Low Grade

B-cell Lymphomas

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This presentation on low grade B-cell lymphomas is meant to provide a general overview of the following:

1. Grading lymphomas: why do we grade lymphomas, and why is it important.
2. Categories: what are the common names/types of low grade lymphomas
3. Normal Lymphoid cells: what are their features?
4. What are characteristics of abnormal lymphoid cells?
5. Examples: at the end of this presentation are a few examples of the immunophenotype of some common low grade B-cell lymphomas.
Lymphomas can be graded as low grade, with slow indolent growth over many years, or intermediate or high grade, with more rapid growth and more aggressive behaviour.

Low grade lymphomas usually have small cells, with rare mitotic figures and few cells "in cycle", that is dividing. In contrast, high grade lymphomas often have large, pleomorphic cells, numerous mitotic figures, and a significant fraction of cells are "in cycle" (which can be demonstrated by Ki-67 immunohistochemistry). This greater fraction of dividing cells accounts for the more rapid growth of the lymphoma.

The focus of this presentation will be on the less aggressive, low grade lymphomas.
WHO classification designates multiple categories of B-cell derived lymphoid neoplasia. Listed here are a small subset of that group, comprising the Mature B-cell derived lymphoid neoplasia, many of which are low grade processes.

Some examples and common features of circulating B-cell lymphoproliferative disorders are:

- Indolent course
- Adult/elderly population
- Proliferation and accumulation of clonal, mature B-lymphocytes

The focus of the following slides will be to take a look at some key flow cytometric characteristics (size of cells, immunophenotype, intensity of various surface markers) used to differentiate among B cell neoplasias.
B-cell lymphoid neoplasia usually presents in one of two ways: 1) as a tissue based lymphoma, or 2) as a circulating lymphoproliferative disorder. Common sites of involvement for each are shown here.
In order to identify the abnormalities that characterize B-cell neoplasia, it is important to understand the immunophenotype of normal B-cells in blood, bone marrow, and lymphoid tissue. There are subtle variations between the three.
Normal B cells in the bone marrow represent a maturational continuum from the earliest progenitor stage to an antigen non-exposed mature B cell ready to be released to the periphery. In this dot plot, the arrow denotes the “direction” of maturation in relationship to the expression of CD10 and CD20. B-cell surface marker expression levels change depending on the B cell maturation stage: the most immature B cells are shown here as CD10 positive, CD20 negative progenitor B cells (shown in red). With maturation, B cells progressively acquire increased CD20 expression on their surface, while losing CD10 expression (shown in green). The most mature B cells in the bone marrow are CD20 positive and CD10 negative. (shown in navy blue).

As the mature B cells leave the bone marrow, they home to peripheral lymphoid tissues, such as lymph nodes, spleen, or mucosa associated lymphoid tissue where upon exposure to antigen they can undergo further expansion ultimately differentiating to memory B cells or plasma cells producing antibodies.
Normal Lymphoid Tissue
This is a schematic diagram of a cross section of a normal lymph node. Lymph nodes have defined architecture with different types of cells present in the various compartments. Primary follicles, which in a reactive lymph node persist as mantle zones surrounding the germinal centers, house more immature B cells, while germinal centers, often prominent in reactive lymph nodes, contain rapidly proliferating B cells, some of which are selected to become memory cells and plasma cells. The nature of the lymph node is fluid and dynamic; lymphoid cells are mobile, and may move between different chains of lymph nodes via the lymphatic system, or circulate into the blood via the lymph node vasculature.
Just as the different cellular compartments comprising the architecture of a reactive lymph node architecture can be identified morphologically, these compartments can also be identified by flow cytometry, by examining their size (forward scatter properties) and differential expression of surface markers.

Thus, T cells present in the paracortex, are small and do not express CD20 surface molecule, B cells in the Mantle zone are similar in size to T cells on the forward scatter axis, but express moderate levels of CD20 surface marker, and finally the germinal center B cells are larger in size and brightly express CD20. (see differential CD20 stain intensity on immunohistochemistry and flow cytometry plot)
Mixed populations of B cells, expressing different levels of B cell markers on their surface, are present in reactive lymphoid tissue. They can be visualized by flow cytometry using the forward scatter properties (for size) and B cell surface markers such as CD19 and CD20.

CD19 is a commonly used B-cell marker; however, when examining non-neoplastic lymphoid tissue, the use of CD20 is particularly advantageous in discriminating between the different B-cell compartments (germinal center cells, mantle cells, paracortical T-cells), more so than CD19. Germinal center cells are easily distinguished by CD20, as they are the large B cells found in reactive lymphoid tissue with bright CD20 expression. CD19 does not highlight germinal center cells nearly as well. Such heterogeneity of size and staining intensities in non-neoplastic lymphoid tissues is normal.
Another marker that can be useful in looking at polyclonal B cells are kappa and lambda immunoglobulins. In a reactive context, one expects to find a mixed population, with some cells expressing kappa and some expressing lambda light chain, in approximate ratio of 3:1. Flow performed on reactive lymph nodes has a greater degree of complexity than peripheral blood flow since B cells found in germinal centers (CD20 bright expressing B cells) have lower level of Kappa and Lambda than cells found in the mantle zones (CD20 moderate cells). The dim expression of surface immunoglobulin is normal on germinal center cells. In cases of florid reactive follicular hyperplasia, the germinal center cell population can be quite dramatically prominent; it is important not to confuse the GC cells with large B-cell lymphoma, which may sometimes exhibit dim surface immunoglobulin.
Flow cytometry can also demonstrate presence of different immunoglobulin heavy chains on the surface of B cells in the peripheral blood and reactive lymphoid tissues. In peripheral blood, most of the circulating B cells have IgM and IgD on their surface (feature of naive, antigen non-exposed B cells), with very few IgG or IgA expressing B cells. In the reactive lymphoid tissues one can find not only a large population of naive CD20-moderately expressing B cells which also express IgM and IgD (found mainly in mantle zones) but also large CD20-bright B cells which have undergone class switching in the germinal centers and now can express IgG on their surface. IgA positive B cells, similar to peripheral blood, are rare in the reactive tissues.
Other surface markers that can be exploited in visualizing normal B cell populations by flow are CD23 and CD10. In the peripheral blood, B cells show a spectrum of CD23 expression and they are negative for CD10. In reactive lymphoid tissue, however, the CD20 moderate B cells which populate mantle zones are CD23 positive, while CD20 bright cells, populating the germinal centers, are CD23 negative. In contrast, CD20 moderate B cells are negative for CD10, while germinal center cells with bright CD20 are also positive for CD10. Normal B cells, in general, are negative for CD5. Both peripheral blood and reactive lymphoid tissue, however, have a sizeable population of admixed CD5 positive T cells.
Some of the important markers that can be utilized in identifying neoplastic B cells by flow are listed here. Most of them are surface markers with “Cluster of Differentiation” or “CD” designation. Another surface marker, most useful in determining clonality of tested population, are surface light chain immunoglobulins (slg) Kappa and Lambda. In addition, intracellular molecules, such as Zap 70 or Bcl-2, can be assayed by flow by staining permeabilized cells with appropriate reagents. Finally, T cell markers can be aberrantly expressed in B cell lymphomas. The next few slides will present the typical immunophenotype of select B cell lymphomas with flow cytometry dot plot examples of each covered entity.
Different characteristics of cells may be explored to identify abnormal lymphoid cells by flow cytometry. Abnormally large cells have increased forward light scatter properties and cells with some surface or cytoplasmic complexity may have increased side scatter properties.

Abnormal expression of antigen (aberrant expression or abnormal antigen intensity) can characterize an abnormal population. Abnormal B-cells may even co-express T-cell or myeloid markers.

Finally, clonal expansion of Kappa or Lambda expressing B cells may be seen, and are an important feature.
Shown are examples of forward scatter/side scatter plots in small cell lymphoma and large cell lymphoma.

The large cell lymphoma cells have significantly greater forward scatter, which is easy to detect and which corresponds to their larger cell volume/size.
In hairy cell leukemia (HCL) the forward scatter and side scatter characteristics of the neoplastic population are slightly greater than those of a normal lymphocyte population, which corresponds to the slightly larger cell size and greater surface complexity of hairy cell leukemia cells. Typically, the side scatter characteristics of HCL cells are increased to the point that they are located in the same region by FSC and SSC that monocytes would be located, and often overlap with normal monocytes. This is an important feature to bear in mind when performing analysis to detect HCL.
Another feature of B-cell neoplasia is a population of B-cells showing expression of an antigen not found in normal B cells. Classic examples of B cell neoplasms with aberrant surface marker expression are mantle cell lymphoma, which has aberrant expression of CD5 on the B-cells (normally CD5 is expressed on T cells), and hairy cell leukemia, which has aberrant expression of CD103 (which is normally seen in a rare normal subset of T-cells in the gut).

Additionally, aberrant expression of myeloid markers and T-cell markers can be seen in B-cell lymphoproliferative disorders.
Some B cell neoplasms have characteristic decrease in some surface antigen expression levels. This property can be explored in flow cytometry to identify such abnormal proliferations.

For example, normal B cells show strong surface expression of B cell markers such as CD22 and CD20. In contrast, in chronic lymphocytic leukemia, the neoplastic cells have characteristically low level of expression of these markers. Characteristically, they are designated as CD22 dim/CD20 dim.
Shown here is an example of hairy cell leukemia, which expresses abnormally bright CD20 surface antigen.

This is in contrast to normal B cells, which have more moderate levels of CD20 on their surface.

In addition, hairy cell leukemia cells also show aberrantly bright expression of CD11c, while CD11c is only minimally expressed at moderate levels on a minor subset of normal B-cells.
Clonal expansion of a neoplastic cell population can be detected by flow cytometry as a B-cell population with homogeneous expression of some surface markers. Restricted expression of either kappa or lambda light chain in otherwise homogeneous population is highly useful to identify a clonal B cell process. Also, aberrant expression of some surface markers not found in normal populations or altered levels of expression of some markers can also be exploited in detecting aberrant neoplastic populations by flow cytometry.

In a normal reactive B cell population, there is generally a slight predominance of kappa- over lambda-expressing B-cells. Individual cases, however, may show an equal distribution of kappa and lambda expressing cells, or even occasionally a a slight lambda predominance. A marked predominance of either kappa or light chain expression may indicate the presence of B-cell neoplasia and should warrant further investigation and analysis.
Shown here in the top row are normal polyclonal B cells (as identified by CD20). They are polyclonal with respect to Kappa and Lambda light chain expression.

Shown in the bottom row is a small population of clonal B-cells (denoted by oval) that are present in a background of polyclonal B-cells. The clonal B-cells are identified by their brighter expression of CD20, and are kappa restricted.
Examples
Chronic lymphocytic leukemia (CLL) is a disease characterized by a proliferation of small mature lymphoid cells. CLL shows dim expression of many antigens. It has normal levels of CD19 expression, characteristically dim expression of CD20 and CD22, and dim to negative expression of FMC7 and CD79b. The CD45 expression is also notably less intense than that of normal lymphoid cells. Additional important markers that allow distinction of CLL from other small B cell neoplasms are CD5, CD11c and CD23, which are present in a typical case of CLL. Importantly, CLL is positive for CD11c and negative for Cyclin D1, features that distinguish it from Mantle cell lymphoma, which is Cyclin D1+ and typically CD11c negative. CLL cells are also negative for CD10 (a marker of germinal center origin lymphomas) and negative for CD103 (a marker useful in Hairy cell leukemia).
This is an example of CLL, which shows co-expression of CD5 and CD19 (top left). The cells show lambda light chain restriction (top middle, top right, bottom right); note the dim expression of lambda surface light chain. The CLL cells demonstrate dim expression of CD20 and CD22, and the dim, variable expression of CD11c (bottom left, bottom middle). CLL cells typically express CD23 (not shown).
Mantle cell lymphoma, similarly to CLL, is a CD5 expressing B cell lymphoma comprised of small cells. In contrast to CLL, however, all the B cell surface markers (CD19, CD20, CD22) CD79b, FMC7 and surface immunoglobulin light chain (sIg) are at moderate levels of expression. In addition, mantle cell lymphoma is positive for Cyclin D1. Typically, CD23 is negative or dim positive and CD11c is negative.
The cells in this example of mantle cell lymphoma also show low forward and side scatter. The lymphoid population gated based on forward scatter and side scatter in the top-most dot plot (Gate 1) includes the mantle cell lymphoma cells (shown in pink in subsequent dot plots) as well as normal small T lymphocytes (shown in blue in subsequent dot plots). Mantle cell lymphoma cells show strong expression of CD22, CD20, CD19, FMC7 and CD79b. They also express CD5. They are negative for CD23, in contrast to CLL. Clonal nature of the population is demonstrated in the bottom right dot plot, where all the mantle cell lymphoma cells are positive for Lambda light chain and negative for Kappa light chain.

Both CLL and MCL express CD19 at moderate intensity. Expression of CD20, however, while present in both, is lower in CLL (CD20 dim+) and higher in mantle cell lymphoma (CD20 moderate+). CD5 is expressed in both neoplasms, while CD23 is positive in CLL and negative in mantle cell lymphoma. Surface light chain restriction is clearly demonstrated in both MCL and CLL.
Follicular lymphoma can be differentiated from other entities by its expression of CD10 in addition to the B cell markers. In rare situations, such as follicular lymphoma of the skin, CD10 may be negative. Follicular lymphoma is negative for CD5, CD25, CD11c, CD103. B cell markers are positive, with characteristic dim expression of CD19.
Shown here are typical morphology and immunophenotype of follicular lymphoma cells. The cells are small and often show nuclear irregularity, indentation or even cleaved nuclei. They typically present as enlarged lymph nodes or masses, but can occasionally be found in the circulations (left column, upper and lower images). CD19 is characteristically dim, with co-expression of other B cell surface markers (CD20 and CD22) at moderate levels. CD10 is positive in follicular lymphoma, however, unlike B lymphoblastic lymphoma, CD34 is negative. Finally, this case of follicular lymphoma shows restricted surface light chain expression.
Hairy cell leukemia has a very characteristic, unique immunophenotype. It has very high level of expression of B cell markers: it is CD20 bright and CD22 bright. In addition, it shows bright expression of CD25, CD11c and surface immunoglobulin. CD103 is a characteristic feature. CD123 is typically brightly and homogeneously expressed. Typically HCL is negative for CD5, CD10 and CD23, although exceptions occur occasionally. However, the other above mentioned features are often so prominent that it is not difficult to distinguish a CD5 expressing HCL from a CLL or MCL.
Cells of hairy cell leukemia are small (see photomicrograph), but unlike the other small cell B cell neoplasms, they have somewhat more open, ground glass-appearing chromatin pattern, moderate amount of cytoplasm and villous, hair-like projections of their cell membrane (hence the name of the leukemia).

By flow cytometry, the HCL cells (denoted by oval) often have slightly increased FSC properties when compared to a typical lymphoid cell. Additionally, they consistently exhibit increased SSC properties than is seen for a typical lymphocyte; in fact, it is in the range of monocytes. The HCL cells are brightly positive for CD20 and CD22 (upper right dot plot). In addition, the cells co-express CD103, CD11c and CD25. Other markers positive in hairy cell leukemia include CD123 (lower right dot plot), which is brightly and homogeneously expressed. Surface immunoglobulin light chain is brightly expressed as well, in a restricted pattern.
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