CHAPTER 23
TUMOR IMMUNOLOGY

While the importance of the immune system's role in normally preventing the development of tumors is questionable at best, it *is* clear that immune responses against tumors often do develop, and can be detected and studied in both experimental and clinical situations. This chapter introduces some of the concepts required to understand the complex relationship between the immune system and tumor development, and a selection of the complex terminology which has characterized this field. Three key points are: (1) *Tumor cells often do express new antigens* ("neo-antigens") which are potential or actual targets for immune recognition. (2) Despite this fact, *the immune system is often ineffective* in eliminating tumors or preventing their growth. (3) Various means may be possible for *modifying anti-tumor immune responses* to render them more effective, and to use *immunological approaches for both diagnosis and treatment.*

NEOPLASIA:

Appearance of a *tumor* (from the Latin word for "swelling") results from ABNORMAL PROLIFERATION of cells, through the loss or modification of normal growth control. Cells which normally do not divide (*e.g*. muscle or kidney cells) may start proliferating, or cells which normally do proliferate (*e.g*. basal epithelial cells or hemopoeitic cells) may begin dividing in an uncontrolled fashion.

If the growth of a tumor remains localized, it can often be removed surgically (if it is accessible), and is therefore relatively harmless; or "benign". In some cases, however, cells from a growing tumor may be capable of intruding into adjacent normal tissue ("*invasive growth"*), and may leave the original site and begin to proliferate in a new location (METASTASIS). These properties distinguish "MALIGNANT" tumors from benign ones.

It is important to understand and reconcile two seemingly contradictory properties of tumors namely their MONOCLONAL ORIGIN *versus* their HETEROGENEITY. While tumors are almost invariably of *monoclonal origin*, mutation and chromosomal instability commonly generate a substantial degree of *heterogeneity* within tumor populations (see IMMUNOMODULATION and IMMUNOSELECTION, below). This fact has important implications for both diagnosis and treatment of cancers.

ORIGINS OF NEOPLASIA:

At least three major factors are known to initiate tumor development; regardless of which of the three is the cause (or if the cause is unknown), the consequence is uncontrolled growth, resulting either from the abnormally high expression of genes which *stimulate* cell proliferation (ONCOGENES), or defective expression of genes which normally *control* proliferation (TUMOR SUPPRESSOR GENES):

1) CHEMICAL CARCINOGENS: Many natural as well as man-made compounds present in our foods and environment are known to be capable of inducing tumors, *i.e.* they are *carcinogenic* (tobacco smoke and char-broiled steaks, for example, both contain complex mixtures of carcinogens).

2) IONIZING RADIATION: X-rays, gamma rays and ultraviolet (UV) radiation can all induce the appearance of cancers by their ability to cause changes in gene expression; We are exposed to these through sunlight (UV), normal background radiation (gamma rays), and clinical sources (gamma rays and X-rays).

3) VIRUSES: Many viruses carry their own *oncogenes*, *i.e.* genes whose products can stimulate neoplastic transformation of the infected cell; these genes may be expressed during a viral infection, and in some cases may be integrated into the genome of an infected cell and be expressed at a later time.

TUMOR-SPECIFIC ANTIGENS (TSA) and TUMOR-ASSOCIATED ANTIGENS (TAA):

As mentioned above, many tumors can be shown to express cell surface antigens which are not expressed in the normal progenitor cells before the neoplastic transformation event. These antigens have been categorized based on their nature and distribution, resulting in a complex collection of acronyms, some of which are defined here:

1) Chemical or radiation-induced tumors each generally express a *unique* neo-antigen, different from other tumors induced by the same or different agent. These have been termed Tumor-Specific Transplantation Antigens, or **TSTA**.

2) Tumors induced by the same virus express antigens *shared* between different tumors. These consist of membrane-expressed *virally encoded antigens*, and have been termed Tumor-Associated Transplantation Antigens, or **TATA** (since they are not, strictly speaking, tumor "specific").

3) **Oncofetal antigens**: These are TATAs which are *more or less* selectively expressed on tumors, but are also shared with some normal fetal or embryonic tissues. Examples include *carcinoembryonic antigen* (CEA, shared with healthy fetal gut tissue), and *alpha-fetoprotein* (AFP, also present in the serum of healthy infants, but decreasing by one year of age). Expression of such antigens by tumors is thought to reflect their reversion to a less fully differentiated state.

IMMUNOLOGICAL SURVEILLANCE:

In its classical form, this term refers to the idea that an important role of the adaptive immune system, particularly cell-mediate immunity (CMI), is to destroy newly appearing neoplastic cells as a result of the new antigens they tend to express. *However, considerable experimental evidence has failed to support this idea*, at least in its simplest form**.** Most importantly, immunosuppressed hosts do *not* generally show the expected high frequency of tumors predicted by this theory, a phenomenon which has been most strikingly illustrated in the *nu/nu* thymus-deficient mouse model, extensively studied for precisely this reason. Those

tumors which *have* been found more frequently in immunosuppressed hosts are usually those induced by viruses, and are best understood simply as the defective immune system's inability to control the viral infection as it normally would. The importance of the adaptive immune system in providing natural protection against neoplasia is therefore highly questionable at best, although NK ("natural killer") cells are still considered as possibly playing such a role. Nevertheless, as we will see, there has been considerable interest in exploiting those anti-tumor immune responses which are known to occur, and direct them toward more effective cancer therapy.

FACTORS WHICH LIMIT ANTI-TUMOR IMMUNE RESPONSES:

If tumors express antigens which can be recognized by the immune system, why are they not normally eliminated, as if they were a foreign tissue graft? Several more or less well-characterized processes have been shown to contribute to the ability of tumors to continue to grow despite the possible or demonstrated presence of a potentially destructive immune response.

TOLERANCE: A state of immunological tolerance may be promoted by the presence of very high levels of tumor antigens, particularly soluble forms which may be shed into the serum by tumor cells (see also Blocking Factor, below).

IMMUNOMODULATION/IMMUNOSELECTION:

Immunomodulation: Antibody binding to a membrane antigen on either normal or neoplastic cells may result in the disappearance of that antigen from the cell surface either by endocytosis (internalization) or by shedding. Once a tumor cell has lost its neoantigen, it becomes invisible to the immune system.

Immunoselection: Variant cells can appear in a tumor population which have lost a particular TATA or TSTA through a mutational event; such a variant will have a selective advantage in the face of an even slightly effective immune response (either humoral or cellular), and cells with the antigen-negative phenotype will therefore tend to progressively take over the population. Note that such a process depends on the presence of *heterogeneity* in a tumor cell population.

ENHANCING ANTIBODIES, IMMUNOSTIMULATION.

Enhancing antibodies: Remember that most nucleated cells (other than lymphocytes, which constitute an important exception) are relatively resistant to complement-mediated lysis, and destruction of grafted tissue is largely the role of cell-mediated immunity. However, if antibodies are present which can bind to neo-antigens, they may effectively "hide" these antigens which might otherwise serve as targets for T-cell-mediated killing, and thus "enhance" the survival of such cells.

Immunostimulation: Antibodies binding to tumor cells may also, in some cases, actually *stimulate* cell growth, presumably through the generation of receptor-mediated proliferative signals. We have previously discussed the fact that antibody directed against a cell surface receptor may act as an agonist with respect to the normal signaling pathway for that receptor (see Chapter 19, AUTOIMMUNITY).

BLOCKING FACTORS: Soluble factors have been described in the serum of tumor-bearing animals as well as in patients which can inhibit an existing immune response from affecting the tumor. The best characterized "blocking factors" have turned out to consist of circulating antigen-antibody complexes containing tumor antigens, which may blind or divert the immune response in ways which are still poorly understood.

IMMUNOSUPPRESSION: Tumor-bearing animals and human tumor patients often exhibit a substantial degree of generalized immunosuppression, resulting directly or indirectly from inhibitory cytokines and other substances secreted by the tumor (Hodgkin's lymphoma is one notable example).

CONCOMITANT IMMUNITY: A mouse bearing a progressively growing tumor may reject a new inoculum of the same tumor at a different site; this rejection is a manifestation of "concomitant immunity", an immune rejection reaction occurring at one site, co-existing with the progressive growth of an antigenically identical tumor elsewhere in the organism. This phenomenon illustrates the importance of *tumor mass* in determining the ultimate outcome of a battle between a tumor and the immune system; a small focus of tumor cells is more susceptible to immune killing than a large and well-vascularized tumor.

ACTUAL AND POTENTIAL APPLICATIONS OF IMMUNOLOGICAL PRINCIPLES TO THE CANCER PROBLEM:

IMMUNODIAGNOSIS

1) *Tumor-associated antigens in serum*. Detection of TSTA/TATA in serum to detect the presence of tumors which are undetectable by conventional methods, or to measure the overall tumor mass and its response to therapy. Assays for determining serum levels of CEA and PSA ("prostate specific antigen"), for example, are widely used for both screening and evaluation of the success of therapy.

2) *Antibody-based therapy*. Use of radioactively labeled antibodies to TSTA, to allow detection and localization of otherwise invisible metastases. While the use of radiolabeled antibodies for such purposes has been proposed and studied for decades, it has not yet advanced to routine clinical application.

IMMUNOTHERAPY

1) **Humoral**: A number of monoclonal antibodies which target tumor antigens have been FDA-approved for therapy, currently representing the most widely used form of immunotherapy. Many are used as unconjugated "naked" antibodies, and their effectiveness derives either from their ability to target tumor cells for destruction by fixing complement and opsonization, or by serving as antagonists for cell surface receptors important for cell proliferation or angiogenesis (growth of blood vessels).

Several decades of research have been devoted to the potential use of anti-tumor antibodies conjugated with toxins (*e.g*. *diptheria toxin*, or the plant toxin *ricin*), or highly radioactive isotopes, thus serving as targeting agents to deliver these toxic molecules to tumor cells. Only two conjugated mAbs, both radioactively labeled, have thus far been approved for human use as therapeutics, and are being studied for use in imaging (to detect otherwise invisible centers of growth). A number of other radioactive mAb conjugates are in various stages of development.

2) **Cell-mediated** (Lymphokine-Activated Killer [LAK] cells). Lymphocytes removed from a tumor-bearing patient can be treated *in vitro* with lymphokines (*e.g*. IL-2), and the resulting "activated" cells re-injected into the patient, in the hope that the tumor cells will be killed by tumor-specific killer T-cells present in the transfused cell population. These lymphocytes can be either obtained from the patient's blood (which hopefully contains some tumor-specific T-cells), or else extracted from a surgically excised tumor ("tumor-infiltrating leukocytes" or "TILs"), presumably consisting of a more highly enriched tumor-specific population. Results in humans have not been as positive as in experimental animals, but attempts continue to developing this approach into an effective therapeutic.

CHAPTER 23, STUDY QUESTIONS:

1. Define the concept of *"Immunological Surveillance"*. What is the strongest evidence regarding its validity?
2. Define and give examples of the terms *"Tumor-Specific Antigen"*, and *"Tumor-Associated Antigen"*.
3. Describe at least three mechanisms by which tumors are known to avoid immune destruction.
4. Describe *two* possible approaches to using antibodies for anti-tumor therapy.