LYMPHOID MALIGNANCIES & NEW IDEAS

LEUKEMIAS AND LYMPHOMAS. Malignancies of B, T, or NK cells are lymphoid; of any other hematopoietic cells, myeloid. We'll only discuss lymphoid malignancies here, and concentrate on the immunology more than the oncology. When many abnormal cells are found in the blood, the condition is leukemia; if in the tissues, including lymph nodes and bone marrow, it is lymphoma. The malignant process is probably always in the tissues, with cells escaping into the blood stream in some conditions. The USA incidence is about 45,000 new cases of leukemia and 75,000 cases of lymphoma per year. 9% of all new cancers will be leukemia, lymphoma, or myeloma; the prevalence is over 1 million.

When lymphoid cells undergo malignant transformation, the predominant cell type usually exhibits a phenotype somewhat similar to that of a normal cell, and we can classify the leukemia on this basis. The Revised European-American Classification of Lymphoid Neoplasms[1] (REAL) was created to include all of the lymphoid malignancies, characterized by both morphology of the cells and immunological markers, and to some extent disease prognosis and response to treatment. We use the WHO classification, more current than REAL but similar, provided here for your convenience. We discuss a few of the more immunologically-interesting conditions.

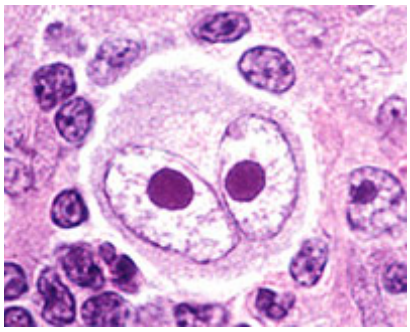
To begin, lymphoid malignancies are classified as either Hodgkin[2] or Non-Hodgkin.

WHO: Hodgkin lymphoma (Hodgkin's disease)

a. Nodular lymphocyte predominant Hodgkin lymphoma

b. Classical Hodgkin lymphoma
- •Nodular sclerosis classical Hodgkin lymphoma
- •Lymphocyte-rich classical Hodgkin lymphoma
- •Mixed cellularity classical Hodgkin lymphoma
- •Lymphocyte-depleted classical Hodgkin lymphoma

HODGKIN LYMPHOMA is a bit mysterious, centering on an abnormal, highly dysregulated malignant cell in lymph nodes (the Reed-Sternberg cell, often binucleate with prominent nucleoli, right) which can usually be identified as a germinal center B cell and may be carrying Epstein-Barr virus (EBV) genes. It is recognized and attacked by normal T cells, but resists apoptosis; the result is a fierce proliferative and inflammatory response in the nodes. ►The Reed-Sternberg cells are usually not more than 1% of the cells total, but they are the only malignant cells. Secondary changes make the interpretation of subsequent events difficult. T cell immunodeficiency often develops. This disease, which carried a 95% mortality rate in 1940, now has a 95% survival rate, due to new forms of chemotherapy and radiation therapy. Cases that resist chemotherapy respond very well to the anti-PD-1 monoclonal antibody nivolumab. The incidence of Hodgkin (9000/year in US) is steady. There is one new case of Hodgkin for every 7 new cases of Non-Hodgkin lymphoma.



Owl eyes

[1] The 2016 revision of the World Health Organization classification of lymphoid neoplasms. 2016. Swerdlow, SH et al. Blood 127:2375-90. https://doi:10.1182/blood-2016-643569.

[2] Thomas Hodgkin, 1798-1866, English pathologist.

WHO: Precursor lymphoid neoplasms

Precursor B-cell neoplasms:

a. **Precursor B-lymphoblastic leukemia/lymphoma**
b. **Precursor T-lymphoblastic leukemia/lymphoma**

LYMPHOID PRECURSOR MALIGNANCIES. Acute lymphoblastic leukemia (ALL) is the most common leukemia of children. In about 85% of cases the cells can be identified as belonging to the B cell lineage not because there is cytoplasmic or surface immunoglobulin expressed, but because the Ig genes are rearranged (these are therefore "null cells," that is, lymphocytes that cannot easily be typed using fluorescent antibodies as either B or T). ALL patients with the B cell precursor form of the disease have a poorer prognosis than those with the T cell form. About 15% of children with ALL have leukemic cells with T cell markers. These children often have a thymoma as well, and it may be that the malignancy originated in the thymus; in the other patients, the bone marrow is the origin.

WHO: Mature B-cell neoplasms

(37 main headings). A selection:

a. B-cell **chronic lymphocytic leukemia** / small lymphocytic lymphoma
b. B-cell prolymphocytic leukemia
c. Lymphoplasmacytic lymphoma (includes **Waldenstrom**)
d. Splenic marginal zone B-cell lymphoma (+/- villous lymphocytes)
e. Hairy cell leukemia
f. Monoclonal gammopathy of undetermined significance (MGUS)
g. Plasma cell myeloma/plasmacytoma (**Multiple myeloma**)
h. Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type
i. Nodal marginal zone lymphoma (+/- monocytoid B-cells)
j. Follicular lymphoma
k. Mantle cell lymphoma
l. Diffuse large cell B-cell lymphoma, Germinal center and activated B cell types
m. Burkitt lymphoma

B CELL NEOPLASMS. Most **Non-Hodgkin lymphomas** are of B cell type, and most of the rest are T cell type. B cell lymphomas have a better prognosis. As you can see, this is a large and heterogeneous category of diseases. It is 45 to 100 times more common in immunosuppressed people than in normals. NHL is the 6th leading cause of cancer death in the USA, and rising at an alarming rate. We do not know the specific causes in most cases.

The most common B cell disease (and about a third of all leukemias in the U.S.) is **chronic lymphocytic leukemia** (CLL), most often seen in older people (mean age 65; men more often than women). It is a malignancy of resting B cells, which are sIg+ but rarely secrete immunoglobulin. ►The prognosis is good, because these cells and their precursors divide slowly and secrete no harmful products such as inflammatory cytokines. Often treatment is more harmful than the disease, and so it is withheld unless complications demanding it develop.

Hairy cell leukemia is another chronic leukemia of B cells, in which the malignant cells have peculiar cytoplasmic projections. Perhaps this relates to their ability to burrow into and replace lymphoreticular tissues such as spleen and bone marrow, leading to splenomegaly, leukopenia and susceptibility to infection. Treatment, as in CLL, can often be delayed. The leukemia responds well to purine analog drugs; sometime the spleen is so large it must be removed.

Burkitt[3] lymphoma is a solid tumor of B cells, quite common in Africa. It is triggered by the Epstein-Barr virus (EBV), which infects B cells and causes their intense proliferation (this is why EBV is used in making hybridomas from B cells in normal donors' blood). People with normal immune systems clear the infected B cells by a killer T cell response; while this is going on the patient feels terrible and may be susceptible to secondary infections, typically Strep throat; the condition is **infectious mononucleosis**. A preexisting relative immunodeficiency (in Africa, originally due to malaria and now also to HIV) may thus predispose to malignancy by impeding removal of the overgrowing B cell population. (Recall, this is what happened to David, the boy in the bubble.) ►Eventually, bad luck makes a chromosomal rearrangement take place in one of the dividing cells, bringing the cellular proto-oncogene *c-myc* close to one of the immunoglobulin chain genes (which have strong promoters and enhancers), and that cell is now truly malignant and independent of further EBV requirements. Burkitt responds well to chemotherapy.

Multiple myeloma is a malignancy of a clone of activated B (plasma) cells, which produce not only immunoglobulin but also several osteoclast activating factors including RANKL which are responsible for extensive bone lesions[4]. The disease may be first recognized in a patient who suffers a fracture without significant trauma (these are called pathological fractures). In the malignant cells, L and H chain synthesis becomes unregulated. Free light chain dimers (**Bence-Jones[5] protein**) are sometimes found in the urine. ►Because of the dysregulation of H and L chain production, the myeloma cell is critically dependent on good proteasome function to remove otherwise toxic misfolded proteins. The myeloma drug Velcade (bortezomib) inhibits the proteolytic sites on the central 20S proteasome subunit, so the cell chokes to death on its own trash. Many other drugs and BRMs are used or in development for multiple myeloma.

Waldenstrom[6] macroglobulinemia is a form of immature plasmacytoma which secretes IgM. Some patients have severe complications from **high serum viscosity**, and thick blood does not clot well so chronic bleeding at the mucous membranes is seen. This condition is reported to respond well to treatment with rituximab, an anti-CD20 monoclonal (CD20 being a common B cell surface marker). Most multiple myeloma patients do not respond significantly to rituximab, probably because CD20 expression is greatly reduced on mature plasma cells. In July 2015 the FDA approved ibrutinib, a Bruton tyrosine kinase inhibitor, for the treatment of Waldenstrom; phase III trials had been impressive (ibrutinib is currently in 195 different trial protocols for a variety of B cell malignancies.)

[3] Denis Burkitt, Irish physician, 1911-1993.
[4] Denosumab, the monoclonal against RANKL, seems like a natural drug for myeloma, but safety concerns have prevented its approval so far, though Phase III trials continue.
[5] Henry Bence Jones, English physician and chemist, 1813-1873.
[6] Jan G. Waldenström, Swedish physician, 1906-1996.

WHO: Mature T-Cell and Natural Killer Cell Neoplasms

(19 main headings). A selection:

a. T cell prolymphocytic leukemia
b. T-cell **large granular lymphocytic** leukemia
c. Aggressive NK-Cell leukemia
d. Adult T cell lymphoma/leukemia (HTLV1+)
e. Extranodal NK/T-cell lymphoma, nasal type
f. Enteropathy-type T-cell lymphoma
g. Hepatosplenic gamma-delta T-cell lymphoma
h. Subcutaneous panniculitis-like T-cell lymphoma
i. Mycosis fungoides/Sézary syndrome
j. Anaplastic large cell lymphoma, T/null cell, primary cutaneous type
k. Peripheral T cell lymphoma, not otherwise characterized
l. Angioimmunoblastic T cell lymphoma
m. Anaplastic large cell lymphoma ALK + or -

WHO: Post-transplant lymphoproliferative disorders (6 classes)
WHO: Histiocytic and dendritic cell neoplasms (9 classes)

T CELL MALIGNANCIES.

The **Sézary**[7] **syndrome** and the closely related cutaneous T cell lymphomas (WHO prefers *mycosis fungoides* but it's less used in the USA and Canada) are T cell tumors that primarily affect the skin. Many cases are associated with the human T-lymphotropic retrovirus HTLV-1. These viruses are also implicated in the very common adult T cell leukemia of the Far East, and are beginning to be seen in the U.S. in drug injecters. The intense inflammatory response that is often seen in the skin suggests that these $CD4^+$ malignant cells can be activated and secreting lymphokines. They have markers of memory T cells.

CANCER STEM CELLS. The leukemias are an especially good model for looking at malignant stem cells, as the normal stem cells are familiar and there exist good surface markers for studying them. Working with leukemias led investigators to suspect that there must be a minor population in a tumor that is truly malignant. In chronic lymphocytic leukemia, for example, the cells in the blood have a nearly normal phenotype and dividing cells are rare; furthermore, they will not give rise to leukemia in immunodeficient mice. But a very small fraction of them may have that property, and the first cancer stem cell was described in acute myelogenous leukemia; it represents only about 0.1% of the total leukemic cell population. The rest of the cells are to a greater or lesser extent differentiated. We understand now that most of our cancer therapies are directed against the differentiating cell compartments, which have surface markers we exploit (like the tumor antigen Her2/Neu) or are in cell cycle; whereas true stem cells divide rarely and in terms of markers are "lineage negative." This probably explains why treatments are commonly only partly effective, and relapses, even after years, are frequent.

[7] Albert Sézary, French dermatologist, 1880-1956.

NEW DIRECTIONS IN IMMUNOLOGY

WHAT'S NEW? Where do you look for the latest, especially if you don't want to read the entire world's primary literature in about 150 immunology journals?

> For medical people and those interested in human immunology, the best nonspecialist sources of updated immunology information are the **New England Journal of Medicine** and **JAMA**. They have frequent reviews of basic science as it applies to clinical medicine, and as you'd expect many of these deal with immunology.
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> No graduate student can afford not to read **Nature**, regardless of the field she or he is in. Especially important is the front material, News and Views, in which hot new topics are discussed as or before they are published. **Science**, the equivalent American journal, is equally good, and most of the job ads are North American.
>
> If you need a review article, look up **Advances in Immunology, Annual Review of Immunology, Trends in Immunology, Immunological Reviews**, and **Current Opinion in Immunology** in the Library. On the web trust .gov and .edu but avoid .com sites, and use your judgement on .org.
>
> And if you don't already know it, learn how to use **Medline** at its user-friendly interface, **PubMed**, at http://www.ncbi.nlm.nih.gov/pubmed?holding=uchsclib from Anschutz Medical Center computers.
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> And wherever you go, you can always call, write, or e-mail JJC; I'm always happy to hear from you. The course website at http://immuno4ever.org is always available and updated whenever I get a moment.

BEHAVIORAL IMMUNOLOGY. This term refers to the study of the connections between the immune system and the brain. There are many, and they go both ways. It's a new and growing area, not highly regarded by traditional immunologists, at least not yet. There is plenty of evidence linking behavior and diseases of the immune response, as well as stress and personality. Much of the evidence is weak, though, and this area needs good research. [The big reference book: Psychoneuroimmunology. Robert Ader, ed., Academic Press, 4th Edition, 2011]

One illustrative experiment, just to give you a taste of this interesting field: Robert Ader showed that you could condition immunosuppression, the way Pavlov conditioned salivation. He gave mice an injection of the immunosuppressive drug cyclophosphamide, and coupled it with a novel stimulus, saccharin, in their drinking water. Of course, such mice make a poor (suppressed) response to antigen given shortly after the cyclophosphamide. If rested a month, and then given another antigen injection and saccharin to drink, the mice made a poor antibody response, even though no further cyclophosphamide had been given. Mice given cyclophosphamide alone, and then a month later given antigen and saccharine, made a normal response (this is the necessary control for residual cyclophosphamide effect) [Ader, R. Conditioned immunomodulation: research needs and directions. Brain Behav Immun. 2003 Feb; 17 Suppl 1:S51-57]. Had Ader shown that you can condition side effects? Does this work in people, or only in mice? I find this actually very interesting, do you?

T CELL RECEPTOR FAMILIES. By way of review: The T cell receptor for antigen (TCR) is made up of two chains, α and β, linked to each other by disulfide bonds. Both α and β chains are inserted in the plasma membrane.

Alpha chain genes are made up by recombining V and J regions, and beta chain genes by V, D, and J genes. Beta chain V genes can be assigned to "families". This means that there are groups of Vβ genes that are closely related to each other, but less closely related to other Vβ genes. From a practical point of view, DNA probes can be made that hybridize only with the members of one Vβ family, so that the particular Vβ family that any given T cell is using can be identified by looking at which probe hybridizes with the beta-chain mRNA in that cell. Approximately 20 Vβ families have been identified in the human.

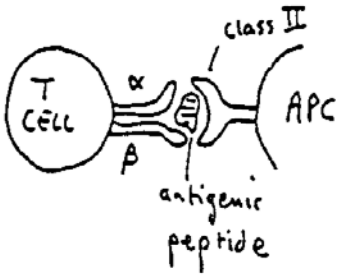
Sequence analysis shows that the members of any Vβ family have different hypervariable regions, especially CDR3, the one most responsible for interacting with antigenic peptides. They are similar mostly in "framework" amino acids, which must be the ones that the family-specific antibody recognizes. This probably means that the 20 families arose by some terrific gene duplication event long ago, so that one primitive Vβ became about 20. These all had plenty of evolutionary time to become quite different from each other, and sometimes to duplicate again, creating families of related V regions. (Some of the original 20 duplicates seem not to have duplicated much again, so we have a number of single- or few-member "families".)

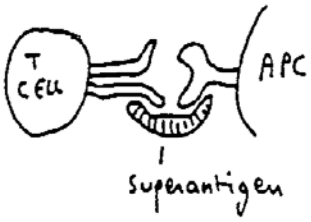
In chronic beryllium disease, several anti-beryllium T cell families can be identified in the blood of most patients, but only one or two of these are able to actually cause serious tissue destruction; they are the ones found in the lungs. Will this help us design therapies that eliminate only the "bad clones"?

The Vβ families remind us that to get T cell immunopathology you need T cell activation, and that depends critically on the trimolecular complex of TCR, peptide, and MHC. We think a lot about unusual MHC alleles that can present self or modified self or certain otherwise-ignored foreign peptides, but we must remember that even if you have that peptide in MHC, you still need a T cell receptor that can recognize it; and thus both the T cell and the MHC are equally important, and both may be therapeutic targets.

SUPERANTIGENS. Microbes make a number of proteins that, when added to T cells isolated from normal blood, cause proliferation and differentiation of many cells. Mitogens, you say? Well, the difference was that not *all* T cells responded; the percentage was variable, from about 3% to maybe 20%. That's way too high for antigens, but low for mitogens, which usually stimulate close to 100% of T cells. These new molecules have the extraordinary, in fact mind-boggling, ability to bind both to MHC Class II molecules, and to the "framework" part of the V region of the β chain of only certain Vβ families (the particular families involved being different for each different ***superantigen***). Look at the picture (next page) to distinguish the essential difference between ordinary antigens and superantigens:

Note that the superantigen binds to amino acids on the framework, not the CDR, of the T cell receptor; but it efficiently bridges the TCR and the antigen-presenting cell, and the result is that the T cell becomes stimulated as if it was recognizing antigen.

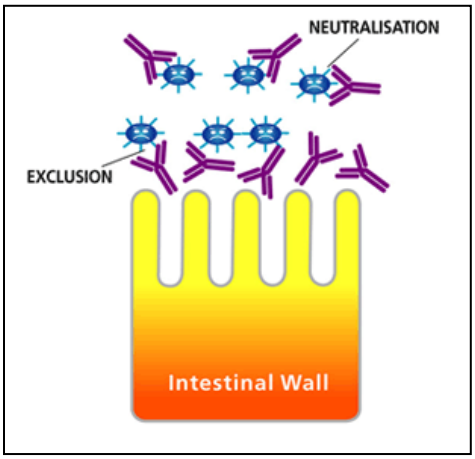




Now, what might this mean for us? Suppose that a bacterium, say *Staph. aureus*, made a superantigen that it released into the blood stream during infection, and that that superantigen bound to TCRs that used members of the Vβ8 and Vβ14 families, and also suppose that together those two families comprise 20% of your T cells. Therefore 20% of your T cells might suddenly become activated, divide, and release lymphokines. This is *much* more than would ever happen in a "normal" immune response. What does, say, too much IL-2 in the system do? It causes a dreadful vascular leakiness syndrome, and shock ("**cytokine storm**"). This is what is believed to have happened to Jim Henson, the Muppeteer, after a minor Staph infection: he went into irreversible shock, which no one knew how to treat (you might want to speculate on how *you'd* consider treating it). It is also what happens in **Toxic Shock Syndrome**; the toxic shock toxin (TSST-1) has been clearly shown to be a superantigen.

At a less dramatic level, chronic bacterial infections like sinusitis or periodontal disease have been associated with a variety of superantigens; these might worsen or prolong inflammation.

TRAVELAN®. An Australian company, Immuron, specializes in immunizing pregnant cows with vaccines for human diseases, and then collecting the colostrum (the milk made in the first ~24 hours after delivery.) Colostrum in cows and humans is very rich in IgA; calves will not live if they don't get colostrum, as Bossy does not pass IgG across the placenta. Fortunately, dairy cows produce a huge excess of IgA, far more than the calves need; the rest can be purified and put into capsules for human oral use. Their first drug, available over the counter in Australia since 2005, is Travelan, from cows immunized with enterotoxigenic *E. coli*, endotoxin, and flagellin; it has been shown to prevent and treat traveler's diarrhea. Immuron have other colostrum products in the pipeline; they talked, a little wildly[8], about products that could be slimed onto surfaces to neutralize microorganisms after a bioterrorism attack.



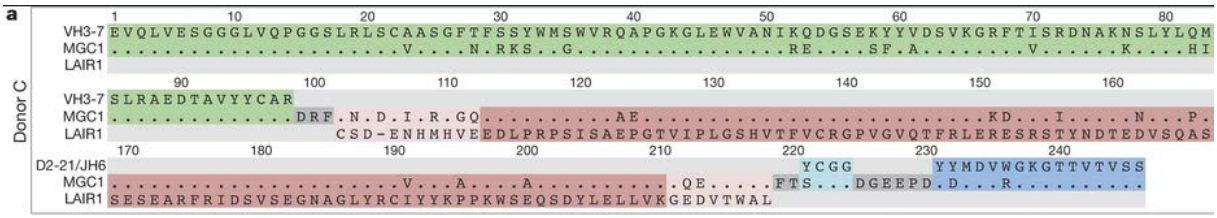
[8] Considering that Clorox would be more effective, cheaper, and could be applied while the Travelan guys were still calling the farm in Woop ("G' day mate, milk the smallpox cow!")

A SUICIDE SWITCH IN TRANSPLANTED CELLS [9]**.** Sometime, when cells are transferred to a human recipient, you end up wishing you hadn't. The example that is most familiar to us in this course is the induction of graft versus host disease after bone marrow or even stem cell transplantation. Malcolm Brenner's group at Baylor published a fix for this situation. In patients who were given T cells along with haploidentical hematopoietic stem cells (which seem to need some T cell urging to reconstitute the recipient, and to cause the "graft versus leukemia" effect), they loaded the T cells with a transgene that created a form of the apoptosis executioner caspase, caspase-9, that could be activated if the patient were given a harmless drug. "A single dose of dimerizing drug, given to four patients in whom GVHD developed, eliminated more than 90% of the modified T cells within 30 minutes after administration and ended the GVHD without recurrence."

This approach is reminiscent of the more-recent "on-off" switch designed for chimeric antigen receptors in tumor immunology.

AN EXTRORDINARY ANTIBODY. Antibodies that can bind to red cells infected with malaria are usually weak and don't lead to the destruction of the red cell and its parasite cargo. A European group[10] isolated some monoclonal antibodies from the B cells of infected people which were highly active against infected cells, binding a malarial antigen called RIFIN. When they sequenced the VH gene in these antibodies, they got a surprise:

"Here we report the isolation of human monoclonal antibodies that recognize erythrocytes infected by different *P. falciparum* isolates and opsonize these cells by binding to members of the RIFIN family. These antibodies acquired broad reactivity through a novel mechanism of insertion of **a large DNA fragment between the V and DJ segments**. The insert, which is both necessary and sufficient for binding to RIFINs, encodes the entire 98 amino acid collagen-binding domain of LAIR1, [a normal human collagen-binding protein.] In each of the two donors studied, the antibodies are produced by a single expanded B-cell clone and carry distinct somatic mutations in the LAIR1 domain that abolish binding to collagen and increase binding to infected erythrocytes. These findings illustrate, with a biologically relevant example, a novel mechanism of antibody diversification by interchromosomal DNA transposition and demonstrate the existence of conserved epitopes that may be suitable candidates for the development of a malaria vaccine."



KEY V_H amino acid sequence. Positions 1-98: V segment. 99-112: 3 junctional a.a's and a LAIR1 intron. 113-210: LAIR1 exon. 211-218: second LAIR1 intron. 221-224: D segment followed by an N region. 235-250: J segment.

[9] Inducible Apoptosis as a Safety Switch for Adoptive Cell Therapy (2011) A. Di Stasi et al. *N Engl J Med* 365:1673-1683. (3 November 2011)

[10] A LAIR1 insertion generates broadly reactive antibodies against malaria variant antigens (2016) Joshua Tan et al. *Nature* 529:105-9.

Learning Objectives for Lymphoid Malignancies & New Ideas

1. Identify a malignant condition in which the cells involved resemble:

activated T cells
resting B cells
activated B cells secreting IgG
activated B cells secreting IgM

2. Discuss the events thought to be necessary for the development of Burkitt lymphoma, including: the virus involved, the nature of the chromosomal translocation, the role of malarial or other infection.

3. Define superantigen, and distinguish it from a mitogen and an antigen in terms of mechanism and approximate fraction of T cells stimulated by each.

4. The vast majority of leukemias and lymphomas occur in cells of the B lineage. Discuss the most likely reasons for this.