Haematology and Blood Transfusion Vol. 29
Modern Trends in Human Leukemia VI
Edited by Neth, Gallo, Greaves, Janka

Immunocytochemical Labelling of Haematological Samples Using Monoclonal Antibodies *

W. N. Erber, A. K. Ghosh, and D. Y. Mason

A. Introduction

Monoclonal antibodies have become increasingly important in the diagnosis and classification of neoplastic blood disorders. Immunofluorescent labelling of cell surface antigens has been the conventional method used for this purpose. However, this method of antigen detection presents a number of drawbacks when used in a routine hospital haematology laboratory: namely that blood or bone marrow samples must be processed within a short time of collection; antigen labelling is not permanent; and cell morphology cannot be visualised.

The present paper describes the method for labelling routine haematological samples by an immunoalkaline phosphatase technique which is used routinely in this laboratory for leukaemia phenotyping. This approach has several practical advantages over conventional immunofluorescent labelling, i.e. staining can be performed on routinely prepared bone marrow and blood smears, samples can be stored for long periods before labelling, cell morphology can be visualised simultaneously with the antigen label and the staining reaction is permanent. As detailed below a wide range of cellular antigens can be detected in marrow and blood smears using this technique.

* University of Oxford, Department of Haematology, John Radcliffe Hospital, Oxford OX3 9DU, UK

B. Methods

Immunoalkaline Phosphatase Labelling

Antigen labelling is performed by the APAAP immunoalkaline phosphatase technique as described elsewhere [1, 2]. The method (shown schematically in Fig. 1) involves primary incubation of blood or bone marrow samples with monoclonal antibody, followed by unlabelled sheep anti-mouse Ig and then monoclonal alkaline phosphatase: anti-alkaline phosphatase (APAAP) complexes. Each incubation step lasts 30 min and is followed by a brief wash in Tris-buffered saline (for 1–5 min). Amplification of the staining is possible by repeating the anti-mouse Ig and APAAP stages (for 10 min each). The alkaline phosphatase reaction is then developed (by incubation for 15 min) in a naphthol-AS-MX/fast red substrate. Slides are washed, counterstained with haematoxylin and mounted in an aqueous medium.

C. Results

Staining of blood and marrow smears using the APAAP technique yields a vivid red reaction on antigen-positive cells. The staining reaction contrasts clearly with the haematoxylin counterstain and there is no background staining of antigen-negative cells. The labelling reactions are equally strong whether they are performed on freshly prepared smears or on smears that have been stored at – 20 °C. The nature of

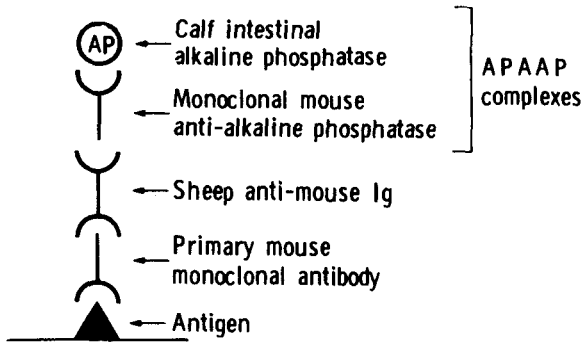

Fig. 1. Schematic representation of the APAAP immunoalkaline phosphatase procedure

the labelled cells can easily be determined because of the preservation of morphological detail.

D. Discussion

Cytological examination of peripheral blood and bone marrow plays a major role in the diagnosis of neoplastic blood disorders. The introduction of monoclonal antibodies in the late 1970s greatly aided the objective identification of cell types. Cellular antigens are conventionally detected by immunofluorescent staining of cells in suspension, however, this method does not enable antigen labelling and cell morphology to be visualised simultaneously.

Immunoalkaline phosphatase labelling of cell smears combines labelling of cellular antigens with visualisation of morphological detail. Preliminary mononuclear-cell separation and washing is unnecessary as staining can be performed on routinely prepared blood and bone marrow smears. Background labelling of the smears (as occurs with peroxidase labelling) is avoided, and the intense red reaction product is easy to visualise. Blood and marrow smears can be stored for long periods (at – 20 °C) prior to labelling. This enables samples to be analysed in batches and also facilitates retrospective analysis of stored cases (e.g. when it is necessary to test a new monoclonal antibody against a panel of different leukaemia cases). Smears sent by post from other hospitals also give staining reactions identical to those of samples processed fresh in this laboratory. Finally, the labelling reaction is permanent, allowing slides to be reviewed after a period of time.

Using these labelling procedures a wide range of cellular antigens, both surface and intracellular, can be detected (as noted in Table 1) in routinely prepared blood and bone marrow smears. APAAP labelling may thus be used to enumerate lymphocyte populations in peripheral blood smears and to detect abnormal T-helper/suppressor ratios [3]. This lends itself to mass screening, e.g. of blood donors. The technique is also very convenient for phenotyping cases of leukaemia and lymphoma. The markers used for this purpose include terminal transferase, HLA-DR, B- and T-cell antigens, and common ALL antigen.

Finally, the APAAP procedure is valuable for detecting metastatic malignant cells in bone marrow smears, since malignant cells can often be demonstrated which are not identifiable on routine haematological examination [4].

Table 1. Cell surface antigens detectable in cell smears by the APAAP technique

T-cell markers[a]	B-cell markers[b]
CD1 (T6)	p95
CD2 (T11)	IgM
CD3 (T3)	IgD
CD4 (T4)	HLA-DR
CD5 (T1)	p135
CD7	
CD8 (T8)	
Miscellaneous	
CALLA	
MY906 (Myeloid)	
IL2-receptor (Tac)	
HC1, HC2 (hairy cells[c])	
Glycophorin	
p150,95 (macrophages, hairy cells[d])	
gp Ib, IIb-IIIa, IIIa (platelet/ megakaryocytes)	
Terminal transferase	

[a] The T-cell markers are indicated according to the "CD" system introduced at the 1st Workshop on Leucocyte Differentiation Antigens (Paris 1982), with their alternative nomenclature in the OKT series shown in parenthesis

[b] p95 (CD19) and p135 (CD22) are B-cell-associated markers detected by, respectively, antibody B4 [5] and Tol5 (DAKO PAN-B)

[c] [6]

[d] [7]

Acknowledgments. This work is supported by grants from the Leukaemia Research Fund and the Cancer Research Campaign. W. N. Erber holds a Rhodes Scholarship.

References

1. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz A, MacDonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 32:219–229
2. Moir DJ, Ghosh AK, Abdulaziz A, Knight P, Mason DY (1983) Immunoenzymatic staining of haematological samples with monoclonal antibodies. Br J Haematol 55:395–410
3. Erber WN, Pinching AJ, Mason DY (1984) Immunocytochemical detection of T and B cell populations in routine blood smears. Lancet I:1042–1045
4. Ghosh AK, Erber WN, Hatton C, Falini B, O'Connor NJ, Osborn M, Mason DY (1985) Detection of metastatic tumour cells in routine bone marrow smears by immuno-alkaline phosphatase labelling with monoclonal antibodies. Br J Haematol (in press)
5. Nadler LM, Anderson KC, Marti G, Bates M, Park E, Daley JF, Schlossman SF (1983) B4, a human B lymphocyte-associated antigen expressed on normal, mitogen-activated, and malignant B lymphocytes. J Immunol 131: 244–250
6. Posnett DN, Chiorazzi N, Kunkel HG (1982) Monoclonal antibodies with specificity for hairy cell leukaemia cells. J Clin Invest 70:254–261
7. Falini B, Schwarting R, Erber WN, Posnett DN, Martelli MF, Grignani F, Zuccaccia M, Gatter KC, Cernetti C, Stein H, Mason DY (1985) The differential diagnosis of hairy cell leukaemia with a panel of monoclonal antibodies. Am J Clin Pathol (in press)