TUMOR IMMUNOLOGY

CANCER. About 601,000 people in the United States will die of cancer in 2017[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: US cancer death *rates* decreased on average 2.1 percent per year from 2002 through 2010. Rates are rising in much of the rest of the world.

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 less for dealing with foreign substances, than 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 it could give rise to a mutant, possibly malignant, clone.

►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 severely immunodeficient) had a 25 to 100-fold increase in tumors relative to healthy controls. People treated with chemotherapy have a risk of developing a secondary leukemia.

2. Activated T cells that recognize tumor-associated antigens can easily be identified. The presence of lymphocytes within the substance a tumor (tumor-infiltrating lymphocytes or TIL), many of which are tumor-specific, is a good prognostic sign.

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

4. In **paraneoplastic syndromes** symptoms develop in a distant organ which does not contain tumor cells. Most of these syndromes have now been found to involve autoimmunity; the patient has responded to an antigen abnormally expressed or overproduced by a tumor, but the symptoms are due to the reaction of antibodies or T cells to another organ that normally expresses that antigen.

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. The tumors, in fact, may largely be virus-induced. 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 (NK) cells, which are not part of the traditional (T and B cell) immune system but can be tumoricidal. We'll discuss NK cells soon.

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

Without much progress in the 90s and 2000s, the ►*immune surveillance hypothesis* fell somewhat into disrepute, and interest waned; but newer results have revolutionized the study and application of immunological principles to cancer. Compared to the traditional modalities of cancer treatment—radiation, chemotherapy, and surgery—immunotherapy promises the long-sought possibility of *specificity*.

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

►**IMMUNOEDITING.** We can 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 and unsupported by evidence. *Something* must be eliminating most cells that get initiated by a mutagenic event.

For the immunological thinker, that something 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 Th1 cells, macrophages, and cytotoxic T cells infiltrate the tumor. If the abnormal clone is successfully eradicated, the process ends.

2. Equilibrium. In many clinically relevant tumors, lymphocytes infiltrate the tumor, but do not fully destroy it. Instead the tumor and lymphocytes exist in equilibrium. This is analogous to the situation with Epstein-Barr virus in the bone marrow, or Varicella (chicken pox) 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 a little for some reason, or further mutations accumulate—can eventually lead to reactivation[3].

3. Escape: the tumor cells break out. It's been known for many years that tumors fight back when the immune system attacks them. For example, it is common to find tumor-specific CTLs surrounding tumor clusters in biopsies. But they aren't effective tumor cell killers. In the past decade it's been found that ►CTL have at least two inhibitory surface receptors ("checkpoints") which, if engaged by corresponding ligands on antigen-presenting cells, signal downregulation of the CTL's cytotoxic activity. These clearly play a normal role in immune regulation. It's easy to imagine that when CTL first arrive, they kill most of the tumor cells, but a few cells that happen to have upregulated the inhibitory ligands escape. They are the ones that grow out, and with time, the entire tumor could be made up of CTL-resistant cells.

The inhibitory checkpoint receptors are **CTLA-4** and **PD-1**. More about them later in these notes (they are also mentioned in the Immunomodulators unit.)

Tumors evolve many immunologic escape mechanisms. Some mutate their tumor-associated antigens until the host does not have T cells against them with highly avid receptors.

[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.

Others make immunosuppressive factors like TGFβ. And almost all, as they progress, reduce the expression of MHC Class I so there is less for CTL to recognize. But it's looking like the checkpoint inhibitory pathway is of overriding importance.

> **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 it can control tumors?

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

Recent amazing progress in analyzing a tumor's genome and exome have helped us understand the antigenic nature of human tumors. Tumors carry two kinds of mutations: ►**drivers** and **passengers**. Driver mutations activate oncogenes or inactivate tumor-suppressor genes, and are the reason the tumor grows. Unfortunately, they are mostly "undruggable" so far, as well as subject to mutation and selection for resistance to the few targeted drugs we've developed. ►Passenger mutations greatly outnumber drivers. They are not causative, but are incidental to the disorder that exists in transformed cells. Human melanoma, for example, is at the high end for such events; one person's tumor harbored *3,000* protein-altering mutations. It is among the passenger mutations that CTL find mutated protein antigens—***neoantigens***—to control tumors.

Although tumors have many mutations, not all will code for neoantigens. Some will be in stretches of protein not easily cut to make peptides of the right length for MHC presentation. Some will not code for "anchor" amino acids with side-groups that interact strongly enough with MHC. Many will not present the changed amino acid in a position that interacts with a T cell receptor. In a technical tour-de-force, a tumor with 4,285 exomic coding variations was predicted to have 170 neo-epitopes (that could bind to MHC); of these, 7 were predicted to actually be immunogenic. And only three could be validated as recognized by CTL[4].

Nevertheless, neoantigens exist, and there is plenty of evidence that the more passenger mutations a tumor has, the more likely it is to be well-controlled by therapies that relieve the CTL from "checkpoint blockade" (below). ►That is, the more antigens, the more CTL, if only they had the chance to do their jobs.

What about **cancer stem cells**, which divide very little and express few lineage-specific antigens? They might escape immunotherapies directed at TRA, allowing the tumor eventually to regrow. A recent study[5] shows they may be particularly sensitive to NK cells—suggesting a possible route to directed therapy?

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[4] Yadav, M. et al. (2014) Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature 515: 572-6.

[5] Ames, E. et al. (2015) NK cells preferentially target tumor cells with a cancer stem cell phenotype. J. Immunol. 195: 4010-9.

OTHER TUMOR ASSOCIATED OR RELATED ANTIGENS.

Microbial gene products. Many tumors are caused by tumor viruses; in humans about 20% of tumors are caused directly or indirectly by viruses. They include HTLV-1 and -2, which have been implicated in Sézary syndrome/mycosis fungoides (described later), as well as a 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. A lot of liver cancer in the developing world is the consequence of 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.

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 give early warning of recurrence.

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 has been used in screening programs, though its utility as a guide for treatment has come into question[6].

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 CAN KILL TUMOR CELLS. All the mechanisms that immunity uses against pathogens should also be available to combat tumors. Remember, of course, that the tumors will be fighting back.

1. Cytotoxic T cells. CTL can recognize TRA presented by MHC class I. Naive T cells are activated in the lymph nodes, not at the tumor site, via interactions with antigen-presenting dendritic cells. Following the initial activating event, the CD8+ T cells undergo clonal expansion and acquire lytic function. Activated TRA-specific T cells leave the lymph node and migrate to the tumor. CTL can kill tumor cells by inducing apoptosis via either the perforin (secretion-based) or Fas-mediated (transmembrane signaling) pathways.

2. Th1 cells. These CD4+ T cells recognize the tumor antigens, make lymphokines, and attract angry M1 macrophages. How can we make people with tumors get this system going better than it is? Tumors frequently protect themselves by creating an environment in which M2, not M1,

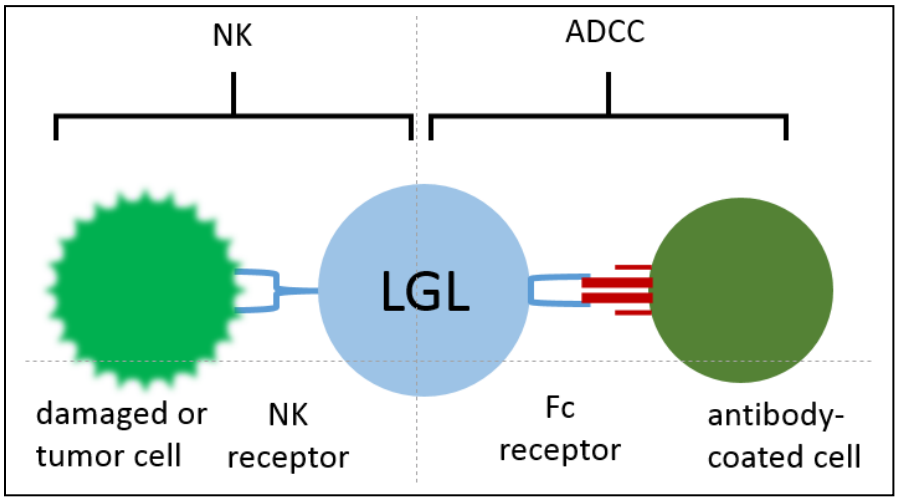
[6] http://www.uspreventiveservicestaskforce.org/Page/Document/UpdateSummaryFinal/prostate-cancer-screening

macrophages are favored. M2 seem to protect tumors, even encourage their growth[7]. A 2015 paper showed that local infusion of IL-21 into breast cancers shifted macrophages from the M2 to the M1 phenotype, with improved response to immunotherapy.

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. They have NK, not T cell, receptors which see a few stress-associated ligands.

Here's what's wonderful about NK cells. As just noted, 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 that CTL do best. What cells might have low MHC expression? ►Many tumor cells downregulate MHC to avoid CTL. But that makes them into NK targets! 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, and so can target antibody-coated cells even if they are not stressed NK targets. When this happens the phenomenon is called **antibody-dependent cell-mediated cytotoxicity, ADCC.** This is a very effective way of killing tumor cells, and it will be discussed more soon (*see* Immunomodulators).



4. 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 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.

[7] This important finding was largely the work of Al Malkinson of the School of Pharmacy, who passed away in 2012.

IMMUNOTHERAPY. Anything you can think of, and by this stage you may 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.

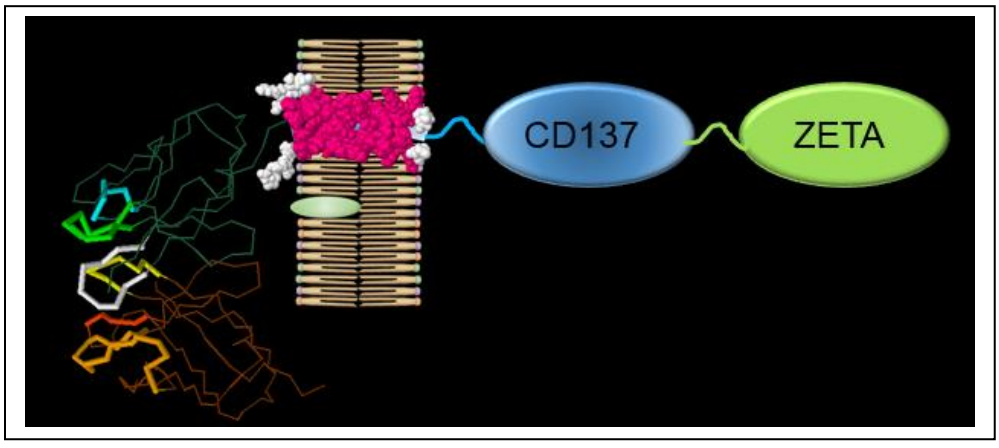
Specific immunization, a.k.a., a tumor vaccine. There hasn't been much success in this area yet, but as more defined TAAs are being identified, keep an eye out for breakthroughs. Initially the vaccines will be therapeutic, not preventative. ►The first FDA-approved vaccine uses the patient's own dendritic cells mixed with a proprietary fusion protein containing a prostate cancer TAA, prostatic acid phosphatase. Called Provenge® (sipuleucel-T), it extended survival in phase III tests. 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[8]. With costly drugs like this, the Incremental Cost Effectiveness Ratio is unfavorable, but if biomarker studies can identify the subset of patients who are most likely to get real benefits, the ICER becomes much more attractive.

Innocent bystander killing. BCG (the tuberculosis vaccine) instilled directly into the bladder on multiple occasions is the treatment of choice for superficial bladder carcinoma. It's likely that lots of M1 ("angry") macrophages, attracted by activated Th1 cells, non-specifically kill the easily-reached tumor cells.

Monoclonal anti-tumor antibodies. There are now about 25 approved monoclonals, some of which have been game changers. *See* Immunomodulators.

Autologous cell therapy. ►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, for which activated T cells upregulate receptors. The patient's immune system may then be partially depleted by irradiation to make "room" for the expanded anti-tumor clones. They are reintroduced into the immune-depleted patient to kill remaining tumor cells. Though championed by Steve Rosenberg at NIH, this approach has failed to get much traction so far.

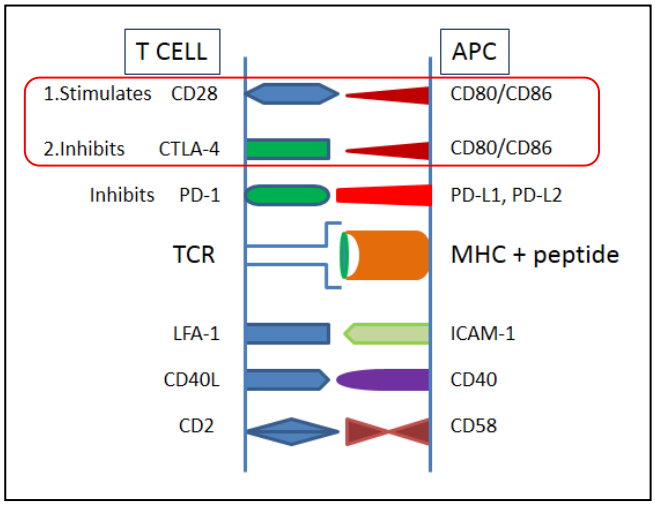
Chimeric antigen receptors. CTL are superb killers, but the MHC-peptide-TCR system is low-affinity and, it seems, too easily circumvented. ►So several groups have re-armed CTL. The stitched-together genes are inserted into the patient's CTL. From left: Fv (V_H and V_L) of an anti-tumor mAb, with its CDRs highlighted; transmembrane region; module that activates killing; module that activates the T cell.



[8] TCR hypervariable regions expressed by T cells that respond to effective tumor vaccines. Jordan, Buhrman, Sprague, Moore, Gao, Kappler, Slansky. Cancer Immunol Immunother (2012) 61:1627–1638. [Fascinating work done in Denver at NJH.]

Inhibitory checkpoint blockade.

When a T cell first engages an APC, it upregulates CD28, which engages CD80 and CD86 on the APC. This results in co-stimulation of the T cell ("co-" meaning, along with TCR engagement of peptide-MHC.) After several days, when the immune response should begin to wind down, CD28 is downregulated and CTLA-4 appears on the T cell surface. It has higher affinity for CD80/86, and its signal is inhibitory; the CTL is, effectively, turned off. Similarly, late in T cell activation, the CTL upregulates another inhibitory checkpoint, PD-1, which is engaged by PD-L1 on the APC. All this is normal, to keep immune responses in check.



►But most tumors learn quickly to express CTLA-4 and PD-1 ligands; these turn off the invading T cells (which are often observed standing helplessly on the outskirts of a tumor.) Perhaps not surprisingly, in many cases the CD80/86 and PD-L1 are not actually on the tumor, ►but on lymphocytes and phagocytes that are in the tumor and probably responding to tumor-derived signals to express these ligands. In any event, the result is that there are CTL near or in the tumor that *could* do the job, but their off-buttons have been pushed.

►So now we have two blocking monoclonal Abs against PD-1; they bind it, and don't allow it to receive the inhibitory signal from PD-L1. They are called **nivolumab** and **pembrolizumab**. There are 3 blocking antibodies against PD-L1 in Phase II/III trials. And there is a blocking monoclonal against CTLA-4, **ipilimumab**, first approved in 2011.

Ipilimumab is effective but quite toxic, as it unleashes immune responses generally, including ones that look like autoimmunity. The anti-PD-1 mAbs are less toxic and also highly effective. In combination with ipilimumab, they are even more effective; there are patients who seem to have been cured. Tested tumors include non-small cell lung cancer, renal carcinoma, melanoma, colorectal cancer, bladder and prostate cancer.

In several studies, response has been related to the extent of PD-L1 expression on tumor-associated cells; the more there is, the better the response. ►Response is also directly correlated with the number of tumor mutations (an index of the number of potential neoantigenic epitopes, see above.)

Recent work strongly suggests that the tumor microenvironment is metabolically hostile to effector CD8 T cells; for example, they don't function well in hypoxia, and compared to the same cells in lymph nodes they have a smaller mitochondrial mass (justifying describing them as "exhausted.") This may largely explain the observation that tumors with the most stroma (non-tumor cells in the lesion) are the least responsive to checkpoint inhibition; pancreatic cancer is an example. Other tumors upregulate enzymes that metabolize away essential amino acids, which T cells need, so they don't work well. Looking for ways to metabolically beef up the patient's CD8 cells within the tumor microenvironment may be the next big thing in developing widely applicable, effective immunotherapy.

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. Define what is meant by checkpoint inhibitory therapy for tumors, and describe their mechanisms of action.

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

9. Define a chimeric antigen receptor.

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

11. Define driver and passenger mutations in cancer, and discuss their roles in tumor control and immunotherapy.