·综　　述·

Flow cytometric detection of immunoglobulin light chain in hematolymphoid immunophenotyping

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关键词: flow cytometry;　immunophenotyping;　light chain restriction
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During B-cell development and maturation, the antigen receptor, which is encoded by the immunoglobulin heavy-(IgH) and light-chain genes, rearrange to associate one of a number of variable, diverse, and joining gene segments. A single mature B-cell expresses an IgH chain and either a kappa or lambda light chain, which is known as allelic or isotypic exclusion. In normal or reactive conditions, lymphoid cells comprise the mixtures of lymphocytes with either kappa or lambda expression. The normal proportion of kappa to lambda (κ/λ ratio) is within the range of 0.5－3.0 in peripheral blood or bone marrow and 1.2－2.7 in lymph nodes[1].

The most useful feature for diagnosing mature B cell neoplasm is light chain restriction or monotypic staining with κ or λ light chain. Currently, it is a common assumption that demonstration of light-chain restriction in a B-lymphocyte population is generally considered proof of monoclonality and indicates malignancy although monotypic B cell populations have been infrequently demonstrated in patients with no definitive evidence of B cell malignancy[2-4]. The most common flow cytometric analysis for determining B cell monotype is the percent κ and λ immunoglobulin light chains.

Because of the importance of light chain restriction in the diagnosis of B cell neoplasm, anti-immunoglobulin antibodies (e.g. anti-κ and anti-λ) are vital tools in the detection of monotypic B cell populations. Accurate determination of surface light chain expression depends on many factors, such as proper washing procedure, lysing solution, type of antibody used, specimen type or lymphoma type[5]. This article will discuss some common problems encountered in flow cytometric(FCM) determination of surface immunoglobulin light chain expression in hematolymphoid immunophenotyping.

1　No or minimal detectable surface light chains

Below are some common reasons why no or minimal detectable surface light chain expression is seen during routine hematolymphoid immunophenotyping.

1.1　Inadequate washing　Inadequate washing is one of the most common causes resulting in no detectable surface light chains. Native antibodies present in patient plasma and other body fluids bind to Fc receptors on the surface of many lymphoid cells and react with externally added anti-immunoglobulin reagents. This bound antibody is called cytophilic antibody. Because of cytophilic antibody, steps must be taken to make sure that light chain staining does not occur because of cytophilic antibody, but because the immunoglobulin is synthesized by and expressed on the surface of the B cells being studied[6].

Washing with phosphate-buffered saline or tissue transport medium (RPMI) before staining with anti-immunoglobulin reagents is required to remove residual plasma or cytophilic antibody. Because B cells generally have lower levels of Fc receptors than do monocytes, granulocytes, and some T cells, combining anti-immunoglobulin reagents with a pan-B antibody (such as anti-CD19) can help ensure that cytophilic antibody bound to the non-B cells will not interfere with analysis of the B cells.

1.2　Inappropriate pre-lyse procedure　Sometimes, harsh prelyse processes to remove red blood cells will injure the membrane integrity of lymphocytes and affect proper anti-immunoglobulin antibodies to bind to surface light chains. Brief incubation of treated cells for 20－30 min at 37 ℃ prior to addition of anti-immunoglobulin reagents will enable the antigen receptor to recycle back to the surface for proper detection.

1.3　Single set of light chain reagents used　Occasionally, neoplastic B cells may express light chain epitopes not readily detected by a single set of light chain reagents. Therefore, incorporation of two sets of light chain reagents into the panels results in the maximum sensitivity of detection of monoclonality. The monoclonal and polyclonal light chain reagents were of equal value in detection of monoclonality[6]. The anti-immunoglobulin reagents commonly used in US are listed in Table 1.

1.4　Lack of surface light chain in some lymphomas　After exclusion of above mentioned causes, absence or minimal expression of surface immunoglobulin light chains in mature B cell proliferation is usually indicative of a B cell neoplasm. The lymphomas commonly without detectable surface light chain expression are follicular lymphoma or diffuse large B cell lymphoma[7]. Other B cell neoplasms that often show no detectable surface light chain expression are plasma cell neoplasms, precursor B cell acute lymphoblastic leukemia/lymphoma and occasionally small lymphocytic lymphoma.

1.5　Benign conditions　Absence or minimal expression of surface immunoglobulin light chains of B cells is also seen in some benign conditions, which illustrate the importance of clinicopathologic correlation in assessing lymphoid proliferation. Normal B cell precursors (hematogones) in bone marrow usually have no detectable surface light chain expression. Occasionally, reactive germinal center B cells in florid follicular hyperplasia, particularly centroblasts will have low or no detectable

surface light chains. In addition, reactive B cells in effusion fluids often show no detectable light chains, likely due to interference of cytophilic antibodies in the body fluids.

Table 1　Anti-human immunoglobulin light chain reagents for FCM

Anti-κ			Anti-λ		
Clone	Isotype	Company	Clone	Isotype	Company
Polyclonal	IgG Goat	Coulter	Polyclonal	IgG Goat	Coulter
HP6062	IgG3 Mouse	Coulter	JDC-12	IgG1 Mouse	Coulter
TB28-2	IgG1 Mouse	BD	1-155-2	IgG1 Mouse	BD
G20-193	IgG1 Mouse	BD	JDC-12	IgG1 Mouse	BD
Polyclonal	IgG Goat	BD	Polyclonal	IgG Goat	BD
Polyclonal	IgG Rabbit	DAKO	Polyclonal	IgG Rabbit	DAKO
Polyclonal	IgG Goat	SEROTEC	FMC63/Mc24-IC6	Cocktail CD19/Lambda	SEROTEC
Polyclonal	IgG Goat	ACCURATE	Polyclonal	K/L cocktail	ACCURATE
HP6062	IgG3	Invitrogen	HP6062	IgG3	Invitrogen
HP6064	IgG2a	Invitrogen	HP6064	IgG2a	Invitrogen

2　Polytypic patterns of light chain expression

In some circumstances, light chain restriction may not be apparent even in the presence of malignant lymphomas.

2.1　Biclonal/bi-phenotypic lymphomas　It has been well documented that some low grade lymphomas, particularly chronic lymphocytic leukemia (CLL)[8-9] as well as marginal zone lymphoma[10] can exhibit biclonal or bi-phenotypic features, which is estimated approximately 1%－2% of incidences. If one clone expresses κ light chain and another expresses λ light chain, the κ or λ light chain restriction may not be apparent by flow cytometric analysis. Therefore, it is very important for the analyst to visually inspect flow plot data, particularly for those cases with high clinical suspicion. Two different clones may have different fluorescent intensities for light chain or CD19, CD20 antigen expressions. In addition, one should design antibody panels so that co-expression of tumor-specific antigens, such as CD5 in CLL can be used to determine monoclonality. In some difficult cases, monoclonality can be confirmed by molecular methods, such as polymerase chain reaction (PCR) or fluorescence in situ hybridization (FISH)[11]. It should be also noted that the neoplastic clones may evolve over time, from a κ predominant clone to a λ predominant clone or vise versa.

2.2　Composite lymphomas　Composite lymphoma is defined as the occurrence of two or more morphologically and immunophenotypically distinct lymphoma clones in a single anatomic site[12]. If one lymphoma expresses κ light chain and another expresses λ light chain, the κ or λ light chain restriction may not be apparent by flow cytometric analysis. This is the similar phenomenon as seen in biclonal or biphenotypic lymphoma. Again, one should look for other clues that may be suggestive of the presence of composite lymphoma, such as the intensities of light chain or CD19, CD20 expression. In addition, one can design antibody panels so that co-expression of tumor-specific antigens, such as CD5 in CLL and mantle cell lymphoma or CD10 in follicular lymphoma can be used to determine monoclonality. As described in bi-clonal of bitypic lymphoma previously, the monoclonality can also be confirmed by molecular methods, such as PCR or FISH[11].

2.3　Dual light chain expression　CLL and aggressive B cell lymphoma have been reported to exhibit dual surface immunoglobulin light-chain expression[13-14]. Therefore, recognition of dual κ/λ light-chain expression on B cells has diagnostic implications in hematolymphoid immunophenotyping. In the presence of dual light-chain expression, a κ/λ ratio will likely be a polytypic pattern, depending on the percent dual expressing cell population. However, the percentages of $\kappa^+/CD19^+$ plus $\lambda^+/CD19^+$ B cells will exceed the total percent $CD19^+$ B cells. This is analogous to the phenomenon seen in the case of T-cell leukemia/lymphoma expressing double $CD4^+/CD8^+$ immunophenotype, in which $CD4^+$ T cells plus $CD8^+$ T cells exceed total number of $CD3^+$ T cells.

The importance of the visual inspection of the flow data plot is critical, especially paying attention to not only the κ and λ ratio but also the sum of κ and λ expressing B cells, which is approximately equal to the total number of mature B cells. It is recommended that if the sum of $\kappa^+/CD19^+$ plus $\lambda^+/CD19^+$ expressing B cells is 10% greater than the total percent $CD19^+$ B cells or if the dual light-chain expressing B cell populations comprise more than 10% of the total number of mature B cells, molecular studies to identify monoclonality may be justified. In addition, when calculating an overall κ and λ ratio, it is reasonable to consider that the dual κ/λ-positive B cell populations are clonally related to the κ expressing B cells.

2.4　No specific binding　On the other hand, it is known that nonspecific antibody interaction through Fc receptors on the cell surface may occasionally produce the results suggestive of

dual light-chain expression. If that is the case, "blocking" the Fc binding sites with excess non-fluorescent-labeled IgG antibodies will reduce or eliminate the nonspecific antibody interactions.

References

[1] Reichard KK, McKenna RW, Kroft SH. Comparative analysis of light chain expression in germinal center cells and mantle cells of reactive lymphoid tissues. A four-color flow cytometric study[J]. Am J Clin Pathol, 2003, 119(1): 130-136.

[2] Kussick SJ, Kalnoski M, Braziel RM, et al. Prominent clonal B-cell populations identified by flow cytometry in histologically reactive lymphoid proliferations[J]. Am J Clin Pathol, 2004, 121(4): 464-472.

[3] Kroft SH. Monoclones, monotypes, and neoplasia pitfalls in lymphoma diagnosis[J]. Am J Clin Pathol, 2004, 121(4): 457-459.

[4] Chen HI, Akpolat I, Mody DR, et al. Restricted κ/λ light chain ratio by flow cytometry in germinal center B cells in Hashimoto thyroiditis[J]. Am J Clin Pathol, 2006, 125(4): 42-48.

[5] Clinical and Laboratory Standards Institute. H43-A2 Clinical cytometric analysis of neoplastic hematolymphoid cells, Approved Guideline-Second Edition[S]. Wayne PA: Clinical and Laboratory Standards Institute, 2007.

[6] Carey JL, McCoy P, Keren DF. Flow cytometry in clinical diagnosis[M]. 2nd ed. Chicago IL: American Society for Clinical Pathology, 2008.

[7] Kaleem Z, Zehnbauer BA, White G, et al. Lack of expression of surface immunoglobulin light chains in B-cell non-Hodgkin lymphomas[J]. Am J Clin Pathol, 2000, 113(3): 399-405.

[8] Hsi ED, Hoeltge G, Tubbs RR. Biclonal chronic lymphocytic leukemia[J]. Am J Clin Pathol, 2000, 113(6): 798-804.

[9] Gonzalez-Campos J, Rios-Herranz E, De Blas-Orlando JM, et al. Chronic lymphcytic leucemia with two cellular populations, a biphenotypic or biclnal disease[J]. Ann Hematol, 1997, 94(1): 135-137.

[10] Delville JP, Heimann P, El Housni H, et al. Biclonal low grade B-cell lymphoma confirmed by both flow cytometry and karyotypic analysis, in spite of a normal kappa/lambda Ig light chain ratio [J]. Am J Hematol, 2007, 82(6): 473-480.

[11] Chang H, Cerny J. Molecular characterization of chronic lymphocytic leukemia with two distinct cell populations: evidence for separate clonal origins[J]. Am J Clin Pathol, 2006, 126(1): 23-28.

[12] Demurtas A, Aliberti S, Bonello L, et al. Usefulness of multiparametric flow cytometry in detecting composite lymphoma: study of 17 cases in a 12-year period[J]. Am J Clin Pathol, 2011, 135(4): 541-555.

[13] Xu DS. Dual surface immunoglobulin light-chain expression in B-cell lymphoproliferative disorders [J]. Arch Pathol Lab Med, 2006, 130(6): 853-856.

[14] Fujiwara T, Ishizawa K, Kohata K, et al. Aggressive B-cell lymphoma with dual surface immunoglobulin light-chain expression [J]. Intern Med, 2007, 46(17): 1458-1461.

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·综　　述·

Principle of panel design, antibody selection, and fluorochrome choice for immunophenotyping of acute leukemia

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After working in the field of clinical flow cytometry for many years, I still continue wondering at its ever changing and evolving nature. Like everything else, clinical flow cytometry comes a long way, and I am sure will keep changing, improving, and evolving. Looking back, we see many changes made: new immuno-markers discovered, manufactured, and applied to clinical practice; new instruments developed and put into market; new recommendations proposed; and test methods standardized and improved. Along the way, the flow cytometry has gained undeniable popularity and has become a standard practice in diagnosing diseases, guiding and monitoring treatment in hematopoietic neoplasia such as leukemia and lymphoma.

1 Panel selection

Focusing on the topic of leukemia panel design, antibody selection and fluorochrome choice, I have reviewed a few well known published papers[1-4], and also the leukemia panels from our own flow lab, to get a better understanding of the rationality in why there is variation among clinical labs in panel design. Up to this point, there is not yet a standard panel for acute leukemia diagnosis regardless of many attempts of recommendations and proposals by international and national experts in the field. The reason for this variation I believe is because different labs operate under different circumstances, which lead them to take different approaches to reach the same goal of providing an accurate diagnosis. In general, there are three types of approaches in current practice: comprehensive panel, minimal screen panel, and directly disease oriented panel. Each has its pros and cons and has its rationality.

Comprehensive panel consists of large panel of reagents to include all cell lines and used in the diagnosis of both leukemia and lymphoma. It tends to be used in reference labs which usually do not have sufficient accessibility to patient information and morphology of the samples. This approach provides extensive immunophenotyping information, saves time to almost e-