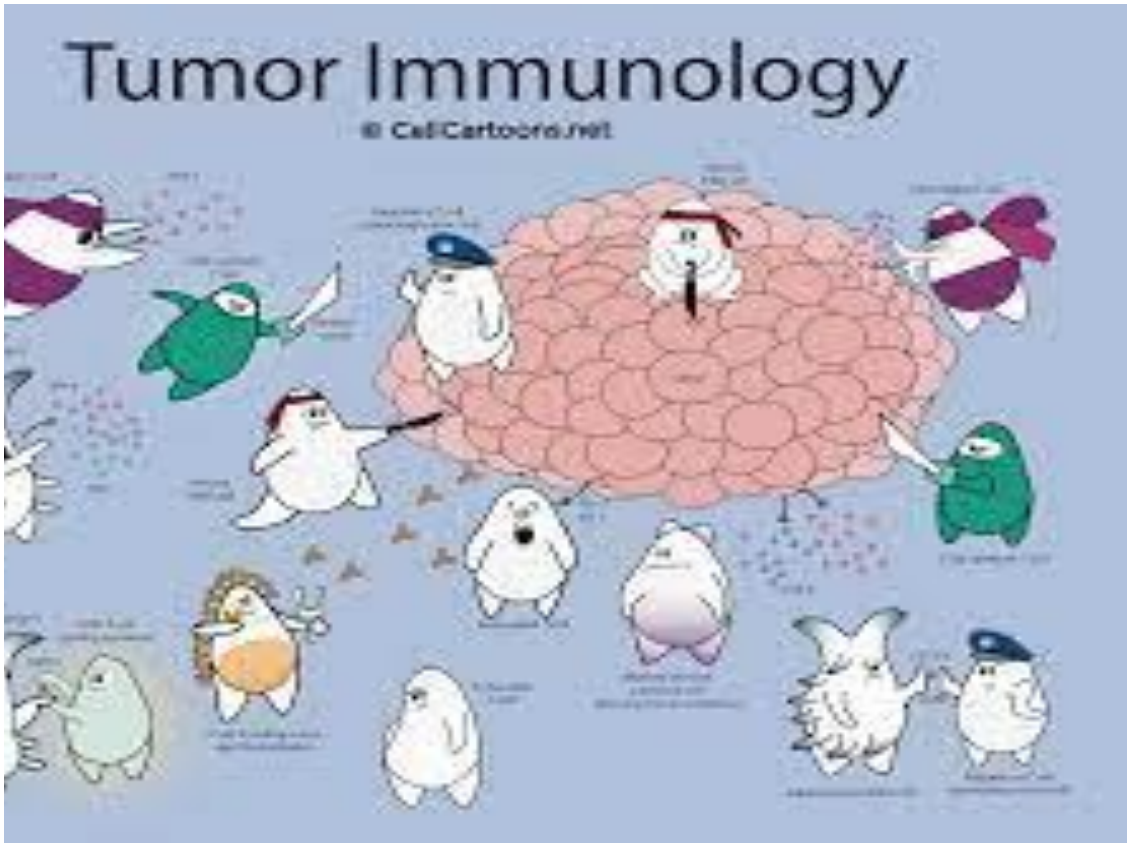
Tumor Immunology



PhD. Zoology **2023-2024**

By

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Lec 1 **Tumor Immunology** **Ph D/ Zoology**

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References:

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Learning objectives

1. To understand how the immune system mounts an immune response against tumors
2. To understand how tumors evade immunity
3. To review strategies to combat tumors based on immunotherapy, including passive and active immunization.
4. To understand tumor marker and diagnosis of tumor marker.

To understand the immune response aganst transplantation.

The most important causes of morbidity and mortality inchildren and adults. The lethality of malignant tumors is due to their uncontrolled growth within normal tissues,causing damage and functional impairment. The malignant phenotype of cancers results from defective regulation of cell proliferation, resistance of the tumor cells to apoptotic death, and the ability of the tumor cells to invade host tissues and metastasize to distant sites.

In addition, reflecting our improved understanding of immune responses against cancers and the therapeutic success of cancer immunotherapy, we now include the ability of tumor cells to evade host immune defense mechanisms as one of the hallmark features of cancer.

The concept of **immune surveillance** of cancer, which was proposed by Macfarlane Burnet in the 1950s, states that a physiologic function of the immune system is to recognize and destroy clones of transformed cells before they grow into tumors and to kill tumors after they are formed. The existence of immune surveillance has been demonstrated by the increased incidence of some types of tumors in immunocompromised experimental animals and humans.

OVERVIEW OF TUMOR IMMUNITY

Several characteristics of tumor antigens and immune responses to tumors are fundamental to an understanding of tumor immunity and for the development of strategies for cancer immunotherapy.

Tumors stimulate specific adaptive immune responses that can prevent or limit the growth and spread of the cancers.

Clinical studies, pathological analyses of tumors ,and animal experiments have all established that although tumor cells are derived from host cells, the tumors elicit immune responses in their hosts. Most evidence indicates that the clinically relevant immune responses involve T cells, and especially CD8+ cytotoxic T lymphocytes (CTLs).Histopathologic studies show that many tumors are surrounded by mononuclear cell infiltrates composed of T lymphocytes and macrophages, and that activated lymphocytes and

macrophages are present in lymph node draining the sites of tumor growth (Fig. 1).Quantitative analyses of these infiltrates in colon cancers and some other tumor types have revealed that higher numbers of T cells, in particular CD8+ CTLs and CD4+ Th1 cells, are associated with a better prognosis than tumors with less of these cells (Fig. 1). The first experimental demonstration that tumors can induce protective immune responses came from studies.

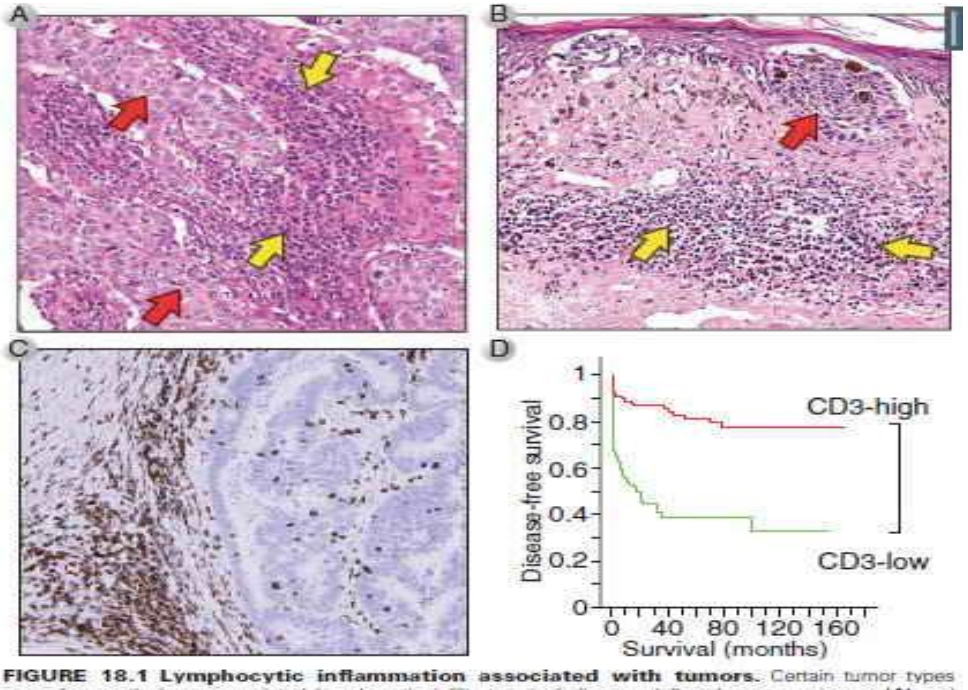


FIGURE 18.1 Lymphocytic inflammation associated with tumors. Certain tumor types more frequently have associated lymphocytic infiltrates, including medullary breast carcinoma **(A)** and malignant melanoma **(B)**. *Red arrows* indicate malignant cells. *Yellow arrows* indicate lymphocyte-rich inflammatory infiltrates. Immunohistochemical staining of resected tumors can be used to enumerate different types of T cells associated with the tumor, such as an infiltrate of $CD8^+$ T cells in a colonic carcinoma. The tumor cells appear blue and the $CD8^+$ T cells brown **(C)**. Increased density of $CD3^+$ T cells at the invasive margin of the tumor, detected in this way, is associated with longer disease-free survival **(D)**. *(C, Courtesy of the Brigham and Women's Hospital Department of Pathology. D, From Galon J, Costes A, Sanchez-Cabo F: Type, density, and location of immune cells within human colorectal tumors predict clinical outcome, Science 313:1960–1964, 2006.)*

of transplanted tumors performed in the 1950s. A sarcoma may be induced in an inbred mouse by painting its skin with the chemical carcinogen

methylcholanthrene (MCA). If the MCA-induced tumor is excised and transplanted into other syngeneic mice, the tumor grows. In contrast, if cells from the original tumor are transplanted back into the original host, the mouse rejects this transplant and no tumor grows. The same mouse that has become immune to its own tumor does not reject MCAinduced tumors produced in other mice, which have different MCA-induced mutations and express different tumor antigens. Furthermore, transfer of T cells from the tumor-bearing animal to a tumor-free animal can impart protective immunity against the tumor. Thus, immune responses to tumors exhibit the defining characteristics of adaptive immunity—namely, specificity, memory, and a key role of lymphocytes. Subsequent work showed that the frequency of spontaneous or MCA-induced tumors in genetically immunodeficient mice is increased compared with immunologically normal mice, further establishing a role of the immune system in tumor immune surveillance. Immunodeficient humans, such as AIDS patients or transplant recipients given immunosuppressant drugs, are at increased risk for developing tumors, many of which are of known viral etiology (reflecting increased susceptibility to virus infection), but also some that are not.

Immune responses frequently fail to prevent the growth of tumors. There may be several reasons why antitumor immunity is unable to eradicate cancers. First, many tumors have developed specialized mechanisms for subverting host immune responses. In fact, established tumors may inhibit immune responses by various mechanisms. We will return to these inhibitory mechanisms later in the chapter. Second, tumor cells lose the expression of

antigens that may be recognized by the host immune system. Even tumors that do elicit effective immune responses may become less immunogenic over time because subclones that do not express immunogenicrapid growth and spread of a tumor may overwhelm the capacity of the immune system to effectively control the tumor, which requires that all the malignant cells be eliminated.

Ineffective adaptive immune responses to cancers can be overcome by therapeutic strategies that stimulate such responses, such that antitumor T cells can be activated to effectively kill tumor cells.

this realization has spurred new directions in cancer immunotherapy in which augmentation of the host antitumor response is the goal of treatment.The existence of specific antitumor immunity implies that tumors must express antigens that are recognized as foreign by the host. The nature and significance of these antigens are described next antigens have a selective survival advantage. Third, the

TUMOR ANTIGENS

The majority of tumor antigens that elicit protective immune responses are

1-neoantigens

2-produced by mutated genes in different tumor cell clones (Fig. 2). Because these antigens are not produced by healthy cells and are therefore not normally present, the immune system is not tolerant to them. Modern next generation sequencing technology has revealed the great diversity of **neoantigens** produced in different tumors. In virus-induced tumors,the tumor antigens are mostly foreign proteins produced by the oncogenic viruses, and the immune response

seen is essentially an antiviral response. Some tumor antigens that elicit protective immunity are normally expressed early in development and are aberrantly expressed in tumors, or are overexpressed in tumors. The modern emphasis on tumor antigens that are the inducers and targets of adaptive immunity has obvious relevance to understanding immune responses to tumors and developing ways of harnessing these responses.

tumor antigen: has been used to encompass many different molecules expressed by tumor cells, whether or not they stimulate protective immune responses.

Neoantigens: Antigens Encoded by Mutated Genes ***The protein neoantigens of tumors are mostly the products of randomly mutated genes ("passenger mutations"),reflecting the genetic instability of cancer cells or, less commonly, products of mutated oncogenes or tumor suppressor genes that are involved in oncogenesis ("drivermutations").***

New DNA sequencing technologies have identified mutated peptides from individual tumors that elicit T cell responses in the tumor patients (Fig. 18.2B). Usually, these neoantigens are produced by point mutations or deletions in genes that are unrelated to the development of the tumors. The encoded proteins generate new MHC-binding peptides that are presented to T cells and are foreign to the immune system since they are not normally present. The neoantigens are often cytosolic or nuclear proteins that are degraded by proteasomes and can be presented on class I major histocompatibility complex (MHC) molecules in tumor cells. After phagocytosis by dendritic cells, they may also enter the class II MHC antigen presentation pathway or be cross-

presented by the class I pathway. The application of new technologies for identifying tumor antigens is being used for the development of tumor vaccines, The same type of tumor in different patients mayexpress different sets of neoantigens. Furthermore, even in a single patient, as a tumor evolves it may acquire new mutations and thus produce new collections of neoantigens.

These findings have led to the concept of "clonal neoantigens," implying variability among tumor cell clones. The identification of these neoantigens is important for following immune responses to tumors in individual patients and for identifying antigens for vaccine development.

Antigens of Oncogenic Viruses

The products of oncogenic viruses function as tumor antigens and elicit specific T cell responses that may serve to eradicate virus-induced tumors. Viruses are implicated in the development of a variety of tumors in humans and experimental animals. Examples in humans include the Epstein-Barr virus (EBV), which is associated with B cell lymphomas and nasopharyngeal carcinoma, and human papillomavirus (HPV), which is associated with carcinomas of the uterine cervix, oropharynx, and other sites. In most of these DNA virus–induced tumors, virus-encoded protein antigens are found in the nucleus, cytoplasm, or plasma membrane of the tumor cells (Fig. 18.2C). These endogenously synthesized viral proteins can be processed and presented by MHC molecules on the tumor cell surface. Some viruses, such as hepatitis B and C, are associated with cancer but are not oncogenic. It is thought they promote tumors by inducing chronic inflammatory reactions in which tumorigenic

growth factors and other signals are generated. The tumor cells may contain viral antigens, but this is highly variable.

The ability of adaptive immunity to prevent the growth of DNA virus–induced tumors has been established by many observations. For instance, EBV-associated lymphomas and HPV-associated cervical cancers arise more frequently in immunosuppressed individuals, such as allograft recipients receiving immunosuppressive therapy and patients with acquired immunodeficiency syndrome (AIDS). The efficacy of virus-specific adaptive immunity to prevent tumors may be due, in large part, to preventing infection and eliminating infected cells, before cancers develop. Vaccination to prevent infection by these viruses also decreases the incidence of virus-associated cancers.

A vaccine against HPV has reduced the incidence of precancerous cervical lesions in vaccinated women. The vaccine is composed of recombinant HPV capsid proteins from the most common oncogenic strains of HPV, which form virus-like particles free of viral genome. Vaccination against hepatitis B virus has reduced the incidence of HBV-associated liver cancer.

Overexpressed Cellular Proteins

Table 4.1 Viral-encoded cancer antigens.

Virus	Virus family/genome	Associated cancer type(s)	Antigen(s)
HPV	Papillomaviridae/DNA	Cervical SCC, oropharyngeal SCC, vulvar SCC, anal and rectal SCC, penile SCC, vaginal SCC	E6, E7
HBV	Hepadnaviridae/DNA	Hepatocellular Carcinoma	HBx
HCV	Flaviviridae/RNA	Hepatocellular Carcinoma, NHL	
EBV	Herpesviridae/DNA	Nasopharyngeal carcinoma, Hodgkin disease, NHL, gastric carcinoma	EBNA1, EBNA3, LMP1, LMP3, gp350
HHV-8	Herpesviridae/DNA	Kaposi sarcoma, primary effusion lymphoma	K12, ORF-gB, ORF-6, ORF-61, ORF-65
HTLV-½	Retroviridae/RNA	Adult T-cell leukemia/lymphoma	Tax, HBz

HPV, Human Papilloma Virus; DNA, Deoxyribonucleic Acid; SCC, Squamous Cell Carcinoma; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; RNA, Ribonucleic Acid; EBV, Epstein Bar Virus; NHL, Non-Hodgkin Lymphoma; EBNA, Epstein Bar Virus Nuclear Antigen; LMP, Latent membrane Protein; HHV, Human Herpes Virus; ORF, Open Reading Frame; HTLV, Human T Cell Lymphotropic Virus.

Viral-encoded cancer antigens: some viral infections are capable of triggering oncogenesis in humans (Table 4.1). They can cause cancer either by governing host cell proliferation, enhancing genomic instability, propagating chronic inflammation, or suppressing the host immune system.57 *Human Papillomavirus* (*HPV*), *Hepatitis B Virus* (*HBV*), *Hepatitis C Virus* (*HCV*), *Epstein Bar Virus* (*EBV*), *Human Herpes Virus* (*HHV*)*−8, Human T-Lymphotropic Virus* (*HTLV*)*−½* collectively attribute to around 1,400,000 cancer cases each year.58 Virus-encoded proteins in the host cell can be presented by MHC class I molecules using the host cell machinery for self-antigen presentation. Transformed cells infected by viruses present virus-encoded antigens that can be recognized by the host's adaptive immune system.

As these antigens are not self-antigens, the immune responses against them are highly specific.57,59,60 Cancer germline antigens: several cancer germline antigens have been discovered so far. Some of the most studied cancer germline antigens are MAGE antigens, GAGE antigens, SSX, BAGE, Cyclin A, KMHN1 and SPA17.35,61-63 Immune response against cancer germline antigens is highly specific.35 Differentiation antigens: differentiation antigens are so-called since the tissue of origin also expresses the antigen. Some remarkable examples are Tyrosinase and Melan-A in melanoma, prostatic-specific antigen (PSA) in prostate cancer, carcinoembryonic antigen (CEA) in colorectal, pancreatic, ovarian and breast cancers, and alpha-fetoprotein in hepatocellular carcinoma.35,37 Immune response against these antigens is of low tumor specificity.35Overexpressed antigens: some of the immune responses against cancer cells are toward proteins whose genes are overexpressed in cancer cells. One noteworthy example is WT1 in acute myelogenous leukemia (AML)

Rejection Antigens

Rejection antigens were originally defined as those responsible for induction of an effective immune response against murine tumors induced by the chemical carcinogen, methylcholanthrene. Similar observations were subsequently made with other chemical carcinogens. Rejection antigens appear to vary among different carcinogens and between individual animals given the same carcinogen, demonstrating that rejection antigens are unique to each individual

tumor. These observations suggested that a mutational mechanism, not necessarily directed to the same genetic target, might be responsible for the generation of antigenic entities easily recognized by the host immune response. At the same time, tumor rejection was shown to be mediated by activated T cells. In vitro studies utilizing tumor-specific T cells also revealed that the recognition of antigenic entities by lymphocytes did correlate with tumor rejection in vivo. It is important to understand that the antigenicity of a tumor may not be sufficient to mediate immunological rejection of the same tumor because they may be only weakly immunogenic and fail to elicit an effective immune response. Tumor Antigens Numerous tumor antigens have been identified in human and mouse cancers as potential targets for antitumor immunotherapies.

These tumor antigens can be further divided into two major categories; tumor-specific antigens and tumor-associated antigens.

Tumor-Specific Antigens

Tumor-specific antigens are defined as gene products that are specifically expressed in tumors, such as the mutant ras oncogene-encoded proteins and the mutant p53 suppressor geneencoded proteins. These mutational changes provide the tumor cells with growth advantages but are not immunogenic. Thus, these tumor-specific antigens are not tumor-rejection antigens. However, they are currently being tested as gene therapy targets in several clinical studies

Tumor-Associated Antigens

Tumor-associated antigens are normal cellular proteins overexpressed in certain cancers. Several of these proteins have been identified and characterized in great detail in human melanoma, which represents the best-studied tumor from an immunological perspective. This interest is largely due to the extensive clinical and biological evidence that the host immune response can have a measurable impact on its natural disease progression. Examples of melanoma tumor-associated antigens are melanoma antigen recognized by T cells-1 (MART-1), GP100, and tyrosinase. These gene products are also lineage-specific, meaning that their expression is limited to melanocyte lineage and their overexpression correlates with neoplastic development.

Melanoma-Associated Antigen

This family of melanoma antigens represents a separate set of melanoma-associated antigens (MAGEs) that result from overexpression of normal cellular genes. These proteins are not lineage-specific, as they are normally expressed in the testes. However, these antigens are tumor-associated because they are often overexpressed not only in melanoma, but in several other epithelial cancers. Results of several clinical studies suggest that well-defined MAGE epitopes can induce effective antitumor responses following injection into melanoma patients

Carcinoembryonic Antigen

Additional studies have led to the identification of tumor-associated antigens in several tumor types. The primary example is carcinoembryonic antigen, an oncofetal antigen, which is overexpressed in adenocarcinomas of the gastrointestinal tract as well as some head and neck

squamous cell carcinomas.

Prostate-Specific Antigen

Another important example is prostate-specific antigen, a protease expressed by prostate gland cells that becomes elevated in hypertrophic and cancerous prostate tissue.

Alpha-Fetoprotein

A third example is alpha-fetoprotein that becomes elevated in patients with cancer of the liver or testes. These tumor-associated antigens are often overexpressed and may provide useful markers for monitoring the course of disease, as they can be measured in serum.

Some tumor antigens are the products of genes that are silenced in normal cells and derepressed in tumor

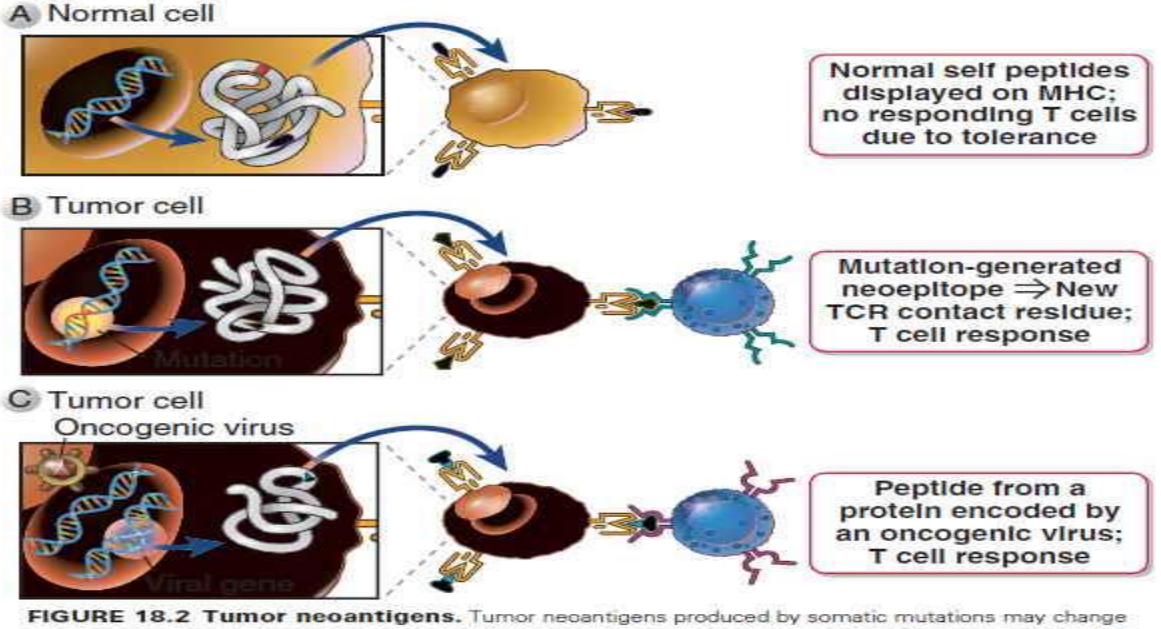


FIGURE 18.2 Tumor neoantigens. Tumor neoantigens produced by somatic mutations may change a self-protein that the patient is tolerant to (**A**) to one with a peptide with a new TCR contact residue that is recogn… T cells (C…

cells, or are proteins made by normal cells but producedin excessive amounts by tumors.

These antigens are not inherently foreign for the host, but nevertheless they

stimulate immune responses. There are several possible explanations for their immunogenicity. Normally, theantigens may be expressed for a limited time or at aparticular location—for example, only during embryonic development or only in immune-privileged cells—sothere is no long-lived immunologic tolerance to these proteins. Expression in a tumor later in life or in locations that are not protected from immune cells may be enough to stimulate immune responses. The amount of antigen produced in a cancer patient may be abnormally high, because of overexpression in each tumor cell or an abundance of tumor cells, and this too may be enough to elicit an active immune response.

Major categories of unmutated tumor antigens that are more abundant in tumors than normal tissues include cancer-testis antigens, proteins encoded by amplified genes, and tissue differentiation antigens (Fig. 18.3). The expression of only some of these structurally unaltered tumor antigens is sufficiently different from expression in normal cells to stimulate protective immunity in patients. However, many of these tumor antigens are targets for antibody therapy and potential candidates for tumor vaccines.

• ***Cancer-testis antigens are proteins expressed ingametes and trophoblasts and in many types of cancers but not in normal somatic tissues*** (see Fig. 3.3A). Thefirst cancer-testis antigens identified were melanoma associated antigens (MAGE). They are expressed in melanomas and many other types of tumors and in normal testis. Subsequently, several other unrelated gene families have been identified that encode melanoma antigens recognized by CTL clones derived from melanoma patients. The MAGE proteins and these other melanoma antigens are silent in most normal tissues, except the testis and placental trophoblast, but they are expressed in a variety of malignant tumors. More than 200 cancer-testis genes in over 40 different gene families have been identified. About half are encoded by genes on the X chromosome and the rest are distributed on the other chromosomes. It has been postulated that in most somatic cells, the genes encoding these proteins are silenced by epigenetic

mechanisms such as methylation of the promoter regions, but the loci are demethylated in cancer cells, allowing the genes to be expressed.

• ***Some proteins are expressed at abnormally high levels in tumor cells because the genes encoding these proteins are amplified***

(Fig. 18.3B).

One example of such a protein is the oncogenic epidermal growth factor variant called Her2/Neu, which is overexpressed in some breast cancers. There is no evidence that this protein elicits protective immune responses in patients, presumably because it is present in normal cells and induces tolerance. A monoclonal antibody targeting

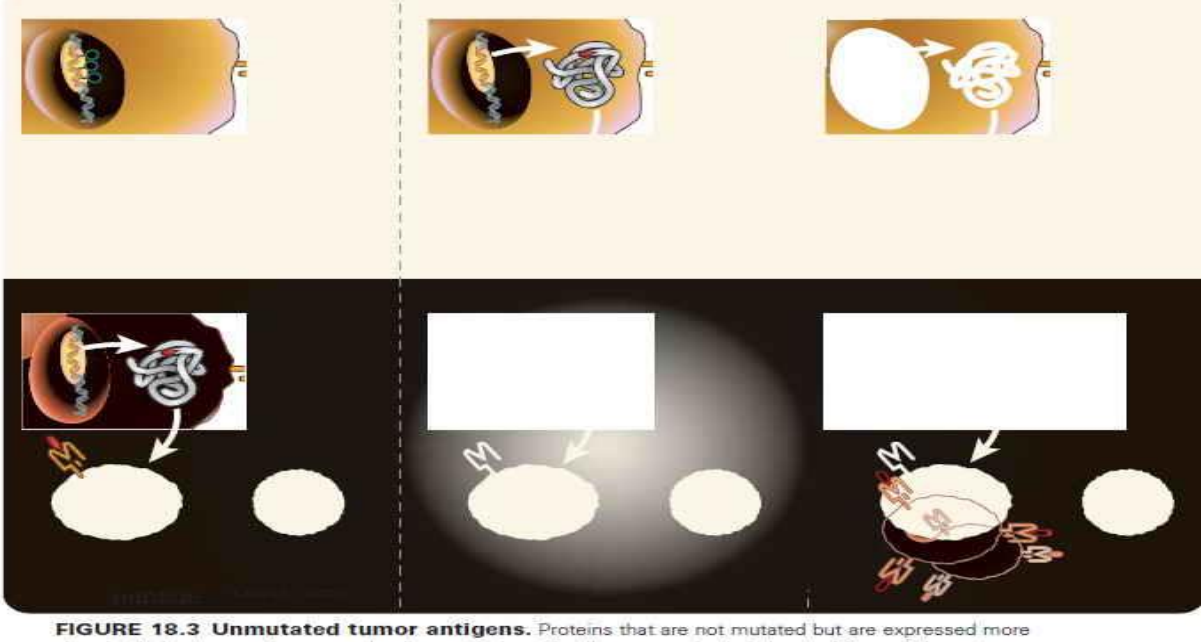


FIGURE 18.3 Unmutated tumor antigens. Proteins that are not mutated but are expressed more abundantly by tumors than normal cells may induce T cell response in their hosts. Many of these tumor antigens include proteins encoded by genes that are normally not expressed all in most cells of adults, because of epigenetic suppression, but are depressed in tumor cells, such as cancer-testis antigens **(A)**. Some tumor antigens may be overexpressed because of gene amplifications, such as the Her2/Neu protein, which is highly expressed in many breast carcinomas **(B)**. Tissue-specific antigens are proteins expressed by both cancer cells and the normal cell types from which tumors are derived, such as tyrosinase made by both melanocytes and malignant melanoma cells. Because of either gene deregulation, or the abundance of the tumor cells, the amount of these proteins is high in the tumors, leading to T cell responses **(C)**.

Her2 is used to treat patients whose tumors show high

Her2 expression.

- ***Differentiation antigens are found normally on tumor***

cells and on the cell types of origin of the tumors but not on cells from other tissues (Fig. 18.3C). Two examples of such differentiation antigens in melanomas are tyrosinase, an enzyme involved in melanin biosynthesis, and MART-1 (also called melan-A), a protein required for melanosome function. Both CD8+ CTLs and CD4+ helper T cells specific for tyrosinase or MART-1 peptides are found in melanoma patients, perhaps because these antigens are expressed at high levels due to the large number of tumor cells. It is, however, possible that in many cases differentiation antigens do not induce immune responses because they are normal self antigens. Even in these situations, differentiation antigens are important in oncology because they aid in accurate diagnosis of tumor types and serve as targets for passive immunotherapy. For example, some lymphomas and leukemias arise from B cells and express surface markers characteristic of this lineage, such as CD20. Antibody and T cell herapies targeted against CD20 are used to treat these cancers.

Other Antigens of Tumors

Many attempts have been made to detect antigens in tumor cells and in the plasma of cancer patients by producing antibodies against tumors and using these as screening reagents. Several classes of tumor antigens have been identified by this approach. It is, however,now clear that most of these antigens are produced even in normal cells, especially under conditions of tissue injury and inflammation. Therefore, the role of these antigens in tumor immunity is uncertain.**Tumor associated antigen(TAA)**

Oncofetal antigens. Oncofetal antigens were the name given to proteins thought to be expressed at high levels in cancer cells and in fetal but not adult tissues. However, their expression in adults is not limited to tumors, but is increased in tissues and in the circulation in various inflammatory conditions, and the antigens are found in small quantities even in normal adult tissues. There is also no evidence that oncofetal antigens are important inducers of antitumor immunity. Thus, their usefulness as tumor markers, targets of antibodies, or vaccine candidates is limited. The two most studied oncofetal antigens are carcinoembryonic **antigen (CEA) and α-fetoprotein (AFP). CEA (CD66) is** a highly glycosylated membrane protein that functions as an intercellular adhesion molecule. High CEA expression is normally restricted to cells in the gut, pancreas, and liver during the first two trimesters of gestation. Its expression is increased in many carcinomas of the colon, pancreas, stomach, and breast, and serum levels are also increased in these patients. Serum CEA can, however, be elevated in the setting of nonneoplastic diseases, such as chronic inflammatory conditions of the bowel or liver, so it is of limited clinical utility. A small clinical trial administering T cells expressing CEA-specific antigen receptors was abandoned because the patients developedsevere colitis, reflecting the expression of CEA in normal tissues. AFP is a circulating glycoprotein normally synthesized and secreted in fetal life by the yolk sac and liver. Fetal serum concentrations can be as high as 2 to 3 mg/mL,but serum concentrations in adults are low. Serum levels of AFP can be elevated in patients with hepatocellular carcinoma, germ cell tumors, and occasionally

gastric and pancreatic cancers. An elevated serum AFP level is sometimes used as an indicator of advanced liver or germ cell tumors or of recurrence of these tumors after treatment.

***Altered Glycolipid and Glycoprotein Antigens*.**

Most human and experimental tumors express higher than normal levels or abnormal forms of surface glycoproteins and glycolipids, including gangliosides, blood group antigens, and mucins. Tumors often have dysregulated expression of the enzymes that synthesize the carbohydrate side chains of mucins, which leads to the appearance of tumor-specific epitopes on the carbohydrate side chains or on the abnormally exposed polypeptide core. Several mucins have been the focus of diagnostic and therapeutic studies. One of these, a mucin called MUC-1, is an integral membrane protein that is normally expressed only on the apical surface of breast ductal epithelium, a site that is relatively sequestered from the immune system. In some carcinomas, however, MUC-1 is expressed in a nonpolarized fashion and contains new, tumor-specific carbohydrate and peptide epitopes detectable by mouse monoclonal antibodies. Whether effective vaccines can be developed with these epitopes remains an open question.

Q/ **Why tumor antigens appear in the body and which are the features they have that allow their recognition as a threatening molecule?**

Answer: mutation. When mutated via chromosomal translocation, point mutation and gene amplification, we say they are activated, and we call them oncogenes. Oncogene theory postulates that when proto-oncogenes are mutated

they become overexpressed or express in the wrong tissues, showing abnormal growth mechanism of somatic cells. Such aberrant proliferation is seen at carcinoma in epithelial cells, sarcoma in muscles and connective tissues like fat and bones, leukemia in blood forming cells, like WBC, and lymphomas in lymphatic system, for instance Tumor antigens appear in the body as a result of a mutation, a gene activation, which is not supposed to happen, or a clonal amplification of a mutated gene product .

Three main routes for tumor antigens formation.

- Via **(1)** Antigens that result from normal cellular gene production, in which something in the process became aberrant or out of place or time and they become amplified clones.
- Via **(2)** Antigens that are the product of mutant gene expression in somatic cells.
- Via **(3)** Antigens encoded by proto-oncogenes that are transformed into effective oncogenes due to viral transformation.

Lec2 **Tumor immunology**

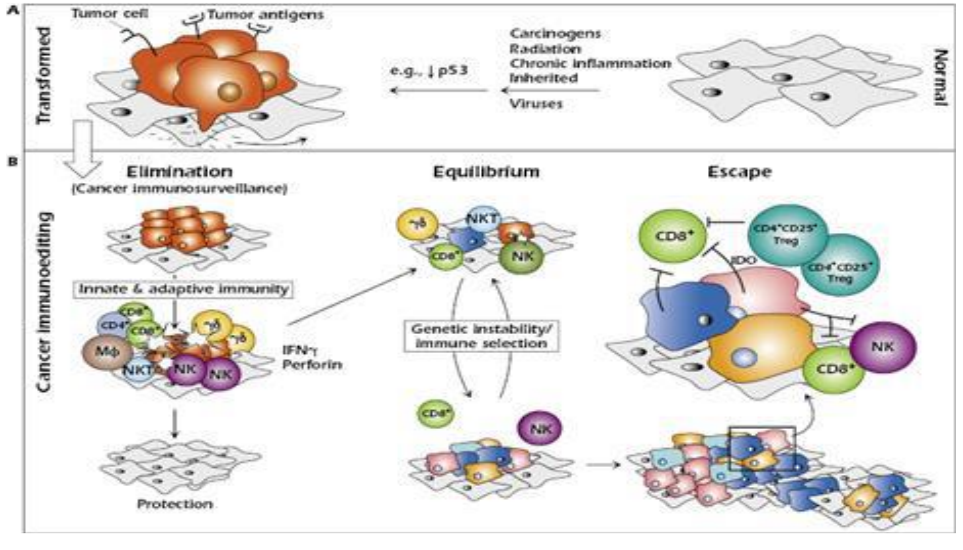
Immunoediting

The immunoediting hypothesis was termed as such after the finding that the immune system may also promote the growth of tumors that are able to escape recognition by the immune system, and therefore escape destruction. This hypothesis helps to "more broadly encompass the potential host-protective and tumor-sculpting functions of the immune system throughout tumor development" Immunoediting is considered a dynamic process consisting of three phases:

(1) Elimination (2) Equilibrium and (3) Escape.

Phase one, elimination, encompasses the classical concept of immunosurveillance where the cells and molecules of both the innate and adaptive immune system work in conjunction to potentially eradicate the developing tumor. These cells and molecules include macrophages, NK cells, NKT cells, CD4+ T cells, CD8+ T cells, and γδ cells.

Fig1:



If the elimination phase is not successful in the destruction of the tumor, the immunoediting system either moves to

phase two, equilibrium, where the tumor cells are chronically maintained over a long period of time or undergo immunologic sculpting by immune "editors". It is this "editing" event that allows for new populations of tumor-cell variants to escape the control of the immune system and subsequently become clinically detectable in the

third phase, escape. Phase two, equilibrium, is the period after tumor destruction has failed, and just before the escape phase where the tumor cells outweigh the balance of the immunological restraints of the equilibrium phase. The equilibrium phase includes NK cells, NKT cells, CD8+ T cells, γδ cells as well as IFNγ and Perforin. The initiation of the cancer immunoediting process occurs early in tumor formation due to the distinct tumor-specific markers or tumor antigens present on the cell surface which generate "danger" signals alerting the immune system to begin phase one.

Both the innate and adaptive immune systems are essential for the effector mechanisms of tumor immunity. The ability of the immune system to act against tumor antigens depends on the type of tumor present and the context of antigen presentation. Studies have shown that the destruction of tumors *in vitro* by the immune effector mechanisms is more likely to be successful if the tumor cells exist as individual cells rather than a solid tumor. This is likely due to the simple fact that tumor cells are easier to attack when dispersed rather than when they are grouped together. Perhaps one of the largest roles in the immune response to

cancer cells is played by the dendritic cells once they detect the “danger signals”. This process is the same whether the dendritic cell encounters the signals due to cellular damage or the invasion of a foreign material or pathogen. Two events normally occur: the dendritic cells produce cytokines that lead to the differentiation of CD4+ TH0 cells into TH1 cells allowing for further cell-mediated immune response or the activated dendritic cells lead to the polarization of TH0 cells into TH2 leading to an antibody response. Both events are theorized to be involved in the destruction of tumor cells.

IMMUNE RESPONSES TO TUMORS

Both innate and adaptive immune responses can be detected in patients and experimental animals, and various immune mechanisms can kill tumor cells in vitro. The challenge for tumor immunologists has been to determine which of these mechanisms may contribute significantly to protection against tumors and to develop therapies that enhance these effector mechanisms in ways that are tumor specific. Recent technical advances in characterizing tumor antigen–specific immune responses, and data from studies of cancer patients treated with recently developed drugs that stimulate T cells have indicated that CTLs are the most important contributors to host immune defense against tumors.

EFFECTOR MECHANISMS IN TUMOR IMMUNITY

The importance of immune effector cells was demonstrated by the results of studies performed in animals following exposure to chemical carcinogens. Immunity was transferred among mice of the same strain through intravenous injection of immune cells, but not through transfer of serum.

Tumor-Specific Cytotoxic T Cells

Subsequent studies have demonstrated the importance of tumor-specific cytotoxic Tcells (CTL) that are MHC-restricted CD8 lymphocytes. CTL kill target cells through recognition of an antigenic epitope bound to an appropriate MHC-I molecule, expressed on the surface of tumor cells.

Until recently, the main focus of antitumor immune responses has been CTL responses based on the expression of MHC-I and lack of MHC-II expression on tumor cells. Numerous animal studies have now demonstrated that CD4 helper T cells (Th) are crucial to antitumor immunity. In mice, of the two functional T helper populations, the Th1 population seems to be more effective in antitumor immune responses, because Th1 cells activate both CTL and APC (i.e., dendritic cells) and favor the switch to immunoglobulin (Ig) G2a synthesis, a biologically potent IgG isotype that enhances antibody dependent cell-mediated cytotoxicity (ADCC) and phagocytosis of tumor cells. Th2 cells assist exclusively the humoral response, which is much less effective as an antitumor protective mechanism. The importance of Th1 cells in the development and activation of CTL has been demonstrated in animal models. In tumor-bearing animals infused with Th1 cells, an increased CTL-mediated antitumor response was observed.

One hypothesized mechanism for this response is that Th1 cells produce cytokines for CTL development and activation. Interferon (IFN)-g, secreted by Th1 cells, may be the key to this effect because it upregulates MHC-I expression on tumor cells, increasing their sensitivity to CTL killing. IFN-g also activates APC, which further stimulates Th1 and CTL responses to mediate tumor cell

killing. Other studies have demonstrated that direct cell–cell contact between Th1 and CTL results in enhanced proliferation and survival during antitumor immune responses.

The direct interaction of costimulatory molecules and receptors on the Th1 cells and CTL, respectively, are suggested to mediate these enhanced responses. The most significant conclusion from numerous studies is that HLA-restricted CTLs are the most effective killers of autologous tumor cells. **APC**, such as dendritic cells, are a central element in antitumor immune responses.

Dendritic cells are the most potent APC known with the unique ability to stimulate naı̈ve T cell responses. Dendritic cells express both MHC-I and MHC-II molecules, which allow presentation of tumor antigen to both CTL and CD4 Th cells. In addition, dendritic cells express costimulatory molecules (i.e., CD80, CD86) required to stimulate the differentiation of CD8T cells into antitumor CTL as well as the activation of Th cells. Importantly, dendritic cells Tumor Immunology have been recognized to confer immunogenicity to antigenic entities that would otherwise have been nonstimulatory and counterproductive to tumor immunity.

Macrophages

In addition to their ability to phagocytize and present tumor antigen, macrophages also have the capacity to kill tumor cells directly through release of toxic soluble molecules, such as lysosomal enzymes, reactive oxygen intermediates, and nitric oxide. Activated macrophages also secrete tumor necrosis factor (TNF) that directly kills tumor cells. Although the mechanism by

which macrophages recognize tumor cells is unknown, the formation of complex, interdigitating interfaces with the target tumor cells creates optimal conditions for tumor cell killing through these toxic soluble molecules.

Antibodies

Antibodies may contribute to killing tumor cells by activating complement or by ADCC, in which Fc receptor-bearing macrophages or natural killer (NK) cells mediate the killing. Elimination of tumor cells by antibodies has been demonstrated in vitro. However, little evidence exists demonstrating that antibodies control tumor growth and spread in vivo. Nonetheless, several monoclonal antibodies are currently being assessed as therapeutics for numerous human cancers.

Cytokines

Cytokines play very important roles as mediators of antitumor immunity. The type and concentration of cytokines, as well as the cytokine receptor expressed, directs the immune response. toward stimulation or tolerance. As discussed earlier, TNF directly kills tumor cells. In addition, Th responses are directed by cytokines. In the presence of **interleukin (IL)-12**, an antitumor Th1 response will develop. However, in the presence of IL-4, a much less effective Th2 response ensues. Finally, several cytokines are known to augment antitumor immunity.

Interleukin-2

IL-2, a well-known activator of T cell immunity, stimulates Th cells to proliferate and become activated following interactions with APC. With the

right sequence of costimulatory signals, the IL-2-induced proliferation of undifferentiated Th cells results in a predominant Th1response, which assists activation and differentiation of CTL, thus promoting the killing of target tumor cells.

Interleukin-12

IL-12, a pleiotropic immunomodulatory cytokine secreted by APC, has an essential role in innate and adaptive antitumor immune responses. In addition to directing toward a Th1response, IL-12 activates Th1 and CTL to secrete IFN-g leading to enhanced cell-mediated antitumorimmune responses.

Granulocyte-Macrophage Colony-Stimulating Factor

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is known to promote the recruitment and activation of dendritic cells and other APC. Following APC activation, tumor antigen can be processed and presented to stimulate antitumor T cells responses (e.g., Th and CTL). The use of these and other immunostimulatory cytokines in anticancer therapies.

GREAT ESCAPE: IMMUNE EVASION OF TUMORS

Despite a reasonable understanding of antitumor effector mechanisms, clinical studies investigating spontaneous antitumor immune responses have yet to lead to reproducible or consistent tumor regression. Thus, the question of why tumors continue to grow and metastasize in immunological competent cancer patients remains unanswered. Several observations have Lathers and Gattoni-Celli demonstrated that tumors evade and actively suppress the immune system. Tumor evasion of the immune system, termed immune escape, may occur through several mechanisms, including

(i) tolerance or anergy induction; (ii) the genetic instability of tumors; (iii) modulation of tumorantigens; and (iv) decreased MHC-I expression. In addition to evasion of the immune system, tumors actively suppress the immune system directly through production of immune suppressive cytokines and indirectly through the induction of immune inhibitory cells.

Tolerance or Anergy Induction

Tumor cells present antigens bound to MHC molecules expressed on their cell surface., costimulation is required to initiate T cell responses. Tumor cells do not express costimulatory molecules (e.g., CD28), a requirement for engagement of the B7 (CD80, CD86) molecules on APC. This results in inadequate T cell activation leading to tolerance or anergy of potential effector cells and iminished antitumor immune responses.

Genetic Instability of Tumors and Modulation of Tumor Antigens

The heterogeneity and instability, both genetically and phenotypically, of tumors may also contribute to their evasion of the immune system. The expression of tumor antigens is frequently lost due to the genetic instability of tumors. From an immunological standpoint, antigen-negative tumors have a distinct growth advantage. Several studies have supported this concept as loss of tumor antigens correlated with increased tumor growth and metastasis.

Decreased Major Histocompatibility Complex-I Expression

In addition to tumor antigen modulation, MHC-I molecules may be downregulated or completely lost. Decreased synthesis or alterations in MHC-I molecules, b2-microgobulin and/or transporter proteins associated with antigen processing and presentation may all contribute to alterations in MHC-I expression on tumor cells. An inability of CTL to recognize tumor antigens and kill tumor cells is a direct consequence of decreased MHC-I expression, as CTL only recognize tumor-derived antigenic epitopes when bound to the extracellular portion of MHC molecules.

Immunity against tumors

Nonspecific and specific, humoral and cellular – influence the growth and progression of tumors

Escape to immune mechanisms

Tumor
– does not present neoantigens that are immunogenic,
– does not express co-stimulating molecules, that activise T cells
– poor cooperation with MHC

Early stages – small amount of antigens., rapid growth – malignant growth – lack of apoptosis - rapid overload of immune system

Some tumors produce
- immunosuppresive substances or
- induce production of suppresor cells or
- antigens that bocks antibodies of T cells reactiong with tumor

Tumor antigens

TAA – tumor associated antigens
- oncofetal antigens – reemergence of embryonal proteins newly produced or present on membranes

AFP – alfafetoprotein,

CEA –carcinoembryonal antigen

TATA – tumor associated transplantation antigens
– neo antigens responssible for rejection
– on virus induced tumors – surface antigens on cells of tumors caused by oncogens from viruses

TSTA – tumor specific transplantation antigens
– na chemically induced tumors – heterogennous antigenic structure (two tumors induced by the same chemical substances or in the same individual have scarecely common specific antigens)

Cancer Immunotherapy

The main strategies for cancer immunotherapy aim to provide antitumor effectors (antibodies and T cells) to patients, actively immunize patients against their tumors, and stimulate the patients′ own antitumor immune responses. At present, most treatment protocols for disseminated cancers, which cannot be cured surgically, rely on chemotherapy and irradiation, both of which

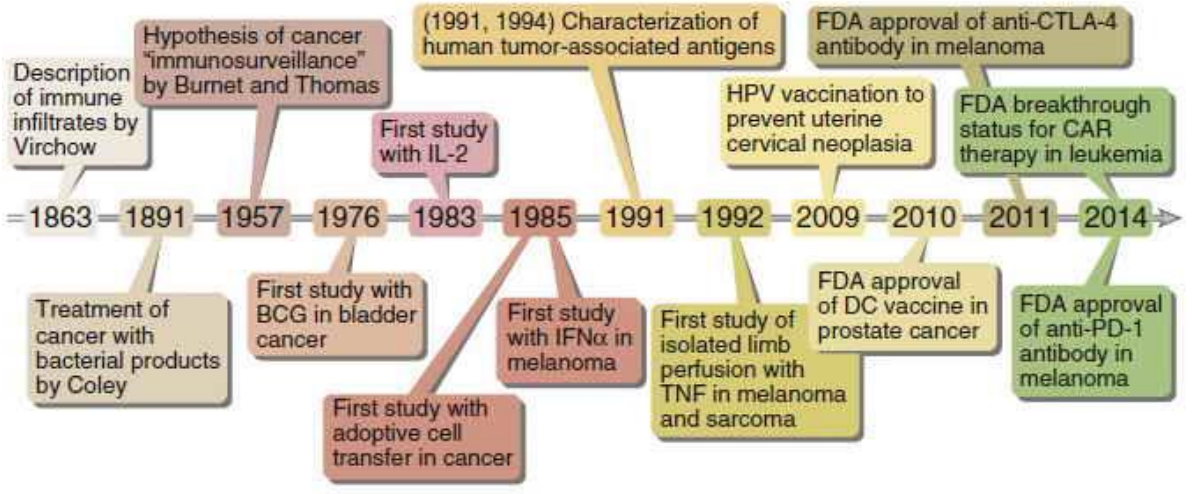


Fig1: History of cancer immunotherapy. Some of the important discoveries in the field of cancer immunotherapy are summarized. (Modified from Lesterhuis et al: Cancer immunotherapy—revisited. *Nat Rev Drug Disc* 10:591, 2011.) *BCG*, Bacillus Calmette-Guerin; *CAR*, chimeric antigen receptor; *CTLA-4*, cytotoxic T-lymphocyte-associated protein 4; *DC*, Dendritic cell; *FDA*, Federal Drug Administration; *HPV*, Human papillomavirus; *IFNα*, Intereron-α; *IL-2*, Interleukin -2; *PD-1*, Programmed cell death protein 1; *TNF*, Tumor necrosis factor.

damage normal nontumor tissues and are associated with serious toxicities. Because the immune response is highly specific, it has long been hoped that tumor-specific immunity may be used to selectively eradicate tumors without injuring the patient. Immunotherapy remains a major goal of tumor immunologists, and many approaches have been tried in experimental animals and in humans. The history of cancer immunotherapy illustrates how the initial, often empirical, approaches have been largely supplanted by rational strategies based on our improved understanding of normal immune responses (Fig. 1).

Passive Immunotherapy

One strategy for tumor immunotherapy relies on various forms of passive immunization, in which immune effectors are injected into cancer patients (Fig. 2, *A*):

• ***Antibody therapy***. Monoclonal antibodies against various tumor antigens have been used in many cancers. The antibodies bind to tumor antigens and activate host effector mechanisms, such as phagocytes or the complement system, that destroy the tumor cells. For example, an antibody specific for CD20, which is expressed on B cells, is used to treat B cell tumors, usually in combination with chemotherapy. Because CD20 is not expressed by hematopoietic stem cells, normal B cells are replenished after the antibody treatment is stopped. Other monoclonal antibodies that are used in cancer therapy may work by blocking growth factor signaling (e.g., anti-Her2/Neu for breast cancer and anti– EGF-receptor antibody for various tumors) or by inhibiting angiogenesis (e.g.,

antibody against the vascular endothelial growth factor for colon cancer and other tumors)

. • ***Adoptive cellular therapy***. T lymphocytes may be isolated from the blood or tumor infiltrates of a patient, expanded by culture with growth factors, and injected back into the same patient. The T cells presumably contain tumor-specific CTLs, which find the tumor and destroy it. This approach, called adoptive cellular immunotherapy, has been tried as a treatment for several types of metastatic cancers, but results have been variable among different patients and **tumors.**

• ***Chimeric antigen receptors***. In a more recent modification of T cell therapy, a chimeric antigen receptor that recognizes a tumor antigen and coupled to intracellular signaling domains is genetically introduced into a patient's T cells, and the cells are expanded ex vivo and transferred back into the patient. Such therapy has shown remarkable efficacy in some leukemias. *Stimulation of Host Antitumor Immune Responses* **The host's immune response against tumors can be promoted by vaccinating with tumor antigens or by blocking inhibitory mechanisms that suppress antitumor immunity.** • *Vaccination.* One way of stimulating active immunity against tumors is to vaccinate patients with their own tumor cells or with antigens from these cells. An important reason for defining tumor antigens is to produce and use these antigens to vaccinate individuals against their own tumors. Vaccines may be administered as recombinant proteins with adjuvants. In another approach, a tumor patient's dendritic cells are expanded in vitro from blood precursors, the dendritic cells

are exposed to tumor cells or a defined tumor antigen, and these tumor-antigen-pulsed dendritic cells are used as vaccines. It is hoped that the dendritic cells bearing tumor antigens will mimic the normal pathway of cross-presentation and will generate CTLs against the tumor cells. Tumor vaccines have achieved only modest success, perhaps because these are therapeutic vaccines that are administered to patients in whom tumors may have established mechanisms that suppress immune responses. Tumors caused by oncogenic viruses can be prevented by vaccinating against these viruses. Two such vaccines

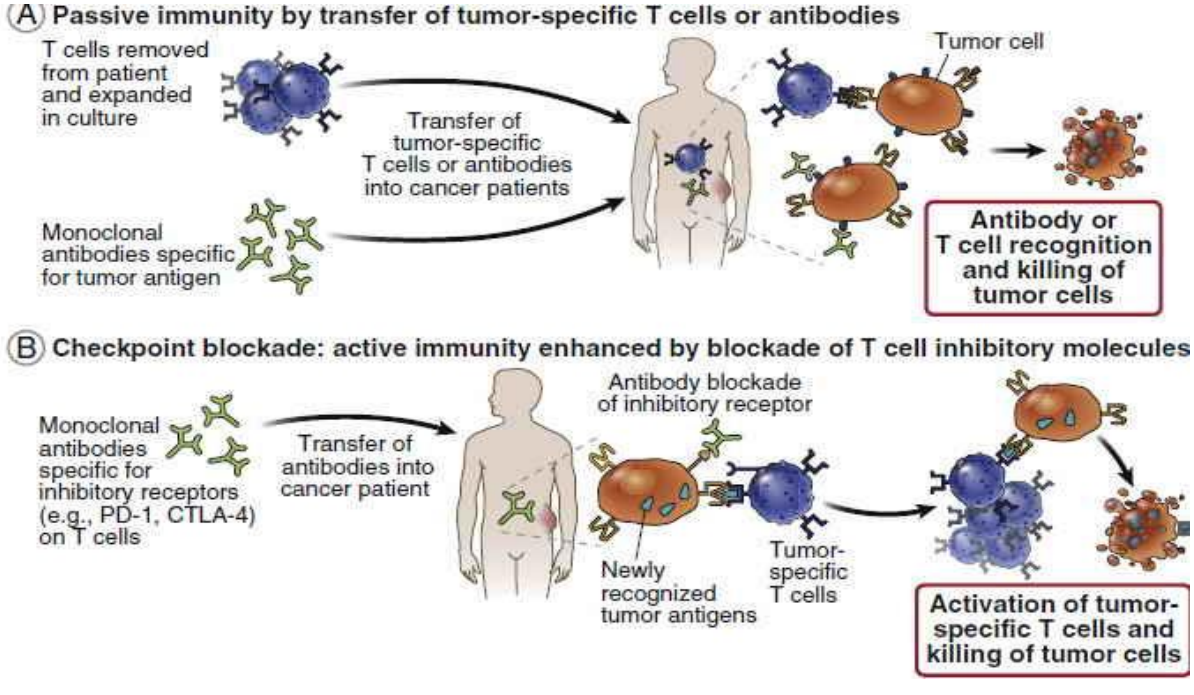


Fig2: Strategies for enhancing antitumor immune responses. A, Transfer of antitumor antibodies or T cells, a form of passive immunity. A variation of T cell therapy is to express in patients' T cells an antibody domain that recognizes a tumor antigen; by attaching signaling domains to the antibody, the T cell is activated upon recognition of the tumor. B, Blockade of inhibitory pathways to boost endogenous antitumor responses. Not shown are tumor vaccines, sometimes given in the form of autologous dendritic cells incubated with tumor cells or their antigens.

that are proving to be remarkably effective are against hepatitis B virus (the cause of a form of liver cancer) and human papillomavirus (the cause of cervical cancer). These are preventive vaccines given to individuals before they are infected, and thus prevent infection (like all preventive vaccines for infections).

• ***Checkpoint blockade***. The realization that tumors activate regulatory mechanisms that suppress immune responses has led to promising recent approaches and a new paradigm in tumor immunotherapy. The principle of this strategy is to boost host immune responses against tumors by blocking normal inhibitory signals for lymphocytes, thus removing the brakes (checkpoints) on the immune response (Fig. 2, *B*). An antibody against CTLA-4 was approved for the treatment of melanoma in 2011. Clinical trials of antibodies that block PD-1 or its ligand PD-L1 have shown impressive efficacy in a variety of cancers, and anti–PD-1 for cancer immunotherapy was approved in 2014. The immune response induced by checkpoint blockade is largely specific for peptides produced by mutated genes in the tumors. Predictably, patients treated with these antibodies, especially anti– CTLA-4, develop manifestations of autoimmunity, because the physiologic function of the inhibitory receptors is to maintain tolerance to self antigens (

• ***Cytokine therapy***. Other ways of boosting antitumor immune responses include treating patients with cytokines that promote lymphocyte activation. The first cytokine to be used in this way was interleukin-2 (IL-2), but its clinical use is limited by serious toxic effects at the high doses that are needed to stimulate antitumor T cell responses. IL-2 also enhances the numbers and functions of

regulatory T cells, which may interfere with antitumor immunity. Many other cytokines have been tried for systemic therapy or local administration at sites of tumors, with mostly unimpressive results thus far.

Imunotherapy of tumors

- Active and passive increase of nonspecific immunity

Active nonspecific – BCG, *Propionibacter acnes*

specific – killed tumor cells and extract, recombinant antigens, idiotypes, costimulationg molecules

Passive nonspecific – LAK cells, cytokines

pecific – antibodies alone or bound on drugs, T cells

- Immunopotentiating substances (modification of biological response) – bacterial products (BCG – activation of macrophages and NK cells via cytokines),
  - synthetic substances (pyran – induction of interferon production)
  - cytokines (interferon, TNF – activation of macrophages)

- Substances activation macrophages and NK cells, stimuliting T lymfocytes and production of cytokines

Lec(4) **Tumor Immunology**

TUMOR-ASSOCIATED ANTIGENS DETECTED BY ANTIBODIES

Few antigens are unique to tumors

There have been many attempts to detect antigens unique to tumors, using either sera from animals deliberately immunized with tumor material (heterologous typing) or sera from tumor-bearing animals or patients (autologous typing). In recent years heterologous typing has relied on monoclonal antibodies (mAbs) and although few molecule uniquely expressed in tumors have been detected, several types of antigen associated with tumors have been identified .

Tumors may express normal differentiation antigens that have a restricted distribution in normal cells

Most tumor cells represent the clonal progeny of a single cell, and cells of that type may be relatively rare. The tumor cells may therefore express antigens present on only few normal cells. The Common Acute Lymphoblastic Leukaemia Angitgen (CALLA or CD10) is an example (Oncofetal antigens are differentiation antigens expressed during fetal development but normally not expressed, or expressed at very low levels, in adult life. Examples are α-fetoprotein (AFP), which is produced by liver cancer cells, and carcinoembryonic antigen (CEA) produced by colon cancer cells and other epithelial tumors.

Normal antigens expressed in tumors may be altered by glycosylation

Glycosylation is altered in many tumors. This may give rise to the expression of new carbohydrate epitopes, such as the Thomsen-Friedenreich antigen, a disaccharide which is usually hidden on normal cells. Aberrant blood groups can also be created in this way. Alternations in glycosylation may also reveal epitopes on the protein backbone that are rarely detected in normal cells. For example, polymorphic epithelial mucins are produced by many normal epithelial cells. They are high-molecular weight glycoproteins with a repeating core peptide carrying the carbohydrate side chains. In epithelial tumors, new peptide epitopescan be detected in the repeating core structure of polymorphic epithelial mucin 1 (MUC 1), but these new epitopes are also detectable in the lactating breast .

Sera from patients with tumors detect widely distributed antigens

Until recently autologous typing was extremely difficult because most human sera are complex and contain many anti-bodies capable of reaching to tumor cells including anti-HLA, Anti-blood group and anti-carbohydrate antibodies. Many antibodies are IgM and of low affinity. Generation of human monoclonal antibodies has been technically difficult and the resulting hybridoma antibodies often detect widely distributed autoantigens, perhaps because they are derived from the pool of natural autoantibody producing B1 cells. The importance of these antibodies in the host response to tumors is unclear.

Serological analysis of human tumor antigens by recombinant cDNA expression expression cloning

More recently SEREX has been developed. Sera from patients are used to screen cDNA expression libraries from fresh tumor material. Isolation of antigens detected only by high titre IgG or IgA antibodies ensues that the method does not detect IgM natural antibodies. The SEREX method has the disadvantage that it may not detect all conformational eqitopes of a protein and does not identify carbohydrate antigens because the bacteria used to express the antigens do not glycosylate them. Nevertheless, over 900 sequences of genes cloned using the SEREX method have already been deposited in a data base set up for this purpose. These include known TSTAs such as MAGE-1 and tyrosinase, sequences identical (or nearly identical) to known genes not previously known to elicit an autoantibody response (e.g. kinectin a transporter associated with Golgi vesicles), and a large group of previously unknown genes . A complete description of the expression patterns of 900 genes in normal and tumor tissue, let alone analysis of their functions, is a major undertaking, but will eventually identify many new targets for immunotherapy.

IMMUNODIAGNOSIS

Antigens need not be tumor specific to be used for diagnosis

Although there are few molecules which are exclusive to tumor cells, antibodies to tumor-associated molecules can be very useful in tumor diagnosis, by either detecting increased amounts of an antigen or the presence of an antigen in an abnormal site.

In vivo

Radio-labeled antibodies against tumor-associated molecules have been used for the detection of tumors , but the method is seldom more sensitive than modern methods of computerized tomography or nuclear magnetic resonance imaging. In addition, immunoscintigraphy has the disadvantage that antibodies need to be freshly labeled for each patient, ad different antibodies are optimal for different tumor types. The development of recombinant multivalent fragments of high-affinity antibodies may improve the sensitivity of immunoscintigraphy in the future.

In vitro

Antibodies are useful for identifying the cell of origin of undifferentiated tumors and for the detection of micrometastases in bone marrow, cerebrospinal fluid, lymphoid organs or elsewhere . There are also immunoassays available for several tumorassociated molecules which can be detected in the serum. These include CAE, AEP and PSA. Raised levels of CEA or AFP may be useful for diagnosis but CEA my be raised in association with several tumor types and both CEA and AFP in some non-malignant conditions so that they are generally more useful in following the course of treatment .

Lec 5 **Tumor immunology**

PRINCIPLES OF LAB TESTS FOR SCREENING, DIAGNOSING, AND MANAGING TUMORS

Screening tests are used in ostensibly normal people to detect occult cancer. Diagnostic tests are those that help determine differential diagnosis, tumor stage, prognosis,and therapy selection. These are two very different functions, and the ability of lab tests to perform all these functions well is still imperfect. Disease prevalence profoundly impacts the test's usefulness.Bayes' theorem probability calculation shows the following:

• A "good" cancer test with 99 percent sensitivity and 95 percent specificity

• will be positive in 99 out of 100 people with disease;

• will be negative in 95 out of 100 people without disease.

• If the cancer rate in the population is 0.1 percent, then 98 percent of positives would be false positives.

At a 1 percent cancer rate, the false-positive rate is still 83 percent.

• Assuming that a clinician can identify this cancer by signs and symptoms 75 percent of the time, if this same test is applied, the false-positive rate is 1.7 percent.

Tests for differential diagnosis, then, generally perform relatively well, because the clinical suspicion of cancer translates to a higher cancer prevalence in the population being tested. The presumptions for screening tests are that a

relatively low number of people being screened actually have cancer and that it would be worse to miss a cancer than to do further testing on a normal person to exclude cancer.

The concept of a normal or reference range doesn't really apply, as it may be difficult to determine with certainty that a reference population does not have cancer, and values from normal and cancerous populations may overlap.

Cutoff values for tumor markers are typically selected above the point at which further testing will be done, so cutoff values for screening tests are generally set with the expectation that there will be an extremely high number of false positives due to low disease prevalence. This is not a benign choice, as additional testing can be invasive, costly, or anxiety-provoking.

Therefore, widespread use of a laboratory test to screen for cancer is justified if 8–10: the tumor is an important health problem for the population being screened;

• there is a recognizable early symptom or marker that can be used for screening with reasonably high sensitivity and specificity;

• it is a tumor for which treatment at an early stage is more successful than at a later stage;

• the screening test is acceptable to the population;

• the costs and benefits of the screening test are acceptable to the population.

The benefits include improved survival time, less radical treatment needed for tumors detected earlier, and reassurance for those with negative results. The costs include longer morbidity in patients whose prognosis is not changed,

Over treatment of questionable diagnoses, misleading reassurance for those with false-negative results, anxiety and possible morbidity from more invasive testing for those with false positive results, the actual physical hazards of the screening tests, and the actual dollar costs of the screening test.8–10 To improve the cost-to-benefit ratio, selected subgroups, such as patients with a family history of a cancer, should be screened when possible instead of the entire population.

Differential diagnosis of tumor type can be done by tissue/cell morphology and detection of tumor markers directly from tumor tissue.

Immunohistochemistry

Can detect expressed antigens using labeled antibodies, and molecular techniques such as fluorescent in situ hybridization (FISH) can detect abnormal gene expression usingnucleic acid probes11 . The requirements for using a tumor marker to facilitate differential diagnosis by the pathologist are less stringent than the requirements for using tests for widespread screening. To be helpful in pathological diagnosis, the marker must be differentially expressed in the tumor of origin and other tumors, which may have a similar appearance histologically.These markers must be combined with other clinical results, because the differentiation that occurs with transformation sometimes can result in loss of the marker. This false-negative situation is relatively common. In addition,the DNA changes that occur with malignant transformation sometimes can cause expression of a marker that is not normally associated with the tumor type in question,although this occurrence is relatively uncommon.Disease management with laboratory tests is typically done with serial determinations of

a tumor marker. A baseline level at initial diagnosis is established. As the disease and treatments progress, additional levels are determined to establish prognosis, monitor the results of therapy, and detect recurrence. This is an area in which many tumor markers are best used clinically, because it is not the absolute value of the tumor marker that is important but rather the upward or downward trend when the marker's biological half-life is considered. Serial determinations done to aid the clinician in making important decisions concerning the therapeutic regimen must be done by the same methodology so that changes are due to actual alterations in the patient, not differences in methods. An idealized model of using tumor markers to guide therapy is shown in **Figure** Problems with prostate-specific antigen (PSA) are a good illustration of the dilemmas associated with tumor markers.

No other tissue in men is known to produce PSA,so it is very specific for the prostate gland and increases in almost all prostate cancers. However, in a healthy person,the amount of PSA produced is directly related to the gland's size, and many men develop benign enlarged prostates asthey age. Further, as men age, they are more likely todevelop prostate cancer, but they are less likely to die fromit. In other words, as men age, there are prostate cancers that can and should be left alone. It is recommended that PSA screening cease once a man's remaining life expectancyis less than 10 years. Great effort has been expended to discriminate between benign prostatic hypertrophy, weakly aggressive cancers, and highly aggressive cancers using PSA. If the free-to-bound ratio of

PSA is low or the rate of PSA is increasing at a rate that exceeds 0.5 ng/mL per year (PSA velocity), this is more associated with cancer and is justification for a biopsy. Due to the fact that an increased PSA does not always indicate a cancerous state or an aggressive cancer that must be treated, the net benefit to the widespread PSA screening currently done in the United States may be questionable.1

LAB TESTS FOR TUMOR MARKER DETECTION

Clinicians screen for the presence of malignancies by a variety of methods. Commonly used tests include stool occult blood and colonoscopy for colorectal carcinoma, Papanicolaou smear for cervical cancer, self-exams for breast and testicular cancer, x-ray mammography for breast cancer, and digital rectal exam for prostate cancer. Laboratory tests can provide important adjunct information to patient histories and physical exams. Broadly, the three types of laboratory methods for cancer screening and diagnosis are gross and microscopic morphology of tumors, detection of antigen/protein tumor markers, and DNA/RNA molecular diagnostics. These techniques are complementary in that many of the DNA changes and subsequent mRNA expression result in the altered antigens/ proteins detected or morphology visualized, so the choice of method often depends on convenience, cost, sensitivity, and specificity.

Pathologists and histology labs process suspected tumor tissue with gross dissection and preparation of slides for microscopic analysis. A variety of special stains, nucleic acid probes, and tumor marker antibodies can be applied to the slides to enhance the visible features. Even so, evaluation of morphology

and staining patterns can be very subjective, and classification categories can be rather broad. Considerable skill is required to accurately diagnose cancer by morphology alone and final diagnosis is often made with supplemental clinical information and additional testing, which is described below.14 Some of the molecular diagnostic techniques that have become increasingly routine include the following:

• **Cytogenetic studies:** Many cancers are associated with particular karyotypes. However, as more precise knowledge of the exact gene defects present in various cancers is gained, testing for the aberrant genes is becoming more prevalent.14 **Nucleic acid amplification techniques:** Polymerase chain reaction (PCR) and its variants increase the inherent level of DNA or RNA, allowing the detection of small populations of cancer cells (including circulating cells in metastasis) and the detection of mutations, deletions, and gene rearrangements/translocations.14 (See Chapter 11 for a complete discussion of PCR.)

• **Fluorescent in situ hybridization (FISH):** Nucleic acid probes capable of binding to sequences of interest are tagged with fluorophors and applied to cells. Cells containing the sequence of interest can be visualized with fluorescent microscopes. Similar techniques using nonfluorescent labels such as enzymes and silver stains are also becoming available. Candidate DNA/RNA sequences for genetic screening of cancers abound, but most are still in the research stage. The *BCR-ABL* translocation associated with chronic myelogenous leukemia is a well-respected marker for this disease,15 and monoclonal expression of B-cell

DNA rearrangement is present almost exclusively in multiple myeloma or other lymphoid malignancy.16 Most other phenotypically related cancers have heterogeneous genetic causes, so universal and reliable genetic abnormalities are not yet described. The future may lie with **microarray** tests that are currently being developed with multiple nucleic acid tests contained on a single chip to allow for simultaneous testing of a sample for multiple genes. One potential use of this technology is detection and semiquantitation of mRNA expression in cells to distinguish patterns (rather than single markers) consistent with cancer.14 Some genetic abnormalities are associated with an increased risk of developing a cancer or with a poorer prognosis. Examples of **susceptibility genes** are the BRCA-1 and BRCA-2 mutations linked with an increased risk of breast, ovarian, and prostate cancers.10 An example of a prognostic marker is overexpression of the Her2/neu oncogene. Breast cancers with this oncogene tend to be more aggressive but will more likely respond to certain therapies (trastuzumab).10 Antigen/protein tumor markers are substances expressed by cancer cells or by the body in response to the presence of cancer. The ideal tumor marker has the following characteristics:

• It must be produced by the tumor or as a result of the tumor and must be secreted into some biological fluid that can be analyzed easily and inexpensively for levels.

• Its circulating half-life must be long enough to permit its concentration to rise with increasing tumor load.

• It must increase to clinically significant levels (above background control levels) while the disease is still treatabl and with few false negatives (sufficient sensitivity).

• The antigen must be absent from or at background levels in all individuals without the malignant disease in question to minimize false-positive test results (sufficient specificity Non-nucleic acid tumor markers generally fall into seven categories: cell surface markers, proteins, oncofetal antigens, carbohydrate antigens, blood group antigens, enzymes/isoenzymes, and hormones.12 Examples are shown in **Table 18–1**. Most are detected by immunologic methods with antibodies to distinct epitopes on the molecules. Prostate-specific antigen (PSA), for example, is an enzyme, but it is typically detected as an antigen. Tumor markers are not always directly associated with the malignant transformation. Often, they are the normal products of the tissue of origin being expressed, and this is more likely if the tumor is well differentiated. For example, endocrine gland tumors often produce generous amounts of hormone that the tissue of origin produces. Although there are scores of possible tumor markers in the literature, less than a dozen have FDA approval as such. However, many non-FDA-approved markers are available to clinicians, with a notation on the lab report stating that results are for research use only. The National Academy of Clinical Biochemistry has developed a set of very useful consensus guidelines regarding the clinical use of tumor markers. They list methods and markers for a variety of purposes that have acceptable evidence of validity. These guidelines recommend very few markers for screening/early

detection and still recommend using adjunct tests or screening high-risk populations. These markers are listed in **Table 18–2**. All other recommended markers have various uses in diagnosis and disease monitoring. Some of the most common and useful markers are listed in **Table 18–3**, along with important noncancerous conditions that cause elevations. The new field of **proteomics** employs **mass spectrometry (MS)** to identify and quantify an array of proteins simultaneously present in a sample. This has given birth to a new field called **oncopeptidomics.** Protein profiling in cancer patients will aid in the discovery of new tumor markers or patterns of protein expression that are consistent with cancer. Oncopeptidomics may allow more subtle increases of tumor markers to have diagnostic significance, since multiple markers can be measured and the overall pattern assessed, but this is currently only at the research stage.

There are some important aspects to laboratory testing for tumor markers. Most tumor markers are detected using antibodies because of the specificity of antibodies and the general reliability of immunoassays. However, there are some important limitations to using antibodies as reagents. Antibodies are directed at specific epitopes, and the antibodies from different manufacturers may vary greatly in terms of what is measured, particularly if monoclonal antibodies are used. This makes it important to use the same method for monitoring patients over time, and clinicians should be aware of this if patients change clinics or laboratories. It also means that if laboratories switch methods, they must provide a transition period during which specimens are measured by both methods and specimens are archived until new data is established for each patient**.**

Table 18–1. Categories of Protein/Antigen Tumor Markers[2,12]

TUMOR MARKER CLASS	EXAMPLES	DISEASE ASSOCIATIONS
Cell surface markers	Estrogen/progesterone receptors	Prognosis for hormone therapy in breast cancer
	CD markers on white blood cells	Clonality and lineage of white blood cell neoplasms
Proteins	Thyroglobulin (TG)	Well-differentiated papillary or follicular thyroid carcinoma
	Immunoglobulins (Ig) and Ig light chains (Bence Jones proteins)	Multiple myeloma and lymphoid malignancies
Oncofetal antigens	Alpha-1-fetoprotein (AFP)	Germ cell carcinoma, hepatocellular carcinoma
	Carcinoembryonic antigen (CEA)	Colorectal carcinoma and some others
Carbohydrate antigens	CA 125	Ovarian cancer
	CA 15-3	Breast cancer
Blood group antigens	CA 19-9 (related to Lewis antigens)	Pancreatic and gastrointestinal cancers
Enzymes/isoenzymes	Prostate-specific antigen (PSA)	Prostate cancer
	Alkaline phosphatase (ALKP)	Bone and liver cancer
	Neuron specific enolase	Neural tissue neoplasms
Hormones	Human chorionic gonadotropin (hCG)	Germ cell carcinoma, trophoblastic tumors
	Calcitonin	Medullary thyroid cancer
	Gastrin	Pancreatic gastrinoma

Table 18–2. Tumor Markers Useful for Cancer Screening Professional Consensus Recommendations from the National Academy for Clinical Biochemistry Lab Medicine Practice Guidelines[10]

CANCER TYPE	MARKER	ADJUNCT TEST	POPULATION RECOMMENDED
Prostate	Prostate-specific antigen (PSA, total and free)	Digital rectal exam	Men over 50 and with at least 10 years of life expectancy
Colorectal	Fecal occult blood	Genetic testing	Subjects over 50 years old for occult blood; genetic testing in high-risk subjects
Liver	Alpha-1-fetoprotein (AFP)	Ultrasound	High-risk subjects
Ovarian	Carbohydrate antigen 125 (CA 125)	Ultrasound	Subjects with family history of ovarian cancer

Table 18–3. Common Tumor Markers[2,10,12,27]

MARKER	CANCER(S)	USES*	NORMAL SOURCES	NONCANCEROUS CONDITIONS WITH ELEVATIONS	COMMENTS
AFP	Nonseminomatous testicular Germ cell Liver	1, 2, 3, 4	Fetal liver and yolk sac, adult liver	Pregnancy, non-neoplastic liver disease	Screening for high-risk populations for liver cancer such as those with liver cirrhosis and chronic hepatitis In germ cell tumors, both AFP and hCG are elevated.
β–2 microglobulin	Lymphocyte malignancies	2	MHC class I	Inflammatory and high cell turnover conditions	Higher levels imply poor prognosis in multiple myeloma.
Calcitonin and Ca++	Familial medullary thyroid carcinoma	N/A	Thyroid	In hypercalcemia, increased calcitonin is expected. Serum Ca++ may be low when calcitonin is elevated in medullary carcinoma.	Can be elevated in other forms of cancer.
CD markers	White blood cell (WBC)	N/A	All WBCs	WBC increase such as infection	An array of CD markers are associated with WBC malignancies.
CEA	Colorectal Breast				Values increased with age and in smokers.
CA125	Ovarian	1, 2, 3, 4	Various	Endometriosis, pelvic	Don't collect specimen

While antibodies are employed for their specificity, it is not absolute. Antibodies will cross-react with similar structures, and this is particularly problematic when the crossreacting substances are in excessive amounts, as can occur in cancer. For example, hCG is made of an alpha subunit and a beta subunit. The alpha subunit is virtually identical to the alpha subunit of luteinizing hormone (LH), and the beta subunits are 80 percent homologous, so epitope choice is quite important. Further, assay configuration influences what is measured. Cancers may produce free alpha and beta chains in addition to intact hCG, so an immunochemical method that relies only on beta chain epitopes to minimize LH interference will measure something completely different than a method that sandwiches intact hCG between an antialpha capture antibody and an antibeta labeled antibody.

By virtue of unchecked growth and aggressive metabolism,some tumors may produce massive amounts of tumor marker molecules. The prozone effect is a wellknown limitation of antibody-based assays in which antigen saturation of antibodies inhibits the cross-linkage required to visualize the reaction. In immunoassays, a similar phenomenon has been called the **high-dose hook effect,**18 and the result is a falsely decreased measurement, as shown in **Figure 18–2.** It is critical that criteria be developed to identify situations in which the hook effect may be present so that specimens can be diluted and accurate results obtained. A related problem of antigen excess in automated systems is specimen carryover, so in addition to diluting the specimen with excessive antigen, the specimen being tested immediately after it may need to be repeated.

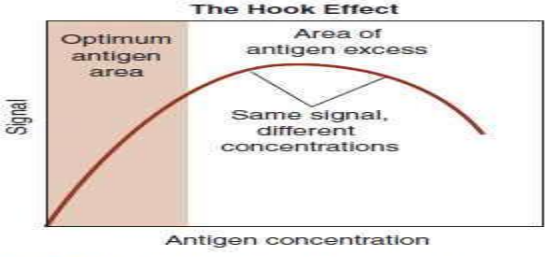


FIGURE 18–2. The high-dose hook effect. Antigen excess can saturate antibodies, and the intended "sandwich" configurations cannot form, leading to a false decrease in signal.

Finally, because many of the antibodies used in immunoassays are animal in origin, heterophile antibodies in specimens can interfere profoundly with results. In a tragic case involving false-positive hCG results from an automated analyzer, several women had unnecessary chemotherapy or hysterectomies for presumed undetected cancer. Although heterophile antibodies are mostly associated with false increases by mechanisms similar to that shown in **Figure 18–3,** false decreases are also possible.Antibody-blocking reagents are commercially available (e.g.,Scantibodies) to block heterophile antibodies in suspiciousspecimens, and many manufacturers are employing blocking agents within the routine reagents. Specimens with nonlinear behavior on dilution or with discrepant resultsusing different methods or after applying antibody-blocking reagents may have heterophile antibodies and should not be reported until the issue is resolved.

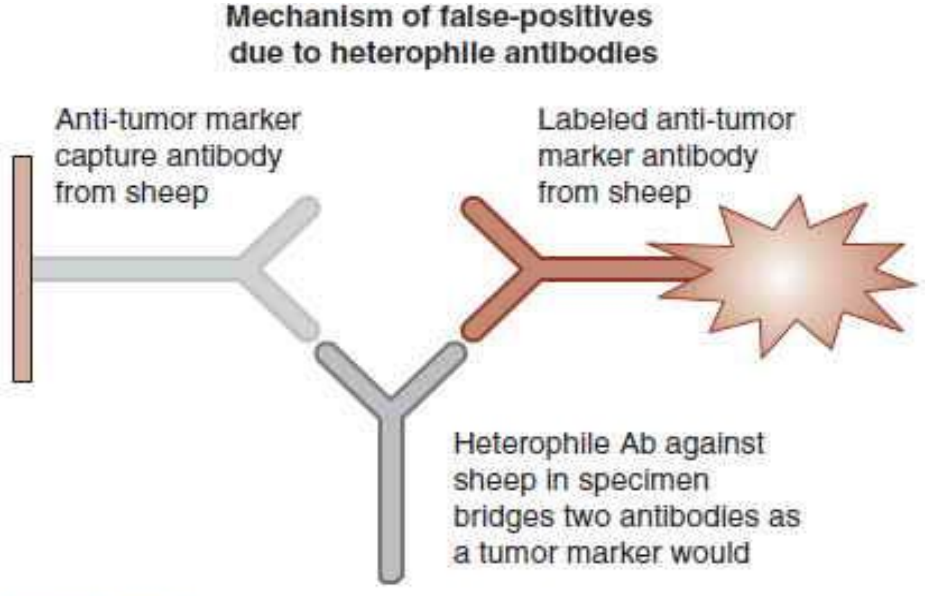


FIGURE 18–3. Heterophile antibody interference. Heterophile antibodies can cause both false decreases and false increases, depending on their reactivity against the antibody species used in an assay. However, as shown, false increases are most likely.

Lec 6

IMMUNE RESPONSES AGAINST TRANSPLANTS

Some of the earliest attempts to replace damaged tissues by transplantation were during World War II, as a way of treating pilots who had received severe skin burns in airplane crashes. It was soon realized that individuals reject tissue grafts from other individuals. Rejection results from inflammatory reactions that damage the transplanted tissues. Studies since the 1940s and 1950s established that graft rejection is mediated by the adaptive immune system, because it shows specificity and memory and it is dependent on lymphocytes (Fig. 10-7). Much of the knowledge

Evidence	Conclusion
Prior exposure to donor MHC molecules leads to accelerated graft rejection	Graft rejection shows memory and specificity, two cardinal features of adaptive immunity
The ability to reject a graft rapidly can be transferred to a naive individual by lymphocytes from a sensitized individual	Graft rejection is mediated by lymphocytes
Depletion or inactivation of T lymphocytes by drugs or antibodies results in reduced graft rejection	Graft rejection requires T lymphocytes

FIGURE 10-7 Evidence indicating that the rejection of tissue transplants is an immune reaction. Clinical and experimental evidence indicates that rejection of grafts is a reaction of the adaptive immune system. *MHC*, Major histocompatibility complex.

about the immunology of transplantation came from experiments with inbred strains of rodents, particularly mice. All members of an inbred strain are

genetically identical to one another and different from the members of other strains. These studies showed that grafts among members of one inbred strain are accepted and grafts from one strain to another are rejected, firmly establishing rejection as a process controlled by the animals' genes. Later experiments defined the nature of the genes that control graft rejection and showed that the products of many of these genes are expressed in all tissues.

As mentioned in Chapter 3, the genes that contributed the most to the rejection of grafts exchanged between mice of different inbred strains were called **major histocompatibility complex (MHC)** genes. The language of transplantation immunology evolved from the experimental studies. The individual who provides the graft is called the **donor,** and the individual in whom the graft is placed is the **recipient** or **host.** Animals that are identical to one another (and grafts exchanged among these animals) are said to be **syngeneic;** animals (and grafts) of one species that differ from other animals of the same species are said to be **allogeneic;** and animals (and grafts) of different species are **xenogeneic.** Allogeneic and xenogeneic grafts, also called **allografts** and **xenografts,** are always rejected by a recipient with a normal immune system. The antigens that serve as the targets of rejection are called alloantigens and xenoantigens, and the antibodies and T cells that react against these antigens are alloreactive and xeno-reactive, respectively. In the clinical situation, transplants are exchanged between allogeneic individuals, who are members of an outbred species who differ from one another (except for identical twins). Most of the following discussion focuses on immune responses to allografts.

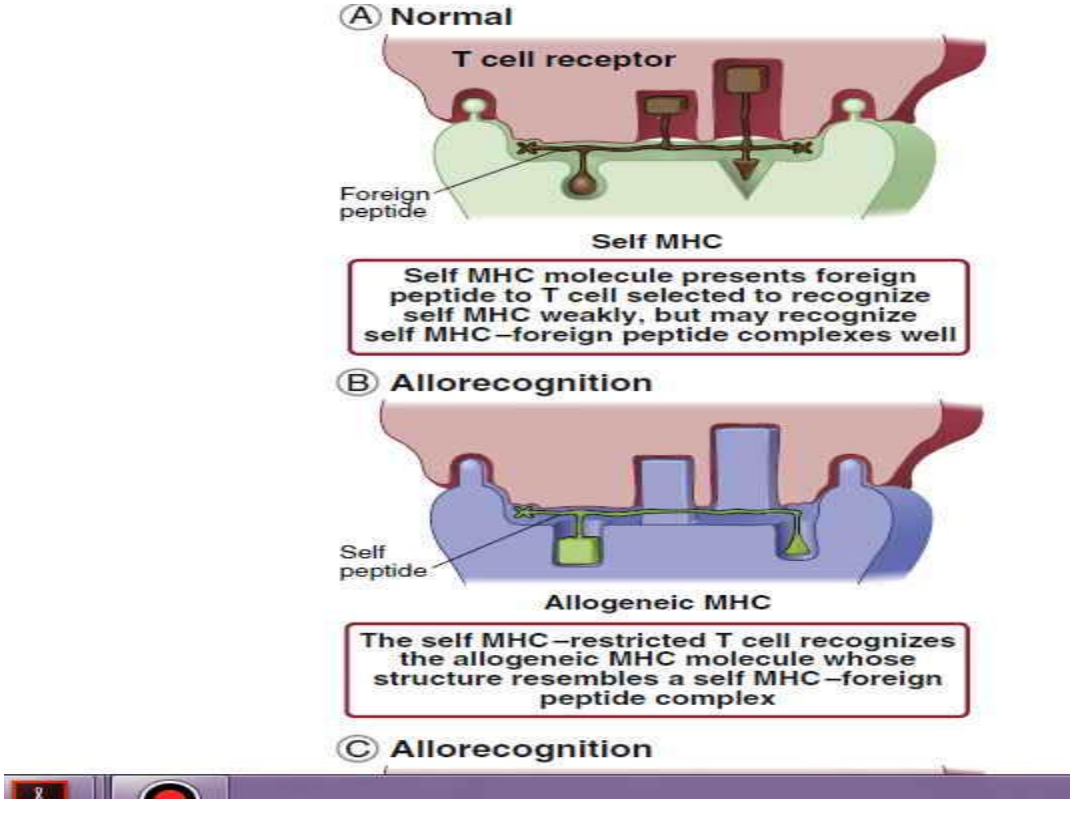
Transplantation Antigens

The antigens of allografts that serve as the principal targets of rejection are proteins encoded in the MHC. Homologous MHC genes and molecules are present in all mammals; the human MHC is called the **human leukocyte antigen** (HLA) complex. It took more than 20 years after the discovery of the MHC to show that the physiologic function of MHC molecules is to display peptide antigens for recognition by T lymphocytes (see Chapter 3). Recall that every person expresses six class I MHC alleles (one allele of HLA-A, -B, and -C from each parent) and usually more than eight class II MHC alleles (one allele of HLA-DQ and -DP and one or two of -DR from each parent, and some combinations of these). MHC genes are highly polymorphic, with over 13,000 HLA alleles among all humans, encoding about 2200 HLA-A proteins, 2900 HLA-B proteins, and 1300 DR B proteins. Because these alleles can be inherited and expressed in virtually any combination, every individual is likely to express some MHC proteins that differ from those of another individual and that therefore appear foreign to another individual′s immune system, except in the case of identical twins. Because each HLA locus is inherited as a block, the chance that two siblings will have the same MHC alleles is 1 in 4.

The response to MHC antigens on another individual's cells is one of the strongest immune responses known. T cell receptors (TCRs) for antigens have evolved to recognize MHC molecules, which is essential for surveillance of cells harboring infectious microbes. As a result of positive selection of developing T cells in the thymus, mature T cells that have some affinity for self MHC molecules survive, and many of these will have high affinity for self

MHC displaying foreign peptides. Allogeneic MHC molecules containing peptides derived from the allogeneic cells may look like self MHC molecules plus bound foreign peptides (Fig. 10-8). Therefore, recognition of allogeneic MHC molecules in allografts is an example of an immunologic cross-reaction.

There are several reasons why recognition of allogeneic MHC molecules results in strong T cell reactions. Many clones of T cells specific for different foreign peptides bound to the same self MHC molecule may cross-react with any one allogeneic MHC molecule, as long as the allogeneic MHC molecule resembles complexes of self MHC plus foreign peptides. As a result, many self MHC–restricted T cells specific for different peptide antigens may recognize any one allogeneic MHC molecule. Also, the process of negative selection in the thymus eliminates cells that strongly



recognize self MHC, but there is no mechanism for selectively eliminating T cells whose TCRs have a high affinity for allogeneic MHC molecules, because these are never present in the thymus. Furthermore, a single allogeneic graft cell will express thousands of MHC molecules, every one of which may be recognized as foreign by a graft recipient′s T cells. By contrast, in the case of an infected cell, only a small fraction of the self MHC molecules on the cell surface will carry a foreign microbial peptide recognized by the host's T cells. The net result of these features of allorecognition is that as many as 0.1% to 1% of all T cells in a normal individual may react against an allogeneic MHC molecule, much more than the 1 in 105 or 106 T cells that recognize any microbial antigen.

Although MHC proteins are the major antigens that stimulate graft rejection, other polymorphic proteins also may play a role in rejection. Non-MHC antigens that induce graft rejection are called minor histocompatibility antigens, and most are normal cellular proteins that differ in sequence between donor and recipient. The rejection reactions that minor histocompatibility antigens elicit usually are not as strong as reactions against foreign MHC proteins. Two clinical situations in which minor antigens are important targets of rejection are blood transfusion and hematopoietic stem cell transplantation, discussed later.

Induction of Immune Responses Against Transplants

In order to elicit antigraft immune responses, alloantigens from the graft are transported by dendritic cells to draining lymph nodes, where they are

recognized by alloreactive T cells (Fig. 10-9). The dendritic cells that present alloantigens also provide costimulators and can stimulate helper T cells as well as alloreactive CTLs. The effector T cells that are generated circulate back to the transplant and mediate rejection.

T cells may recognize allogeneic MHC molecules in the graft displayed by donor dendritic cells in the graft, or graft alloantigens may be processed and presented by the host's dendritic cells (Fig. 10-10). These two pathways of presentation of graft antigens have different features and names.

• *Direct allorecognition.* Most tissues contain dendritic cells, and when the tissues are transplanted, the dendritic cells are carried in the graft. When T cells in the recipient recognize donor allogeneic MHC molecules on graft dendritic cells, the T cells are activated; this process is called **direct recognition** (or direct presentation) of alloantigens. Direct recognition stimulates the development of alloreactive T cells (e.g., CTLs) that recognize and attack the cells of the graft.

• *Indirect allorecognition.* If graft cells (or alloantigens) are ingested by recipient dendritic cells, donor alloantigens are processed and presented by the self MHC molecules on recipient APCs. This process is called **indirect recognition** (or indirect presentation) and is similar to the cross-presentation of tumor antigens discussed earlier. If alloreactive CTLs are induced by the indirect pathway, these CTLs are specific for donor alloantigens displayed by the recipient's self MHC molecules on the recipient's APCs, so they cannot recognize and kill cells in the graft (which, of course, express donor MHC molecules). When graft alloantigens are recognized by the indirect pathway, the subsequent rejection of the graft likely is mediated mainly by alloreactive CD4+ T cells. These T cells may enter the graft together with host APCs, recognize graft antigens that are picked up and displayed by these APCs, and secrete cytokines that injure the graft by an inflammatory reaction.

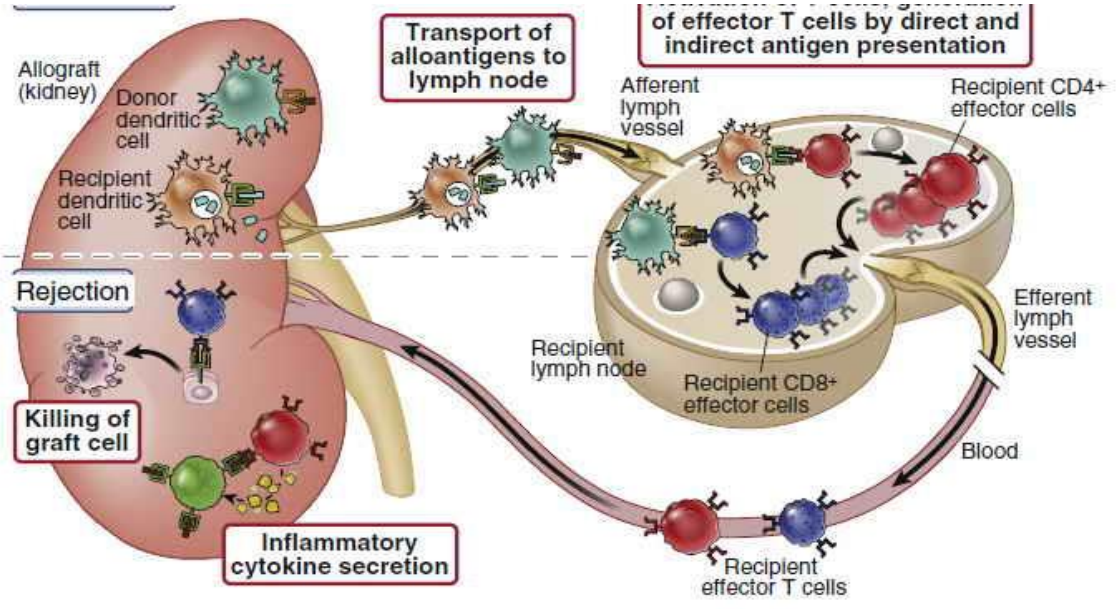


FIGURE 10-9 **Immune response against transplants.** Graft antigens that are expressed on donor dendritic cells or captured by recipient dendritic cells are transported to peripheral lymphoid organs where alloantigen-specific T cells are activated (the sensitization step). The T cells migrate back into the graft and destroy graft cells (rejection). Antibodies are also produced against graft antigens and can contribute to rejection (not shown). The example shown is that of a kidney graft, but the same general principles apply to all organ grafts.

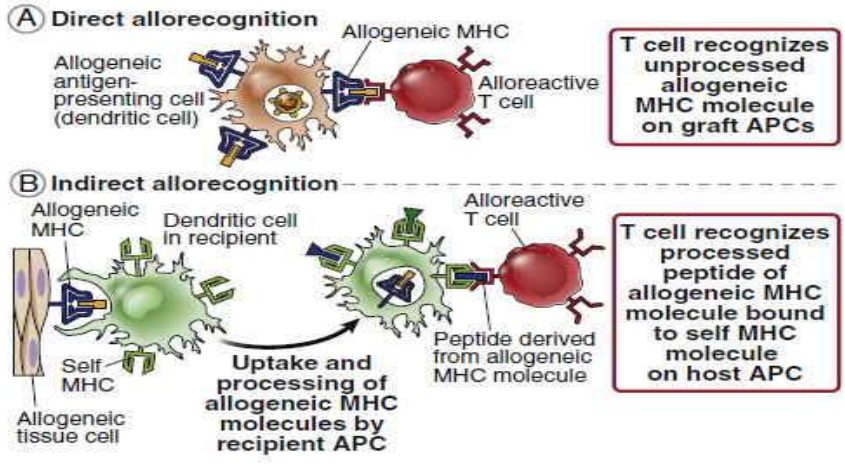


FIGURE 10-10 **Direct and indirect recognition of alloantigens.** **A,** Direct alloantigen recognition occurs when T cells bind directly to intact allogeneic major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs) in a graft, as illustrated in Figure 10-8. **B,** Indirect alloantigen recognition occurs when allogeneic MHC molecules from graft cells are taken up and processed by recipient APCs, and peptide fragments of the allogeneic MHC molecules are presented by recipient (self) MHC molecules. Recipient APCs also may process and present graft proteins other than allogeneic MHC molecules.

We do not know the relative importance of the direct and indirect pathways of allorecognition in the rejection of allografts. The direct pathway may be most important for CTL-mediated acute rejection, and the indirect pathway may play a greater role in chronic rejection, as described later.

T cell responses to allografts require costimulation, but which stimuli in grafts enhance the expression of costimulators on APCs is unclear. As with tumors, graft cells may undergo necrosis, perhaps in the period of ischemia before the transplant is done, and substances released from the injured and dead cells activate APCs by innate immune mechanisms. As we discuss later, blocking costimulation is one therapeutic strategy for promoting graft survival.

The **mixed lymphocyte reaction** (MLR) is an in vitro model of T cell recognition of alloantigens. In this model, T cells from one individual are cultured with leukocytes of another individual, and the responses of the T cells are assayed. The magnitude of this response is proportional to the extent of the MHC differences between these individuals and is a rough predictor of the outcomes of grafts exchanged between these individuals.

Although much of the emphasis on allograft rejection has been on the role of T cells, it is clear that alloantibodies also contribute to rejection. Most of these antibodies are helper T cell– dependent high-affinity antibodies. In order to produce alloantibodies, recipient B cells recognize donor alloantigens and then process and present peptides derived from these antigens to helper T cells (that may have been previously activated by recipient DCs presenting the same donor

alloantigen), thus initiating the process of antibody production. This is a good example of indirect presentation of alloantigens, in this case by B lymphocytes.

Immune Mechanisms of Graft Rejection

Graft rejection is classified into hyperacute, acute, and chronic, on the basis of clinical and pathologic features (Fig. 10-11). This historical classification was devised by clinicians based on rejection of kidney allografts, and it has stood the test of time remarkably well. It also has become apparent that each type of rejection is mediated by a particular type of immune response.

• **Hyperacute rejection** occurs within minutes of transplantation and is characterized by thrombosis of graft vessels and ischemic necrosis of the graft. Hyperacute rejection is mediated by circulating antibodies that are specific for antigens on graft endothelial cells and that are present before transplantation. These preformed antibodies may be natural IgM antibodies specific for blood group antigens, or they may be antibodies specific for allogeneic MHC molecules that are induced by exposure to allogeneic cells due to previous blood transfusions, pregnancy, or organ transplantation. Almost immediately after transplantation, the antibodies bind to antigens on the graft vascular endothelium and activate the complement and clotting systems, leading to injury to the endothelium and thrombus formation. Hyperacute rejection is not a common problem in clinical transplantation, because every donor and recipient are matched for blood type and potential recipients are tested for antibodies against the cells of the prospective donor. (The test for antibodies is called a cross-

match.) However, hyperacute rejection is the major barrier to xenotransplantation,

• **Acute rejection** occurs within days or weeks after transplantation and is the principal cause of early graft failure. Acute rejection is mediated by T cells and antibodies specific for alloantigens in the graft. The T cells may be CD8+ CTLs that directly destroy graft cells or CD4+ cells that secrete cytokines and induce inflammation, which destroys the graft. T cells may also react against cells in graft vessels, leading to vascular damage. Antibodies contribute especially to the vascular component of acute rejection. Antibody-mediated injury to graft vessels is caused mainly by complement activation by the classical pathway. Current immunosuppressive therapy is designed mainly to prevent and reduce acute rejection by blocking the activation of alloreactive T cells.

Chronic rejection is an indolent form of graft damage that occurs over months or years, leading to progressive loss of graft function. Chronic rejection may be manifested as fibrosis of the graft and by gradual narrowing of graft blood vessels, called graft arteriosclerosis. In both lesions, the culprits are believed to be T cells that react against graft alloantigens and secrete cytokines, which stimulate the proliferation and activities of fibroblasts and vascular smooth muscle cells in the graft. Alloantibodies also contribute to chronic rejection. Although treatments to prevent or curtail acute rejection have steadily improved, leading to better 1-year survival of transplants, chronic rejection is refractory to most of these therapies and is becoming the principal cause of graft failure.

Prevention and Treatment of Graft Rejection

The mainstay of preventing and treating the rejection of organ transplants is immunosuppression, designed mainly to inhibit T cell activation and effector functions (Fig. 10-12). The development of immunosuppressive drugs launched the modern era of organ transplantation, because these drugs made it feasible to transplant organs from donors that were not HLA-matched with recipients, especially in situations when such matching was impractical, such as transplantation of heart, lung, and liver.

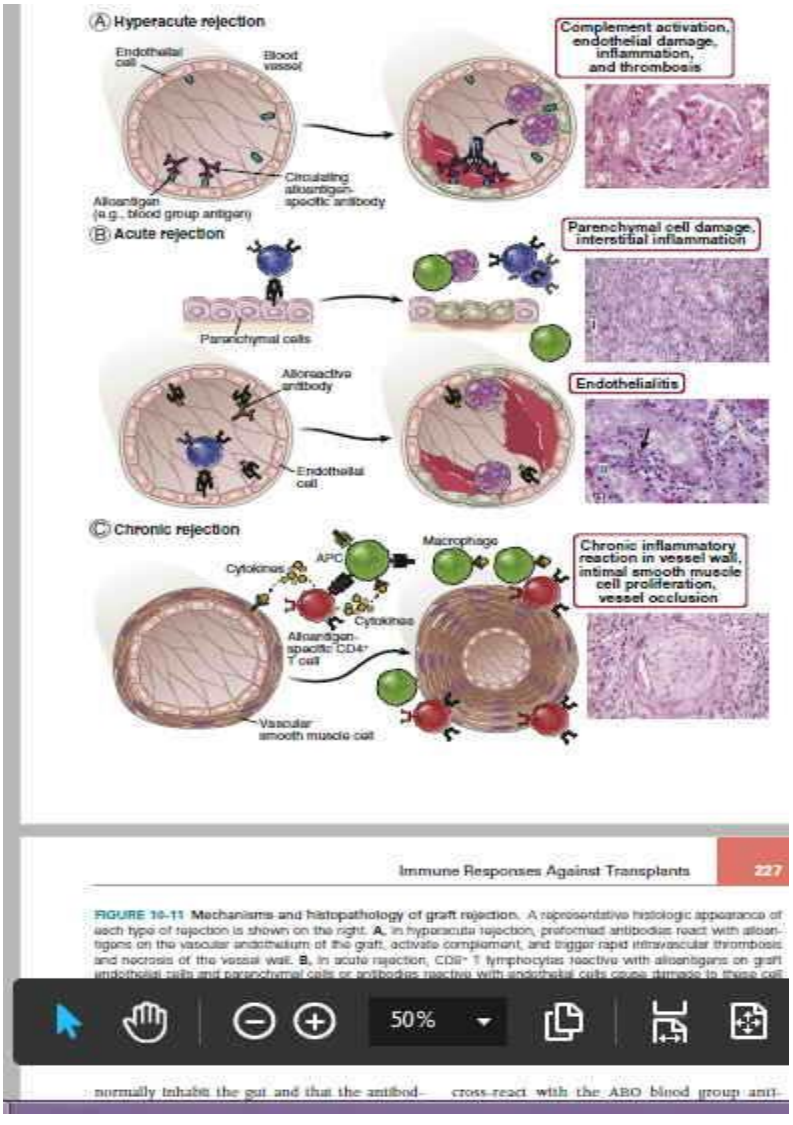
One of the most useful classes of immunosuppressive drugs in clinical transplantation has been the calcineurin inhibitors cyclosporine and tacrolimus (FK506), which function by blocking the phosphatase calcineurin. This enzyme is required to activate the transcription factor NFAT (nuclear factor of activated T cells), and blocking its activity inhibits the transcription of cytokine genes in the T cells. Cyclosporine was the first clinically useful immunosuppressive drug that inhibited the major mediators of graft rejection, T cells. Another widely used drug is rapamycin, which inhibits a kinase called mTOR required for T cell activation. Many other immunosuppressive agents are now used as adjuncts to or instead of calcineurin and mTOR inhibitors (see Fig. 10-12).

All of these immunosuppressive drugs carry the problem of nonspecific immunosuppression (i.e., the drugs inhibit responses to more than the graft). Therefore, patients receiving these drugs as part of their post-transplantation treatment regimen become susceptible to infections, particularly infections by intracellular microbes, and demonstrate an increased incidence of cancer, especially tumors caused by oncogenic viruses.

The matching of donor and recipient HLA alleles by tissue typing had an important role in minimizing graft rejection before cyclosporine became available for clinical use. Although MHC matching is critical for the success of transplantation of some types of tissues (e.g., hematopoietic stem cell transplants) and improves survival of other types of organ grafts (e.g., renal allografts), modern immunosuppression is so effective that HLA matching is not considered necessary for many types of organ transplants (e.g., heart and liver), mainly because the number of donors is limited and the recipients often are too sick to wait for well-matched organs to become available.

The long-term goal of transplant immunologists is to induce immunological tolerance specifically for the graft alloantigens. If this is achieved, it will allow graft acceptance without shutting off other immune responses in the host. Experimental and clinical attempts to induce graft-specific tolerance are ongoing.

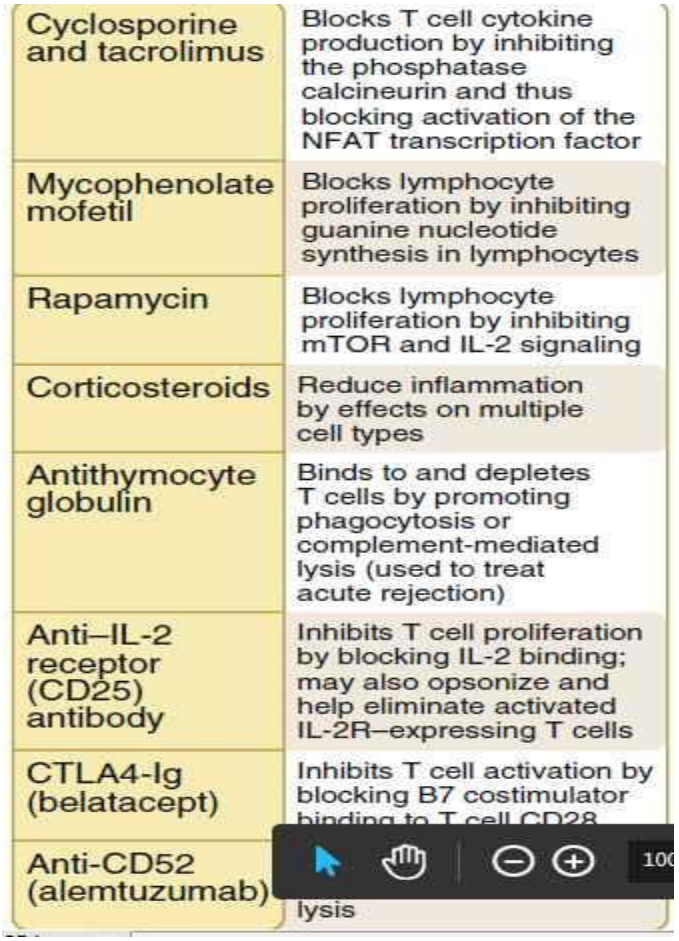
A major problem in transplantation is the shortage of suitable donor organs. **Xenotransplantation** has been considered a possible solution for this problem. Experimental studies show that hyperacute rejection is a frequent cause of xenotransplant loss. The reasons for the high incidence of hyperacute rejection of xenografts are that individuals often contain antibodies that react with cells from other species and the xenograft cells lack regulatory proteins that can inhibit human complement activation. These antibodies, similar to antibodies against blood group antigens, are called natural antibodies because their production does not require prior exposure to the xenoantigens. It is thought that these antibodies are produced against bacteria that normally inhabit the gut and that the antibodies cross-react with cells of other species. Xenografts also are subject to acute rejection, much like allografts but often even more severe than rejection of allografts. Because of the problem of rejection, and difficulty in procuring organs from animals that are evolutionarily close to humans, clinical xenotransplantaion remains a distant goal



matching also prevents rejection of transplanted stem cells by natural killer cells, which are inhibited by recognition of self MHC molecules .If mature allogeneic T cells are transplanted with the stem cells, these mature T cells can attack the recipient's tissues, resulting in a clinical reaction called **graft-versus-host disease.** Because HLA matching is always done for these transplants, this reaction is likely directed against minor histocompatibility antigens. The same reaction is exploited to kill leukemia cells, and hematopoietic stem cell

transplantation is now commonly used to treat leukemias resistant to chemotherapy. NK cells in the marrow inoculum may also contribute to the destruction of leukemia cells.

Even if the graft is successful, recipients often are severely immunodeficient while their immune systems are being reconstituted. Despite these problems, hematopoietic stem cell transplantation is a successful therapy for a wide variety of diseases affecting the hematopoietic and lymphoid systems.



Cyclosporine and tacrolimus	Blocks T cell cytokine production by inhibiting the phosphatase calcineurin and thus blocking activation of the NFAT transcription factor
Mycophenolate mofetil	Blocks lymphocyte proliferation by inhibiting guanine nucleotide synthesis in lymphocytes
Rapamycin	Blocks lymphocyte proliferation by inhibiting mTOR and IL-2 signaling
Corticosteroids	Reduce inflammation by effects on multiple cell types
Antithymocyte globulin	Binds to and depletes T cells by promoting phagocytosis or complement-mediated lysis (used to treat acute rejection)
Anti–IL-2 receptor (CD25) antibody	Inhibits T cell proliferation by blocking IL-2 binding; may also opsonize and help eliminate activated IL-2R–expressing T cells
CTLA4-Ig (belatacept)	Inhibits T cell activation by blocking B7 costimulator binding to T cell CD28
Anti-CD52 (alemtuzumab)	lysis

FIGURE 10-12 Treatments for graft rejection. Agents used to treat rejection of organ grafts and their mechanisms of action. Like cyclosporine, tacrolimus (FK506) is a calcineurin inhibitor, but it is not as widely used. *CTLA4-Ig*, Cytotoxic T lymphocyte–associated protein 4–immunoglobulin (fusion protein); *IL*, interleukin; *NFAT*, nuclear factor of activated T cells.