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 2017 USA incidence is estimated to be 62,000 cases of leukemia, 80,000 of lymphoma, and 30,000 of myeloma. Of all new cancers, 9.7% will be leukemia, lymphoma, or myeloma; the prevalence is 1.3 million.

When lymphoid cells undergo malignant transformation, the predominant cell type usually exhibits a phenotype similar to that of a normal cell, and we can classify the leukemia on this basis. The Revised European-American Classification of Lymphoid Neoplasms¹ (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 information. We’ll discuss a few of the more immunologically-interesting conditions.

To begin with, lymphoid malignancies are classified as either Hodgkin² or Non-Hodgkin.

<table>
<thead>
<tr>
<th>WHO: Hodgkin lymphoma (Hodgkin’s disease)</th>
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<tr>
<td>a. Nodular lymphocyte predominant Hodgkin lymphoma</td>
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<td>b. Classical Hodgkin lymphoma</td>
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<td>• Nodular sclerosis classical Hodgkin lymphoma</td>
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<td>• Lymphocyte-rich classical Hodgkin lymphoma</td>
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<td>• Mixed cellularity classical Hodgkin lymphoma</td>
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<td>• Lymphocyte-depleted classical Hodgkin lymphoma</td>
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HODGKIN LYMPHOMA centers on a 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 well to the anti-PD-1 monoclonal antibody nivolumab. Brentuximab vedotin (Adcetris™) is an immunotoxin anti-CD30, which is expressed on Reed-Sternberg cells; the mAb is linked to the toxic auristatin E by a protease-cleavable linker.


² Thomas Hodgkin, 1798-1866, English pathologist.
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: Precursor lymphoid neoplasms

Precursor B-cell neoplasms:
- Precursor B-lymphoblastic leukemia/lymphoma
- Precursor T-lymphoblastic leukemia/lymphoma

WHO: Mature B-cell neoplasms

(37 main headings). A selection:
- B-cell chronic lymphocytic leukemia / small lymphocytic lymphoma
- B-cell prolymphocytic leukemia
- Lymphoplasmacytic lymphoma (includes Waldenstrom)
- Splenic marginal zone B-cell lymphoma (+/- villous lymphocytes)
- Hairy cell leukemia
- Monoclonal gammopathy of undetermined significance (MGUS)
- Plasma cell myeloma/plasmacytoma (Multiple myeloma)
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type
- Nodal marginal zone lymphoma (+/- monocytoid B-cells)
- Follicular lymphoma
- Mantle cell lymphoma
- Diffuse large cell B-cell lymphoma, Germinal center and activated B cell types
- Burkitt lymphoma

B CELL NEOPLASMS. Most Non-Hodgkin lymphomas are of B cell type, and most of the rest are T cell type. 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. Because B cells use the DNA mutating AID (activation-induced cytidine deaminase) in both class switching and somatic hypermutation, they are more susceptible to off-target mutation than T cells, which do neither process.

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 slg+ 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. The leukemia responds well to purine analog drugs; sometime the spleen is so large it must be removed.

**Burkitt** is a solid tumor of B cells, quite common in Africa. The endemic African form 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. (HIV-associated and sporadic Burkitt are less strongly-linked to EBV.) Burkitt responds well to chemotherapy, with 70-80% long-term survival.

**Multiple myeloma** is a malignancy of a clone of activated B (plasma) cells, which produce not only immunoglobulin but also several osteoclast-activating cytokines including RANKL which are responsible for extensive bone lesions. 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* 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. Elotuzumab (against CS1/SLAMF7) and daratumumab (against CD-38) preferentially target myeloma cells.

**Waldenstrom** 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. Waldenstrom 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. Ibrutinib is currently in 237 different trial protocols for a variety of B cell malignancies.

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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.
T CELL MALIGNANCIES.

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. Extramedullary 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 -

The **Sézary** 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. They have markers of tissue-resident memory T cells. 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 seen in the U.S. in drug injectors. The intense inflammatory response that often accompanies the skin invasion suggests that these CD4+ malignant cells can be activated and secreting lymphokines.

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

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, while the majority of abnormal cells are actually no longer dividing. 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. 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. In acute myelogenous leukemia (AML), Craig Jordan (now at AMC) showed that Bcl-2 is upregulated in the small population of leukemic stem

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8 A good analysis (2015) of The Cancer Stem Cell Gamble: http://science.sciencemag.org/content/347/6219/226.full
cells. His group showed these cells were wiped out by exposure to a Bcl-2 inhibitor. Bcl-2 is a well-known inhibitor of apoptosis, but in this case it seems to work by blocking oxidative phosphorylation, which, surprisingly, leukemic stem cells use, while normal HSC prefer glycolysis for energy. Dan Pollyea (also at AMC) and others showed stunning survival, and possible cures, in previously untreatable elderly patients with AML using a Bcl-2 inhibitor, venetoclax.\(^9\)

**OTHER 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](https://www.nejm.org) and [JAMA](https://www.jama.com). They have frequent reviews of basic science as it applies to clinical medicine, and as you’d expect many of these deal with immunology. No graduate student can afford not to read [Nature](https://www.nature.com), 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](https://www.sciencemag.org), the equivalent American journal, is equally good, and most of the job ads are North American.


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](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](https://www.aderlab.com)

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?

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AN EXTRAORDINARY TRANSPLANTATION ACHIEVEMENT. A 7-year old boy had junctional epidermolysis bullosa, which had denuded 80% of his skin surface. Some surviving skin was cultured and transformed with a retroviral vector expressing the full-length LAMB3 cDNA, the gene that is truncated in this condition\textsuperscript{10}. Enough skin was grown to cover most of his surface. Two years later the boy is in public school, and plays soccer; without this treatment he would surely have died. Aside from being a miraculous result, this story raises a question: full-length LAMB3 would have been foreign to his immune system. Why was the skin not rejected?

TRAVELAN\textsuperscript{®}. An Australian company, Immuuron, 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 \textit{E. coli}, endotoxin, and flagellin; it has been shown to prevent and treat traveler’s diarrhea.

SHINGRIXTM. The “HZ/su” or GSK1437173A varicella zoster vaccine (Shringrix\textsuperscript{TM}) is a subunit vaccine manufactured by GSK containing 50 μg of recombinant VZV glycoprotein E (gE) formulated with AS01B adjuvant. It was developed based on preliminary work here at CU (as was the first VZV vaccine, Zostavax). It’s so good that the CDC recommends it for everyone over 50, even if they’ve had the older vaccine. And it’s almost impossible to find for sale, the demand is so great!

FLU NEWS. The constantly-varying antigens that we respond to in our yearly flu shots are located on the bulb at the end of the trimeric hemagglutinin “mushroom” spike that protrudes from the virus. Other flu antigens, that don’t vary, are on other proteins—M and NP—as well as on other parts of the mushroom. The Israeli vaccine maker BiondVax says these antigens are common to all 40,000 flu isolates in the NIH database. The company has been conducting trials in Europe of M-001, a peptide vaccine with 9 highly conserved epitopes. It has done well in Phase 1 and 2 trials, and entered a Phase 3 trial in 2018 in Eastern Europe. The NIH began a Phase 2 trial in the US in May\textsuperscript{11}.

\textsuperscript{10} Regeneration of the entire human epidermis using transgenic stem cells. 2017. T. Hirsch et al. https://www.nature.com/articles/nature24487
\textsuperscript{11} https://clinicaltrials.gov/ct2/show/NCT03058692
FAR OUT FLU NEWS. This just in: A group from Scripps in La Jolla and Janssen in the Netherlands and Belgium have described a novel approach to flu prevention. They identified 2 epitopes on the influenza A spike, and 2 on the influenza B spike, that are highly conserved. They then immunized llamas with peptides containing these epitopes, and isolated 4 specific antibodies. Llama antibodies are single-chain, ending in a Vh domain. The 4 domains were stitched together into a “multidomain antibody,” that was then inserted into a harmless (?) adeno-associated virus vector. This, when squirted up the noses of mice, really looks like it worked against any flu strain. For example (figure) the nasty 1968 Hong Kong virus\(^\text{12}\). How long would the effect last, I wonder, in people?

Above: On the left, the 4 Vh regions of the 4 antibodies; in the center, the 4 fused to a human IgG1 Fc (this is the multidomain antibody the used in their in vivo mouse protection assays.) On the right, the familiar structure of a murine antibody to one fly epitope.

SUPERANTIGENS. Common bacteria, including Staph and Strep, make a variety of virulence factors called superantigens. They have the surprising ability to bind to both the TCR and Class II MHC, outside the regions containing the CDRs and the peptide-binding groove, in such a way that the T cell receives a strong activation signal, no matter what its antigen specificity is. In the figure, SEB is staphylococcal enterotoxin B. Since huge numbers of Th cells in the body can be stimulated, the result can be a horrendously cytokine release syndrome. One such syndrome is Toxic Shock, due to a Staphylococcal superantigen called TSST-1. It’s what killed Jim Henson, the Muppet inventor. Superantigens are involved in many infectious syndromes.

Each superantigen prefers to bind TCRs of particular groups based on sequences in the framework around the CDR. Thus one might activate T cells of the \(V\beta8\) and \(V\beta14\) families. But that could be 20% of all your Th cells!

\(^{12}\) http://science.sciencemag.org/content/362/6414/598.long
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.