Original Article

Lymphocyte subsets phenotype in patients with infectious mononucleosis

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Summary:

Background: Infectious mononucleosis (IM) is a lymphoproliferative disease caused primarily by the Epstein-Barr virus (EBV) infection. The initial viral infection by EBV occurs in B lymphocytes and is followed by an extensive proliferation of T lymphocytes. Previous studies on immunity to EBV (including IM) have mainly focused on activation of peripheral blood T cells, which are responsible for the lymphocytosis in blood during acute IM.

Patients and Methods: Indirect immunofluorescence technique analysis was performed to detect the percentage of CD3, CD4, CD8, CD19, and CD56 positive lymphocytes.

Results: Our results on the phenotype of T cells in samples from patients with infectious mononucleosis showed that there is a significant increase in percentage of CD8+ T-cells when compared with healthy group. In addition, increased in percentage of CD3 T-cells and moderately increase in CD56 (NK) cells. CD4 and CD19 percentage were significantly decrease in comparison with healthy control.

Conclusion: Acute infectious mononucleosis is characterized by a marked increase in the percentage of CD3 and CD8 T- cells with a slight increase in CD56 percentage. A marked decrease in CD19 percentage and CD4/CD8 ratio was noticed.

Keywords: Infectious mononucleosis, CD-Marker profile and EBV.

Fac Med Baghdad 2009; Vol. 51, No.2
Received Aug. 2008
Accepted Sep. 2008

Introduction:

Infectious mononucleosis (IM) is an acute infection characterized by high fever, sore throat and lymphadenopathy especially in the cervical lymph nods. It is mainly caused by Epstein - Barr virus (EBV), a gamma herpes virus that is believed to infect 90% of the world's population. It's most common presentation is a flue-like illness called Infectious mononucleosis, which usually resolves on it's own, but can also be caused by the Cytomegalovirus (CMV), Herpes simplex viruses (HSV)-1 and 2, Varicella- zoster virus (VZV) and Human herpes viruses (HHV) -6, 7 and 8. These viruses are members of one family Herpesviridae, all of them share properties including a genome of double-stranded linear DNA core surrounded by icosahedra nucleocapsid symmetry, and a viral envelops (1) (2). They also share the biological properties of latency and reactivation, which cause recurrent infections in the host (2). The cellular adaptive immune defense against IM involves both nonspecific and antigen-specific phases. Eradication of infection is likely dependent upon classical CD4+ and CD8+ cytotoxic T-lymphocyte (CTL) responses. These cells express a large number of different molecules on their surface, named CD markers. These markers have the capacity to receive and transmit signals from the environment

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And to execute effectors function. In both human disease and murine experimental infection, cellular immunity is determined by the polarization of CD4+ T cell subsets (3), (4) and (5). The CD4+ and CD8+ glycoproteins characterize the two main subpopulations of T- lymphocytes. The CD4+ T cells are involved in the regulation function (helper/ inducer) of the immune response and the CD8+ Tcells have suppressive and cytotoxic activity. T- cell function releasing involves the respective recognition of CD4 and CD8 by class 11 and class 1 HLA structures which represent their natural ligands (5). The count of CD4+ and CD8+ T lymphocytes in the peripheral blood is a major test in the hematological follow-up of the diseases with immune response had shown atypical cells. Persons with severe cellular immunodeficiency might develop fulminant mononucleosis or monoclonal B cell malignancy. By comparison, individuals with intact immune function could control the proliferative potential of EBV-infected lymphocyte and get the proper regulation of replication within pharyngeal epithelia, thus preventing the emergence of lymphoproliferative disorders and cancer (6). After 2-3 weeks, in typical IM patients, Lymphocytosis might reach 50% of total peripheral WBCs, of which 10-20% or more were seen as atypical cells (7). In the first week of illness atypical cells proved to be T-cells, mainly supressor/cytotoxic CD8 subset. These cells could be stimulated in a cytotoxic response against EBV infected B cells that expresses lymphocyte- determined membrane antigen (7). So, part of IM might reflect a rejection reaction

against virally converted lymphocyte (8). Atypical lymphocytes had shown to be neither MHC- restricted nor EBV specific. They could act in a responsible way to prevent the unchecked expansion of EBV transformed B lymphocytes (7). Also, atypical lymphocytes had been reported to be seen in blood of patients with CMV, adeno virus, rubella and Toxoplasma gondii (7) .T cell immunity plays an important role in the clinicopathology of EBV associated diseases. An EBV- induced IM is a common self-limiting disease. However, other EBV-associated diseases, including chronic active EBV infection (CAEBV), NK cell lymphoma (NKL) and Hodgkins lymphoma (HL), exhibit distinct clinical features (9).

Patients and Methods:

Subjects: The present study included 25 children with infectious mononucleosis (IM). Clinical diagnosis of IM was confirmed based on fever, lymphadenopathy, splenomegaly, lymphocytosis (> 50%), atypical lymphocytes (> 10%) in blood smears and the elevated levels of IgM antibody against EBV capsid antigen. The control group consists of 25 age-matched healthy children.

Methods: Lymphocye separation the isopaque-ficol technique originally described by Boyum (10) in1968 was used for Lymphocyte separation was done as following: Two and a half ml of blood which was collected in EDTA tubes were diluted with phosphate buffer saline (PBS) complete medium 1:1 v/v. Then after dispense 4ml of lymphocyte separation medium (lymph prep) into a 10ml centrifuge tube. 2.5 ml of the diluted blood sample was carefully layered on the top of the separation medium and centrifuged for 30 minutes at 2500 rpm at 20ºc, after centrifugation, the lymphocytes formed a grey-colored layer at the interface of the blood plasma and the separation medium. Using Pasteur pipette, the supernatant blood plasma aspirated down to the upper surface of the lymphocyte layer and the layer of lymphocytes aspirated with a sterile Pasteur pipette. Then the lymphocyte was transferred to a centrifuge tube containing at least three times the volume of PBS and suspend evenly. Then lymphocyte suspension was centrifuged at 1000 rpm for 10 minutes and the supernatant was aspirated and discarded and the cell pellet was resuspended in fresh PBS complete medium by gentle drawing the cells in and out of a Pasteur pipette, then repeat (but, at 1500 rpm) centrifugation and resuspension in PBS complete medium another two times, then resuspend in the medium for IFT.

Indirect fluorescent antibody test (IFAT) for counting lymphocyte subsets A-Principle:Indirect fluorescent antibody test for the detecting of CD-antigen depends on two steps, the first step leads to the binding of primary antibody to specific cell antigen. The second step allows the detection of specific CD-antigen when anti-mouse immunoglobulin IgG fluorescinated conjugates is added to be examined by immunofluorescent microscope. The positive samples show an apple green fluorescent corresponding to areas of cell surface where primary antibody bound.

B- Assay procedure, according to the information supplied by Slaper-Cortenbach Company.

C-Calculation of the results: Slides were examined under 40X-magnification of a fluorescent microscope. Their dark green staining identified positively labeled cells. Two hundred cells were counted to determine percentage of reactivity of the tested monoclonal antibodies figure 1.

Statistical analysis: Data have been analyzed statistically using UPSS program version 10. Results were expressed using simple statistical parameters such as mean and standard deviation. Analysis of quntitation data was done using t-test and ANOVA. Acceptable level of significance was considered to be less than 0.05.

Results:

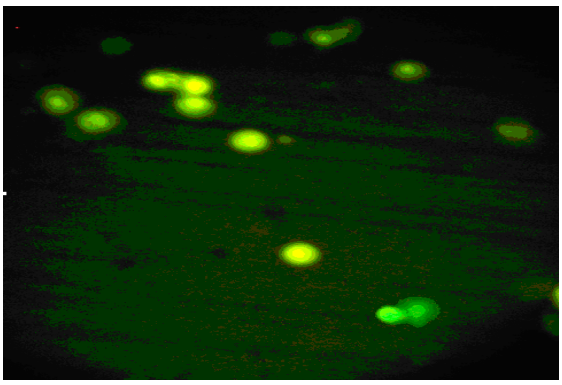

Figure (1): Slides of indirect immunofluorescence microscope at 490 nm. Positive cells give green-apple when stained with FITC-labeled antibodies after exposure to UV-light to see lymphocytes. (400 x)

Lymphocyte phenotype by IFAT showed that there was a significant increase in the percentage of CD3 and CD8 in IM group (83.5 ± 6.5) and (54.9 ± 7.8) respectively in comparison with healthy group (72.2 ± 5.8) and (23.9±3.6) respectively ($p< 0.0001$). A significant decrease in the percentage of CD4 and CD19 in IM group (20.03± 5.6) and (6.4 ±1.6) ($p< 0.0001$) respectively when compared with healthy group (34.2±5.4) and (9.5±1.4) respectively. Percentage of CD56 showed no significant increase (9.4±1.9) in comparison with healthy group (8.3±1.04) ($P=0.028$). CD4/CD8 ratio was (0.36) significantly lower than healthy group (1.43). See figures (2, 3, 4, 5 and 6)

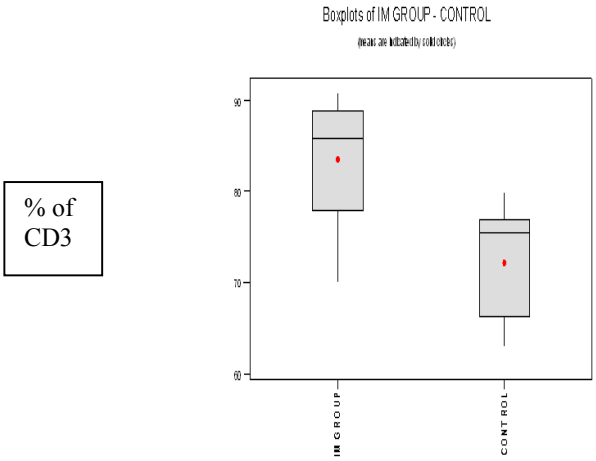

Figure (2): Percentage of CD3 in infectious mononucleosis group and healthy control group.

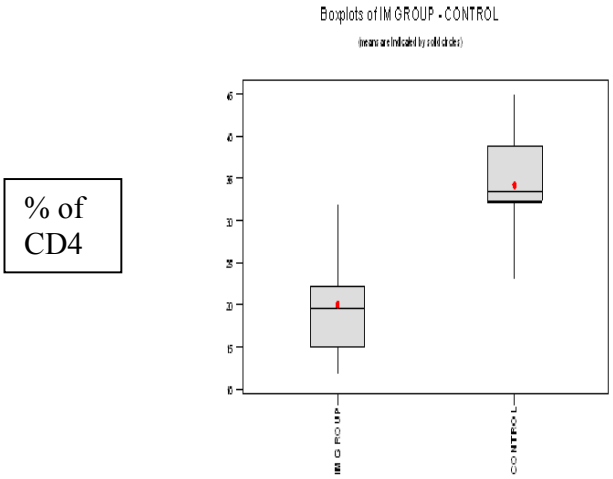

Figure (4): Percentage of CD4 in infectious mononucleosis group and healthy control group.

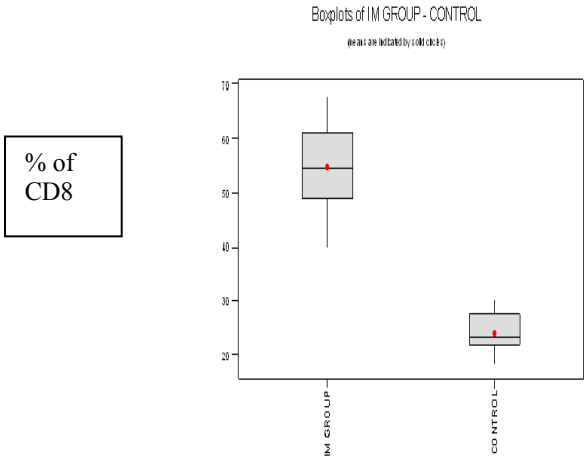

Figure (3): Percentage of CD8 in infectious mononucleosis group and healthy control group.

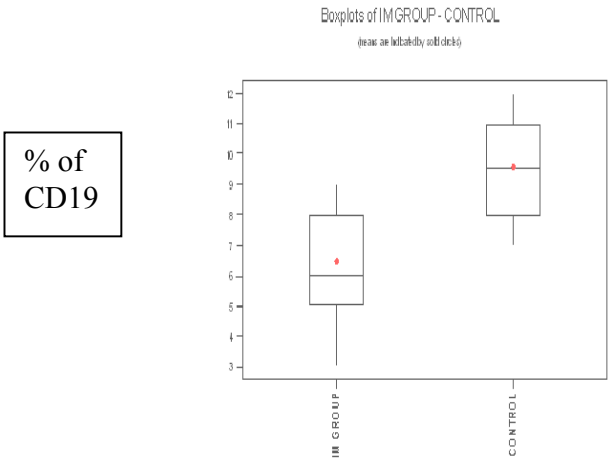

Figure (5): Percentage of CD19 in infectious mononucleosis group and healthy control group.

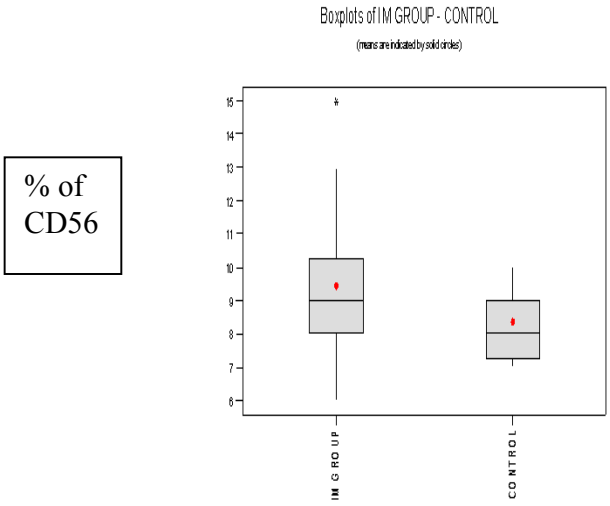

Figure (6): Percentage of CD56 in infectious mononucleosis group and healthy control group.

Discussion:

The obtained results showed that infectious mononucleosis caused by EBV significantly changes the percentages and absolute counts of several peripheral blood lymphocyte subpopulations. 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 (8), (11), (12) and (13). In the present study, much higher increased percentage of CD8+T cells in EBV-infected patients group in comparison with healthy control. This could be attributed to the fact that MHC antigens are important for T-cell activation, if the antigen presenting cells (APCs) presented the antigen in association with MHC Class1. Also, this may activate Th1 and macrophage to secret IL-2 which increase CD8+ division. CD8+T cells have a cytotoxic/ suppressor function of killing infected cells. They also release lymphokines like TNF-alpha and INF-gamma. Higher increase in the percentage of T cells (CD3) in EBV group was obtained when compared with healthy control group. The reason of elevated percentage of CD3 cells in IM as with CD8+ after expansion of lymphocytes with IL-2 (14).Our results showed, no significant difference was detected in the percentage of CD56 between IM and healthy control group. The reasons of these criteria are due to CD56 cells which are important during the early phase of infections, especially in certain viral infections (15), (16), and (17). NK cell (CD56) produces a group of cytokines and chemokines, namely, IFN-gamma, lymphotactin, macrophage inflammatory protein-1α (MIP-1α), MIP-1β, and RANTES from subset of NK cells. IFN-γ plays a key role in the initial antiviral response because it induces a number of functional effects on macrophages, namely, induction of MHC Class 1 expression, increased Ag presentation, production of antimicrobial oxygen and nitrogen intermediates, and release of IL-12. By IL-12, the CD56 cells produced IFN-γ contribute to the differentiation of T cells into Th1 and T cytotoxic cells (TC cells) (18) (19). The percentage of CD19 (B cell) in IM group was much lower than that in healthy control group. Such a finding may be due to higher affinity to infection between the receptor CD21 of B cells and gp350 of the EBV leads to phagocyte of the infected cells .Also, it might be due to CD4+ T cells polarization towards Th1which in turn directs the response towards cell mediated immunity, while Th2 is directed towards antibody production (20). CD4 count was found to show lowest level in IM group However, it showed high level in healthy control and such a result agrees with other previous studies (8),(21)and (22) which revealed that percentages of CD4+ was significantly decreased. Lowering of CD4+ may result from cell apoptosis resulting in selective detection of IL-2 and IFN-γ and protein oxidation by the oxygen free radicals. A number of studies have shown that there is a much greater propensity for detectable colonel expansions to develop in the CD8 than the CD4 compartment over time in healthy donors, and some of these have now been clearly linked to ongoing responses to persistent viral infections such as EBV (23). In IMLS, CD4+/CD8+ ratio was significantly lowest IM group than those in healthy control group. This agrees with other studies (8), (11), (21)and(14) which reported the reversed ratio (CD4+/CD8+) and high increase proportions of activated HLA-DR+ CD4+ and CD8+T lymphocytes in the peripheral blood.

Conclusion:

Acute infectious mononucleosis is characterized by a marked increase in the percentage of CD3 and CD8 T-cells with a slight increase in CD56 percentage. A marked decrease in CD19 percentage and CD4/CD8 ratio was noticed.

References:

1- *Cohen, J.I. (2003). Epstein-Barr virus infection. N. Eng. J. Med., 343: 481- 492.*

2- Mocarski,E.S. (1996). Cytomegaloviruses and their replication. In: Fields, B.; Knipe, D. and Hawley, P. (Eds.). Virology, 3rd ed., vol.2, Lippincott-Raven, Phil89-Locksley, R.M.; Reiner, S.L.; Hatam,F.; Littman, D.R. and Killeen, N.(1992). Helper T cells with out CD4: Control of leishmaniasis in CD4 deficient mice .Science. 261: 1448-1451.

3-Locksley, R.M.; Reiner, S.L.; Hatam,F.; Littman, D.R. and Killeen, N.(1992). Helper T cells with out

CD4: Control of leishmaniasis in CD4 deficient mice .Science, 261: 1448-1451.

4- Jones, D. E.; Elioso, M. M. and Scott, P. (1998) .Host susceptibility factors to cutaneous leishmaniasis. Front. Biosci., 15 (3): 1171-1180 (Medline Abst).

5- Sommer, F.; Meixner, M.; Mannherz, M.; Ogilvie, AL.; Rolling hoof, M. and Lohoff, M. (1998). Analysis of cytokine patterns produced by individual CD4+ lymph node cells during experimental murine Leishmaniasis in resistant and susceptible mice . Int Immunol., 10 (2):1853-1861(Medline Abst).

6- Strauss, S.E. (1993) .Epstein Barr virus infection: Biology, Pathogenesis and Management. Ann. Intern. Med., 118:45-48.

7- Andiman, W.A.(1989) .Epstein-Barr virus .In Lennette, E.H. and Schmidt, N. J. (Eds.) 'Diagnostic procedures for viral, ricketsial and Chlamydia infections, 6th ed. Am. Pub. Health Press, Washington D.C: 407-452.

8- Zidovec-Lepej, S.; Vince, A.; Rakusic, S.; Dakovic-Rode, O.; Soniki, Z. and Jeren,T. (2003).). Center for Disease Control (CDC) Flow cytometry panel for human immunodeficiency virus infection allows recognition of infectious mononucleosis caused by EBV or CMV. Croatian. Med. J., 44 (6): 702-706.

9-Ohshima, K.; Karube, K.; Hamasaki, M.; Tutiga, T. and Kikuchi, M. (2003). Differential chemokine receptor and cytokine expression in Epstein-Barr virus-associated lymphoproliferative diseases. Leuk-lymphoma., 44 (8):1367-1378.

10- Boyum, A. (1968). Separation of leukocytes from blood and bone marrow. Scand. j. Clin. Lab. Invest.,21., (Suppl 97):

11- Hudnall, S.D.; Patel, J.; Schwab, H. and Martinez, J. (2003) .Comparative immunophenotypic features of EBV-positive and EBV-negative atypical lymphocytosis . Cytometry., 55B (1): 22-28.

12- Callan, M. F. (2003). The evolution of antigen-specific CD8+T cell responses after natural primary infection of human with EBV. Viral. Immunol.,16 (1): 3-16.

13- Lynne, J. E.; Schmid, I.; Matud, J. L. and Shlian, D.M. (1998). Major expansions of select CD8+ subsets in acute EBV infection : Comparison with chronic human immunodeficiency virus disease. J. Infec. Dis., 1083-1087.

14- Williams, H.; MacAulay, K.; Macsween, K.F.; Gallacher, N.J. and Crawford, D.H. (2005). The immune response to primary EBV infection: a role for natural killer cells.Br. J. Haematol., 129 (2): 266-274.

15- Bancroft, G.J. (1993). The role of natural killer cells in innate resistance to infection. Cure. Open. Immunol., 5: 503-508.

16- French, A. R. and Yokoyama, W.M. (2003). Natural killer cells and viral infections. Cure. Opin. Immunol.,15:45-50

17- Yokoyama, W.M. and Scalzo, A.A. (2002). Natural killer cell activation receptors in innate immunity to infection . Microbes . Infect., 4:1513-1516.

18- Mosmann, T.R. and Sad, S. (1996). The expanding universe of T cell subsets: Th1 and Th2 and more . Immunol. Today.,17: 138

19- Brigitte, G.; Dorner, Hamish, R.C.; Anthony, R.; French, and Wayne, M. (2004). Coordinate expression of cytokines by NK cells during murine cytomegalovirus infection. J Immunol., 172: 3119-3131.

20- Sarhan, G.M. (1985). Epidemiological and clinical studies on cutaneous leishmaniasis. M.Sc. thesis. Coll Med.Baghdad Univ.

21- Precopio, M.L.; Sullivan, J.L.; Willard, C.; Somasundaran, M. and Luzuriaga, K. (2003). Differential kinetics and specificity of EBV-specific CD4+ and CD8+T cells during primary infection .Immunol., 170 (5):2590-2598.

22- Hislop, A.D.; Annels, N.E.; Gudgeon, N. H.; Leese, A.M. and Rickinson, A.B. (2002). Epitope-specific evolution of human CD8+T cell responses from primary to persistent phases of EBV infection. J.Exp. Med., 195 (7):893-905.

23- Silins, S.L.; Cross, S.M.; Krauer, K.G.; Moss, D.J.; Schunidt, C. W. and Misko, I.S. (1998). A functional link for TCR expansions in healthy adults caused by persistent Epstein-Barr infection. J. Clin. Invest.,102:1551-1556.