Immunophenotypic Profile in Acute Infectious Mononucleosis Mimicking Malignant Lymphoproliferative Disorder: A Case Report and Review of Literature

Prashant Tembhare · Manisha Ramani ·
Keerti Syed · Amar Das Gupta

Received: 26 August 2009 / Accepted: 26 April 2010 / Published online: 30 September 2010
© Indian Society of Haematology & Transfusion Medicine 2010

Abstract Infectious mononucleosis is characterized by an intensive lymphoproliferation with atypical forms which sometimes resemble with acute leukemia or malignant lymphoproliferative diseases. Flow cytometric analysis of lymphocytes shows a typical phenotype but unawareness of it may lead to misdiagnosis of malignant lymphoproliferative diseases. Herewith we present an immunophenotypic profile in a case of acute infectious mononucleosis and review of literature.

Keywords Immunophenotyping · Acute infectious mononucleosis

Introduction

Infectious mononucleosis (IM) is a disease in children and adolescents. It is common mainly in countries with temperate and cold climate. Patients usually present with fever, sore throat, lymphadenopathy, often hepatosplenomegaly [1]. The diagnosis of IM is based on clinical presentation and supportive laboratory (hematological and biochemical) findings. It usually is characterized by relative and absolute

lymphocytosis. At least a subset (>10%) of the lymphocytes is atypical, called as Downey-type cells [2], which morphologically mimic neoplastic atypical lymphoid cells. Differential diagnosis of IM and atypical lymphocytosis usually includes acute infection with a host of viruses such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), toxoplasma, human immunodeficiency virus type 1 and 2 (HIV-1/2), human herpes virus 6, hepatitis viruses as well as lymphoproliferative diseases [3].

Flow cytometric (FC) immunophenotyping is a method of choice in the diagnosis of lymphoproliferative disorders. However, T-cell neoplasms are the difficult to diagnose by FC owing to their lack of readily identifiable markers of clonality and the phenotypic spectrum of overlap between reactive and neoplastic T-cell proliferations [4]. The intense activated T cell-mediated immune response in IM caused by EBV shows a phenotype similar to that of T cell neoplastic lymphoproliferative disorder. Unawareness of this fact may lead to misdiagnosis of IM as T cell lymphoma or leukemia [4]. We present a data from FC analysis of a case of acute IM caused by EBV in a 11 year-old girl mimicking high grade lymphoid malignancy.

Case Report

An 11-year-old girl presented with the history of high grade fever, weakness, anorexia for 2 weeks. On clinical examination she had generalized lymphadenopathy with mild splenomegaly. Complete blood count revealed WBC count $22 \times 10^9/L$ with lymphocytes 74.5%, hemoglobin 126 g/L and platelet count $252 \times 10^9/L$. Peripheral blood smear showed lymphocytosis with 52% atypical lymphoid cells mimicking neoplastic lymphoblasts/lymphoma cells. The cells were 4–6 times the diameter of adjacent small

P. Tembhare (✉) · M. Ramani · K. Syed · A. D. Gupta
Hematology, Coagulation and Flow Cytometry, Super Religare
Laboratories Ltd, Mumbai, India
e-mail: docprt@gmail.com

M. Ramani
e-mail: flowcytometry@srl.in

K. Syed
e-mail: keertikomai@yahoo.com

A. D. Gupta
e-mail: adasgupta@srlworld.com

Fig. 1 Peripheral blood smear showing atypical lymphocytes (Downy cells). Leishman stain 100×

Table 1 List of antibodies along with the fluorochromes and clones

Antibody	Fluorochrome	Clone
CD3	FITC/PC5	UCHT1/SK7
CD4	PE (RD1)	SFC112T4D11
CD5	FITC	BL1a
CD8	ECD	SFC121Thy2D3
CD10	FITC	ABL1
CD16	PE	3G8
CD19	PE	J4.119
CD34	PE	S81
CD45	PC-5	J33
CD56	PE	N901
HLADR	PE	L243

lymphocytes and had a moderate amount of deep to pale blue cytoplasm with large nuclei (Fig. 1).

Immunophenotyping was performed on Cytomics FC-500 flow cytometer (Beckman Coulter, USA). The sample was processed with lyse and wash technique and the cells were stained with a panel of antibodies shown in Table 1. A minimum ten thousand events were acquired and analyzed using CD45/SSC gating strategy. Majority (85%) of gated lymphoid cells showed strong but heterogeneous CD3 expression, out of which 16% cells were CD4+ and 63% cells were CD8+ T cells (CD4 + /CD8 + ratio = 1:4) indicating proliferation of CD8+ T cells. These cells on further analysis showed weak CD5 expression (down regulation of CD5) and strong HLADR as well as CD38 expression (Fig. 2). The cells were negative for CD10, CD16, CD19, CD34, and CD56. Considering clinical history and these hematological finding we

suggested a diagnosis of reactive lymphocytosis secondary to viral infection, possibly acute infectious mononucleosis. Serologic testing for IM was done by using an enzyme immunoassay (IBL, Hamberg, Germany) for qualitative and quantitative detection of IgM antibodies against the Epstein Barr Virus nuclear antigen (EBNA-1) from the serum of patient. The test detected significantly high titer of IgM antibodies against the EBNA-1 which confirmed the diagnosis of IM caused by EBV. Patient was given supportive treatment and recovered completely.

Discussion

Infectious mononucleosis represents an uncommon benign self-limiting lymphoproliferative disorder characterized by primary EBV infection of B lymphocytes and massive proliferation of activated cytotoxic T cells [5]. An intense T cell-mediated immune response is seen within oropharyngeal lymphoid tissues and also leads to increased (>10%) atypical lymphocytes (Downy cells) in the peripheral blood [4–6]. In the present case, peripheral blood smear showed lymphocytosis (85%) with a large number of (52%) atypical lymphocytes mimicking high grade lymphoid malignancy (as shown in Fig. 1).

Acute EBV infection induces a vigorous CD8+ T cell immune response, which is believed to be responsible for the control of viral replication and establishment of latency [5, 6]. Callan et al. have shown that the majority of EBV-specific T cells have an activated/memory phenotype and express HLA-DR, CD38, low levels of CD45RA and have decreased expression of homing receptor CD62L [7, 8]. Increased numbers of CD8+ cytotoxic-suppressor T cells have also been seen in other viremias, including HIV and CMV infection, as well as in hepatitis C [4, 6]. The CD8+ T cells in acute IM have a CD45RO+/CD45RA– phenotype, consistent with memory T lymphocytes rather than naive T cells. This implies that recruitment of T cells most likely occurs during the latent subclinical phase which provides time for clonotypic amplification and selection [4]. The present case showed markedly increased CD8+ T cells which strongly expressed T cell activation markers like HLADR and CD38. The percentage of helper CD4+ T cells was relatively decreased and the ratio of CD4+ to CD8+ T-cells was completely reversed.

Patients with EBV-IM usually exhibit oligoclonal or monoclonal T cell proliferation, which may mimic T cell lymphoproliferative disorder (T-LPD) [9]. The diverse group of T cell neoplasms is often difficult to characterize and diagnose [10]. Flow Cytometric Immunophenotyping has become a widely used tool for the analysis of both B and T cell lymphoid populations [10]. Clonality of T-lymphocytes is very difficult to determine even by FC

Fig. 2 Flow cytometric analysis on peripheral blood sample. Analysis is performed on lymphocytes (green events) gated (gate A and B) in plot SSC vs. CD45 and SSC vs. FSC (plots A and B). Plots C to I are analyzed on A + B gated events (green events). Majority (85%) of these were T cells with heterogeneous expression CD3 (plots C, G

& H) and down regulation of CD5 expression (plot D). Out of total CD3+ cells CD4+ cells were 16.5% and CD8+ cells were 61.4% (plot F). These cells also expressed T cell activation markers i.e. HLADR and CD38 (plots G & H). CD10, CD19 and CD34 were negative (plots I & J)

because of lack of clonality markers like kappa or lambda light chains in B-cell lymphoproliferative disorders. However, aberrant antigen expression detected by FCM represents a useful diagnostic clue to diagnose a T-cell lymphoproliferative disorder. Down regulation of markers like CD7 and less commonly CD5/CD3 is one of the antigenic aberrations in T-cell lymphoproliferative disorders [4, 10–12]. However, T-cell antigenic aberrancy must be interpreted with caution as also can be seen in nonneoplastic T-cell disorders. Down-regulation or absence of CD7 expression on T-cells can be seen in a variety of reactive conditions, including inflammatory dermatoses and rheumatoid arthritis [13, 14]. In the case presented here, immunophenotypic analysis of the T cells showed partial down regulation of CD5 and heterogeneous expression of CD3. Combination of these immunophenotypic findings with atypical morphology might be misinterpreted as a malignant T-cell neoplasm, a diagnosis that may be further supported (erroneously) by the

demonstration of a clonal population by reflex molecular studies. Many cases of acute infectious mononucleosis, as well as other viremias, harbor T cell clones. A clue to the correct diagnosis under these circumstances is the fact that peripheral T cell neoplasms often are negative for the activation antigen HLA-DR. However, it may be expressed heterogeneously in T-cell lymphoproliferative disorders [4]. If the HLA-DR+ T-cell population displays antigenic down regulation of CD7 and also of CD5, it is important to correlate these results of immunophenotyping with the peripheral blood smear findings and with appropriate viral serologic markers to rule out the possibility of IM. In present case the lymphocytes showed good expression of HLADR along with partial down regulation of CD5. Serological testing has shown IgM antibodies against EBV1 antigen for EBV with significant titer confirming the diagnosis of acute IM due to EBV infection.

The patients of IM many times may not require any specific treatment [15] and even if required, it responds

very well to intravenous immunoglobulin, interferons, ganciclovir and prednisolone [9, 15], suggesting restoration of immune system and eradication of the clonal T cells. Thus, careful clinicopathological correlation is warranted in the interpretation of immunophenotyping and clonality data in T cell proliferation in association with EBV-IM to avoid erroneous diagnosis of T-LPD and hence exposure to hazardous as well as expensive chemotherapy.

References

1. Karcheva M, Lukanov Tz, Gecheva S, Slavcheva V, Veleva G, Nachev R (2008) Infectious mononucleosis-diagnostic potentials. In: Journal of IMAB-Annual Proceeding (scientific papers) Book 1. Peytchinski Gospodin Iliev, Pleven, pp 9–13
2. Malik UR, Oleksowicz L, Dutcher JP, Ratch H, Borowitz MJ, Wiernik PH (1996) Atypical clonal T cell proliferation in infectious mononucleosis. *Med Oncol* 13:207–213
3. Ana S, Lepej I, Vince A, Rakušić SA, Rode OD, Sonicki Z, Jeren T (2003) Center for disease control (cdc) flow cytometry panel for human immunodeficiency virus infection allows recognition of infectious mononucleosis caused by epstein-barr virus or cytomegalovirus. *Croat Med J* 44:702–706
4. Weisberger J, Cornfield D, Gorczyca W, Liu Z (2003) Down-regulation of pan-T-cell antigens, particularly CD7, in acute infectious mononucleosis. *Am J Clin Pathol* 120:49–55
5. Verbeke CS, Wenthe U, Bergler WF, Zentgraf H (2000) Characterization of the expanded T cell population in infectious mononucleosis: apoptosis, expression of apoptosis-related genes, and Epstein Barr Virus (EBV) status. *Clin Exp Immunol* 120:294–300
6. Hudnall SD, Patel J, Schwab H, Martinez J (2003) Comparative immunophenotypic features of EBV-positive and EBV-negative atypical lymphocytosis. *Cytometry B Clin Cytom* 55:22–28
7. Callan MF, Tan L, Annels N, Ogg GS, Wilson JD, O'Callaghan CA et al (1998) Direct visualization of antigen-specific CD8+ T cells during primary immune response to Epstein-Barr Virus in vivo. *J Exp Med* 187:1395–1402
8. Macallan DC, Wallace D, Irvine A, Asquith B, Worth A, Ghattas H, Zhang Y (2003) Rapid turnover of T cells in acute infectious mononucleosis. *Eur J Immunol* 33:2655–2665
9. Lin MT, Chang HM, Huang CJ, Chen WL, Lin CY, Chuang SS (2007) Massive expansion of EBV+monoclonal T cells with CD5 down regulation in EBV-associated haemophagocytic lymphohistiocytosis. *J Clin Pathol* 60:101–103
10. Craig FE, Foon KA (2008) Flow cytometric immunophenotyping for hematologic neoplasms. *Blood* 111:3941–3967
11. Gorczyca W, Weisberger J, Liu Z, Tsang P, Hossein M, Wu CD et al (2002) An approach to diagnosis of T-cell lymphoproliferative disorders by flow cytometry. *Cytometry B* 50:177–190
12. Jamal S, Picker L, Aquino DB, McKenna RW, Dawson DB, Kroft SH (2001) Immunophenotypic analysis of peripheral T-cell neoplasms: a multiparameter flow cytometric approach. *Am J Clin Pathol* 116:512–526
13. Moll M, Reinhold U, Kukel S, Abken H, Müller R, Oltermann I et al (1994) CD7-negative helper T cells accumulate in inflammatory skin lesions. *J Invest Dermatol* 102:328–332
14. Smith KJ, Skelton HG, Chu WS, Yeager J, Angritt P, Wagner KF (1995) Decreased CD7 expression in cutaneous infiltrates of HIV-1+ patients. *Am J Dermatopathol* 17:564–569
15. Tattevin P, Tulzo YL, Minjolle S, Person A, Chaplain JM, Arvieux C et al (2006) Increasing incidence of severe Epstein-Barr Virus-related infectious mononucleosis: surveillance study. *J Clin Microbiol* 5:1873–1874