EBV-linked Immunophenotypes of Burkitt's Lymphoma Cell Lines Defined by 5-color Flow Cytometric Analysis

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Introduction. Burkitt's lymphoma (BL) is an aggressive B-cell lymphoma, characterised by a high degree of proliferation of malignant cells. Epstein-Barr virus (EBV) is implicated in the pathogenesis of the endemic BL. The EBV-positive BL-derived cell lines initially maintain the original tumour phenotype of EBV infection (latency I, LatI), but most of them drift toward a lymphoblast phenotype of EBV latency III (LatIII) during *in vitro* culturing.

The aim. The aim of the present work was to characterise the phenotypes of B cells in BL cell lines and to verify whether particular cell subsets correlate with the type of EBV infection (LatI and LatIII).

Materials and methods. The phenotype analysis of two EBV-negative and eleven EBV-positive (three of LatI and eight of LatIII) BL cell lines was performed by polychromatic flow cytometry (FC), based on expression pattern of CD19, CD10, CD38, CD27, and CD5 markers. Cells were scored using a FACSAriaII analyser equipped with 3 lasers (BD; Becton, Dickinson and Company, NJ, USA), and data were processed using Diva6.2 software (BD). The same approach was applied for the characterisation of B-cell subpopulations (immature, naïve, memory, and plasma cells) in peripheral blood (PB) of 8 healthy donors.

Reverse transcription (RT)–duplex-PCR analysis was carried out using primers that define the EBV *EBNA1* transcripts derived from Qp and from Wp/Cp promoters, and the EBV *EBNA2* and *LMP1* transcripts. The presence of EBV DNA in PB of donors was evaluated by PCR (single and nested).

Results. Using RT–duplex-PCR of EBV genes, *EBNA1* (derived from Qp and from Wp/Cp promoters) and *LMP1*, we confirmed the presence of EBV and the EBV latency types in 13 BL cell lines, two EBV negative and eleven EBV positive (three of LatI and eight of LatIII).

For a phenotype profiling of BL cell lines, analysis of cell surface expression of CD19, CD10, CD38, CD27, and CD5 was carried out. In EBV$^-$ and LatI BL cell lines, all cells showed the CD19$^+$CD10$^+$CD38$^+$ phenotype. In contrast, in three LatIII cell lines that were established by a single cell cloning of the original BL tumour-derived cell lines; more than 90% of the cells were CD19$^+$CD10$^-$ CD38$^+$. The original BL tumour-derived cell lines of LatIII, i.e. Jijoye M13, Raji, BL18, Akuba, and BL16, consisted of the CD19$^+$CD10$^+$ and CD19$^+$CD10$^-$ cell subsets in proportion of 84–28% and 17–72% of the cells, respectively.

The BL cell lines phenotypes were compared with the phenotypes of B-cell subpopulations circulating in PB of healthy donors. EBV DNA was not detected in the PB leukocytes of any of the 8 donors analysed, using single PCR, thus excluding the involvement of EBV in B cell activation.

Conclusions. The phenotype analysis of cells in EBV-negative and EBV-positive (both of LatI and LatIII) BL-tumours derived cell lines defined two cell subsets, CD19$^+$CD10$^+$ and CD19$^+$CD10$^-$, in LatIII cell lines; in both cell subsets, the CD27 and CD5 cell surface expression was detected in a small proportion of the cells. A proportion of the cells in LatIII BL cell lines display the phenotype of peripheral blood circulating mature B cells (CD19$^+$CD10$^-$CD38$^+$ CD27$^+$ CD5$^-$). This observation may suggest that expression of the EBV LatIII genes may promote the differentiation of malignant cells.

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