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Expression of bcl-2 protein in infectious mononucleosis patients — a pilot study

Ekspresja białka bcl-2 u pacjentów z mononukleozą zakaźną — badania pilotowe

INTRODUCTION

Epstein-Barr Virus — a Herpes virus — is an etiological agent of infectious mononucleosis (IM). Chronic active EBV infection, Burkitt lymphoma, nasopharyngeal carcinoma, Hodgkin's disease, peripheral T cell lymphoma and NK cell leukemia/lymphoma are other diseases associated with EBV infections. The majority of cells infected by EBV are lymphocytes. EBV-infected cells are eliminated by cytotoxic T cells as well as NK cells on apoptotic pathway [3].

Apoptosis — an active mode of cell death — must be controlled very strictly. Bcl-2 family proteins play a crucial role in this process. It includes pro-apoptotic proteins (e.g. bax, bcl-x_S, bad, bid) and anti-apoptotic proteins (e.g. bcl-2, bcl-x_L). A decision between life and death of a cell is caused by balance between them. Bcl-2 protein is mainly located in mitochondria. It plays a key role in regulation of mitochondrial transmembrane potential as well as secretion such proapoptotic factors as caspase-9, Apaf-2, cytochrome c and AIF. All of these factors can activate a key enzyme in apoptotic machinery — caspase-3 [2, 6].

The aim of the present study was to evaluate expression of bcl-2 protein in B and T lymphocytes in IM patients.

MATERIAL AND METHODS

In the present study 7 fresh diagnosed, non-treated IM patients were included as well as 25 healthy blood donors. Peripheral blood lymphocytes were isolated on ficol centrifugation. Staining of monoclonal antibodies anti-bcl-2 (Fitc), CD3 (PE) and CD19 (Cy5) (DAKO or BD) were performed as described elsewhere [4, 5]. Briefly,

after staining with surface antibodies for 30 min. in temperature of 40 the cells were fix in 0.25% paraformaldehyde for 15 min. in room temperature. Then the cells were permabilised in 70% methanol for 60 min. in 4°C and stained with monoclonal antibodies anti-bcl-2 (30 min., 4°C). Just after staining cells were analysed by flow cytometry (FACSalibur, BD) — all healthy blood donors, or Laser Scaning Cytometer (CompuCyte) — all IM patients after previous cytocentrifugation. LSC is a unique cells counter which shares futures of flow and image cytometer (see [1, 2] for details). Contouring for LSC measurements in this paper was based on fluorescence of CD3 or CD19 antigens.

RESULTS

The mean bcl-2 fluorescence of CD3+ cells was 392558±162440 (median: 336303) in IM patients. The mean bcl-2 fluorescence of CD19+ cells in these same patients was 422148±162440 (median: 379092). The fluorescence of bcl-2 was higher in B than T cells in all studied patients. In healthy blood donors mean fluroscence of bcl-2 protein was higher in CD3+ than in CD19+ cells (subsequently 108±10.4; 102±8.7). Although the data obtained from LSC (IM patients) and flow cytometry (healthy blood donors) are incomparable, the variances between T and B lymphocytes are instrumentally independent.

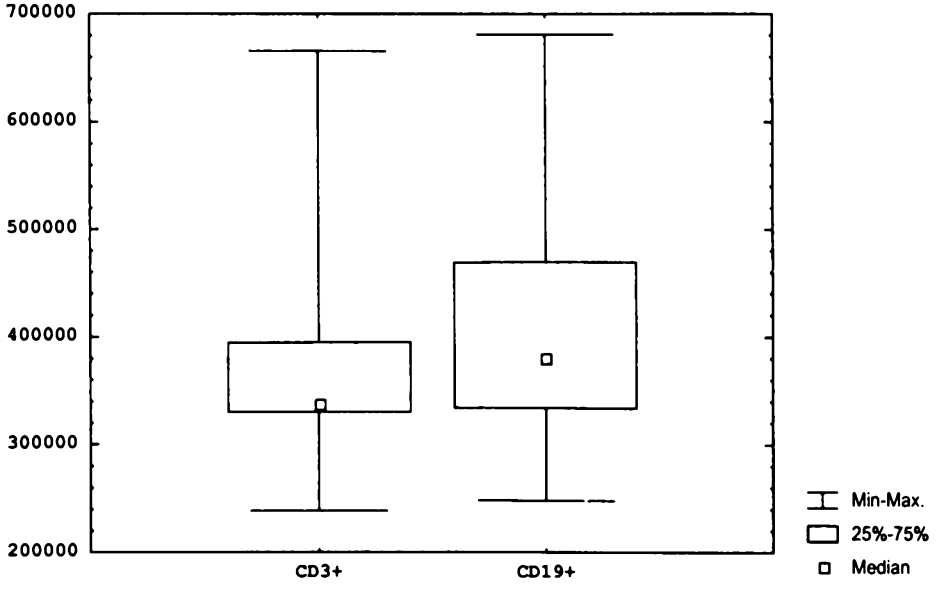

Fig. 1. Comparison of bcl-2 protein expression on CD3+ and CD19+ cells in infectious mononucleosis patients

DISCUSSION

Cell mediated immunity to primary EBV infection includes a cytotoxic T cell response that is induced by EBV — associated proteins (e.g. latent membrane protein). These cytotoxic T cells and NK cells induce apoptosis of EBV-infected lymphocytes. It could be done in two different ways: the first Fas-based and the second perforin/granzyme-based. The Fas-FasL interactions seem to be key ones in IM. This thesis is supported by the fact that the expression of perforin and granzyme is very low in these patients [3]. Moreover, about 30% of IM lymphocytes have Fas antigents on their surfaces. Low expression of FasL antigen on their surfaces suggests that this expression is not induced by EBV infection [3].

One of the most common ways of preventing apoptosis by cells is high level of antiapoptotic proteins. In our study we showed that B cells have higher level of bcl-2 protein than T cells. We also showed that the situation is different with healthy individuals (T cells have more bcl-2 then B cells) [4]. We also showed that a similar situation like in IM patients takes place in B Chronic Lymphocytic Leukaemia [5]. All these findings could suggest that increased expression of bcl-2 protein in IM B cells could be involved in prolongation of their life span. Moreover, it could be EBV-related.

In conclusion we want to state that increased expression of bcl-2 protein in B cells of IM patients probably is one of important immune surveillance factors. There is a need for further studies which will evaluate how closely it is connected with EBV-infection.

REFERENCES

1. *Bedner E. et al.*: Analysis of apoptosis by Laser Scaning Cytometry. Cytometry, 1999; 35: 181-195.
2. *Bedner E. et al.*: Translocation of Bax to mitochondria during apoptosis mesured by Laser Scaning Cytometry. Cytometry, 2000; 41: 83-88.
3. *Ohshima K. et al.*: CD 95 (Fas) ligand expression of Epstein–Barr virus (EBV)- infected lymphocytes: A possible mechanism of immune evasion in chronic active EBV infection. Pathol Internation., 1999; 49: 9-13.
4. *Rolinski J. et al.*: Expression of the mitochondrial bcl-2 oncoprotein and CD95 antigen in peripheral blood lymphocytes of healthy volunteers. Annales Univer Mariae Curie-Sklodowska, 1998; 52:74-78.
5. *Rolinski J. et al.*: Differential expression of the intercellular bcl-2 oncoprotein and the Fas (CD95) antigen in B and T cells in patients with B chronic lymphocytic leukaemia and in normal individuals. Central Europ. J. Immunol., 1998; 23: 37-44.
6. *Scaffidi C. et al.*: Apoptosis signaling in lymphocytes. Curr. Opin. Immunol., 1999; 11: 277-285.

STRESZCZENIE

Mononukleoza zakaźna jest chorobą powodowaną przez wirus Epstein–Barr'a. Kluczową rolę w kontrolowaniu liczby komórek zakażonych pełnią limfocyty T cytotoksyczne oraz komórki

NK. Są one w stanie indukować apoptozę zakażonych komórek. Kluczowe znaczenie dla możliwości indukcji apoptozy ma ekspresja białek z rodziny bcl-2. W obecnej pracy stwierdziliśmy wyższą ekspresję białka bcl-2 w limfocytach B, niż T u pacjentów z mononukleozą zakaźną. Wykazaliśmy również, że u zdrowych dawców krwi ekspresja ta jest wyższa w limfocytach T, niż B. Otrzymane wyniki mogą sugerować, że podwyższona ekspresją białka bcl-2 może być jednym z mechanizmów obrony przed układem immunologicznym przez komórki zakażone wirusem Epstein–Barr'a.