cells, NK cell lines, and HPSCs. CAR-NK has adopted the basic structural framework of CAR-T, that is, chimeric antigen receptors mainly composed of extracellular, hinge, transmembrane and intracellular domains, as well as the transfection methods. The extracellular domain can bind tightly to tumor-associated antigens expressed on the surface of tumor cells, which determines the specificity of CAR structures. Single-chain variable fragments (ScFvs) are the most currently used ectodomains for CARs. The hinge domain is the connecting sequence between the extracellular domain to the transmembrane domain, which endowed CAR with adequate orientation and flexibility to bind to tumor antigens and are expected to impact the CAR-NK activities. The transmembrane domain lies between the

hinge and the intracellular signaling domain, including CD3ζ, HLA-A2, or CD28 molecules. The structure of the intracellular signaling domain determines the intensity of the CAR-NK activation signal, which contains the immunoreceptor tyrosine-activated motifs (ITAMs). The majorities of current CAR endo-domains contain an activation region derived from CD3ζ, which is the most classical intracellular domain including three ITAMs. In order to increase the proliferation and cytotoxicity of CAR modified effector cells, co-stimulatory protein receptors such as CD28, 4-1BB, CD134, ICOS are added to the cytoplasmic tail [10, 13, 14].

The first CAR used in NK cells is a CD4-CD3ζ (CD4ζ) fusion receptor [15]. The CD4ζ chimeric receptor is biochemically and functionally active and can guide human NK cells efficiently to kill either HIV-infected $CD4^+$T cells or NK-resistant tumor cells expressing gp120 in vitro, indicating CAR structure can be successfully expressed on NK cells, leading to efficient retargeting. Subsequently, more attempts were taken to enhance the anti-tumor ability of NK cells [15]. CAR-NK cells have been evaluated for the treatment of hematological cancers and solid tumors in preclinical studies with numerous ideal results. Based on these findings, the clinical translation of CARNK cells has witnessed significant interests. Although CAR-NK therapy is still under clinical evaluation, NK cells possess several advantages over T cells in being engineered to express CARs and used for cancer treatment. First, NK cells are easy to be isolated and have a relatively short lifespan. Therefore, the risk of overexpansion of transferred CAR-NK cells in patients is relatively low. Second, the cytokines secreted by NK cells mainly include IFN-γ and GM-CSF, which are relatively safer than those released by activated CAR-T cells, e.g., TNF-α and IL-6. In particular, the proinflammatory cytokines produced by CART cells may cause life-threatening cytokine release syndrome (CRS), a most common and severe side effect of CAR-T therapy. Third, CAR-NK cells could trigger the lysis of target cells in both CAR-dependent and CAR-independent manners, which further potentiating their killing activity. In addition, CAR-NK therapy is expected to be less expensive, considering that NK cells can be derived from PBMCs, NK cell lines and hPSCs. However, T cells used for CAR-T therapy are required to be autologous [10, 15, 16].

Currently, cancer immunotherapy strategies are dominated by immune cells. However, there are limitations when using immune cells directly for the treatment of cancers. For instance, immune cells can penetrate hardly into the solid tumor, leading to unsatisfactory therapeutic effects. Moreover, the cost for producing, preserving and transporting clinical grade living immune cells is high, posing a challenge to wide applications in clinics. In recent years, EVs, a nano-sized vesicle naturally secreted by many different types of cells including NK cells, have gradually been proposed and studied, providing a new cell-free immunotherapy avenue (Figure 1.1) [10].

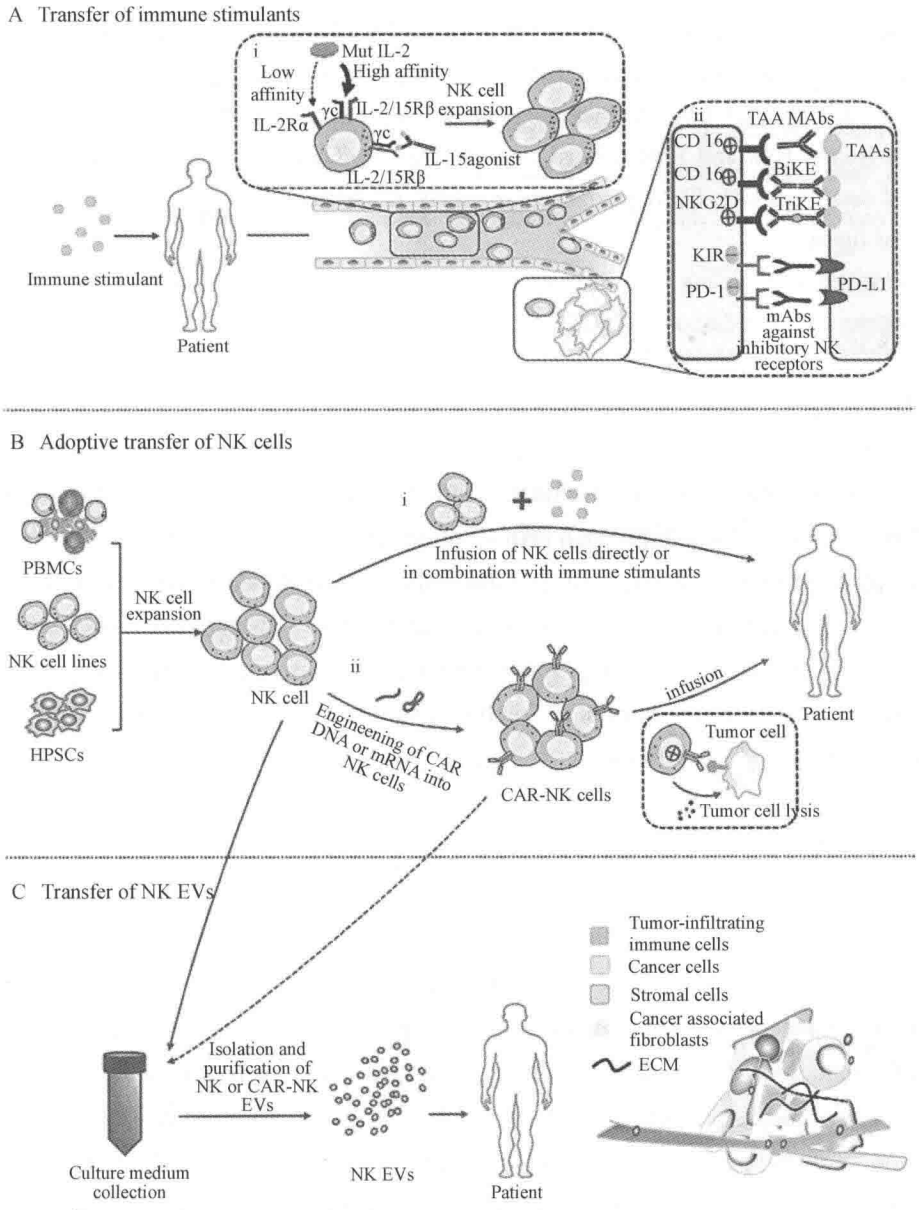


**Figure 1.1 Various NK cell-based immunotherapy approaches.** (A) Administration of stimulatory cytokines and antibodies to patients triggers activation and expansion of the autologous NK cells and enhance their cytotoxicity. (i) Cytokine. "Super" agonist of IL-2 improves its affinity for IL-2/15Rβ. Arrow width indicates expected intensity of IL-2 signaling. The "super" agonist of IL-15 mimics the physiological trans-presentation of IL-15 to NK cells without the involvement of antigen-presenting cells. (ii) Antibodies. Binding of CD16 to the Fc portion of TAA mAbs leads to NK cells activation and ADCC. Application of BiKE or TriKE target to CD16 or NKG2D (on NK cells) and tumor antigens promotes the formation of immune synapses between NK cells and tumor cells. mAbs against inhibitory receptors on NK cells facilitate NK cytotoxicity. (B) Adoptive transfer of NK cells. (i) NK cells obtained from PBMCs, NK cell lines or hPSCs can be infused into patients directly or in conjunction with

immune stimulants. (ii) NK cells are designed to express CAR, which are then allowed to expand ex vivo before being transfused back into the patient. (C) Infusion of engineered NK cells and NK/CAR-NK cell derived EVs. Culture medium of expanded NK cells or CAR NK cells can be exploited to isolate EVs and then infused into the patient. TAA, Tumor-associated antigen; PD-L1, Programmed death ligand-1; ECM, Extracellular matrix. (Please scan the QR code on the Preface to get original color figures.)

Source: Hu W, Wang G, Huang D, Sui M, Xu Y. "Cancer Immunotherapy Based on Natural Killer Cells: Current Progress and New Opportunities". *Front Immunol*, 2019, 10:1205.

### *1.3.2 Progress of Activated Immune Cells* [17]

A lot of studies have shown that self-antigen or tumor antigen-specific $CD4^+$ and $CD8^+$ T cells are present in healthy individuals. How such self- or tumor-reactive T cells are controlled in healthy or tumor-bearing individuals remains to be determined. Mechanisms for the maintenance of immunological self-tolerance (i.e., unresponsiveness to self-antigens) not only prevent autoimmunity but also hamper effective tumor immunity because many tumor antigens recognized by autologous lymphocytes are normal self-antigens or quasi-self-antigens with genetic mutations. This is one reason why it is difficult to elicit strong tumor immunity in cancer-bearing patients by cancer vaccine alone. It also suggests that effective tumor immunity can be evoked by breaching a certain mechanism(s) of immunological self-tolerance systemically or locally in tumor tissues.

On the one hand, among the various mechanisms of immunological self-tolerance, immune suppression by endogenous $Foxp3^+CD25^+CD4^+$ Treg cells is essential and indispensable as illustrated by spontaneous autoimmune disease development when Treg cells are rendered deficient. For example, mutations of the gene encoding the Treg-specific transcription factor Foxp3 impair Treg cell development and cause a fatal multi-organ autoimmune disease called immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome. Depletion of $Foxp3^+CD25^+CD4^+$ Treg cells by a variety of methods is also able to cause similar autoimmune diseases in otherwise normal rodents.

On the other hand, it is now well substantiated that a large number of Treg cells infiltrate into tumor tissues of various cancers and their abundant presence is often associated with poor clinical prognosis. Experimentally, the role of Treg cells in tumor immunity was first demonstrated by an attempt to determine a common basis between tumor immunity and autoimmunity. Removal of Treg cells using cell-depleting anti-CD25 antibody, either by *in vivo* antibody administration to mice or transfer of cell suspension depleted *in vitro* of $CD25^+$ Treg cells into histocompatible T-cell-deficient mice, effectively eradicated a variety of inoculated syngeneic tumors. The mice

showed an increase of tumor-infiltrating $CD8^+$ T cells with strong tumor-specific killing activity, and upon re-challenge with the same tumor cells, exhibited more rapid rejection than the primary rejection, indicating the establishment of tumor-specific immunity. These studies have thus demonstrated that the removal of Treg cells is able to evoke effective anti-tumor immunity by abrogating immunological unresponsiveness to syngeneic tumors, albeit it may also cause autoimmunity, especially if Treg cells are depleted systemically.

The T-cell receptor (TCR) repertoire of Treg cells is broad and skewed to a certain extent to recognizing self-antigens. That is, in the course of T-cell selection in the thymus, a developing Treg cell exhibits a higher TCR affinity than a conventional T (T conv) cell for the MHC/self-peptide ligand that selects both. Assuming that TCR recognition of peptides is cross-reactive (and degenerate) and a particular TCR is able to recognize a million different peptides of 10 amino acid length, the TCR repertoire of Treg cells as well as T conv cells is broad and able to recognize a wide spectrum of self and non-self-antigens including quasi-self-tumor antigens. Given the antigen-primed state of endogenous Treg cells (as illustrated by higher level expression of T-cell accessory molecules such as LFA-1), it is reasonable to assume that Treg cells recognizing a particular self- or tumor- antigen are more easily activated than naive T conv cells recognizing the same antigen, ensuring Treg-mediated dominant tolerance.

Treg cells would be able to control not only T cells but also B cells, NK cells, dendritic cells (DCs), and macrophages via humoral and cell-cell contact mechanisms. A variety of molecules are involved in Treg-mediated suppression mechanisms, including CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), IL-2, IL-10, TGF-β, IL-35, GITR (glucocorticoid-induced TNF receptor), LAG3 (lymphocyte-activation gene 3), granzyme B, adenosine, and cAMP (Figure 1.2). Given that ectopic Foxp3 expression in T conv cells would be able to confer Treg-like suppressive activity, the molecule(s) mediating a core suppressive mechanism may well be controlled by Foxp3. In addition, among various mechanisms of Treg-dependent suppression, those important for maintaining self-tolerance (i.e., the suppression mechanisms whose impairment causes autoimmune disease) have the most impact on tumor immunity. On these assumptions, there are only a few molecules whose expression is controlled by Foxp3 directly or indirectly and whose deficiency abrogates Treg-suppressive function and causes severe autoimmune diseases. The candidates include IL-2, IL-2 receptor subunits, and CTLA-4. Foxp3 indeed controls the expression of these molecules and deficiencies of IL-2, CD25 (IL-2 receptor α-chain), and CD122 (IL-2 receptor β-chain), or CTLA-4 produces similar autoimmune diseases as observed in Foxp3 deficiency.