Quantification of CD34 epitopes in AML (please read the reply ELT23)

Haematologica 2007; 88:(8)e110-e111

Although the conclusions drawn by Maynadié et al. in Haematologica (87: 795-803, 2002) are of interest, the methodology used to generate the data casts doubt upon them. Early studies[1] revealed that some epitopes of the mucin-like CD34 antigen were sensitive to cleavage with sialidase and O-sialoglycoprotein endopeptidase (OSGE) from P. haemolytica. Antibodies that bound to epitopes requiring the presence of negatively charged sialic acid residues were designated class I antibodies. Those reactive with epitopes resistant to sialidase but sensitive to OSGE were designated class II, while mAbs to epitopes insensitive to both enzymes were designated class III.[2] Class I mAbs fail to detect the glycoforms of CD34 expressed on some leukemias and leukemia-derived cell-lines[1,3] suggesting that some subsets of normal CD34+ cells may escape detection by class I mAbs. The lower avidity of class I mAbs and their inability to retain reactivity after conjugation with negatively charged fluorochromes, such as FITC, further reduces their utility in immunodiagnosis and the enumeration of CD34+ cells for transplantation.[4] More recent studies[4,5] demonstrated that the class II epitope is determined by the linear stretch of amino acids 10 through 16 flanked by two likely negatively charged (sialylated) O-glycosylated threonine residues at the far N-terminus of the molecule. Thus, QBEnd10 does not *investigate glycosylations* as claimed but rather detects an invariant epitope present on all glycoforms of CD34. Several studies have compared the efficiency of class II (QBEnd10) and class III (HPCA2) mAbs labeled with FITC and concluded that the class III reagents detected a greater number of CD34+ cells in acute leukemia samples, normal marrow and cytokine mobilized peripheral blood. These results and the conclusions drawn therefrom were made without taking epitope characteristics into account and confounded expectations based on detailed knowledge of the latter.[4,5] Furthermore, these studies (Maynadié *et al.*[6,7]) utilized unsophisticated single parameter flow methods and isotype controls to measure low-frequency CD34+ cells in samples. The location of the class II epitope between clusters of sialylated O-linked glycans has major implications for the use of negatively charged conjugates of class II mAb such as used by Maynadié et al. When phycoerythrin (PE) and FITC conjugates of class II and class III CD34 mAbs were compared on the same samples using state-of-the-art multiparameter flow cytometry, all except the class II FITC conjugates generated concordant data. Together, these studies emphasize the need to use not only a CD34 mAb clone that detects all glycoforms of this mucin-like molecule, but also one that retains high specificity and avidity of binding after conjugation to the chosen fluorochrome.[4,8] Through their use of QBEnd10FITC the authors may have arrived at incorrect results, i.e., erroneously low fluorescence (FL) intensities and percentages of blasts reactive with this conjugate. However, due to the time frame of their study, the authors may not have been aware of these issues. A second problem concerns the comparison of results obtained with an unlabeled class I CD34 mAb using indirect immunofluorescence (which amplifies the detection of bound mAb) with those of directly conjugated reagents. Consequently, comparing FL intensity data from two different techniques may not be valid. A third problem is formed by the assignment of molecules of equivalent soluble fluorochrome (MESF) FITC units to results of FL intensity measurements after calibration of the FITC scale using a system that is not spectrally matched with FITC (i.e., Immunobrite [Beckman-Coulter]). This approach would only have been valid if the Immunobrite beads had been calibrated against spectrally matched beads.[9] This calibration should have been performed on each individual instrument used in the study, as matching of spectra between calibrators and samples is required to normalize the responses to FL signals between the various instruments that may have barrier filters with different characteristics.[10] Maynadié et al. did not provide any information as to whether or not this approach has been followed. Finally, selection of malignant blasts using forward (FSC) and sideward (SSC) light scatter characteristics may be problematic on samples in which malignant blasts constitute a minority of the leukocytes. It was claimed (based on 18 samples with a reported blast percent of only 16+/- 29%) that AML M3 blasts or those with a t(15;17) translocation expressed higher levels of class III than class I CD34 epitopes. The use of CD45 vs. SSC *blast* gating would have allowed more reliable selection of AML blasts on such samples, as these are mostly CD45^{dim}, SSC.[low/intermediate; 4]

R. Sutherland,[1] M. Keeney,[2] J.W. Gratama[3]
[1]Department of Medical Oncology and Hematology, The University Health Network, Toronto, Canada; [2]Department of Hematology, London Health Sciences Center, London, Canada; [3]Department of Clinical and Tumor Immunology, Daniel den Hoed Kliniek, Rotterdam, the Netherlands.
Correspondence: D. Robert Sutherland, Department of Medical Oncology and Hematology, The University Health Network, Toronto, Ontario M5G 2M9, Canada.
Tel; (416) 946 2218 (office), 416 946 5591 (lab), Fax; 416 946 6546.

References

1. Sutherland DR, Keating A. The CD34 antigen: structure, biology, and potential clinical applications. Journal of Hematotherapy 1992; 1: 115-29.
2. Sutherland DR, Marsh JCW, Davidson J, Baker MA, Keating A, Mellors A. Differential sensitivity of CD34 epitopes to cleavage by Pasteurella haemolytica glycoprotease: implications for purification of CD34-positive progenitor cells. Experimental Hematology 1992; 20: 590-9.
3. Greaves MF, Titley I, Colman SM, Buhring H-J, Campos L, Castoldi GL, et al. Report on the CD34 cluster Workshop. In:

Schlossman S., et al eds. Leukocyte Typing V. Oxford University Press, 1995; 840-6.
4. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. Journal of Hematotherapy 1996; 3: 213-26.
5. Lanza R, Healy L, Sutherland DR. Structural and functional features of the CD34 antigen: an update. Journal of Biological Regulators and Homeostatic Agents 2001; 15: 1-13.
6. Lanza F, Moretti S, Castagnari B, Latorraca A, Rigolin GM, Bardi A, Castoldi G. CD34+ leukemic cells assessed by different CD34 monoclonal antibodies. Leukemia & Lymphoma 1995; 18 Suppl 1: 25-30.
7. Steen R, Tjonnfjord G, Gaudernack G, Brinch L, Egeland T. Differences in the distribution of CD34 epitopes on normal hemopoietic progenitor cells and leukaemic blast cells. British Journal of Haematology 1996; 94: 597-605.
8. Gratama JW, Keeney M Sutherland DR. Enumeration of CD34+ hematopoietic stem and progenitor cells. In: Robinson JP et al eds. Current Protocols in Cytometry. New York: Wiley & Sons 1999: Unit 6.4: 1-22.
9. Schwartz A, Marti GE, Poon R, Gratama JW, Fernandez-Repollet E. Standardizing flow cytometry: a classification system of fluorescence standards used for flow cytometry. Cytometry 1998; 33: 106-14.
10. Schwartz A, Fernandez Repollet E, Vogt R, Gratama. Standardizing flow cytometry: construction of a standardized fluorescence calibration plot using matching spectral calibrators. Cytometry 1996; 26: 22-31.