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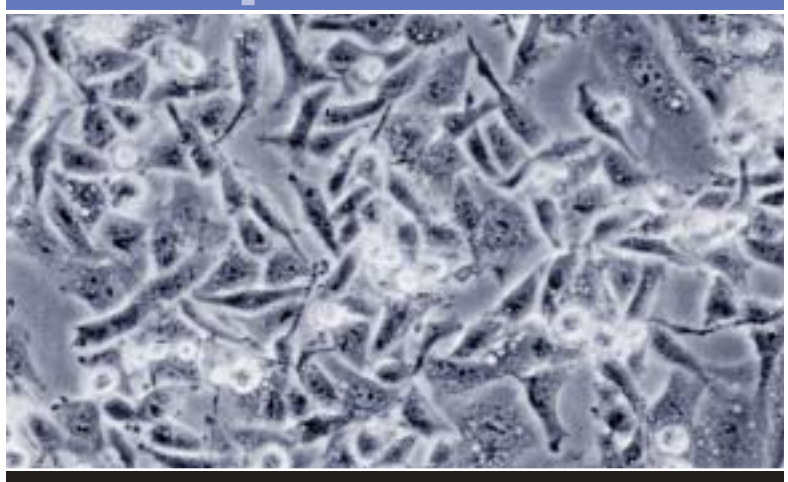
chapter 22

Cancer and the Immune System



Cancerous melanoma cells.

As the death toll from infectious disease has declined in the Western world, cancer has become the second-ranking cause of death there, led only by heart disease. Current estimates project that one person in three in the United States will develop cancer, and that one in five will die from it. From an immunologic perspective, cancer cells can be viewed as altered self-cells that have escaped normal growth-regulating mechanisms. This chapter examines the unique properties of cancer cells, paying particular attention to those properties that can be recognized by the immune system. The immune responses that develop to cancer cells, as well as the methods by which cancers manage to evade those responses, are then described. The final section describes current clinical and experimental immunotherapies for cancer.

Cancer: Origin and Terminology

In most organs and tissues of a mature animal, a balance is usually maintained between cell renewal and cell death. The various types of mature cells in the body have a given life span; as these cells die, new cells are generated by the proliferation and differentiation of various types of stem cells. Under normal circumstances, the production of new cells is regulated so that the number of any particular type of cell remains constant. Occasionally, though, cells arise that no longer respond to normal growth-control mechanisms. These cells give rise to clones of cells that can expand to a considerable size, producing a tumor, or **neoplasm.**

A tumor that is not capable of indefinite growth and does not invade the healthy surrounding tissue extensively is **benign.** A tumor that continues to grow and becomes progressively invasive is **malignant;** the term *cancer* refers specifically to a malignant tumor. In addition to uncontrolled growth, malignant tumors exhibit **metastasis;** in this process, small clusters of cancerous cells dislodge from a tumor, invade the blood or lymphatic vessels, and are carried to other tissues, where they continue to proliferate. In this way a primary tumor at one site can give rise to a secondary tumor at another site (Figure 22-1).

Malignant tumors or cancers are classified according to the embryonic origin of the tissue from which the tumor is derived. Most (>80%) are **carcinomas,** tumors that arise from endodermal or ectodermal tissues such as skin or the epithelial lining of internal organs and glands. The majority of cancers of the colon, breast, prostate, and lung are carcinomas. The **leukemias** and **lymphomas** are malignant tumors of hematopoietic cells of the bone marrow and account for about 9% of cancer incidence in the United States. Leukemias proliferate as single cells, whereas lymphomas tend to grow as tumor masses. **Sarcomas,** which arise less frequently (around 1% of the incidence in the United States), are derived from mesodermal connective tissues such as bone, fat, and cartilage.

Malignant Transformation of Cells

Treatment of normal cultured cells with chemical carcinogens, irradiation, and certain viruses can alter their morphology and growth properties. In some cases this process, referred to as **transformation,** makes the cells able to produce tumors when they are injected into animals. Such cells

VISUALIZING CONCEPTS

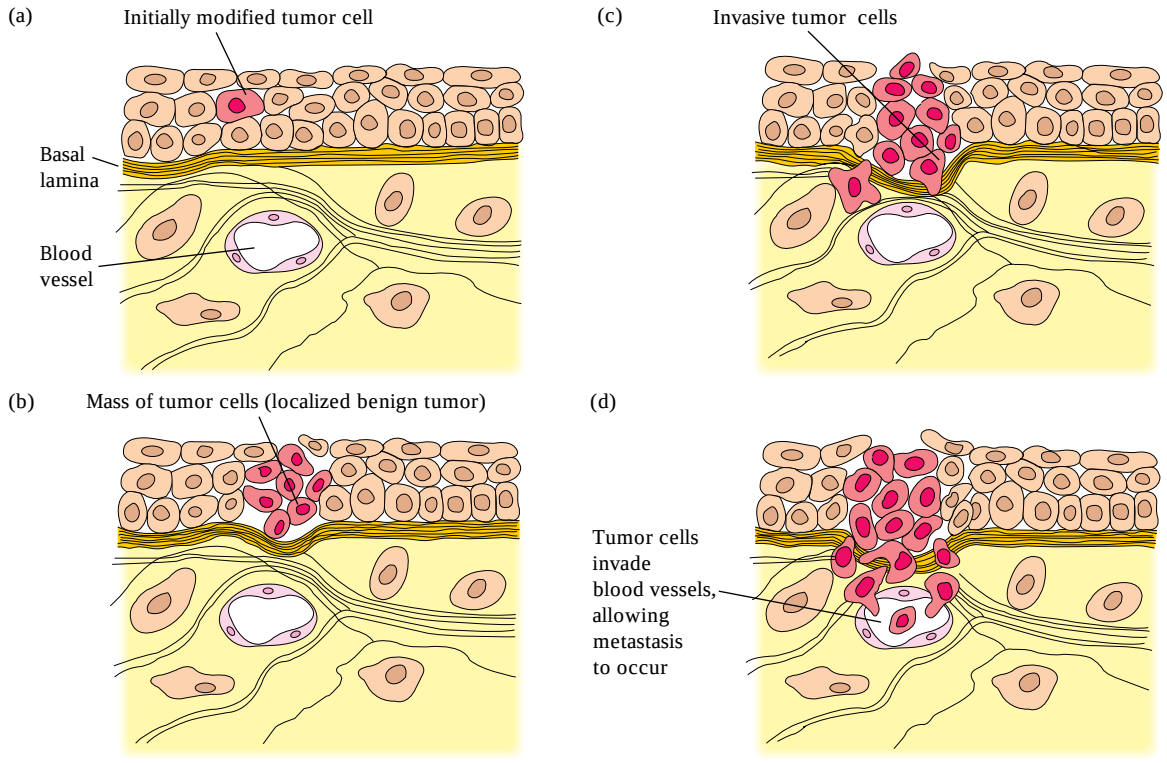


FIGURE **22-1** Tumor growth and metastasis. (a) A single cell develops altered growth properties at a tissue site. (b) The altered cell proliferates, forming a mass of localized tumor cells, or benign tumor. (c) The tumor cells become progressively more invasive, invading the underlying basal lamina. The tumor is now classified as malignant. (d) The malignant tumor metastasizes by generating small clusters of cancer cells that dislodge from the tumor and are carried by the blood or lymph to other sites in the body. *[Adapted from J. Darnell et al., 1990,* Molecular Cell Biology, *2d ed., Scientific American Books.]*

are said to have undergone malignant transformation, and they often exhibit properties in vitro similar to those of cancer cells. For example, they have decreased requirements for growth factors and serum, are no longer anchorage-dependent, and grow in a density-independent fashion. Moreover, both cancer cells and transformed cells can be subcultured indefinitely; that is, for all practical purposes, they are immortal. Because of the similar properties of cancer and transformed cells, the process of malignant transformation has been studied extensively as a model of cancer induction.

Various chemical agents (e.g., DNA-alkylating reagents) and physical agents (e.g., ultraviolet light and ionizing radiation) that cause mutations have been shown to induce transformation. Induction of malignant transformation with chemical or physical carcinogens appears to involve multiple steps and at least two distinct phases: initiation and promotion. Initiation involves changes in the genome but does not, in itself, lead to malignant transformation. After initiation, promoters stimulate cell division and lead to malignant transformation.

The importance of mutagenesis in the induction of cancer is illustrated by diseases such as xeroderma pigmentosum. This rare disorder is caused by a defect in the gene that encodes a DNA-repair enzyme called UV-specific endonuclease. Individuals with this disease are unable to repair UV-induced mutations and consequently develop skin cancers.

A number of DNA and RNA viruses have been shown to induce malignant transformation. Two of the best-studied are SV40 and polyoma. In both cases the viral genomes,

which integrate randomly into the host chromosomal DNA, include several genes that are expressed early in the course of viral replication. SV40 encodes two early proteins called large T and little T, and polyoma encodes three early proteins called large T, middle T, and little T. Each of these proteins plays a role in the malignant transformation of virus-infected cells.

Most RNA viruses replicate in the cytosol and do not induce malignant transformation. The exceptions are retroviruses, which transcribe their RNA into DNA by means of a reverse-transcriptase enzyme and then integrate the transcript into the host's DNA. This process is similar in the cytopathic retroviruses such as HIV-1 and HIV-2 and in the transforming retroviruses, which induce changes in the host cell that lead to malignant transformation. In some cases, retrovirus-induced transformation is related to the presence of **oncogenes,** or "cancer genes," carried by the retrovirus.

One of the best-studied transforming retroviruses is the **Rous sarcoma virus.** This virus carries an oncogene called v-*src*, which encodes a 60-kDa protein kinase (v-Src) that catalyzes the addition of phosphate to tyrosine residues on proteins. The first evidence that oncogenes alone could induce malignant transformation came from studies of the v-*src* oncogene from Rous sarcoma virus. When this oncogene was cloned and transfected into normal cells in culture, the cells underwent malignant transformation.

Oncogenes and Cancer Induction

In 1971, Howard Temin suggested that oncogenes might not be unique to transforming viruses but might also be found in normal cells; indeed, he proposed that a virus might acquire oncogenes from the genome of an infected cell. He called these cellular genes **proto-oncogenes,** or **cellular oncogenes** (c-*onc*), to distinguish them from their viral counterparts (v-*onc*). In the mid-1970s, J. M. Bishop and H. E. Varmus identified a DNA sequence in normal chicken cells that is homologous to v-*src* from Rous sarcoma virus. This cellular oncogene was designated c-*src*. Since these early discoveries, numerous cellular oncogenes have been identified.

Sequence comparisons of viral and cellular oncogenes reveal that they are highly conserved in evolution. Although most cellular oncogenes consist of a series of exons and introns, their viral counterparts consist of uninterrupted coding sequences, suggesting that the virus might have acquired the oncogene through an intermediate RNA transcript from which the intron sequences had been removed during RNA processing. The actual coding sequences of viral oncogenes and the corresponding proto-oncogenes exhibit a high degree of homology; in some cases, a single point mutation is all that distinguishes a viral oncogene from the corresponding proto-oncogene. It has now become apparent that most, if not all, oncogenes (both viral and cellular) are derived from cellular genes that encode various growth-controlling proteins. In addition, the proteins encoded by a particular oncogene and its corresponding proto-oncogene appear to have very similar functions. As described below, the conversion of a proto-oncogene into an oncogene appears in many cases to accompany a change in the level of expression of a normal growth-controlling protein.

Cancer-Associated Genes Have Many Functions

Homeostasis in normal tissue is maintained by a highly regulated process of cellular proliferation balanced by cell death. If there is an imbalance, either at the stage of cellular proliferation or at the stage of cell death, then a cancerous state will develop. Oncogenes and tumor suppressor genes have been shown to play an important role in this process, by regulating either cellular proliferation or cell death. Cancer-associated genes can be divided into three categories that reflect these different activities, summarized in Table 22-1.

INDUCTION OF CELLULAR PROLIFERATION

One category of proto-oncogenes and their oncogenic counterparts encodes proteins that induce cellular proliferation. Some of these proteins function as growth factors or growth-factor receptors. Included among these are *sis*, which encodes a form of platelet-derived growth factor, and *fms*, *erbB*, and *neu*, which encode growth-factor receptors. In normal cells, the expression of growth factors and their receptors is carefully regulated. Usually, one population of cells secretes a growth factor that acts on another population of cells that carries the receptor for the factor, thus stimulating proliferation of the second population. Inappropriate expression of either a growth factor or its receptor can result in uncontrolled proliferation.

Other oncogenes in this category encode products that function in signal-transduction pathways or as transcription factors. The *src* and *abl* oncogenes encode tyrosine kinases, and the *ras* oncogene encodes a GTP-binding protein. The products of these genes act as signal transducers. The *myc*, *jun*, and *fos* oncogenes encode transcription factors. Overactivity of any of these oncogenes may result in unregulated proliferation.

INHIBITION OF CELLULAR PROLIFERATION

A second category of cancer-associated genes—called **tumor-suppressor genes,** or anti-oncogenes—encodes proteins that inhibit excessive cell proliferation. Inactivation of these results in unregulated proliferation. The prototype of this category of oncogenes is *Rb*, the retinoblastoma gene. Hereditary retinoblastoma is a rare childhood cancer, in which tumors develop from neural precursor cells in the immature retina. The affected child has inherited a mutated *Rb* allele; somatic inactivation of the remaining *Rb* allele leads to tumor growth. Probably the single most frequent genetic abnormality in human cancer is mutation in *p53*, which encodes a nuclear phosphoprotein. Over 90% of small-cell lung cancers and

TABLE 22-1 Functional classification of cancer-associated genes

Type/name	Nature of gene product
CATEGORY I: GENES THAT INDUCE CELLULAR PROLIFERATION	
Growth factors	
sis	A form of platelet-derived growth factor (PDGF)
Growth-factor receptors	
fms	Receptor for colony-stimulating factor 1 (CSF-1)
erbB	Receptor for epidermal growth factor (EGF)
neu	Protein (HER2) related to EGF receptor
erbA	Receptor for thyroid hormone
Signal transducers	
src	Tyrosine kinase
abl	Tyrosine kinase
Ha-*ras*	GTP-binding protein with GTPase activity
N-*ras*	GTP-binding protein with GTPase activity
K-*ras*	GTP-binding protein with GTPase activity
Transcription factors	
jun	Component of transcription factor AP1
fos	Component of transcription factor AP1
myc	DNA-binding protein
CATEGORY II: TUMOR-SUPRESSOR GENES, INHIBITORS OF CELLULAR PROLIFERATION*	
Rb	Suppressor of retinoblastoma
p*53*	Nuclear phosphoprotein that inhibits formation of small-cell lung cander and colon cancers
DCC	Suppressor of colon carcinoma
APC	Suppressor of adenomatous polyposis
NF1	Suppressor of neurofibromatosis
WT1	Suppressor of Wilm's tumor
CATEGORY III: GENES THAT REGULATE PROGRAMMED CELL DEATH	
bcl-2	Suppressor of apoptosis

* The activity of the normal products of the category II genes inhibits progression of the cell cycle. Loss of a gene or its inactivation by mutation in an indicated tumor-suppressor gene is associated with development of the indicated cancers.

over 50% of breast and colon cancers have been shown to be associated with mutations in *p53*.

REGULATION OF PROGRAMMED CELL DEATH

A third category of cancer-associated genes regulates programmed cell death. These genes encode proteins that either block or induce apoptosis. Included in this category of oncogenes is *bcl-2*, an anti-apoptosis gene. This oncogene was originally discovered because of its association with B-cell follicular lymphoma. Since its discovery, *bcl-2* has been shown to play an important role in regulating cell survival during hematopoiesis and in the survival of selected B cells and T cells during maturation. Interestingly, the Epstein-Barr virus contains a gene that has sequence homology to *bcl-2* and may act in a similar manner to suppress apoptosis.

Proto-Oncogenes Can Be Converted to Oncogenes

In 1972, R. J. Huebner and G. J. Todaro suggested that mutations or genetic rearrangements of proto-oncogenes by carcinogens or viruses might alter the normally regulated function of these genes, converting them into potent cancer-causing oncogenes (Figure 22-2). Considerable evidence supporting this hypothesis accumulated in subsequent years. For example, some malignantly transformed cells contain multiple copies of cellular oncogenes, resulting in increased production of oncogene products. Such amplification of cellular oncogenes has been observed in cells from various types of human cancers. Several groups have identified c-*myc* oncogenes in homogeneously staining regions (HSRs) of chromosomes from can-

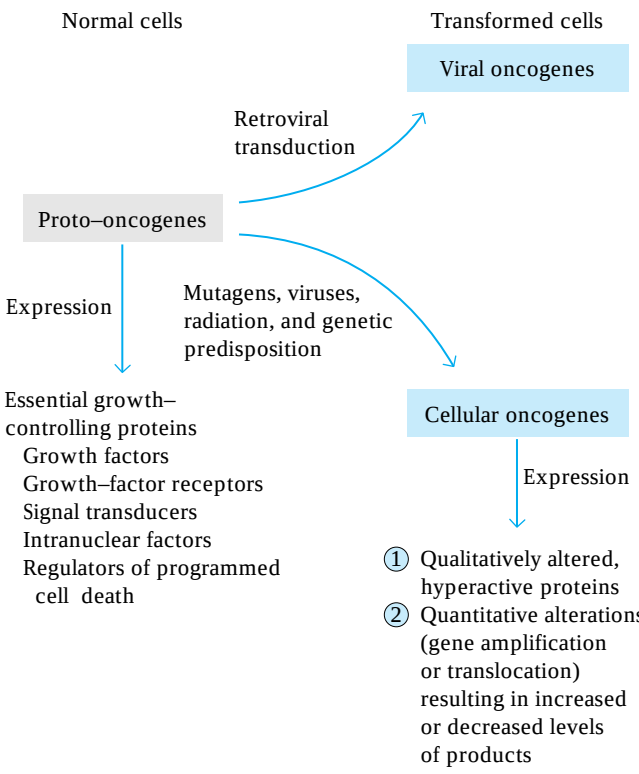


FIGURE **22-2** Conversion of proto-oncogenes into oncogenes can involve mutation, resulting in production of qualitatively different gene products, or DNA amplification or translocation, resulting in increased or decreased expression of gene products.

cer cells; these HSRs represent long tandem arrays of amplified genes.

In addition, some cancer cells exhibit chromosomal translocations, usually the movement of a proto-oncogene from one chromosomal site to another (Figure 22-3). In many cases of Burkitt's lymphoma, for example, c-*myc* is moved from its normal position on chromosome 8 to a position near the immunoglobulin heavy-chain enhancer on chromosome 14. As a result of this translocation, synthesis of the c-Myc protein, which functions as a transcription factor, increases.

Mutation in proto-oncogenes also has been associated with cellular transformation, and it may be a major mechanism by which chemical carcinogens or x-irradiation convert a proto-oncogene into a cancer-inducing oncogene. For instance, single-point mutations in c-*ras* have been detected in a significant fraction of several human cancers, including carcinomas of the bladder, colon, and lung. Some of these mutations appear to reduce the ability of Ras to associate with GTPase-stimulating proteins, thus prolonging the growth-activated state of Ras.

Viral integration into the host-cell genome may in itself serve to convert a proto-oncogene into a transforming oncogene. For example, avian leukosis virus (ALV) is a retrovirus that does not carry any viral oncogenes and yet is able to transform B cells into lymphomas. This particular retrovirus has been shown to integrate within the c-*myc* proto-oncogene, which contains three exons. Exon 1 of c-*myc* has an unknown function; exons 2 and 3 encode the Myc protein. Insertion of AVL between exon 1 and exon 2 has been shown in some cases to allow the provirus promoter to increase transcription of exons 2 and 3, resulting in increased synthesis of c-Myc.

A variety of tumors have been shown to express significantly increased levels of growth factors or growth-factor

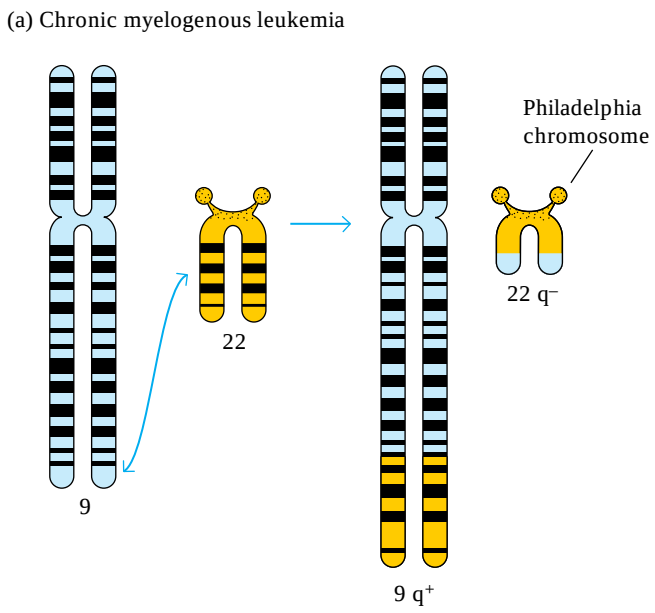


FIGURE **22-3** Chromosomal translocations in (a) chronic myelogenous leukemia (CML) and (b) Burkitt's lymphoma. Leukemic cells from all patients with CML contain the so-called Philadelphia chromosome, which results from a translocation between chromosomes 9 and 22. Cancer cells from some patients with Burkitt's lymphoma exhibit a translocation that moves part of chromosome 8 to chromosome 14. It is now known that this translocation involves c-*myc,* a cellular oncogene. Abnormalities such as these are detected by banding analysis of metaphase chromosomes. Normal chromosomes are shown on the left, and translocated chromosomes on the right.

receptors. Expression of the receptor for epidermal growth factor, which is encoded by c-*erbB*, has been shown to be amplified in many cancer cells. And in breast cancer, increased synthesis of the growth-factor receptor encoded by c-*neu* has been linked with a poor prognosis.

The Induction of Cancer Is a Multistep Process

The development from a normal cell to a cancerous cell is usually a multistep process of clonal evolution driven by a series of somatic mutations that progressively convert the cell from normal growth to a precancerous state and finally a cancerous state.

The presence of myriad chromosomal abnormalities in precancerous and cancerous cells lends support to the role of multiple mutations in the development of cancer. This has been demonstrated in human colon cancer, which progresses in a series of well-defined morphologic stages (Figure 22-4). Colon cancer begins as small, benign tumors called adenomas in the colorectal epithelium. These precancerous tumors grow, gradually becoming increasingly disorganized in their intracellular organization until they acquire the malignant phenotype. These well-defined morphologic stages of colon cancer have been correlated with a sequence of gene changes involving inactivation or loss of three tumor-suppressor genes (*APC, DCC,* and *p53*) and activation of one cellular proliferation oncogene (K-*ras*).

Studies with transgenic mice also support the role of multiple steps in the induction of cancer. Transgenic mice expressing high levels of Bcl-2 develop a population of small resting B cells, derived from secondary lymphoid follicles, that have greatly extended life spans. Gradually these transgenic mice develop lymphomas. Analysis of lymphomas from these mice has shown that approximately half have a c-*myc* translocation to the immunoglobulin H-chain locus. The synergism of Myc and Bcl-2 is highlighted in double-transgenic mice produced by mating the *bcl-2*$^+$ transgenic mice with *myc*$^+$ transgenic mice. These mice develop leukemia very rapidly.

Tumors of the Immune System

Tumors of the immune system are classified as lymphomas or leukemias. Lymphomas proliferate as solid tumors within a lymphoid tissue such as the bone marrow, lymph nodes, or thymus; they include Hodgkin's and non-Hodgkin's lymphomas. Leukemias tend to proliferate as single cells and are detected by increased cell numbers in the blood or lymph. Leukemia can develop in lymphoid or myeloid lineages.

Historically, the leukemias were classified as acute or chronic according to the clinical progression of the disease. The acute leukemias appeared suddenly and progressed rapidly, whereas the chronic leukemias were much less aggressive and developed slowly as mild, barely symptomatic diseases. These clinical distinctions apply to untreated leukemias; with current treatments, the acute leukemias often have a good prognosis, and permanent remission can often be achieved. Now the major distinction between acute and chronic leukemias is the maturity of the cell involved. Acute leukemias tend to arise in less mature cells, whereas chronic leukemias arise in mature cells. The acute leukemias include **acute lymphocytic leukemia (ALL)** and **acute myelogenous leukemia (AML);** these diseases can develop at any age and have a rapid onset. The chronic leukemias include **chronic lymphocytic leukemia (CLL)** and **chronic myelogenous leukemia (CML);** these diseases develop slowly and are seen in adults.

A number of B- and T-cell leukemias and lymphomas involve a proto-oncogene that has been translocated into the immunoglobulin genes or T-cell receptor genes. One of the best characterized is the translocation of c-*myc* in Burkitt's lymphoma and in mouse plasmacytomas. In 75% of Burkitt's lymphoma patients, c-*myc* is translocated from chromosome 8 to the Ig heavy-chain gene cluster on chromosome 14 (see

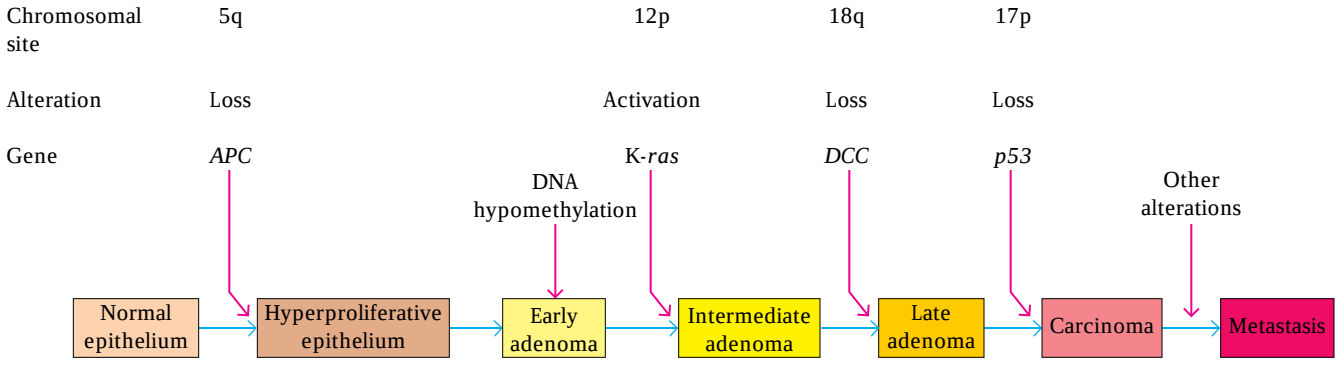


FIGURE **22-4** Model of sequential genetic alterations leading to metastatic colon cancer. Each of the stages indicated at the bottom is morphologically distinct, allowing researchers to determine the sequence of genetic alterations. *[Adapted from B. Vogelstein and K. W. Kinzler, 1993,* Trends Genet. *9:138.]*

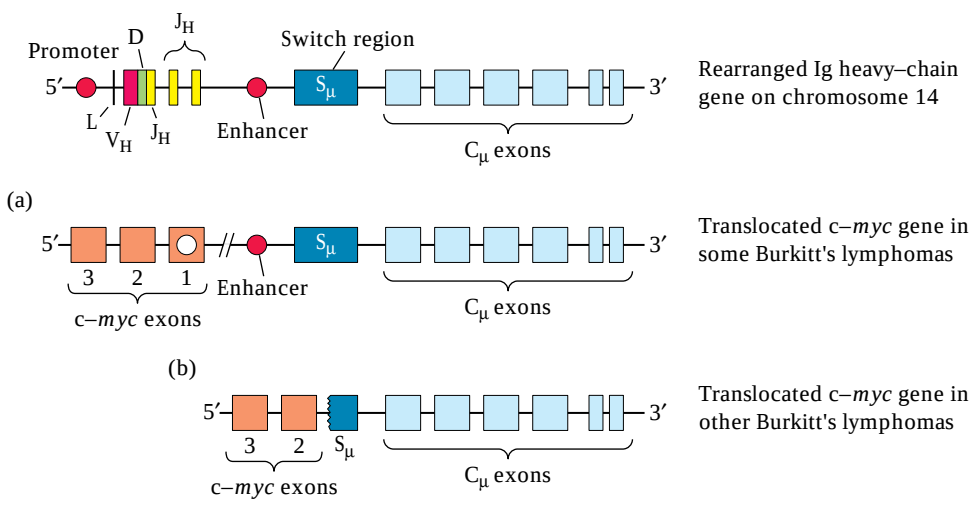


FIGURE **22-5** In many patients with Burkitt's lymphoma, the c-*myc* gene is translocated to the immunoglobulin heavy-chain gene cluster on chromosome 14. In some cases, the entire c-*myc* gene is inserted near the heavy-chain enhancer (a), but in other cases, only the coding exons (2 and 3) of c-*myc* are inserted at the S_μ switch site (b). Only exons 2 and 3 of c-*myc* are coding exons. Translocation may lead to overexpression of c-Myc.

Figure 22-3b). In the remaining patients, c-*myc* remains on chromosome 8 and the κ or γ light-chain genes are translocated to a region 3′ of c-*myc*. Kappa-gene translocations from chromosome 2 to chromosome 8 occur 9% of the time, and γ-gene translocations from chromosome 22 to chromosome 8 occur 16% of the time.

Translocations of c-*myc* to the Ig heavy-chain gene cluster on chromosome 14 have been analyzed, and, in some cases, the entire c-*myc* gene is translocated head-to-head to a region near the heavy-chain enhancer. In other cases, exons 1, 2, and 3 or exons 2 and 3 of c-*myc* are translocated head-to-head to the S_μ or S_α switch site (Figure 22-5). In each case, the translocation removes the *myc* coding exons from the regulatory mechanisms operating in chromosome 8 and places them in the immunoglobulin-gene region, a very active region that is expressed constitutively in these cells. The consequences of enhancer-mediated high levels of constitutive *myc* expression in lymphoid cells have been investigated in transgenic mice. In one study, mice containing a transgene consisting of all three c-*myc* exons and the immunoglobulin heavy-chain enhancer were produced. Of 15 transgenic pups born, 13 developed lymphomas of the B-cell lineage within a few months of birth.

Tumor Antigens

The subdiscipline of tumor immunology involves the study of antigens on tumor cells and the immune response to these antigens. Two types of tumor antigens have been identified on tumor cells: **tumor-specific transplantation antigens (TSTAs)** and **tumor-associated transplantation antigens (TATAs).** Tumor-specific antigens are unique to tumor cells and do not occur on normal cells in the body. They may result from mutations in tumor cells that generate altered cellular proteins; cytosolic processing of these proteins would give rise to novel peptides that are presented with class I MHC molecules, inducing a cell-mediated response by tumor-specific CTLs (Figure 22-6). Tumor-associated antigens, which are not unique to tumor cells, may be proteins that are expressed on normal cells during fetal development when the immune system is immature and unable to respond but that normally are not expressed in the adult. Reactivation of the embryonic genes that encode these proteins in tumor cells results in their expression on the fully differentiated tumor cells. Tumor-associated antigens may also be proteins that are normally expressed at extremely low levels on normal cells but are expressed at much higher levels on tumor cells. It is now clear that the tumor antigens recognized by human T cells fall into one of four major categories:

- Antigens encoded by genes exclusively expressed by tumors
- Antigens encoded by variant forms of normal genes that have been altered by mutation
- Antigens normally expressed only at certain stages of differentiation or only by certain differentiation lineages
- Antigens that are overexpressed in particular tumors

Many tumor antigens are cellular proteins that give rise to peptides presented with MHC molecules; typically, these antigens have been identified by their ability to induce the proliferation of antigen-specific CTLs or helper T cells.

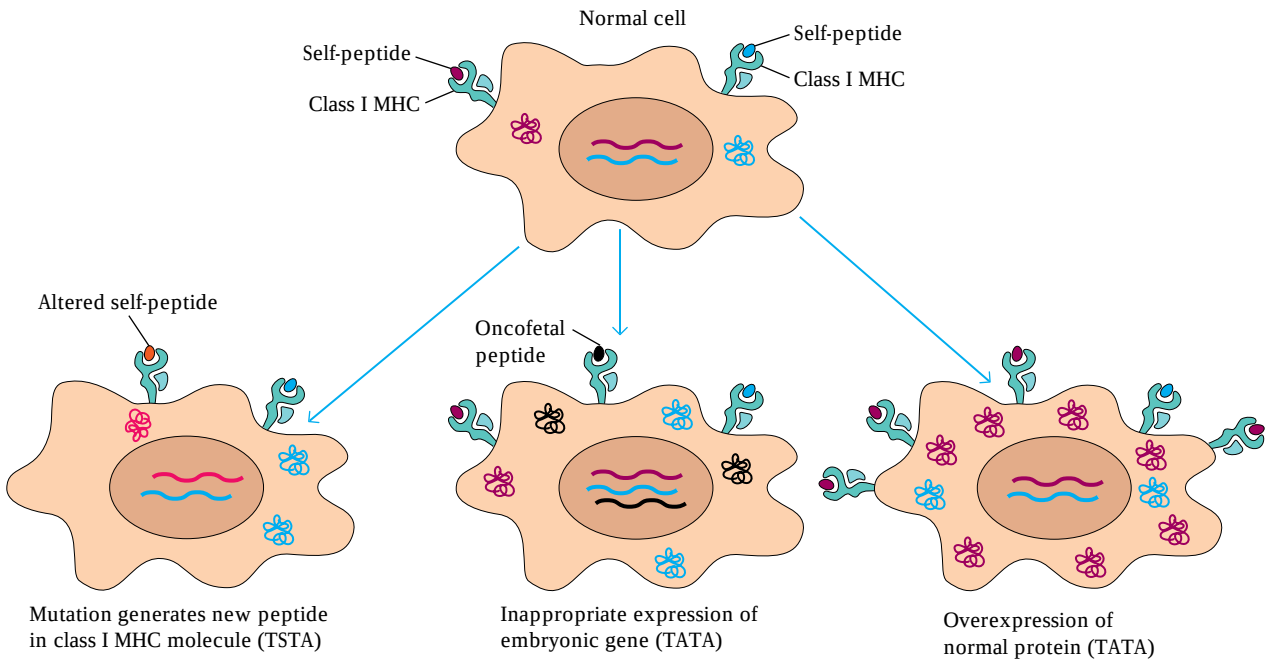


FIGURE **22-6** Different mechanisms generate tumor-specific transplantation antigens (TSTAs) and tumor-associated transplantation antigens (TATAs). The latter are more common.

Some Antigens Are Tumor-Specific

Tumor-specific antigens have been identified on tumors induced with chemical or physical carcinogens and on some virally induced tumors. Demonstrating the presence of tumor-specific antigens on spontaneously occurring tumors is particularly difficult because the immune response to such tumors eliminates all of the tumor cells bearing sufficient numbers of the antigens and in this way selects for cells bearing low levels of the antigens.

CHEMICALLY OR PHYSICALLY INDUCED TUMOR ANTIGENS

Methylcholanthrene and ultraviolet light are two carcinogens that have been used extensively to generate lines of tumor cells. When syngeneic animals are injected with killed cells from a carcinogen-induced tumor-cell line, the animals develop a specific immunologic response that can protect against later challenge by live cells of the same line but not other tumor-cell lines (Table 22-2). Even when the same chemical carcinogen induces two separate tumors at different sites in the same animal, the tumor antigens are distinct and the immune response to one tumor does not protect against the other tumor.

The tumor-specific transplantation antigens of chemically induced tumors have been difficult to characterize because they cannot be identified by induced antibodies but only by their T-cell–mediated rejection. One experimental approach that has allowed identification of genes encoding some TSTAs is outlined in Figure 22-7. When a mouse tumorigenic cell line (tum^+), which gives rise to progressively growing tumors, is treated in vitro with a chemical mutagen, some cells are mutated so that they no longer are capable of growing into a

TABLE **22-2** Immune response to methyl-cholanthrene (MCA) or polyoma virus (PV)*

Transplanted killed tumor cells	Live tumor cells for challenge	Tumor growth
CHEMICALLY INDUCED		
MCA-induced sarcoma A	MCA-induced sarcoma A	–
MCA-induced sarcoma A	MCA-induced sarcoma B	+
VIRALLY INDUCED		
PV-induced sarcoma A	PV-induced sarcoma A	–
PV-induced sarcoma A	PV-induced sarcoma B	–
PV-induced sarcoma A	SV40-induced sarcoma C	+

*Tumors were induced either with MCA or PV, and killed cells from the induced tumors were injected into syngeneic animals, which were then challenged with live cells from the indicated tumor-cell lines. The absence of tumor growth after live challenge indicates that the immune response induced by tumor antigens on the killed cells provided protection against the live cells.

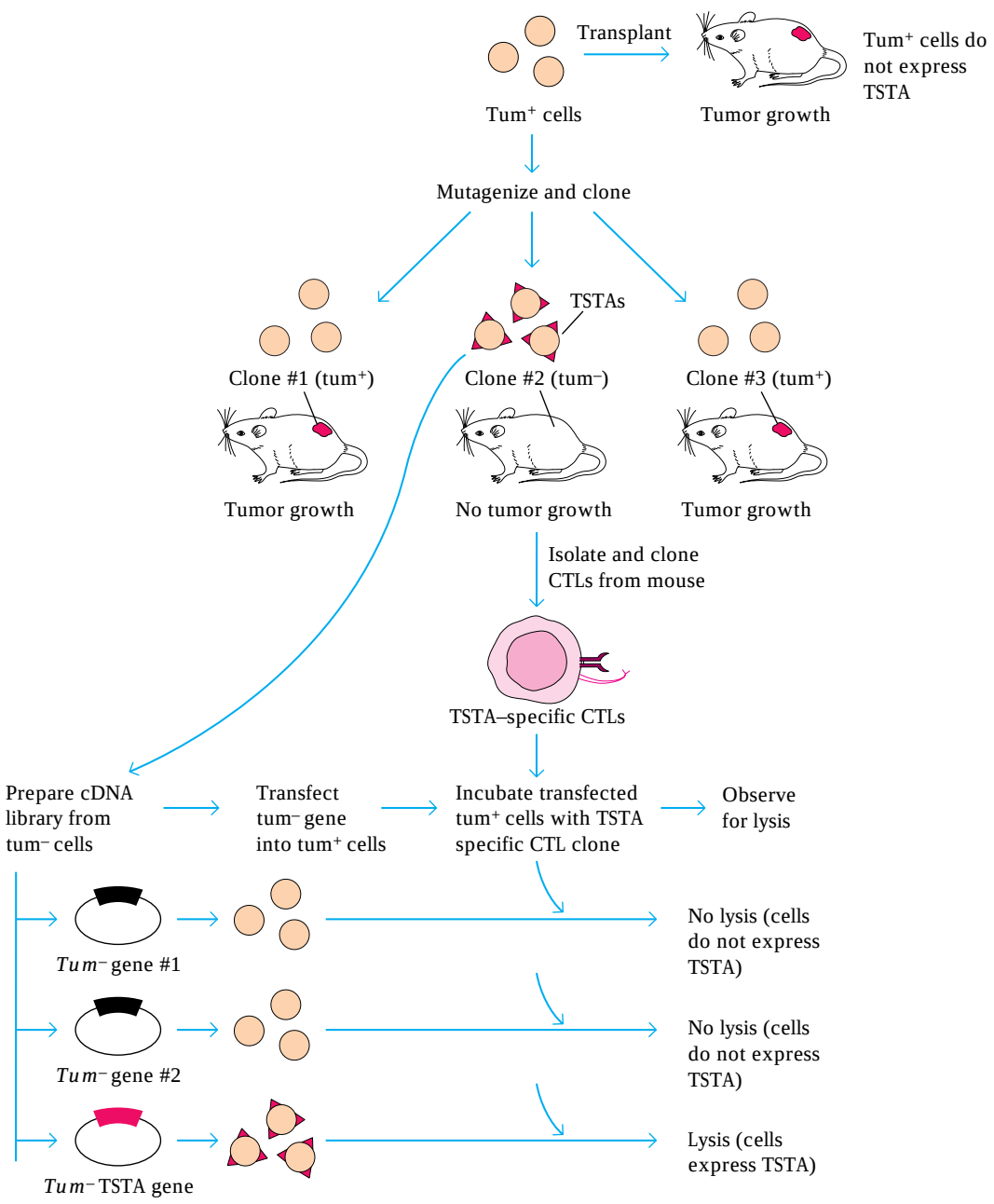


FIGURE 22-7 One procedure for identifying genes encoding tumor-specific transplantation antigens (TSTAs). Most TSTAs can be detected only by the cell-mediated rejection they elicit. In the first part of this procedure, a nontumorigenic (tum^-) cell line is generated; this cell line expresses a TSTA that is recognized by syngeneic mice, which mount a cell-mediated response against it. To isolate the gene encoding the TSTA, a cosmid gene library is prepared from the tum^- cell line, the genes are transfected into tumorigenic tum^+ cells, and the transfected cells are incubated with TSTA-specific CTLs.

tumor in syngeneic mice. These mutant tumor cells are designated as tum^- variants. Most tum^- variants have been shown to express TSTAs that are not expressed by the original tum^+ tumor-cell line. When tum^- cells are injected into syngeneic mice, the unique TSTAs that the tum^- cells express are recognized by specific CTLs. The TSTA-specific CTLs destroy the tum^- tumor cells, thus preventing tumor growth. To identify the genes encoding the TSTAs that are expressed on a tum^- cell line, a cosmid DNA library is prepared from the tum^- cells. Genes from the tum^- cells are transfected into the original tum^+ cells. The transfected tum^+ cells are tested for the expression of the tum^- TSTAs by their ability to activate cloned CTLs specific for the tum^- TSTA. A number of diverse TSTAs have been identified by this method.

In the past few years, two methods have facilitated the characterization of TSTAs (Figure 22-8). In one method, peptides bound to class I MHC molecules on the membranes of the tumor cells are eluted with acid and purified by high-pressure liquid chromatography (HPLC). In some cases, sufficient peptide is eluted to allow its sequence to be deduced by Edman degradation. In a second approach, cDNA libraries are prepared from tumor cells. These cDNA libraries are transfected transiently into COS cells, which are monkey kidney cells transfected with the gene that codes for the SV40 large-T antigen. When these cells are later transfected with plasmids containing both the tumor-cell cDNA and an SV40 origin of replication, the large-T antigen stimulates plasmid replication, so that up to 104–105 plasmid copies are produced per cell. This results in high-level expression of the tumor-cell DNA.

The genes that encode some TSTAs have been shown to differ from normal cellular genes by a single point mutation. Further characterization of TSTAs has demonstrated that many of them are not cell-membrane proteins; rather, as indicated already, they are short peptides derived from cytosolic proteins that have been processed and presented together with class I MHC molecules.

Tumor Antigens May Be Induced by Viruses

In contrast to chemically induced tumors, virally induced tumors express tumor antigens shared by all tumors induced by the same virus. For example, when syngeneic mice are injected with killed cells from a particular polyoma-induced tumor, the recipients are protected against subsequent challenge with live cells from any polyoma-induced tumors (see Table 22-2). Likewise, when lymphocytes are transferred from mice with a virus-induced tumor into normal syngeneic recipients, the recipients reject subsequent transplants of all syngeneic tumors induced by the same virus. In the case of both SV40- and polyoma-induced tumors, the presence of tumor antigens is related to the neoplastic state of the cell. In humans, Burkitt's-lymphoma cells have been shown to express a nuclear antigen of the Epstein-Barr virus that may indeed be a tumor-specific antigen for this type of tumor. Human papilloma virus (HPV) E6 and E7 proteins are found in more than 80% of invasive cervical cancers—the clearest example of a virally encoded tumor antigen. Consequently, there is great interest in testing as vaccine candidates the HPVs that are strongly linked to cervical cancer, such as HPV-16.

The potential value of these virally induced tumor antigens can be seen in animal models. In one experiment, mice immunized with a preparation of genetically engineered polyoma virus tumor antigen were shown to be immune to subsequent injections of live polyoma-induced tumor cells. In another experiment, mice were immunized with a vaccinia-virus vaccine engineered with the gene encoding the polyoma-tumor antigen. These mice also developed immunity, rejecting later injections of live polyoma-induced tumor cells (Figure 22-9).

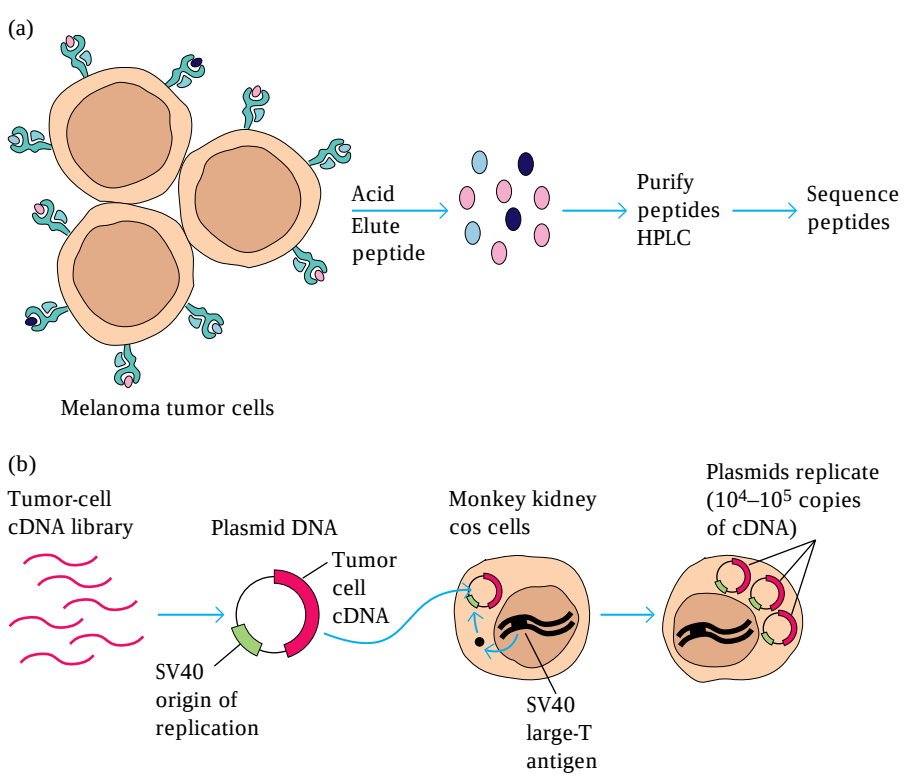


FIGURE 22-8 Two methods used to isolate tumor antigens that induce tumor-specific CTLs. See text for details.

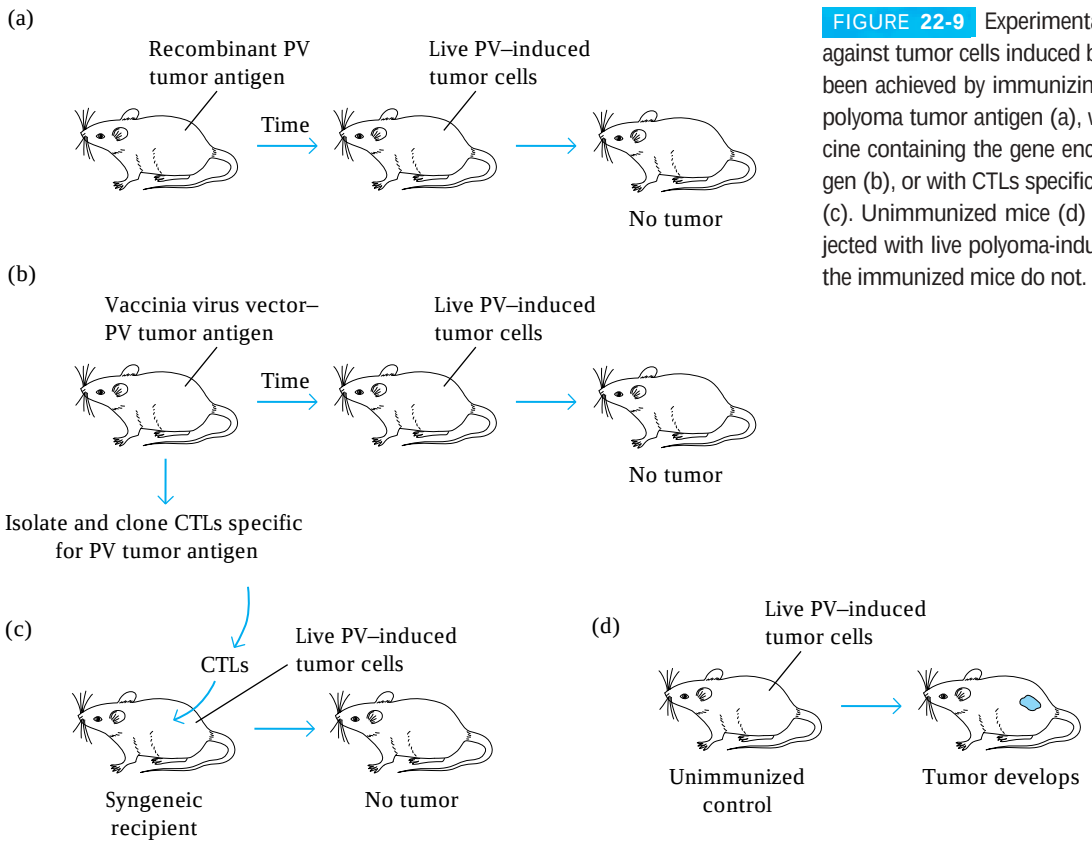


FIGURE 22-9 Experimental induction of immunity against tumor cells induced by polyoma virus (PV) has been achieved by immunizing mice with recombinant polyoma tumor antigen (a), with a vaccinia vector vaccine containing the gene encoding the PV tumor antigen (b), or with CTLs specific for the PV tumor antigen (c). Unimmunized mice (d) develop tumors when injected with live polyoma-induced tumor cells, whereas the immunized mice do not.

Most Tumor Antigens Are Not Unique to Tumor Cells

The majority of tumor antigens are not unique to tumor cells but also are present on normal cells. These tumor-associated transplantation antigens may be proteins usually expressed only on fetal cells but not on normal adult cells, or they may be proteins expressed at low levels by normal cells but at much higher levels by tumor cells. The latter category includes growth factors and growth-factor receptors, as well as oncogene-encoded proteins.

Several growth-factor receptors are expressed at significantly increased levels on tumor cells and can serve as tumor-associated antigens. For instance, a variety of tumor cells express the epidermal growth factor (EGF) receptor at levels 100 times greater than that in normal cells. An example of an over-expressed growth factor serving as a tumor-associated antigen is a transferrin growth factor, designated p97, which aids in the transport of iron into cells. Whereas normal cells express less than 8,000 molecules of p97 per cell, melanoma cells express 50,000–500,000 molecules of p97 per cell. The gene that encodes p97 has been cloned, and a recombinant vaccinia virus vaccine has been prepared that carries the cloned gene. When this vaccine was injected into mice, it induced both humoral and cell-mediated immune responses, which protected the mice against live melanoma cells expressing the p97 antigen. Results such as this highlight the importance of identifying tumor antigens as potential targets of tumor immunotherapy.

ONCOFETAL TUMOR ANTIGENS

Oncofetal tumor antigens, as the name implies, are found not only on cancerous cells but also on normal fetal cells. These antigens appear early in embryonic development, before the immune system acquires immunocompetence; if these antigens appear later on cancer cells, they are recognized as nonself and induce an immunologic response. Two well-studied oncofetal antigens are **alpha-fetoprotein (AFP)** and **carcinoembryonic antigen (CEA).**

Although the serum concentration of AFP drops from milligram levels in fetal serum to nanogram levels in normal adult serum, elevated AFP levels are found in a majority of patients with liver cancer (Table 22-3). CEA is a membrane glycoprotein found on gastrointestinal and liver cells of 2- to 6-month-old fetuses. Approximately 90% of patients with advanced colorectal cancer and 50% of patients with early colorectal cancer have increased levels of CEA in their serum; some patients with other types of cancer also exhibit increased CEA levels. However, because AFP and CEA can be found in trace amounts in some normal adults and in some noncancerous disease states, the presence of these oncofetal antigens is not diagnostic of

TABLE 22-3 Elevation of Alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) in serum of patients with various diseases

Disease	No. of patients tested	% of patients with high AFP or CEA levels*
AFP > 400 μ/ML		
Alcoholic cirrhosis	NA	0
Hepatitis	NA	1
Hepatocellular carcinoma	NA	69
Other carcinoma	NA	0
CEA > 10 ng/ml		
CEA > 10 MG/ML		
Cancerous		
Breast carcinoma	125	14
Colorectal carcinoma	544	35
Gastric carcinoma	79	19
Noncarcinoma carcinoma	228	2
Pancreatic carcinoma	55	35
Pulmonary carcinoma	181	26
Noncancerous		
Alcoholic cirrhosis	120	2
Cholecystitis	39	1
Nonmalignant disease	115	0
Pulmonary emphysema	49	4
Rectal polyps	90	1
Ulcerative colitis	146	5

*Altough trace amounts of both AFP and CEA can be found in some healthy adults, none would have levels greater than those indicated in the table.

tumors but rather serves to monitor tumor growth. If, for example, a patient has had surgery to remove a colorectal carcinoma, CEA levels are monitored after surgery. An increase in the CEA level is an indication of resumed tumor growth.

ONCOGENE PROTEINS AS TUMOR ANTIGENS

A number of tumors have been shown to express tumor-associated antigens encoded by cellular oncogenes. These antigens are also present in normal cells encoded by the corresponding proto-oncogene. In many cases, there is no qualitative difference between the oncogene and proto-oncogene products; instead, the increased levels of the oncogene product can be recognized by the immune system. For example, as noted earlier, human breast-cancer cells exhibit elevated expression of the oncogene-encoded Neu protein, a growth-factor receptor, whereas normal adult cells express only trace amounts of Neu protein. Because of this difference in the Neu level, anti-Neu monoclonal antibodies can recognize and selectively eliminate breast-cancer cells without damaging normal cells.

TATAs ON HUMAN MELANOMAS

Several tumor-associated transplantation antigens have been identified on human melanomas. Five of these—MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2—are oncofetal-type antigens. Each of these antigens is expressed on a significant proportion of human melanoma tumors, as well as on a number of other human tumors, but not on normal differentiated tissues except for the testis, where it is expressed on germ-line cells. In addition, a number of differentiation antigens expressed on normal melanocytes—including tyrosinase, gp100, Melan-A or MART-1, and gp75—are overexpressed by melanoma cells, enabling them to function as tumor-associated transplantation antigens.

Several of the human melanoma tumor antigens are shared by a number of other tumors. About 40% of human melanomas are positive for MAGE-1, and about 75% are positive for MAGE-2 or 3. In addition to melanomas, a significant percentage of glioma cell lines, breast tumors, non-small-cell lung tumors, and head or neck carcinomas express MAGE-1, 2, or 3. These shared tumor antigens could be exploited for clinical treatment. It might be possible to produce a tumor vaccine expressing the shared antigen for treatment of a number of these tumors, as described at the end of this chapter.

Tumors Can Induce Potent Immune Responses

In experimental animals, tumor antigens can be shown to induce both humoral and cell-mediated immune responses that result in the destruction of the tumor cells. In general, the cell-mediated response appears to play the major role. A number of tumors have been shown to induce tumor-specific CTLs that recognize tumor antigens presented by class I MHC on the tumor cells. However, as discussed below, expression of class I MHC molecules is decreased in a number of tumors, thereby limiting the role of specific CTLs in their destruction.

NK Cells and Macrophages Are Important in Tumor Recognition

The recognition of tumor cells by NK cells is not MHC restricted. Thus, the activity of these cells is not compromised by the decreased MHC expression exhibited by some tumor cells. In some cases, Fc receptors on NK cells can bind to antibody-coated tumor cells, leading to ADCC. The importance of NK cells in tumor immunity is suggested by the mutant mouse strain called beige and by **Chediak-Higashi syndrome** in humans, as described in the Clinical Focus in Chapter 14. In each case, a genetic defect causes marked impairment of NK cells and an associated increase in certain types of cancer.

CLINICAL FOCUS

Cancer Vaccines Promise Hope for the Future

THE realization that the vertebrate immune system evolved to distinguish self from non-self led to the notion that our immune system could recognize a tumor as foreign. In fact, a major research effort amongst cancer immunologists during the latter half of the 20th century was the identification and characterization of tumor specific molecules, the so-called tumor antigens. This area of research was met with skepticism. First of all, the existence of tumor-specific antigens was questionable; many antigens were identified as tumor specific only to find that other cells also expressed these antigens. Secondly, early investigations in the field of tumor immunology necessarily employed animal models that may or may not be relevant to human cancers. However, with advances in biotechnology, genomics, and proteomics, coupled with our increased understanding of the cellular interactions in the immune system, tumor immunology now offers us the promise of new drugs that will aid in the treatment of cancer. We now understand that tumor-associated antigens do exist and that focusing the cellular arm of the immune system toward the recognition these proteins is a rational approach to the development of a cancer vaccine.

One of the best-studied tumor-immunity models is melanoma. Melanoma has evolved as a model system for several reasons. First of all and paradoxically, most human cancers are difficult to establish in tissue culture, making it difficult to develop *in vitro* systems for experimental manipulation. Melanoma is relatively easy to adapt to tissue culture, which has led to the identification of several tumor-associated antigens, some of which are unique to melanoma (see Table 22-5). These observations are enhanced by the ability to create cDNA libraries (see Chapter 23) from tumor cells. The cDNAs can be transfected into target cells expressing the appropriate MHC molecules and then used as targets for CTL-mediated killing. Once CTL reactivity is recognized, the transfected cDNA can be isolated and identified as a potential tumor antigen. The ability to isolate genes encoding tumor-associated antigens provides us with the opportunity to use these proteins as immunogens for the induction of tumor-specific responses. Additionally, the identification of tumor-associated proteins allows us to identify peptides that elicit anti-tumor responses.

Over the past few years, several biotech companies have devised strategies for the development of vaccines against melanoma as well as other cancers. These strategies have one thing in the common; the induction of a cell-mediated response to tumor-associated antigens. Antigens are derived from individual patient tumors or established tumor cell lines. The use of patient-derived tumors is appealing for obvious reasons. The response to that tumor should, in theory, be uniquely directed only at tumor antigens and not other, potentially allotypic, determinants. However, such individualized therapy could be very expensive and time-consuming. In this scenario, the tumor would be biopsied or surgically removed, placed into culture, and then used as an immunogen. Establishing a primary tumor in culture is not easy, even for melanoma, and the procedure can take several weeks. The time factor, coupled with the realization that many tumors are not easy to grow *in vitro,* places this into the category of "designer therapies" that may or may not be feasible under the reality of managed health care today.

The use of established tumors as the source of the immunogen is much more accessible in cost and practicality. Samples from several tumors can be grown in culture and protein extracts prepared and frozen, providing a source of immunogen for many patients. In addition to reduced costs, this strategy also allows careful assessment of the immunogenicity of the tumor antigens found in the cultured cells. It is possible that some tumors may express higher levels of tumor-associated antigens and be more immunogenic than others. Indeed one biotech company in California, CancerVax (www.CancerVax.com), has derived three cell lines that express high levels of over 20 tumor-associated antigens. Additionally, these cells express MHC class I alleles which are represented in the majority of individuals in the population, meaning that intracellular antigens will be presented properly. Cells are irradiated to render them incapable of cell division and used as irradiated whole cells for immunization. The advantages of this approach lies in the ability to standardize the immunogen as well as reducing the cost.

Antigen presentation is a critical feature of any immunization strategy and one way to enhance to immunization against tumor antigens is to manipulate the fashion in which the antigen is presented. Professional antigen-presenting cells such as dendritic cells are excellent candidates to employ in vaccination protocols. Several companies have developed novel uses for dendritic cells in cancer therapy. Dendreon (www.Dendreon.com), a Seattle-based company, first isolates dendritic-cell precursors from patient blood, then introduces the immunogen into the dendritic cells and returns the antigen-pulsed dendritic cells to the bloodstream of the cancer patient. This company, through genomics-based drug discovery, has identified tumor-associated antigens prevalent on a wide variety of cancers. Thus the

(continued)

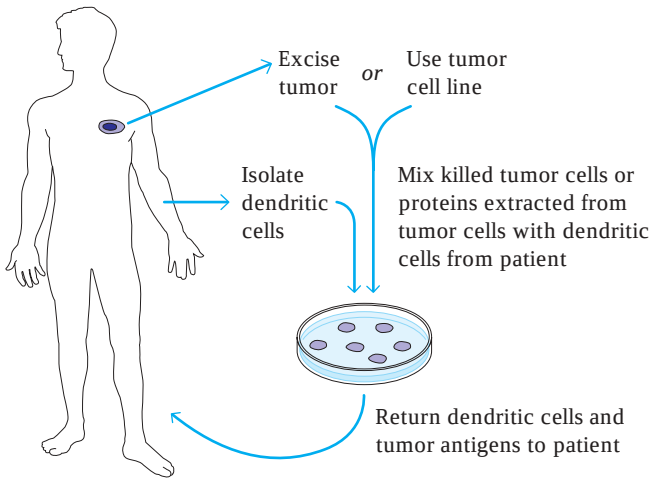
CLINICAL FOCUS

(continued)

dendritic-cell therapy can be tailored to a variety of different tumors. A variation on this theme currently is being tested by Genzyme Molecular Oncology (www.genzymemolecularoncology.com). Their approach also uses dendritic cells, but rather than employing already-defined antigens, clinical trials are underway where dendritic cells from the patient are fused, using polyethylene glycol, with inactivated tumor cells taken from the same patient. The advantage of this technique is that the hybrid cell has the antigen-presenting capability of a dendritic cell but also contains the antigens from the patient's tumor cells. The dendritic cell processes these tumor antigens and efficiently presents the processed antigen to the immune system of the patient.

A different but equally promising approach to the design of cancer vaccines comes from observations made many years ago that tumor cells are immunogenic—animals injected with killed tumor cells do not grow tumors when challenged with live tissue. When the basis of this protective immunogenicity was explored, it was found that heat-shock proteins (HSPs) are critical in providing protection. Furthermore HSPs were found to carry immunogenic peptides, thus acting as molecular chaperones. But how do HSP/peptide complexes in tumor tissue prime the host immune system? Recent data demonstrate that HSPs bind CD91, a receptor found primarily on APCs such as dendritic cells as well as on macrophages. In this scenario, HSP/peptide complexes from tumor cells bind the CD91 on APCs, are internalized, and unexpectedly, the antigen is processed and is thought to re-emerge as peptide/MHC class I complexes on the APC, resulting in the priming of a $CD8^+$ T-cell response. This would not be the predicted response, since the exogenous antigens are almost uniformly presented by class II MHC molecules. However, an impressive amount of experimental data demonstrates that HSPs isolated and purified from tumor tissue are a potent inducer of tumor-specific CTLs. These observations have led to phase III clinical trials conducted by Antigenics (www.antigenics.com) of HSP/antigen complexes as immunogens for kidney cancer as well as melanoma. The mechanism by which HSP/antigen complexes bound to CD91 are delivered to the class I presentation machinery is not well understood, but it is clear that HSP/antigen complexes, when presented to APCs, result in the vigorous activation of $CD8^+$ T cells.

The promise for cancer vaccines appears very bright. Genomics and proteomic methodologies provide novel tools for identifying tumor antigens. Additionally, there is a variety of approaches available to engage the immune system to respond to tumor antigens. The past decade has seen a rapid increase in the number of biotech companies directed at identifying cancer vaccines, and the number of companies in phase II or phase III clinical trials invites an air of optimism about this area of clinical research.



Cancer vaccine design. Tumor cells are removed from the patient and placed in culture. Alternatively, established tumor-cell lines are chosen and placed into culture. Tumor cells are inactivated and mixed with dendritic cells from the patient and injected back into the patient as immunogens. An alternate approach is to prepare extracts or antigens from the tumor cells and inject these, in addition to dendritic cells, into the patient.

Numerous observations indicate that activated macrophages also play a significant role in the immune response to tumors. For example, macrophages are often observed to cluster around tumors, and their presence is often correlated with tumor regression. Like NK cells, macrophages are not MHC restricted and express Fc receptors, enabling them to bind to antibody on tumor cells and mediate ADCC. The antitumor activity of activated macrophages is probably mediated by lytic enzymes and reactive oxygen and nitrogen intermediates. In addition, activated macrophages secrete a

cytokine called tumor necrosis factor (TNF-α) that has potent antitumor activity. When TNF-α is injected into tumor-bearing animals, it has been found to induce hemorrhage and necrosis of the tumor.

IMMUNE SURVEILLANCE THEORY

The immune surveillance theory was first conceptualized in the early 1900s by Paul Ehrlich. He suggested that cancer cells frequently arise in the body but are recognized as foreign and eliminated by the immune system. Some 50 years later, Lewis Thomas suggested that the cell-mediated branch of the immune system had evolved to patrol the body and eliminate cancer cells. According to these concepts, tumors arise only if cancer cells are able to escape immune surveillance, either by reducing their expression of tumor antigens or by an impairment in the immune response to these cells.

Among the early observations that seemed to support the immune surveillance theory was the increased incidence of cancer in transplantation patients on immunosuppressive drugs. Other findings, however, were difficult to reconcile with this theory. Nude mice, for example, lack a thymus and consequently lack functional T cells. According to the immune surveillance theory, these mice should show an increase in cancer, instead, nude mice are no more susceptible to cancer than other mice. Furthermore, although individuals on immunosuppressive drugs do show an increased incidence of cancers of the immune system, other common cancers (e.g., lung, breast, and colon cancer) are not increased in these individuals, contrary to what the theory predicts. One possible explanation for the selective increase in immune-system cancers is that the immunosuppressive agents themselves may exert a direct carcinogenic effect on immune cells.

Experimental data concerning the effect of tumor-cell dosage on the ability of the immune system to respond also are incompatible with the immune surveillance theory. For example, animals injected with very low or very high doses of tumor cells develop tumors, whereas those injected with intermediate doses do not. The mechanism by which a low dose of tumor cells "sneaks through" is difficult to reconcile with the immune surveillance theory. Finally, this theory assumes that cancer cells and normal cells exhibit qualitative antigen differences. In fact, as stated earlier, many types of tumors do not express tumor-specific antigens, and any immune response that develops must be induced by quantitative differences in antigen expression by normal cells and tumor cells. However, tumors induced by viruses would be expected to express some antigens encoded by the viral genome. These antigens are qualitatively different from those expressed by normal tissues and would be expected to attract the attention of the immune system. In fact, there are many examples of specific immune responses to virally induced tumors.

Nevertheless, apart from tumors caused by viruses, the basic concept of the immune surveillance theory—that malignant tumors arise only if the immune system is somehow impaired or if the tumor cells lose their immunogenicity, enabling them to escape immune surveillance—at this time remains unproved. In spite of this, it is clear that an immune response can be generated to tumor cells, and therapeutic approaches aimed at increasing that response may serve as a defense against malignant cells.

Tumor Evasion of the Immune System

Although the immune system clearly can respond to tumor cells, the fact that so many individuals die each year from cancer suggests that the immune response to tumor cells is often ineffective. This section describes several mechanisms by which tumor cells appear to evade the immune system.

Anti-Tumor Antibodies Can Enhance Tumor Growth

Following the discovery that antibodies could be produced to tumor-specific antigens, attempts were made to protect animals against tumor growth by active immunization with tumor antigens or by passive immunization with antitumor antibodies. Much to the surprise of the researchers, these immunizations did not protect against tumor growth; in many cases, they actually enhanced growth of the tumor.

The tumor-enhancing ability of immune sera subsequently was studied in cell-mediated lympholysis (CML) reactions in vitro. Serum taken from animals with progressive tumor growth was found to block the CML reaction, whereas serum taken from animals with regressing tumors had little or no blocking activity. K. E. and I. Hellstrom extended these findings by showing that children with progressive neuroblastoma had high levels of some kind of blocking factor in their sera and that children with regressive neuroblastoma did not have such factors. Since these first reports, blocking factors have been found to be associated with a number of human tumors.

In some cases, antitumor antibody itself acts as a blocking factor. Presumably the antibody binds to tumor-specific antigens and masks the antigens from cytotoxic T cells. In many cases, the blocking factors are not antibodies alone but rather antibodies complexed with tumor antigens. Although these immune complexes have been shown to block the CTL response, the mechanism of this inhibition is not known. The complexes also may inhibit ADCC by binding to Fc receptors on NK cells or macrophages and blocking their activity.

Antibodies Can Modulate Tumor Antigens

Certain tumor-specific antigens have been observed to disappear from the surface of tumor cells in the presence of serum antibody and then to reappear after the antibody is no longer present. This phenomenon, called antigenic modulation, is readily observed when leukemic T cells are injected into mice previously immunized with a leukemic T-cell antigen (TL antigen). These mice develop high titers of anti-TL

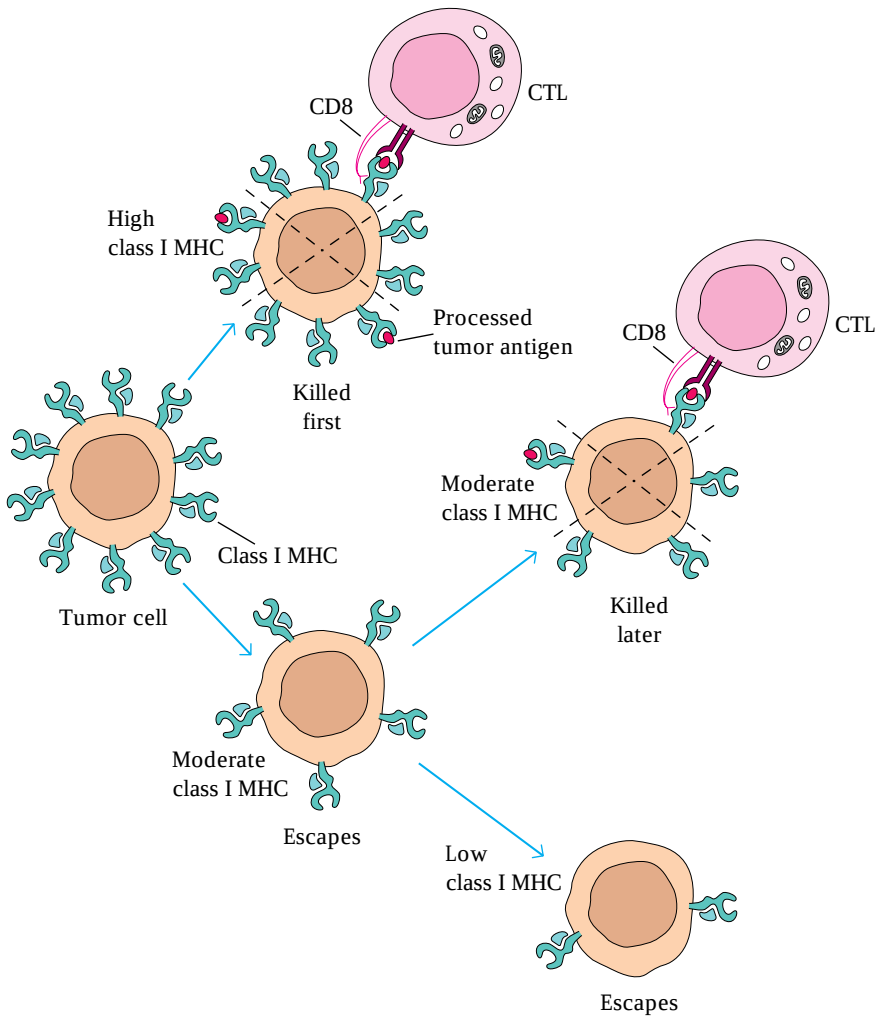


FIGURE **22-10** Down-regulation of class I MHC expression on tumor cells may allow a tumor to escape CTL-mediated recognition. The immune response may play a role in selecting for tumor cells expressing lower levels of class I MHC molecules by preferentially eliminating those cells expressing high levels of class I molecules. With time, malignant tumor cells may express progressively fewer MHC molecules and thus escape CTL-mediated destruction.

antibody, which binds to the TL antigen on the leukemic cells and induces capping, endocytosis, and/or shedding of the antigen-antibody complex. As long as antibody is present, these leukemic T cells fail to display the TL antigen and thus cannot be eliminated.

Tumor Cells Frequently Express Low Levels of Class I MHC Molecules

Since $CD8^+$ CTLs recognize only antigen associated with class I MHC molecules, any alteration in the expression of class I MHC molecules on tumor cells may exert a profound effect on the CTL-mediated immune response. Malignant transformation of cells is often associated with a reduction (or even a complete loss) of class I MHC molecules, and a number of tumors have been shown to express decreased levels of class I MHC molecules. The decrease in class I MHC expression can be accompanied by progressive tumor growth, and so the absence of MHC molecules on a tumor is generally an indication of a poor prognosis. As illustrated in Figure 22-10, the immune response itself may play a role in selecting tumor cells with decreased class I MHC expression.

Tumor Cells May Provide Poor Co-Stimulatory Signals

T-cell activation requires an activating signal, triggered by recognition of a peptide–MHC molecule complex by the T-cell receptor, and a co-stimulatory signal, triggered by the interaction of B7 on antigen-presenting cells with CD28 on the T cells. Both signals are needed to induce IL-2 production and proliferation of T cells. The poor immunogenicity of

many tumor cells may be due in large part to lack of the co-stimulatory molecules. Without sufficient numbers of antigen-presenting cells in the immediate vicinity of a tumor, the T cells will receive only a partial activating signal, which may lead to clonal anergy.

Cancer Immunotherapy

Although various immune responses can be generated to tumor cells, the response frequently is not sufficient to prevent tumor growth. One approach to cancer treatment is to augment or supplement these natural defense mechanisms. Several types of cancer immunotherapy in current use or under development are described in this concluding section.

Manipulation of Co-Stimulatory Signals Can Enhance Immunity

Several research groups have demonstrated that tumor immunity can be enhanced by providing the co-stimulatory signal necessary for activation of CTL precursors (CTL-Ps). When mouse CTL-Ps are incubated with melanoma cells in vitro, antigen recognition occurs, but in the absence of a co-stimulatory signal, the CTL-Ps do not proliferate and differentiate into effector CTLs. However, when the melanoma cells are transfected with the gene that encodes the B7 ligand, then the CTL-Ps differentiate into effector CTLs.

These findings offer the possibility that B7-transfected tumor cells might be used to induce a CTL response in vivo. For instance, when P. Linsley, L. Chen, and their colleagues injected melanoma-bearing mice with $B7^+$ melanoma cells, the melanomas completely regressed in more than 40% of the mice. S. Townsend and J. Allison used a similar approach to vaccinate mice against malignant melanoma. Normal mice were first immunized with irradiated, B7-transfected melanoma cells and then challenged with unaltered malignant melanoma cells. The "vaccine" was found to protect a high percentage of the mice (Figure 22-11a). It is hoped that a similar vaccine might prevent metastasis after surgical removal of a primary melanoma in human patients.

Because human melanoma antigens are shared by a number of different human tumors, it might be possible to generate a panel of B7-transfected melanoma cell lines that are typed for tumor-antigen expression and for HLA expression. In this approach, the tumor antigen(s) expressed by a patient's tumor would be determined, and then the patient would be vaccinated with an irradiated B7-transfected cell line that expresses similar tumor antigen(s).

Enhancement of APC Activity Can Modulate Tumor Immunity

Mouse dendritic cells cultured in GM-CSF and incubated with tumor fragments, then reinfused into the mice, have been shown to activate both TH cells and CTLs specific for the tumor antigens. When the mice were subsequently challenged with live tumor cells, they displayed tumor immunity. These experiments have led to a number of approaches aimed at expanding the population of antigen-presenting cells, so that these cells can activate TH cells or CTLs specific for tumor antigens.

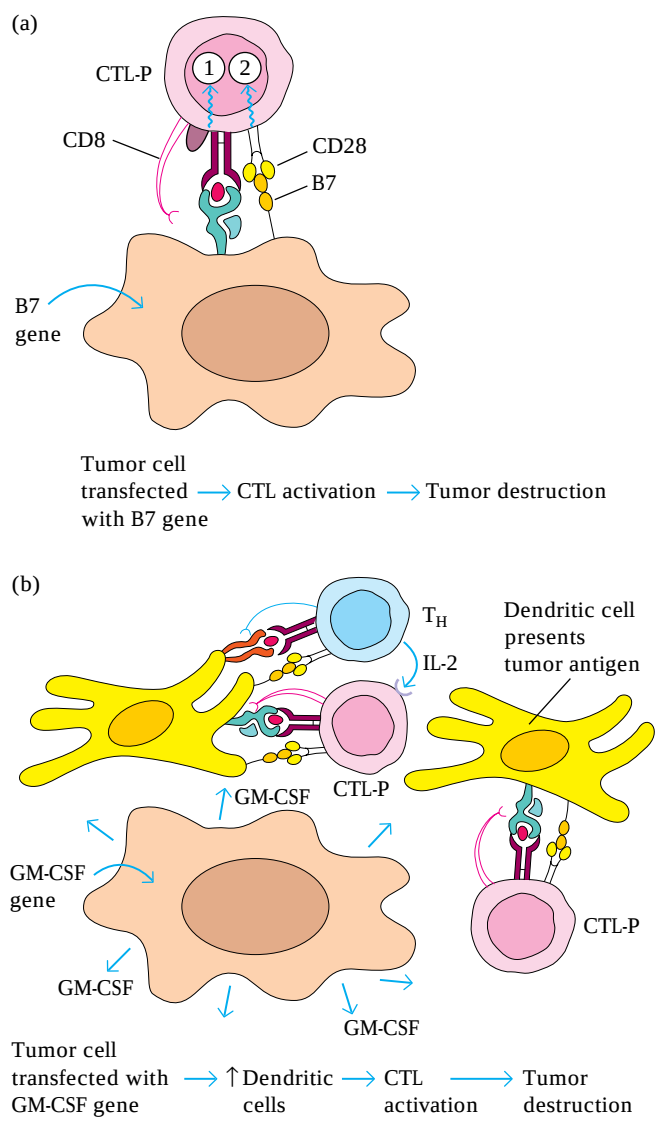


FIGURE 22-11 Use of transfected tumor cells for cancer immunotherapy. (a) Tumor cells transfected with the B7 gene express the co-stimulatory B7 molecule, enabling them to provide both activating signal (1) and co-stimulatory signal (2) to CTL-Ps. As a result of the combined signals, the CTL-Ps differentiate into effector CTLs, which can mediate tumor destruction. In effect, the transfected tumor cell acts as an antigen-presenting cell. (b) Transfection of tumor cells with the gene encoding GM-CSF allows the tumor cells to secrete high levels of GM-CSF. This cytokine will activate dendritic cells in the vicinity of the tumor, enabling the dendritic cells to present tumor antigens to both TH cells and CTL-Ps.

One approach that has been tried is to transfect tumor cells with the gene encoding GM-CSF. These engineered tumor cells, when reinfused into the patient, will secrete GM-CSF, enhancing the differentiation and activation of host antigen-presenting cells, especially dendritic cells. As these dendritic cells accumulate around the tumor cells, the GM-CSF secreted by the tumor cells will enhance the presentation of tumor antigens to TH cells and CTLs by the dendritic cells (Figure 22-11b).

Another way to expand the dendritic-cell population is to culture dendritic cells from peripheral-blood progenitor cells in the presence of GM-CSF, TNF-α, and IL-4. These three cytokines induce the generation of large numbers of dendritic cells. There is some hope that, if these dendritic cells are pulsed with tumor fragments and then reintroduced into the patient, they can activate TH and TC cells specific for the tumor antigens. Whether these hopes are justified will be determined by further investigation.

A number of adjuvants, including the attenuated strains of *Mycobacterium bovis* called bacillus Calmette-Guerin (BCG) and *Corynebacterium parvuum*, have been used to boost tumor immunity. These adjuvants activate macrophages, increasing their expression of various cytokines, class II MHC molecules, and the B7 co-stimulatory molecule. These activated macrophages are better activators of TH cells, resulting in generalized increases in both humoral and cell-mediated responses. Thus far, adjuvants have shown only modest therapeutic results.

Cytokine Therapy Can Augment Immune Responses to Tumors

The isolation and cloning of the various cytokine genes has facilitated their large-scale production. A variety of experimental and clinical approaches have been developed to use recombinant cytokines, either singly or in combination, to augment the immune response against cancer. Among the cytokines that have been evaluated in cancer immunotherapy are IFN-α, β, and γ; IL-1, IL-2, IL-4, IL-5, and IL-12; GM-CSF; and TNF. Although these trials have produced occasional encouraging results, many obstacles remain to the successful use of this type of cancer immunotherapy.

The most notable obstacle is the complexity of the cytokine network itself. This complexity makes it very difficult to know precisely how intervention with a given recombinant cytokine will affect the production of other cytokines. And since some cytokines act antagonistically, it is possible that intervention with a recombinant cytokine designed to enhance a particular branch of the immune response may actually lead to suppression. In addition, cytokine immunotherapy is plagued by the difficulty of administering the cytokines locally. In some cases, systemic administration of high levels of a given cytokine has been shown to lead to serious and even life-threatening consequences. Although the results of several experimental and clinical trials of cytokine therapy for cancer are discussed here, it is important to keep in mind that this therapeutic approach is still in its infancy.

INTERFERONS

Large quantities of purified recombinant preparations of the interferons, IFN-α, IFN-β, and IFN-γ, are now available, each of which has shown some promise in the treatment of human cancer. To date, most of the clinical trials have involved IFN-α. Daily injections of recombinant IFN-α have been shown to induce partial or complete tumor regression in some patients with hematologic malignancies such as leukemias, lymphomas, and myelomas and with solid tumors such as melanoma, Kaposi's sarcoma, renal cancer, and breast cancer.

Interferon-mediated antitumor activity may involve several mechanisms. All three types of interferon have been shown to increase class I MHC expression on tumor cells; IFN-γ has also been shown to increase class II MHC expression on macrophages. Given the evidence for decreased levels of class I MHC molecules on malignant tumors, the interferons may act by restoring MHC expression, thereby increasing CTL activity against tumors. In addition, the interferons have been shown to inhibit cell division of both normal and malignantly transformed cells in vitro. It is possible that some of the antitumor effects of the interferons are related to this ability to directly inhibit tumor-cell proliferation. Finally, IFN-γ directly or indirectly increases the activity of TC cells, macrophages, and NK cells, all of which play a role in the immune response to tumor cells.

TUMOR NECROSIS FACTORS

In some instances, the tumor necrosis factors TNF-α and TNF-β have been shown to exhibit direct antitumor activity, killing some tumor cells and reducing the rate of proliferation of others while sparing normal cells (Figure 22-12). In the presence of TNF-α or TNF-β, a tumor undergoes visible hemorrhagic necrosis and regression. TNF-α has also been shown to inhibit tumor-induced vascularization (angiogenesis) by damaging the vascular endothelial cells in the vicinity of a tumor, thereby decreasing the flow of blood and oxygen that is necessary for progressive tumor growth.

IN VITRO–ACTIVATED LAK AND TIL CELLS

Animal studies have shown that lymphocytes can be activated against tumor antigens in vitro by culturing them with x-irradiated tumor cells in the presence of IL-2 and added tumor antigens. These activated lymphocytes mediate more effective tumor destruction than untreated lymphocytes when they are reinjected into the original tumor-bearing animal. It is difficult, however, to activate in vitro enough lymphocytes with antitumor specificity to be useful in cancer therapy.

While sensitizing lymphocytes to tumor antigens by this method, S. Rosenberg discovered that, in the presence of high

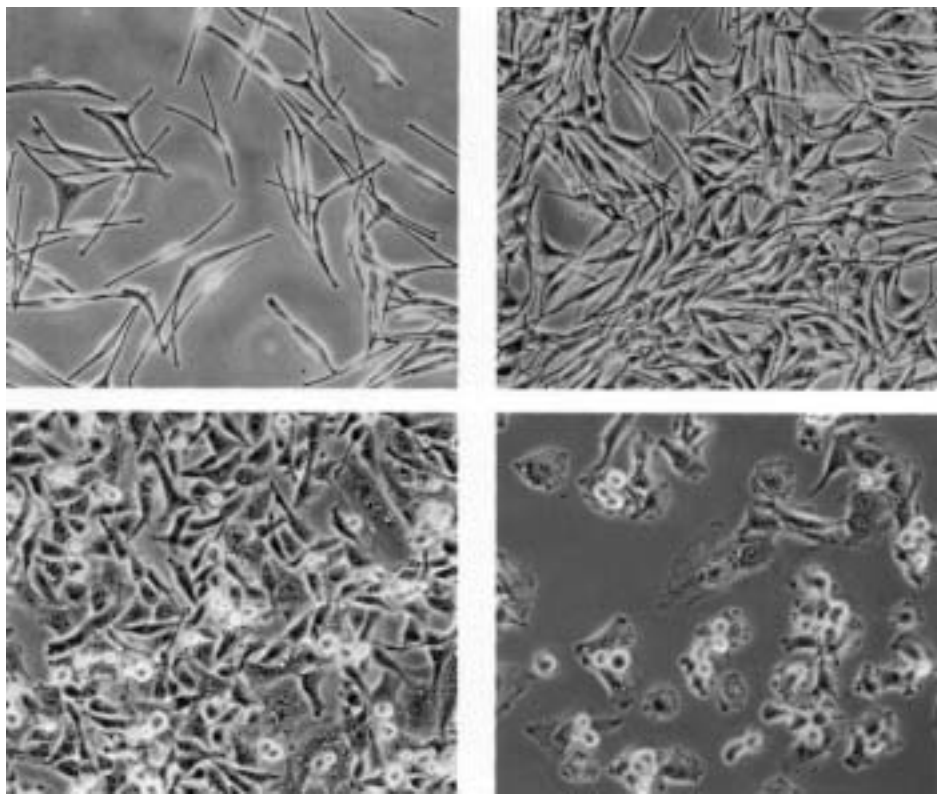


FIGURE **22-12** Photomicrographs of cultured normal melanocytes (*top*) and cultured cancerous melanoma cells (*bottom*) in the presence (*left*) and absence (*right*) of tumor necrosis factor (TNF-α). Note that, in the presence of TNF-α, the cancer cells stop proliferating, whereas TNF-α has no inhibitory effect on proliferation of the normal cells. *[From L. J. Old, 1988,* Sci. Am. ***258****(5):59.]*

concentrations of cloned IL-2 but without the addition of tumor antigens, large numbers of activated lymphoid cells were generated that could kill fresh tumor cells but not normal cells. He called these cells **lymphokine-activated killer (LAK) cells.** In one study, for example, Rosenberg found that infusion of LAK cells plus recombinant IL-2 into tumor-bearing animals mediated effective tumor-cell destruction (Figure 22-13). LAK-cell populations are typically >90% activated NK cells. However, small numbers of TCR-bearing cells are present in LAK populations and it is possible that these may also contribute to their tumoricidal activity.

Because large numbers of LAK cells can be generated in vitro and because these cells are active against a wide variety of tumors, their effectiveness in human tumor immunotherapy has been evaluated in several clinical trials. In these trials, peripheral-blood lymphocytes were removed from patients with various advanced metastatic cancers and were activated in vitro to generate LAK cells. In an early study, patients were then infused with their autologous LAK cells together with IL-2. In this trial, which involved 25 patients, cancer regression was seen in some patients. Subsequently, a more extensive trial with 222 patients resulted in complete regression in 16 patients. However, a number of undesirable side effects are associated with the high levels of IL-2 required for LAK-cell activity. The most noteworthy is vascular leak syndrome, in which lymphoid cells and plasma emigrate from the peripheral blood into the tissues, leading to shock.

Tumors contain lymphocytes that have infiltrated the tumor and presumably are taking part in an antitumor response. By taking small biopsy samples of tumors, one can obtain a population of these lymphocytes and expand it in vitro with IL-2. These activated **tumor-infiltrating lymphocytes** are called **TILs.** Many TILs have a wide range of antitumor activity and appear to be indistinguishable from LAK cells. However, some TILs cells have specific cytolytic activity against their autologous tumor. These tumor-specific TILs are of interest because they have increased antitumor activity and require 100-fold lower levels of IL-2 for their activity than LAK cells do. In one study, TIL populations were expanded in vitro from biopsy samples taken from patients with malignant melanoma, renal-cell carcinoma, and small-cell lung cancer. The expanded populations of TILs were reinjected into autologous patients together with continuous infusions of recombinant IL-2. Renal-cell carcinomas and malignant

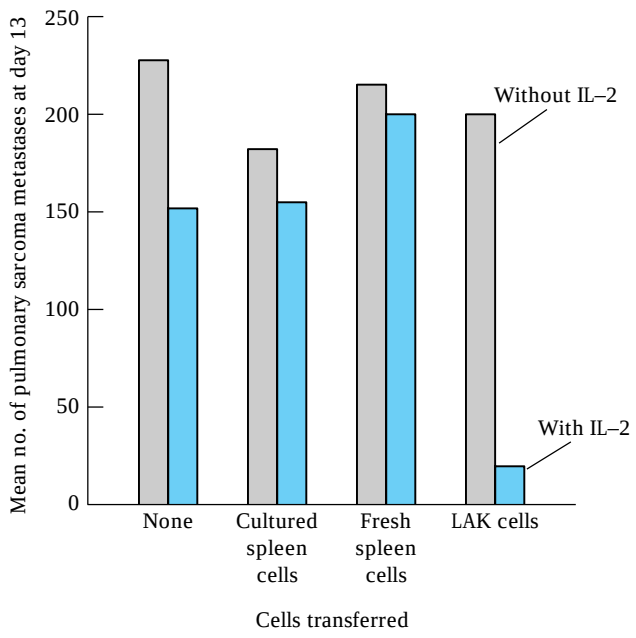


FIGURE **22-13** Experimental demonstration of tumor-destroying activity of LAK cells plus IL-2. Spleen cells or LAK cells, in the presence or absence of recombinant IL-2, were infused into mice with pulmonary sarcoma. The animals were evaluated 13 days later for the number of pulmonary sarcoma metastases. The LAK cells were prepared by isolating lymphocytes from tumor-bearing animals and incubating them in vitro with high concentrations of IL-2. Note that LAK cells caused tumor regression only when IL-2 was also infused. *[Data from S. Rosenberg et al., 1988,* Ann. Int. Med., ***108:****853.]*

melanomas showed partial regression in 29% and 23% of the patients, respectively.

Monoclonal Antibodies Are Effective in Treating Some Tumors

Monoclonal antibodies have been used in various ways as experimental immunotherapeutic agents for cancer. For example, anti-idiotype monoclonal antibodies have been used with some success in treating human B-cell lymphomas and T-cell leukemias. In one remarkable study, R. Levy and his colleagues successfully treated a 64-year-old man with terminal B-cell lymphoma. At the time of treatment, the lymphoma had metastasized to the liver, spleen, bone marrow, and peripheral blood. Because this was a B-cell cancer, the membrane-bound antibody on all the cancerous cells had the same idiotype. By the procedure outlined in Figure 22-14, these researchers produced mouse monoclonal antibody specific for the B-lymphoma idiotype. When this mouse monoclonal anti-idiotype antibody was injected into the patient, it bound specifically to the B-lymphoma cells, because these cells expressed that particular idiotype. Since B-lymphoma cells are susceptible to complement-mediated lysis, the monoclonal antibody activated the complement system and lysed the lymphoma cells without harming other cells. After four injections with this anti-idiotype monoclonal antibody, the tumors began to shrink, and this patient entered an unusually long period of complete remission.

However, this approach requires that a custom monoclonal antibody be raised for each lymphoma patient. This is prohibitively expensive and cannot be used as a general therapeutic approach for the thousands of patients diagnosed each year with B lymphoma. Recently, Levy and his colleagues have used direct immunization to recruit the immune systems of patients to an attack against their B lymphoma. In a clinical trial with 41 B-cell lymphoma patients, the genes encoding the rearranged immunoglobulin genes of the lymphomas of each patient were isolated and used to encode the synthesis of recombinant immunoglobulin that bore the idiotype typical of the patient's tumor. Each of these Igs was coupled to keyhole limpet hemocyanin (KLH), a mollusk protein that is often used as a carrier protein because of its efficient recruitment of T-cell help. The patients were immunized with their own tumor-specific antigens, the idiotypically unique immunoglobulins produced by their own lymphomas. About 50% of the patients developed anti-idiotype antibodies against their tumors. Significantly, improved clinical outcomes were seen in the 20 patients with anti-idiotype responses, but not in the others. In fact, 2 of these 20 experienced complete remission.

Despite its promise, the anti-idiotypic approach is by its very nature patient-specific. A more general monoclonal-antibody therapy for B-cell lymphoma is based on the fact that most B cells, whether normal or cancerous, bear lineage-distinctive antigens. One such determinant, CD20, has been the target of intensive efforts; a monoclonal antibody to it, raised in mice and engineered to contain mostly human sequences, has been useful in the treatment of B-cell lymphoma (see Clinical Focus, Chapter 5). Aside from CD20, a number of tumor-associated antigens (Table 22-4) are being tested in clinical trials for their suitability as targets for antibody-mediated anti-tumor therapy.

A variety of tumors express significantly increased levels of growth-factor receptors, which are promising targets for anti-tumor monoclonal antibodies. For example, in 25 to 30 percent of women with metastatic breast cancer, a genetic alteration of the tumor cells results in the increased expression of HER2, an epidermal-growth-factor–like receptor. An anti-HER2 monoclonal antibody was raised in mice and the genes encoding it were isolated. Except for the sequences encoding the antibody's CDRs, the mouse Ig sequences were replaced with human Ig counterparts. This prevents the generation of human anti-mouse antibodies (HAMAs) and allows the patient to receive repeated doses of the "humanized" anti-HER2 in large amounts (100 milligrams or more). Preparations of this antibody, called Herceptin, are now commercially available for the treatment of HER2-receptor–bearing breast cancers (see Clinical Focus, Chapter 5). Monoclonal antibodies also have been used to prepare tumor-

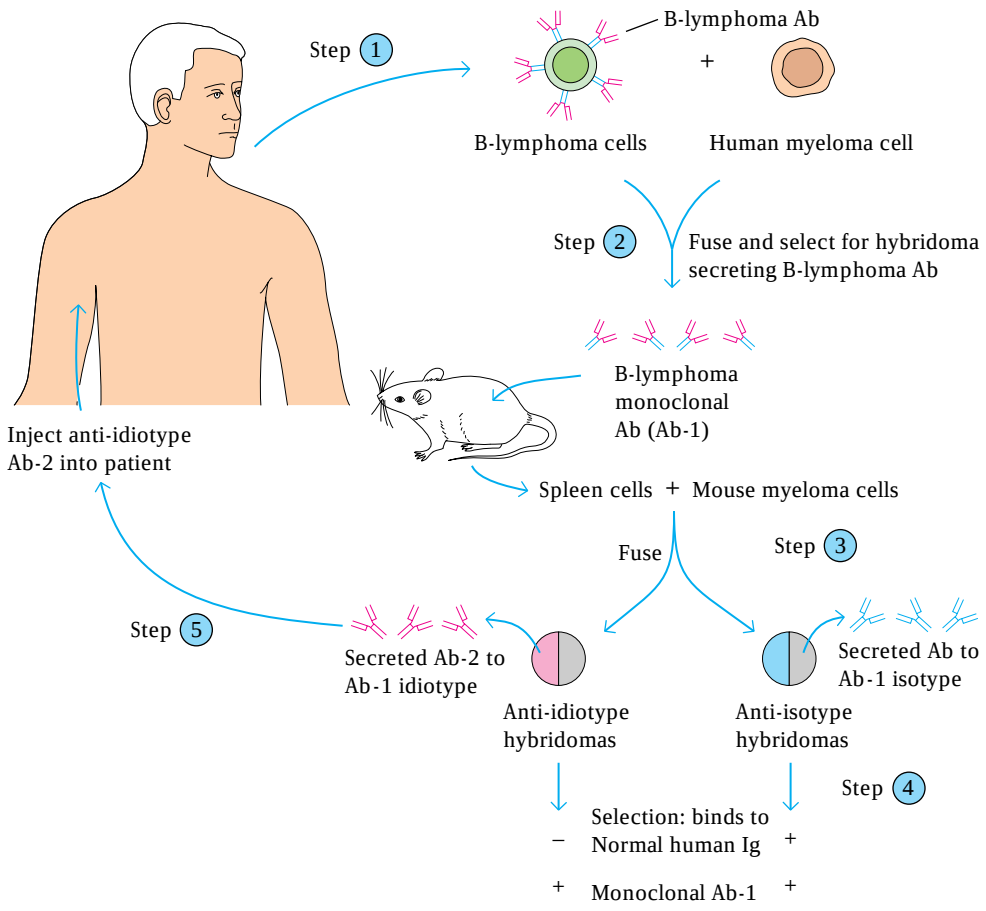


FIGURE **22-14** Treatment of B-cell lymphoma with monoclonal antibody specific for idiotypic determinants on the cancer cells. Because all the lymphoma cells are derived from a single transformed B cell, they all express membrane-bound antibody (Ab-1) with the same idiotype (i.e., the same antigenic specificity). In the procedure illustrated, monoclonal anti-idiotype antibody (Ab-2) against the B-lymphoma membrane-bound antibody was produced (steps 1–4). When this anti-idiotype antibody was injected into the patient (step 5), it bound selectively to B-lymphoma cells, which then were susceptible to complement-plus-antibody lysis.

specific anti-tumor agents. In this approach, antibodies to tumor-specific or tumor-associated antigens are coupled with radioactive isotopes, chemotherapy drugs, or potent toxins of biological origin. In such "guided missile" therapies, the toxic agents are delivered specifically to tumor cells. This focuses the toxic effects on the tumor and spares normal tissues. Reagents known as **immunotoxins** have been constructed by coupling the inhibitor chain of a toxin (e.g., diphtheria toxin) to an antibody against a tumor-specific or tumor-associated antigen (see Figure 4-23). In vitro studies have demonstrated that these "magic bullets" can kill tumor cells without harming normal cells. Immunotoxins specific for tumor antigens in a variety of cancers (e.g., melanoma, colorectal carcinoma, metastatic breast carcinoma, and various lymphomas and leukemias) have been evaluated in phase I or phase II clinical trials. In a number of trials, significant numbers of leukemia and lymphoma patients exhibited partial or complete remission. However in a number of cases, the clinical responses in patients with larger tumor masses were disappointing. In some of these patients, the sheer size of the tumor may render most of its cells inaccessible to the immunotoxin.

SUMMARY

- Tumor cells differ from normal cells in numerous ways. In particular, changes in the regulation of growth of tumor cells allow them to proliferate indefinitely, then invade the underlying tissue, and eventually metastasize to other tissues (see Figure 22-1). Normal cells can be transformed in vitro by chemical and physical carcinogens and by transforming viruses. Transformed cells exhibit altered growth properties and are sometimes capable of inducing cancer when they are injected into animals.
- Proto-oncogenes encode proteins involved in control of normal cellular growth. The conversion of proto-oncogenes to oncogenes is one of the key steps in the induction of most human cancer. This conversion may result from mutation in

TABLE 22-4 Some tumor-associated antigens under examination as potential targets for monoclonal-antibody therapy

Tumor antigen	Tumor type	Target antigen
	LYMPHOID CELL-SURFACE MARKERS	
T-cell marker	T-cell leukemia/lymphoma	CD5
B-cell marker	B-cell lymphoma	CD20
Hematopoietic-cell marker	Acute myeloblastic leukemia	CD45
Anti-idiotype	B-cell lymphoma	Immunoglobulin
	NONLYMPHOID TISSUE MARKERS	
Cell-surface antigens		
Carcinoembryonic antigen (CEA)	Colon cancer (some others)	Glycoprotein
MUC1	Breast cancer	Glycoprotein
Gangliosides such as GD2 and GD3	Neuroectodermal tumors	Glycolipids associated with neural tissue
Growth-factor receptors		
Epidermal growth-factor receptor (EGFR)	Some lung, head, neck, and breast tumors	EGF-binding cell surface protein
HER2 (and EFG-like receptor)	Breast and ovarian tumors	Cell-surface EGF-binding protein with homology to EGFR

SOURCE: Adapted from Scott and Welt, 1997, *Curr. Opin. Immunol.* **9:**717.

an oncogene, its translocation, or its amplification (see Figure 22-2).

- A number of B- and T-cell leukemias and lymphomas are associated with translocated proto-oncogenes. In its new site, the translocated gene may come under the influence of enhancers or promoters that cause its transcription at higher levels than usual (see Figure 22-5).
- Tumor cells display tumor-specific antigens and the more common tumor-associated antigens. Among the latter are oncofetal antigens, (see Table 22-3) and increased levels of normal oncogene products (see Figure 22-6). In contrast to tumor antigens induced by chemicals or radiation, virally encoded tumor antigens are shared by all tumors induced by the same virus.
- The tumor antigens recognized by T cells fall into one of four major categories: antigens encoded by genes with tumor-specific expression; antigens encoded by variant forms of normal genes that have been altered by mutation; certain antigens normally expressed only at certain stages of differentiation or differentiation lineages; antigens that are overexpressed in particular tumors.
- The use of a variety of genetic, biochemical, and immunological approaches has allowed the identification of several tumor-associated antigens (see Table 22-4). In many cases the antigen is expressed on more than one type of tumor. Common tumor antigens offer hope for the design of better therapies, detection, and monitoring, and have important implications for the possibility of anti-tumor immunization.
- The immune response to tumors includes CTL-mediated lysis, NK-cell activity, macrophage-mediated tumor destruction, and destruction mediated by ADCC. Several cytotoxic factors, including TNF-α and TNF-β, help to mediate tumor-cell killing. Tumors may evade the immune response by modulating their tumor antigens, by reducing their expression of class I MHC molecules, and by antibody-mediated or immune complex-mediated inhibition of CTL activity.
- Experimental cancer immunotherapy is exploring a variety of approaches. Some of these are the enhancement of the co-stimulatory signal required for T-cell activation (see Figure 22-11a), genetically engineering tumor cells to secrete cytokines that may increase the intensity of the immune response against them (see Figure 22-11b), the therapeutic use of cytokines (see Figure 22-12), and ways of increasing the activity of antigen-presenting cells.
- A number of encouraging clinical results have been obtained with therapy using monoclonal antibodies against tumor-associated and (in a few cases) tumor-specific antigens (see Figure 22-14). Coupling of antibodies against

TABLE **22-5** Tumor-associated and tumor-specific antigen peptides recognized by human T cells

Human tumor	Protein	Peptide
Many melanomas, esophageal carcinomas, non small-cell lung carcinomas and hepatocellular carcinomas	MAGE-1	EADPTGHSY and SAYGEPRKL
Melanoma	Tyrosinase	MLLAVLYCL, YMNGTMSQV, YMDGTMSQV, and others
Colon cancer	Carcinoembryonic antigen (CEA)	YLSGANLNL
Breast and ovarian cancer	HER2/NEU	KIFGSLAFL
Head and neck squamous-cell carcinoma	Caspase 8	FPSDSWCYF
Chronic myeloid leukemia	*bcr-abl* fusion protein (product of a fusion of an Ig gene with the *abl* gene)	ATGFKQSSKALQRPVAS
Prostatic cancer	PSA	FLTPKKKLQCV and VISNDVCAQV

SOURCE: Adapted from B. Van Den Eynde and P. van der Bruggen, 1996, *Curr. Opin. Immunol.* **9**:684.

tumor antigens with toxins, chemotherapeutic agents, or radioactive elements is being examined. The expectation is that such strategies will focus the toxic effects of these agents on the tumor and spare normal cells their deleterious effects.

- Key elements in the design of strategies for vaccination against cancer are the identification of significant tumor antigens by genetic or biochemical approaches; the development of strategies for the effective presentation of tumor antigens; and the generation of activated populations of helper or cytotoxic T cells.

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USEFUL WEB SITES

http://www.oncolink.upenn.edu/

Oncolink is a site that offers comprehensive information about many types of cancer. It is a good source of information about cancer research and advances in cancer therapy. The site is regularly updated and it includes many useful links to other resources.

http://www.cancer.org/index_4up.html

This is the Web site of the American Cancer Society. It contains a great deal of information on the incidence, treatment, prevention of cancer. The site also highlights significant achievements in cancer research.

http://www.cytopathnet.org/

A good resource for information on the cytological examination of tumors and on matters related to staining patterns that are typical of the cell populations found in a number of cancers.

Study Questions

CLINICAL FOCUS QUESTION You are an oncologist and wish to treat a patient with one of the newly available cancer vaccines, but the only tumor from this patient is preserved in formaldehyde. Can you still use a vaccine? If so, what type of vaccine is available for your use? If you have a tumor sample containing living cells, are there other types of vaccines available?

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.

a. Hereditary retinoblastoma results from overexpression of a cellular oncogene.
b. Translocation of c-*myc* gene is found in many patients with Burkitt's lymphoma.
c. Multiple copies of cellular oncogenes are sometimes observed in cancer cells.
d. Viral integration into the cellular genome may convert a proto-oncogene into a transforming oncogene.
e. All oncogenic retroviruses carry viral oncogenes.
f. The immune response against a virus-induced tumor protects against another tumor induced by the same virus.
g. LAK cells are tumor specific.

2. You are a clinical immunologist studying acute lymphoblastic leukemia (ALL). Leukemic cells from most patients with ALL have the morphology of lymphocytes but do not express cell-surface markers characteristic of mature B or T cells. You have isolated cells from ALL patients that do not express membrane Ig but do react with monoclonal antibody against a normal pre-B-cell marker (B-200). You therefore suspect that these leukemic cells are pre-B cells. How would you use genetic analysis to confirm that the leukemic cells are committed to the B-cell lineage?

3. In a recent experiment, melanoma cells were isolated from patients with early or advanced stages of malignant melanoma. At the same time, T cells specific for tetanus-toxoid antigen were isolated and cloned from each patient.

a. When early-stage melanoma cells were cultured together with tetanus-toxoid antigen and the tetanus toxoid–specific T-cell clones, the T-cell clones were observed to proliferate. This proliferation was blocked by addition of chloroquine or by addition of monoclonal antibody to HLA-DR. Proliferation was not blocked by addition of monoclonal antibody to HLA-A, -B, -DQ, or -DP. What might these findings indicate about the early-stage melanoma cells in this experimental system?
b. When the same experiment was repeated with advanced-stage melanoma cells, the tetanus-toxoid T-cell clones failed to proliferate in response to the tetanus-toxoid antigen. What might this indicate about advanced-stage melanoma cells?
c. When early and advanced malignant melanoma cells were fixed with paraformaldehyde and incubated with processed tetanus toxoid, only the early-stage melanoma cells could induce proliferation of the tetanus-toxoid-T-cell clones. What might this indicate about early-stage melanoma cells?
d. How might you confirm your hypothesis experimentally?

4. What are three likely sources of tumor antigens?

5. Various cytokines have been evaluated for use in tumor immunotherapy. Describe four mechanisms by which cytokines mediate antitumor effects and the cytokines that induce each type of effect.

6. Infusion of transfected melanoma cells into cancer patients is a promising immunotherapy.

a. Which two genes have been transfected into melanoma cells for this purpose? What is the rationale behind use of each of these genes?
b. Why might use of such transfected melanoma cells also be effective in treating other types of cancers?