Clinical Applications of Molecular Haematology: Flow Cytometry in Leukaemias and Myelodysplastic Syndromes

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Abstract

Flow cytometry is semi-automated study of antigen profile of cells using the Scatchard principle of antigen-antibody binding and fluorochrome-based detection systems. Flow cytometric evaluation of cellular proteomics has become an integral part of the laboratory diagnosis and classification of haematopoietic neoplasms. Recent technical advances in lasers, monoclonal antibodies, fluorochromes, and computer-based color compensation algorithms have expanded the usefulness of flow cytometry. Detection of minimal residual disease by flow cytometry in leukaemias and lymphomas is incorporated in many treatment protocols. Finding of aberrant maturation pattern of granulocytes offers a sensitive screening tool for early diagnosis of myelodysplastic syndromes. Detailed proteomic analysis of leukemias is helping more precise prognostic and biological stratification.

INTRODUCTION

Initial flow cytometers were built for biological warfare during the world war II, to test the efficiency of filter masks in removing bacteria or fungi. The light source was the headlight of Ford model T automobile. When the research was declassified and published in 1947, a young engineer, Wallace Coulter promptly acquired a patent for his particle counting machine in 1953. The next major boost to the flow cytometer technology came in 1975, when Dr. George Köhler, a post-doctoral fellow working in Dr. Cesar Milstein’s laboratory in Cambridge, UK, accidentally discovered the hybridoma technic for manufacturing monoclonal antibodies. Now that the major technical hurdle was overcome, advances in laser, optics, hydraulics and computer technology have made flow cytometry cheaper and less labor/skill intensive technic. A search of National Library of Medicine database for word “flow cytometry” showed the number of publications rose from 0 in 1979 to over 7000 in 2006 (Fig. 1).

Flow cytometer records physical properties and fluorescence of cells (Fig. 2). If we use antibodies tagged with different coloured fluorochrome dyes, the flow cytometer can tell us if the appropriate antigens are present on the cells.

Flow cytometry has revolutionized how we diagnose, treat, prognosticate and monitor acute leukaemias.

However, its role in chronic myeloproliferative disorders and myelodysplastic states is still evolving.

Flow Cytometry in quantitation of blasts of acute leukaemia and myelodysplastic syndromes

In myelodysplasias as well as in early acute leukaemias, it is sometimes difficult to assess percentage of blasts, due either to blood contamination of the marrow specimen, or hypogranular morphology of the granulocyte precursors. Flow cytometric assessment of blasts is precise and correlated better with overall survival than morphologic blast count.

Flow cytometry in classification and subtyping of acute leukaemia

Roughly a quarter to a third of the leukemias can not be confidently diagnosed as myeloid or lymphoid by
morphology alone. Flow cytometry provides objective and unequivocal diagnosis in these cases. In addition to myeloid and lymphoid designation, flow cytometry also identifies the cases with expression of multiple lineages, enabling the assignment of biphenotypic leukaemia. Several international groups have defined criteria for biphenotypic leukaemia.

Flow cytometry is very useful in subtyping of acute leukaemias according to the WHO classification. Minimally differentiated acute myeloid leukaemias can only be identified by flow cytometry. Acute megakaryocytic leukaemias express platelet associated glycoproteins CD41 and 61, and von Willebrand/factor VIII antigens, although one should exclude platelet adhesion onto the blast cells. Pure acute erythroleukaemias may express alpha glycophorin (CD235) and transferring receptor (CD71). In addition, characteristic immunophenotypic pattern has been identified in patients with acute myeloid leukaemias with recurrent chromosomal abnormalities. Acute promyelocytic leukaemias with t(15;17) are typically CD9+/HLADR-/CD34+/CD117-. Acute myeloid leukaemias with t(8;21) are CD13+/CD34+/CD19+/CD56, those with inv(16) are CD34bright/CD13bright/CD33dim, while those with 11q23 translocations are CD4+/CD56+/CD34-. These highly sensitive and specific immunophenotypic patterns uncover cryptic genetic abnormalities that otherwise would have gone undetected.

Flow cytometry in prognostication of acute leukaemia

Flow cytometric expression of multidrug resistance proteins MDRI, LRP and MRP have been correlated with induction failure, shorter relapse free periods and shorter overall survival. Coexpression of antigens of other lineage in adult acute leukaemias e.g. CD56 in myeloid leukaemias correlates with aggressive disease and is incorporated into many treatment algorithms.

Flow Cytometry in treatment of haematologic neoplasms

Several antibodies are being used in therapy of haematologic malignancies, including anti-CD33 for myeloid leukaemias, anti-CD52 and anti-CD20 for lymphoid neoplasms etc. Flow cytometric demonstration in the neoplastic cells of bright expression of appropriate antigens is necessary prerequisite to the initiation of immunotherapy.

Flow cytometry in monitoring of acute leukaemia

The molecular technics for monitoring of pediatric acute lymphoblastic leukaemia are well established in the therapy protocols. However, adult myeloid leukaemias do not have uniform or identifiable molecular lesions that lend themselves to molecular monitoring. Flow cytometry can identify "blast-specific" patterns in up to 90% of patients, and is routinely used to monitor minimal residual disease of 1 in 10^4 leukaemic cells.

Flow cytometry in myelodysplastic and myeloproliferative disorders

The incidence of myelodysplastic syndrome is increasing worldwide, especially the early cases. The morphologic abnormalities in these cases are often minimal and subtle, and staining variations between the laboratories make assessment of referred-in cases for hypogranularity etc. difficult. The cytogenetic abnormalities may be absent in half to three-quarter of cases. Flow cytometry helps in these cases by objective demonstration of hypogranularity and also abnormal patterns of antigen expression in neutrophils and other granulocytes. In more advanced cases of myelodysplastic syndromes, flow cytometric counting of blast cells is more precise and correlates with overall survival.

Flow cytometric protein profiling of leukemias

Recent demonstration of usefulness of gene expression profiling in acute leukaemias has spurred interest in the profiling the translation products of these genes. Such protein profiling is technically easier than gene-profiling, and provides functional and translational information to complement gene-profiling.

REFERENCES


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**Announcement**

**23rd Annual Conference of Indian Rheumatology Association**

The 23rd Annual Conference of Indian Rheumatology Association (IRA) is being organized during the 26-29 Oct. 2007 at Khajuraho. Abstract of scientific papers are invited. Prizes will be given for best papers and poster presentations.

For further details please contact: Organising Secretary, Indian Rheumatology Association Conference 2007 Col (Prof.) Ved Chaturvedi, MD, DM, HOD, Rheumatology and Clinical Immunology, Army Hospital (Research and Referral), New Delhi 110010.

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You can also visit our website www.iracon2007.com for detail information.

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**Announcement**

**Golden Jubilee Medicine Update 2007** will be held on 9th - 13th December 2007 at Auditorium Maulana Azad Medical College, New Delhi.

It will include clinical case presentation with Discussions, Workshops, Lecturer, Seminars, Symposia, etc.

Early Bird Registration: Rs. 500/- upto 30th September 2007

Rs. 750/- upto 30th November 2007

Rs. 1000/- after 30th November 2007

Rs. 1500/- Spot Registration

For further details contacts: Dr. R Dewan, Organizing Chairperson, Professor and Head, Department of Medicine, MAMC, NewDelhi. Tel.: 91-11-23236437

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