TUMOR IMMUNOLOGY

CANCER. Over 565,000 people in the United States died of cancer in 2008[1]. It is the second leading cause of death, after heart disease. After accidents, it is the second leading cause of years of life lost. However, we finally seem to be doing some things right: cancer death *rates* decreased on average 2.1 percent per year from 2002 through 2008, up from the annual decrease of 1.1 percent per year from 1993 through 2002. That's in the US; rates are climbing in the rest of the world, according to WHO.

Metazoans have existed for about 600,000,000 years, and cancer has probably been a problem from the beginning, because as soon as cells have sociology, there will be sociopaths. It has been estimated that a human will undergo up to 10^{16} mitoses in a lifetime[2]. Mutations happen at a rate of 10^{-8}/base-pair/mitosis; that would yield about 3000 mutations/hr. About one of these per hour is potentially oncogenic. What happens to those cells?

IMMUNE SURVEILLANCE. In 1959 Lewis Thomas suggested that the adaptive immune response evolved not so much for dealing with foreign substances, but as a way of detecting changes in the body's own cell surfaces. These changes, he reasoned, would probably be due to damage or mutation. The true role of the immune system, especially of T cells, would be to constantly monitor the surfaces of cells in the body; if one was detected as abnormal, that cell would be destroyed before a mutant, possibly malignant, clone developed.

This idea was incredibly ahead of its time, since the T cell's preoccupation with "self" was not discovered for almost 20 years. Looked at in this light, the development of cancer could be seen as a failure of immunity. Is there any evidence to support such a view? Yes.

Evidence for cancer immune surveillance:

1. People with immunodeficiencies, particularly of T cells, have a higher incidence of tumors, e.g. AIDS patients have a higher rate of Kaposi's sarcoma, Burkitt lymphoma, and a few other tumors. Organ transplant recipients taking old-line immunosuppressive drugs (and therefore immunodeficient) had a 25 to 100-fold increase in tumors relative to healthy controls. People treated with chemotherapy may have a 14-fold increased risk of developing secondary leukemia.

2. Activated T cells that recognize tumor-associated antigens can easily be identified. The presence of lymphocytes in a tumor (tumor-infiltrating lymphocytes or TIL) is a good prognostic sign.

3. A small percentage of tumors, mainly melanomas and some lymphomas, spontaneously regress, presumably due to an immunologic response.

There are limitations to the hypothesis, however. First: the tumors that immunodeficient and immunosuppressed people get are not a random sample of all the tumors that can happen; rather, they tend to be tumors of the lymphoid system, and of the skin, but rarely lung or breast. Second: Nude mice (mice with no thymus) should get tumors very readily, but in fact spontaneous tumors are rare in these mice. Why? Probably because these mice have very high levels of natural killer

[1] To see the statistics, go to the American Cancer Society web site at http://www.cancer.org and click on Cancer Facts and Figures.
[2] Weinberg RA. 2007. Book review in Nature 449:978-981.

(NK) cells, which are not part of the traditional (T and B cell) immune system but can be quite tumoricidal. We'll address NK cells soon.

So the *immune surveillance hypothesis*, which engendered a lot of excitement about tumor immunology, fell somewhat into disrepute, and interest waned; but newer ideas have revived the study and application of immunological principles to cancer. Compared to the traditional modalities of cancer treatment—radiation, chemotherapy, and surgery—immunotherapy promises the new concept of *specificity*.

> **ASK YOURSELF:** In what way are radiotherapy, chemotherapy, and surgery not specific compared to immunotherapy?

IMMUNOEDITING. One may think about the role of the immune system in neoplastic development as a series of stages in a process that has been called "immunoediting."

1. Elimination. Going from 1 cell to 1 gram of tumor (10^9 cells) is 31 generations. That would take only 30 days if the tumor cell cycle were 24 hours. But epidemiology suggests it takes more like 20 years! That would imply a cycle time of 240 days, which is unreasonable. *Something* must be eliminating most cells that get initiated by a mutagenic event.

For an immunologist, that thing would be immune surveillance. The idea is that when a clone becomes malignant its most likely fate is to be recognized as abnormal by both the innate and adaptive immune systems, and thus eliminated. Tumor cells exhibit a variety of metabolic abnormalities compared to normal cells, and these can lead to the expression of DAMPs which activate innate immunity. Cytokine secretion and antigen presentation on dendritic cells activate T cells, and so macrophages and cytotoxic T cells infiltrate the tumor. If the abnormal clone is successfully eradicated, the process ends.

2. Equilibrium. In most clinically relevant tumors, lymphocytes infiltrate the tumor, but do not fully destroy it. Instead the tumor and lymphocytes exist in equilibrium. This may be analogous to the situation with Epstein-Barr virus in the bone marrow, or Varicella in dorsal root ganglia; as long as the immune response is strong the virus is kept in latency. But biologic equilibria are dynamic, and changing conditions—the host's immunity drops for some reason, or further mutations accumulate in residual tumor cells—can eventually lead to reactivation[3].

3. Escape: the tumor cells fight back. Years ago the Hellstroms in Seattle did some interesting experiments to investigate the question: If a patient's tumor regresses (goes away), did the immune system have something to do with it? (We don't think that surgery and irradiation, or drugs, could remove every last cell).They studied patients with malignant melanoma. Some had apparently been cured by therapy, and so were *regressors*; most had growing tumors and were *progressors*.

> EXPERIMENT 1: T cells from the blood of regressors were added to a layer of their melanoma cells growing in culture. Result: The T cells killed the tumor cells. Conclusion: Good; as expected if the immune system really helped these people get rid of their tumors.
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> EXPERIMENT 2: T cells from patients with progressively growing tumors were added in culture to their melanoma cells, as a control. Result: The T cells killed the tumor cells.

[3] Fatal Melanoma Transferred in a Donated Kidney 16 Years after Melanoma Surgery. 2003. RM MacKie, R Reid, B Junor. *N Engl J Med* 348:567-568.

Conclusion: Surprise! This was unexpected in light of the results in Expt. 1. Other controls quickly showed that this killing was tumor-specific in both Expts. 1 and 2; both groups really had killer T cells against melanoma; normals don't.

So why did the tumors keep on growing in progressor patients who had developed CTL against them? The answer seems to be that the tumor cells had devised a means to **escape** this surveillance.

EXPERIMENT 3: T cells from either group were added to melanoma cells, as before, but now in the presence of serum from progressor patients. Result: Now, no killing. Conclusion: The serum contains **blocking factors**. These were shown to be specific, only blocking the killing of melanoma cells by melanoma-specific killer T cells.

ASK YOURSELF: What do you think blocking factors might be? Consider that they must specifically block the killing of a target by its killer cell.

The existence of blocking factors (not demonstrated in every tumor type, incidentally) is instructive: It reminds us that the tumor and the host are co-evolving systems, and when we see a clinically-relevant tumor in a patient, we know it has already come up with a variety of tricks to avoid immunity. Blocking factors include shed tumor antigen (or antigen-MHC complexes) and sometimes antibody against tumor antigens, which don't harm the tumor while shielding it from T cells.

Tumors evolve many escape mechanisms. Some modify their tumor-associated antigens (see below) until the host does not have T cells against them with highly avid receptors. Others make immunosuppressive factors like TGFβ. And almost all, as they progress, reduce the expression of MHC Class I so there is less and less for CTL to recognize.

ASK YOURSELF: Tumors are almost always monoclonal (that is, derived from a single cell). It has been shown, however, that the tumors seen in immunosuppressed and immunodeficient patients are often polyclonal. What do you think this might mean?

SOME BASIC QUESTIONS. Are tumor cells antigenically different from the normal cells from which they arise? Can the immune response recognize these antigens and respond in such a way as to control tumor growth? Is there evidence that it does so under normal circumstances? Can we manipulate the immune system so that we can control tumors?

TUMOR ANTIGENS. All tumor cells can be shown to have antigens that are not readily found on the corresponding normal cell. Often they *are* found on normal cells, but in much lower quantities; they are overexpressed or abnormally expressed by the tumor. Such antigens are called **tumor-associated antigens** (TAA). A subclass of TAA are those that can be recognized by the immune system, in a way that leads to the destruction of the tumor. Such antigens are called **tumor rejection antigens**.

Viral gene products. Many tumors are known to be caused by tumor viruses; in humans about 20% of tumors are caused directly or indirectly by viruses. Especially noteworthy are HTLV-1 and -2 that have been strongly implicated in Sézary syndrome/mycosis fungoides (discussed later), as well as the similar epidemic lymphoma in Japan and the Caribbean. Cervical cancer (human papilloma virus) is currently the best-known virally-induced tumor in humans; one hopes the HPV vaccine will make it less well known. Quite a lot of liver cancer in the developing world follows a hepatitis virus infection. Epstein Barr Virus can induce Burkitt

lymphoma and nasopharyngeal carcinoma. The presence of the bacterium *Helicobacter pylori* is associated with gastric carcinomas.

Mutant gene products. Chemical and physical mutagens can lead to cellular transformation. Mutated proteins will be processed and presented to the immune system. Since the mutations contributing to the development of tumors are not always identical from patient to patient, immunotherapy designed against these antigens may not be as generalizable as might be with viral or normal gene products. These antigens are called **tumor-specific antigens**.

Normal gene products.

> **Oncofetal antigens** are made in normal fetal tissues. They are not found in the normal tissues of adults, but can be re-expressed in the tumor. The most familiar is **carcinoembryonic antigen** (CEA), found in the blood of patients with colon carcinoma and other cancers. There are commercially available kits to detect CEA in blood. They should *not* be used as a routine screening test. Why not? Too many false positives. The proper use of CEA measurement comes when you have a high index of suspicion of colon cancer; or, when such a cancer has been removed, to confirm complete excision (levels fall to 0 and remain there) or to warn of recurrence.
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> **Differentiation antigens.** These lineage-specific antigens can be greatly overexpressed in tumors, and they represent the most frequently identified TAA. The best studied are those from malignant melanoma (tyrosinase, gp100, MelanA/MART-1). In 30% of breast and ovarian cancers overexpression of the human EGFR-2 gene product (HER-2/neu) is observed. Therapeutic antibody and T cell responses to HER-2/neu can be induced. Prostate-specific antigen (PSA) appears in the blood of many men with prostate cancer, and its detection is used in screening programs, though its utility as a guide for treatment has recently come into question[4].
>
> **Clonal antigens.** Expressed *uniquely* on the malignant clone. The most familiar example would be the idiotype of the surface immunoglobulin in monoclonal B cell malignancies, or of the TCR in T cell malignancies.

HOW THE IMMUNE SYSTEM KILLS TUMOR CELLS. The immune system has at its disposal all the mechanisms with which we are already familiar, and more.

1. Cytotoxic T cells. These CD8+ T cells (CTL) are probably the most important cells in tumor resistance, and they're every right-thinking immunologist's favorite cell. CTL can recognize TAA presented by MHC class I. Naive T cells are activated in the lymph nodes, not at the tumor site, via interactions with antigen-presenting cells such as dendritic cells. Following the initial activating event, the CD8+ T cells undergo clonal expansion and acquire lytic function. Activated TAA-specific T cells leave the lymph node and migrate to the tumor. CTL can kill tumor cells by inducing apoptosis via either perforin or Fas-mediated pathways. The CTL also secrete IFNγ upon engagement of their TCR, which attracts and stimulates macrophages.

However, patient-derived T cells that recognize TAAs are often ineffective in controlling tumor growth. An important study by Lee et al. examined the biological properties of T cells specific

[4] http://www.uspreventiveservicestaskforce.org/uspstf08/prostate/prostaters.htm

for melanoma antigens from patients[5]. Although the T cells divided in the presence of tumor cells, they did not produce cytokines nor were they cytotoxic. One assumes that this abnormal behavior is not inherent in the T cell, but is induced by the tumor.

2. Th1 cells. These CD4+ T cells recognize the tumor antigens, make lymphokines, and attract angry macrophages. How can we make people with tumors get this system going better than it is? Some T cells, perhaps Th1, as well as macrophages, make cytokines called **TNF** and **lymphotoxin** that kill certain tumor cells more readily than normal cells. Although it is very impressive in the test tube, we do not yet know its role in the body. Could lymphotoxin or TNF become new antitumor drugs? Several companies have been testing them, although drug delivery of these poisons will be a problem.

Tumors frequently protect themselves by creating an environment in which M2, not M1, macrophages are favored. M2 seem to protect tumors, even encourage their growth.

3. Natural Killer (NK) cells. NK cells look like large lymphocytes, but have peculiar granules in their cytoplasm, so they are usually called **LGL**s (large granular lymphocytes). They do not need to come from an immunized host to recognize and destroy quite a wide range of tumors, mostly of hematopoietic origin. NK cells provide a link between the innate and adaptive immune systems since they have the lethal tendencies of CTL, and the pattern recognition of innate immunity.

Here's what's wonderful about NK cells. They have receptors of broad specificity for "stress" markers such as might be expressed on a growth-dysregulated cell. They also have receptors for MHC class I (unlike TCR, the receptors are not clonally variable; they bind just about any MHC Class I, with or without a peptide in it). Binding of MHC class I *suppresses* NK cells, so they don't waste effort trying to kill cells with a lot of MHC Class I; that, after all, is a job for CTL. What cells might have low MHC expression? Some virus-infected cells, as we've mentioned. And many tumor cells downregulate MHC to avoid CTL. But between CTL and NK cells the immune system gets tumors coming and going. It's amazing we ever get tumors.

The NK cell is very versatile. It also has Fc receptors for IgG. Thus if a tumor cell has IgG on its surface, the NK cell can bind (as would a neutrophil, say). But because the NK cell is a "killer", it sends the sort of signals CTL do into the target, which dies by apoptosis. When this happens the phenomenon is called **antibody-dependent cell-mediated cytotoxicity, ADCC.** This is a very effective way of killing tumor cells in the test tube, providing you have some specific anti-tumor antibody and normal blood mononuclear cells (which includes NK cells) to add. It probably is important in people, too; it has been reported that patients with bladder cancer develop better ADCC killing of their tumor cells than any other modality.

4. Macrophages and neutrophils: can be activated in vitro with foreign products (e.g., bacteria) to kill tumor cells. Much of this antitumor activity can be attributed to TNF. However, tumors frequently learn to subvert macrophages and even recruit them to support tumor growth!

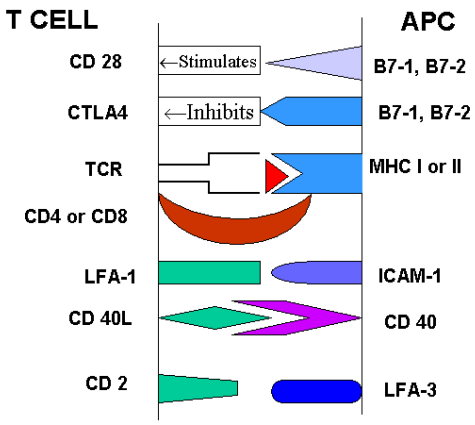
5. Antibody and complement. An antibody response is commonly made in tumor-bearing hosts, but it is not commonly effective. Opsonization of tumor cells by antibody and complement can kill some leukemias *in vitro*, but a strong B cell response to tumor antigens does not seem to correlate with resistance to the tumor. We see tumors that have survived immunoediting; the

[5] PP Lee, et al. (1999) Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. Nature Medicine 5:677-685.

cells of the tumor that survive are likely to have downregulated antigen expression as much as they can. Others have become resistant to complement, or can inactivate it.

IMMUNOTHERAPY. Anything you can think of, and by this stage you ought to be able to come up with all kinds of strategies, might be applied to tumors; remember that there's nothing as specific, and nearly nothing as powerful at cell-killing, as the immune system.

ASK YOURSELF: Can you suggest a target for a blocking reagent (ipilimumab) in this diagram from our T cell lectures?



Specific immunization, a.k.a., a tumor vaccine. There hasn't been much success in this area yet, but as more defined TAAs are being prepared, keep an eye out for breakthroughs. Initially the vaccines will be therapeutic, not preventative. The most interesting vaccine experiments use the patient's own dendritic cells mixed with tumor extracts or purified antigens as highly potent immunogens. One such treatment is the first to be approved (April 2010) by the FDA; it combines the patient's own dendritic cells with a proprietary fusion protein containing the prostate cancer TAA prostatic acid phosphatase. Called Provenge® (sipuleucel-T), it extended survival in phase III tests, but a series of immunizations costs $93,000. Some people are designing improved epitopes with higher affinity to MHC or to the TCR, or both, than the ones that the tumor itself chooses to use[6].

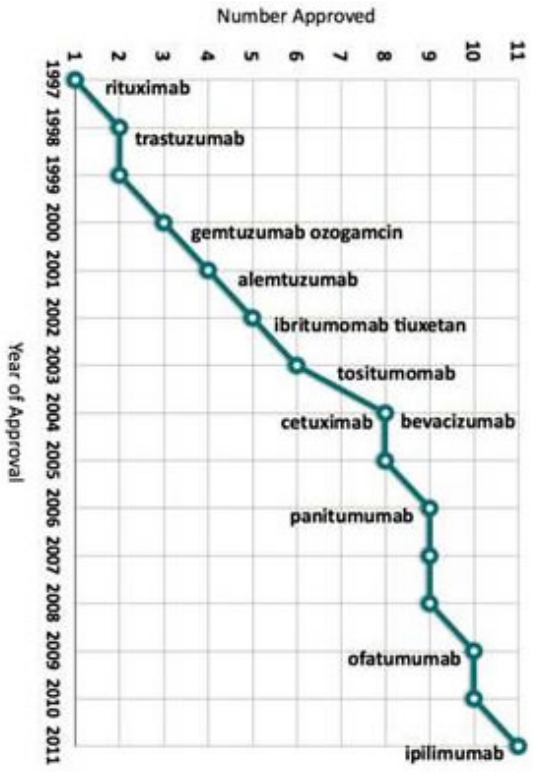
Increase RES activity. This works—either because a turned-on reticuloendothelial system pours out more, and angrier, macrophages, or these cells can more efficiently clear the blood of blocking factors, giving the CTL a chance to go to work. BCG (Bacille Calmette-Guéran, the TB vaccine used in many countries) and some biological response modifiers like interferons work this way. Perhaps there will be ways to switch tumor-supporting M2 macrophages back to M1's that attack the tumor cells.

Innocent bystander killing. BCG is injected directly into the tumor. A ferocious delayed-type hypersensitivity reaction to BCG ensues, and the tumor cells are killed the way lung is killed in TB. This is used to some degree in cutaneous tumors like melanoma, and BCG instilled directly into the bladder is the treatment of choice for superficial bladder carcinoma.

Passive antibody therapy. Antibody to TAAs should be useful, and quite a few monoclonal antibodies (mAb) are already available. They can be used as-is (possibly they activate complement, and the tumor is lysed or phagocytosed; more likely they invoke ADCC), or they can be tagged with a poison such as ricin, or diphtheria toxin, or a radioisotope (such modified antibodies are called **immunotoxins**). At least one mAb is available with either an imaging or a

[6] McMahan, RH at al. (2006) Relating TCR-peptide-MHC affinity to immunogenicity for the design of tumor vaccines J. Clin. Invest. 116:2543-255 [Fascinating work done in Denver at NJH.]

therapeutic radioisotope attached. Herceptin (trastuzumab), a mAb to the HER2/neu surface growth factor receptor on some breast and stomach cancers, has proved its efficacy. A mAb to VEGF (vascular endothelial growth factor) is making quite a stir, too.



Targets

rituximab: CD20

trastuzumab: HER2/neu EGFR

gemtuzumab: CD33 (withdrawn 2010)

alemtuzumab: CD52

ibritumomab: CD20
tositumomab: CD20

cetuximab: EGFR
bevacizumab: VEGF-A

panitumumab: EGFR

ofatumumab: CD20

ipilimumab: CTLA-4

You might think the following approach seems unlikely to work, but it has[7]: A group coupled together two single-chain engineered antibodies, one against CD19 and one against CD3. This construct can bind T cells via their CD3 to CD19+ B cell lymphoma cells. Small doses given to lymphoma patients resulted in some cases in complete clearance of the tumor cells!

Adoptive cell transfer therapy. This technology utilizes cells from the patient's immune system to destroy cancer cells that cannot be surgically removed. Cells from the immune system that have potential to fight the tumor are isolated from the patient's blood, tumor, or lymph nodes. Cells directly from the tumor are called **tumor-infiltrating lymphocytes** (**TIL**). The T cells are expanded greatly in culture using cytokines such as IL-2. The patient's immune system may then be partially destroyed by irradiation to make "room" for the expanded anti-tumor clones. They are reintroduced into the immune-depleted patient to kill remaining tumor cells.

[7] Bargou et al. (2008) Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. Science 321: 974-977.

Learning objectives for Tumor Immunology

1. State the concept of the Immune Surveillance theory. Discuss whether data from immunosuppressed and immunodeficient patients support the theory.

2. Describe the concept of immunoediting.

3. Describe tumor-associated antigens (TAA), and compare and contrast TAA from viral, mutant, and normal gene products.

4. Define carcinoembryonic antigen (CEA) and discuss its usefulness in screening for, diagnosis, and follow-up of colon cancer.

5. Compare and contrast the roles of CTL and NK cells in killing tumors cells, with special reference to the amount of MHC Class I expressed by the tumor.

6. Discuss possible reasons for the low incidence of spontaneous tumors in nude mice.

7. Summarize the Hellstrom experiments using cells from tumor- bearing and cured patients to kill tumor target cells. Indicate which patients had blocking factors. Discuss the possible nature of blocking factors.

8. Discuss the principles underlying antibody or T cell methods that might be used as treatments of tumors.

9. Describe two mechanisms by which BCG treatment may cause tumor regression.

10. Discuss prospects and problems concerning the use of monoclonal antibodies in the diagnosis or treatment of cancer.

11. Describe the nature and therapeutic use of tumor-infiltrating lymphocytes, TIL, in adoptive cellular transfer therapy.