

Multiparametric flow cytometry analysis of peripheral blood B cell trafficking differences among Epstein-Barr virus infected and uninfected subpopulations

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Aims. Epstein-Barr virus (EBV) targets predominantly B cells and these cells could acquire new phenotype characteristics. Here we analyzed whether EBV-infected and -uninfected B cells from healthy subjects differ in proportion of dominant phenotypes, maturation stage, and homing receptors expression.

Methods. EBV-infected and -uninfected cells were identified by flow cytometry using fluorophore-labeled EBV RNA-specific DNA probes combined with fluorophore-labeled antibody to surface lineage markers, integrins, chemokine receptors, and immunoglobulin isotypes, including intracellular ones.

Results. Our results show that the trafficking characteristics of EBER^{pos} B cells are distinct from EBER^{neg} B cells with most dominant differences detected for $\alpha 4\beta 1$ and $\alpha 4\beta 7$ and CCR5 and CCR7. EBV-positive cells are predominantly memory IgM⁺ B cells or plasmablasts/plasma cells (PB/PC) positive for IgA or less for IgM. In comparison to uninfected B cells, less EBV-positive B cells express $\alpha 4\beta 7$ and almost no cells express $\alpha 4\beta 1$. EBV-positive B cells contained significantly higher proportion of CCR5⁺ and CCR7⁺ cells in comparison to EBV-negative cells. *In vitro* exposure of blood mononuclear cells to pro-inflammatory cytokine IL-6 reduces population of EBV-positive B cell.

Conclusion. Although EBV-infected B cells represent only a minor subpopulation, their atypical functions could contribute in predisposed person to development abnormalities such as some autoimmune diseases or tumors. Using multi-parameter flow cytometry we characterized differences in migration of EBV-positive and -negative B cells of various maturation stage and isotype of produced antibodies particularly different targeting to mucosal tissues of gastrointestinal and respiratory tracts.

Key words: Epstein-Barr virus, cell trafficking, IgM, IgA, naïve B cells, memory B cells, plasma blast, plasma cell

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INTRODUCTION

EBV infection has been reported to be involved in the development of various human diseases of infectious (infectious mononucleosis), malignant (nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin lymphoma, and others), and autoimmune (systemic lupus erythematosus, multiple sclerosis, and inflammatory bowel disease) nature¹⁻⁷. EBV infects predominantly B cells but these cells do not express specific surface markers to be distinguished from healthy ones. Combination of *in situ* hybridization with flow cytometry clearly enables identification of EBV-infected cells in population of thousand to millions of peripheral blood mononuclears. Moreover, it is possible to specify their phenotype, immunoglobulin isotype, and

their migration profile by identification of surface-exposed or intracellular molecules such as chemokine receptors, integrins or selectins, surface and intracellular immunoglobulin isotype and lineage-specific markers. Changes in expression of different markers between EBV-infected and healthy cells and changes in their function could help to reveal the etiological aspects of various diseases suspicious of association with EBV infection.

By multicolor flow cytometry phenotyping of EBV-positive and EBV-negative cells based on EBV RNA hybridization and antibody detection of lineage markers CD19, CD27, CD38, CD138, surface and intracellular immunoglobulins IgA, IgG, IgM, IgD, integrins $\alpha 4\beta 1$ and $\alpha 4\beta 7$, L-selectin and chemokine receptors CCR5, CCR7, CCR9, and CCR10 the differences in phenotypes, surface

and intracellular isotypes and migration characteristics were analyzed.

Because inflammatory stimulation of EBV-infected cells has been proposed to play a role in the EBV-associated diseases, peripheral blood mononuclear cells (PBMC) were exposed *in vitro* to IL-6, that is known as an effective B cell activator⁸⁻¹⁰, to analyze whether stimulation of EBV-infected B cells change their behavior, phenotypes and immunoglobulin isotypes expressed.

MATERIAL AND METHODS

Reagents

All chemicals, except where mentioned, were purchased from Sigma (St. Louis, MO). Tissue-culture media and media-supplements were purchased from Invitrogen (Carlsbad, CA).

PBMC isolation

Peripheral blood from healthy donors was obtained from the Department of Transfusion Medicine, University Hospital Olomouc. Peripheral blood mononuclear cells (PBMCs) were isolated by standard protocol described elsewhere with several modifications. Shortly, 25 mL of EDTA-anticoagulated blood was mixed with sterile PBS (Biosera) at 3:2 ratio. The blood was applied by overlaying on the Histopaque 1077 (Sigma) and centrifuged at 400 x g, RT for 30 min. The obtained PBMCs were washed twice with PBS for 20 min at RT using centrifugation with RCF = 200 x g.

IL-6 stimulation of PBMC

PBMC were transferred to complete growth medium (RPMI 1640, 10% FBS, 1% PenStrep) with IL-6 at concentration 10 ng/mL and incubated for 7 days at 37 °C, 5% CO₂.

Cell staining for FC analysis

Isolated PBMCs were stained in three steps: cell surface molecules and antibodies staining, intracellular antibodies staining, and at the end hybridization with DNA probes. Surface staining was performed after blocking the cells with 10% heat inactivated human serum in PBS (Biosera, Nuaille, France) for 10 min at RT. Surface molecules were labeled using selected of following mAbs: Anti-CD19-PE, Anti-CD19-eFluor780, Anti-CD19-eFluor610, Anti-CD27-PE-Cy7, Anti-CD38-PE-Cy5, Anti-CD138-PE-Cy7, Anti-IgM-FITC, Anti-IgD-FITC, Anti-β1 integrin-PE, Anti-β7 integrin-PE-Cy7, Anti-CD62L-PE-Cy5, Anti-CCR7-PE-Cy7, Anti-CCR9-PE (e-Bioscience, San Diego, CA), Anti-CD27-APC-H7, Anti-CCR5-PE-Cy5, Anti-IgG-PE-Cy5, Anti-CCR10-PE, Anti-α4 integrin-PE-Cy5 (BD Biosciences, San Jose, CA), Anti-IgA-biotin, Anti-IgG-biotin (Jackson ImmunoResearch, West Grove, PA). In addition, Streptavidin-FITC (Southern Biotech, Birmingham, AL), Streptavidin-TRITC (Southern Biotech, Birmingham, AL), Streptavidin - Pacific Orange

(ThermoFisher Scientific, Waltham, MA) was used in case of biotin-labeled mAb. Viability was analyzed by Fixable Viability Dye-eFluor780 and Fixable Viability Dye-eFluor450 (e-Bioscience, San Diego, CA). All mAbs were diluted in PBS + 10% FBS and PBMCs were stained by different combinations of above antibodies for 20 min in the dark at RT. After washing, cells were fixed by 4% paraformaldehyde (EMS, Hatfield, PA) for 10 min at RT, and permeabilized with 0.5% Tween 20 (Serva, Heidelberg, Germany) in PBS for 10 min at RT. If required, permeabilized cells were stained for intracellular immunoglobulins using Anti-IgA-biotin, Anti-IgG-biotin and Streptavidin-FITC diluted in PBS + 0.25% Tween 20 + 5% FBS. Here, cells were incubated in the dark for 30 min at RT. Fluorophores used for staining were selected based on their stability at higher temperatures and higher salt concentrations used in subsequent hybridization procedure.

In situ hybridization was performed as follow: mAb-stained cells were washed in adapting buffer containing formamide (31.25 mM NaCl, 6.25 mM Na₂EDTA, 62.5 mM Tris-HCl pH 7.5 and 37.5% formamide) and then incubated with EBER-1-specific hybridization DNA probes diluted in hybridization solution (10% Dextran sulfate, 10 mM NaCl, 30% formamide, 0.1% Sodium pyrophosphate, 0.2% polyvinylpyrrolidone, 5 mM Na₂EDTA, and 50 mM TRIS-HCl, pH 7.5) at 42 °C for 1 h in the dark.

Three independent EBER-1 probes were used for hybridization. Probes were synthesized as conjugated either with Cy5 or Cy3 (Generi Biotech, Hradec Kralove, Czech Republic), based on antibodies used in parallel for staining. The sequences are as follows:

5'-AAACATGCGGACCACCAGCTGGTAC-3',
5'-AAGACGGCAGAAAGCAGAGTCTGGG-3',
5'-AAACCTCTAGGGCAGCGTAGGTCCT-3'

After the hybridization, cells were washed using 0.5% Tween 20 in PBS at 42 °C for 10 min, centrifuged, and subsequently washed for 30 min at the same conditions. Cells were subsequently used for flow cytometry analyses.

Flow cytometry analysis

Two different instruments were used for FACS analysis. The Canto II cytometer (BD Biosciences) equipped with blue (488 nm) and red (633 nm) laser and DIVA software and the SONY Spectral Analyzer (SONY Biotechnologies) equipped with blue (488 nm) and violet (405 nm) laser and SP6800 software. More detailed analyses were performed by FlowJo software (Tree Star Inc., Ashland, OR).

Statistics

Statistical analysis was performed utilizing one-way ANOVA model used for multiple group comparisons with Tukey's post-hoc test. All statistical analyses were done by GraphPad Prism v.5 software (GraphPad Software, La Jolla, CA).

RESULTS

Phenotypes and immunoglobulin isotypes distribution on EBV infected B-cells

EBV-infection of B cells was detected by EBV-specific DNA probes EBER, conjugated with cy3 or cy5 fluorophores. The expression of selected surface and intracellular receptors or immunoglobulins was analyzed for individual cell together with *in situ* hybridization with EBER to distinguish subsets of EBV-infected and uninfected B cells by flow cytometry.

Three different groups – naïve and memory B cells and plasmablasts/plasma cells were identified by CD27, CD38, and CD138 surface markers. CD19⁺ CD27^{dim} CD38^{dim} cells represent naïve B cells; CD19⁺ CD27⁺ represent memory B cells, and CD19^{dim} CD27⁺⁺, CD38⁺ represent plasmablasts/plasma cells (PB/PC). Plasma cells, in addition to PB are CD138⁺, but in healthy individuals PC are almost absent in peripheral blood^{11,12}.

PBMC were stained for CD19, CD27, CD38, and CD138 using specific antibodies and for EBER using specific DNA probe. Cells were analyzed by flow cytometry. Data are means \pm SD. Multiple groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test, *** $P < 0.001$

Although the total number of EBV-infected (EBER^{pos}) CD19⁺ cells in peripheral blood is very low – 0.03% of total CD19⁺ cells, the distribution of the phenotype within EBV-infected cells or EBV-negative cells is significantly distinct (Fig. 1). In the group of EBV-negative cells the naïve B cells population dominates followed by memory B cells. In contrast, in the population of EBV-infected cells, memory and PB/PC dominates whereas naïve B cells represent minority.

Analysis of surface immunoglobulin expression on CD19⁺ cells (Fig. 2) confirmed substantial differences between uninfected and EBV-infected cells. In uninfected peripheral blood B cell population, surface IgM⁺ and

IgD⁺ cells dominate, followed by surface IgG⁺ and IgA⁺ (Fig. 2A). In contrast, in the population of EBV-infected B cells, less dominant majority represent IgM⁺, followed by IgG⁺ and IgA⁺ (Fig. 2B). Negligible number of EBV-positive cells were IgD⁺. If we analyzed percentage of EBV-positive cells for individual surface immunoglobulin (sIg) isotype positive population (sIgA, sIgG, sIgM, or sIgD), the highest percentage of EBV positivity was identified for surface IgA⁺ B cells, followed by IgG⁺ and IgM⁺ (Fig. 2C). This corresponds to previously published data obtained by PCR analysis of PBMC (ref.¹³). Furthermore, the low percentage of EBER^{pos} IgD⁺ B cells (Fig. 2B) indicates that EBER^{pos} IgM⁺ B cell are not IgD⁺ and thus are not naïve but rather memory B cells. Naïve B cells are considered to be double positive for IgM⁺ + IgD⁺ (ref.^{14,15}). Furthermore memory B cells were CD27^{dim} whereas naïve B cells were CD27^{dim} (data not shown) in accordance with general literature^{16,17}.

PBMC were stained with fluorophore-labeled antibodies specific to CD19 and surface immunoglobulins of IgA, IgG, IgM, and IgD isotype and hybridized with EBER DNA probe specific to EBV-encoded small RNAs. Cells were analyzed on flow cytometry. Data are means \pm SD. Multiple groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test, *** $P < 0.001$, * $P < 0.05$.

EBER positive PB/PC are mainly intracellularly positive for IgA

Results presented in the (Fig. 1) indicated that half of the EBER^{pos} CD19⁺ cells are memory and half of them are PB/PC. Despite the fact that the percentage of intracellularly positive cells in PBMC is very low – approx. 1% (Fig. 1,3A), the proportion of individual immunoglobulin isotype positive cells differs for EBER^{pos} and EBER^{neg}. In EBER^{neg} PB/PC the most abundant population of intracellular immunoglobulin (iIg) positive cells are iIgM⁺, followed by iIgA⁺ and iIgG⁺ (Fig. 3A) In contrast, in the

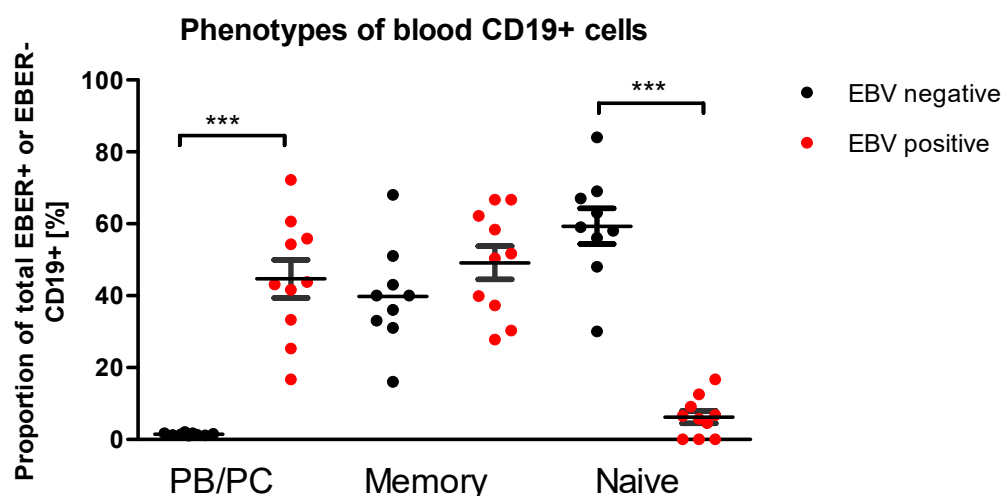


Fig. 1. Proportion of naïve and memory B cells and PB/PC in peripheral blood are different for EBV-infected and uninfected cells.

population of EBER^{pos} PB/PC the iIgA⁺ population significantly dominates over others iIg isotypes (Fig. 3B).

PBMC were stained with fluorophore-labeled antibodies against surface CD19, CD27, CD38 and intracellular iIgA, iIgG, iIgM followed by *in situ* hybridization with EBER DNA probe specific to EBV-encoded small RNAs. A) Population of EBER^{neg} CD19^{dim} CD27⁺⁺, CD38⁺ representing PB/PC was analyzed for distribution of individual iIg cells. B) Population of EBER^{pos} PB/PC was analyzed for distribution of individual iIg cells. Data are means \pm SD. Multiple groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

EBV-infected B cells keep expression of integrin $\alpha 4\beta 7$ but reduce the expression of $\alpha 4\beta 1$ leading to their preferential targeting to gut mucosa

Because peripheral blood EBER^{pos} CD19⁺ cells exhibit dominantly memory or PB/PC phenotype, we analyzed migration destination of such cells by analyzing surface integrin and chemokine expression.

Analysis of $\alpha 4\beta 1$ positive or $\alpha 4\beta 7$ positive cells, and L-selectin positive cells from CD19⁺ population is shown in Fig. 4A.

PBMC were stained with fluorophore-labeled antibodies against CD19, sIgG, sIgA, $\alpha 4\beta 7$, $\alpha 4\beta 1$, L-selectin, and hybridized with EBER DNA probe specific to EBV-encoded small RNAs. A) Expression of $\alpha 4\beta 7$, $\alpha 4\beta 1$, and L-selectin in population of all B cells to EBV infection (EBER^{neg} cells are in black, EBER^{pos} cells are in red).

Analogous analysis was performed for B) sIgA⁺ and C) for sIgG cells. Data are means \pm SD. Multiple groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test, * $P < 0.05$; *** $P < 0.001$.

The percentage of $\alpha 4\beta 1$ ⁺ and $\alpha 4\beta 7$ ⁺ B cells differ significantly between EBV-positive and negative populations (Fig. 4A) indicating that EBV affects substantially migratory behavior of peripheral blood B cells. Although EBV-negative B cells are dominantly $\alpha 4\beta 7$ ⁺, typical for gut homing PB, EBV-positive population contains significantly less $\alpha 4\beta 7$ ⁺ cells. Even more, EBV positivity is associated with almost total loss of $\alpha 4\beta 1$ ⁺ cells, whereas EBV negative B cells population contains about 15% of $\alpha 4\beta 1$ ⁺ cells. Furthermore, we determined integrin expression on sIgA⁺ or sIgG⁺ cells relative to EBV positivity (Fig. 4B,C). In the case of sIgA⁺ population, EBV positivity is associated with insignificant increase in $\alpha 4\beta 7$ ⁺ cells but total absence of $\alpha 4\beta 1$ ⁺ cells which implicates limited migration of EBV-infected peripheral blood IgA⁺ cells to upper respiratory tract, bronchus, lungs, tonsils, and Waldeyer's ring¹⁸⁻²⁰. Less pronounced changes were detected for sIgG⁺ cells. Observed differences between EBV-positive and negative cells for integrin expression on total B cell population, sIgA⁺, and sIgG⁺ cells (Fig. 4A versus Fig. 4B,C) indicate involvement of sIgM cells especially in EBV-negative B cells population.

When analyzing B cells for L-selectin relative to EBV infection, no significant difference was observed between EBV -positive and -negative B cells with mean 80% of positive B cells.

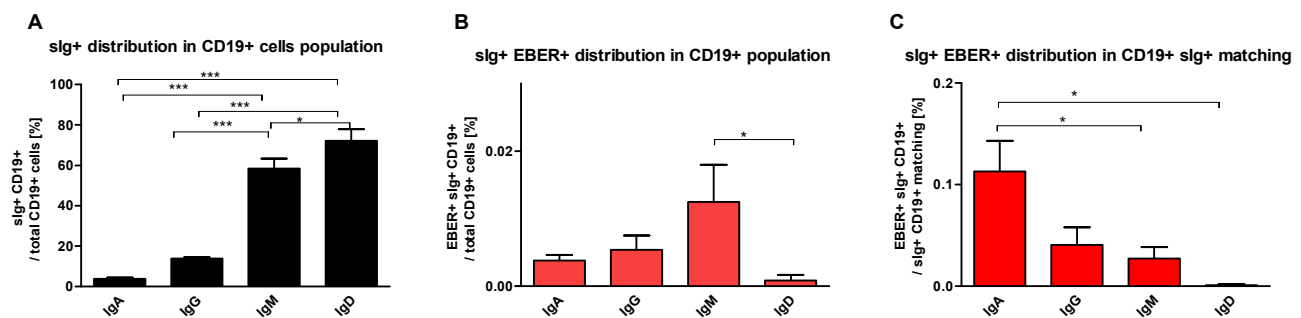


Fig. 2. Differences in surface immunoglobulin expression on CD19⁺ cells uninfected and infected with EBV.

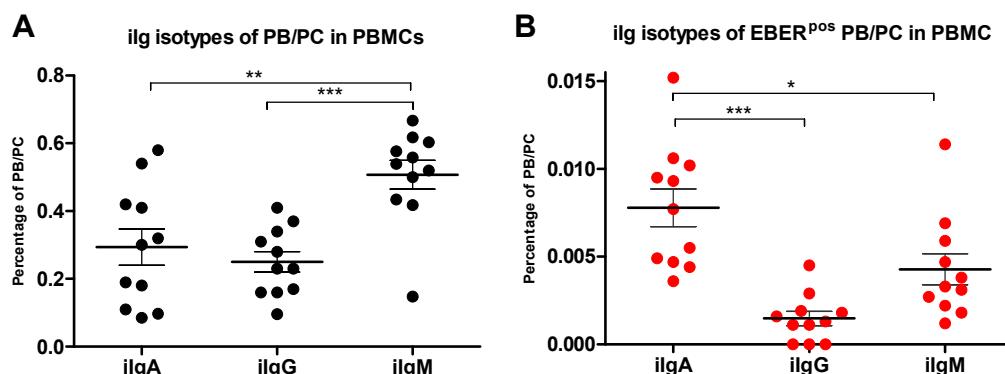


Fig. 3. EBER^{pos} PB/PC cells are predominantly of iIgA⁺.

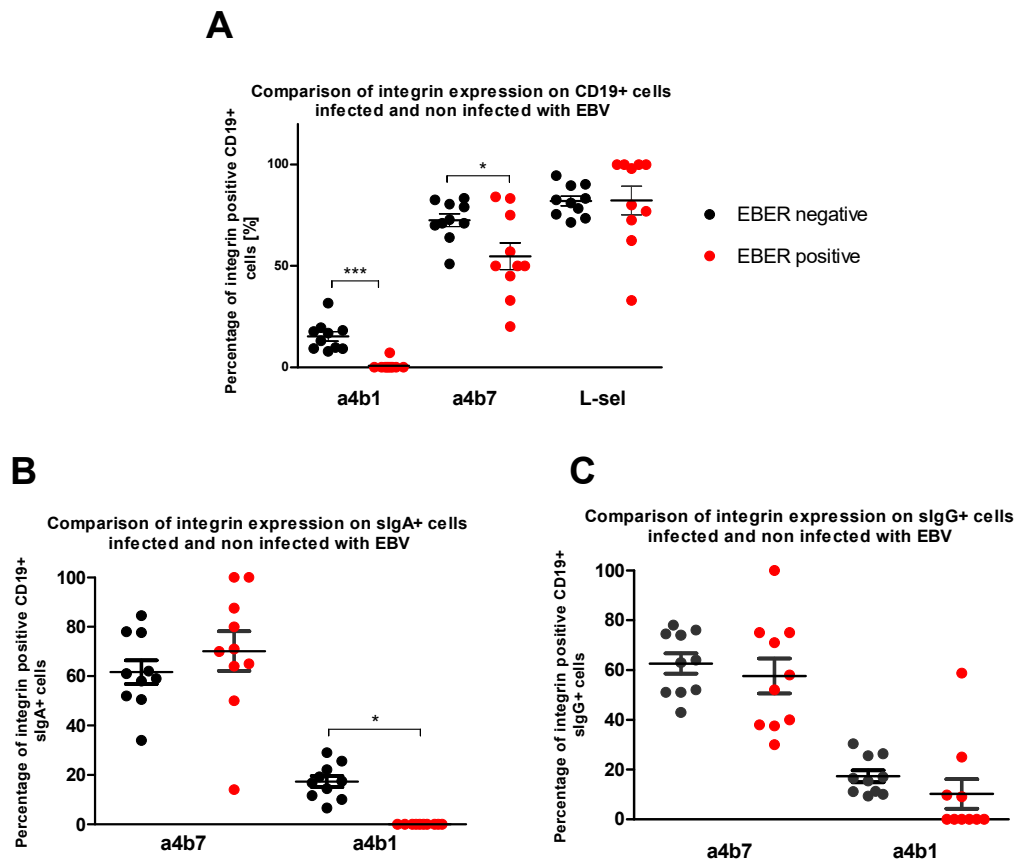


Fig. 4. Integrin molecules expression in total B-, sIgA⁺- and sIgG⁺- cells relative to EBV infection.

Chemokine receptor expression

In addition to integrins and L-selectin, B cells were analyzed for the expression of CCR5, CCR7, CCR9, CCR10 chemokine receptors depending to EBV positivity and negativity. EBV-positive B cells contained significantly higher proportion of CCR5⁺ and CCR7⁺ cells in comparison to EBV-negative cells. The changes of the percentage of CCR9⁺ and CCR10⁺ cells relative to EBV infection was only modest and not significant (Fig. 5).

PBMC were stained using fluorophore-labeled antibodies specific to CD19, CCR5, CCR7, CCR9, and CCR10 and hybridized with EBV DNA probe specific to EBV-encoded small RNAs. Cells were analyzed by flow cytometry. EBER^{pos} cells are in red, EBER^{neg} cells are in black. Data are means \pm SD. Multiple groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test, * $P < 0.05$; *** $P < 0.001$.

B cells response to IL-6 in EBV-positive and EBV-negative cells

IL-6 stimulates predominantly CD19⁺ CD27⁺⁺ CD38⁺ CD138⁺ with low level of surface immunoglobulin (sIg^{dim}) and high level of intracellular immunoglobulin (iIg⁺) expression corresponding to plasmablasts and/or plasma cells (PB/PC) (data not shown). After IL-6 stimulation, the population of EBER⁺ B cells is substantially reduced (Fig. 6A) indicating that in healthy subjects IL-6 stimulates preferentially EBV-negative cells of which

IgA-producing plasma cells are the most effective IL-6 responders (Fig. 6B). When analyzing EBER⁺ subpopulation of PB/PC after IL-6 exposure, variable reduction was observed for individual isotype (iIgA, iIgG, iIgM) positive PB/PC. The most substantial reduction was observed for iIgM, followed by IgG and IgA (Fig. 6C). Thus IgA-producing EBV-positive PB/PC are the only subpopulation of EBV-infected PB/PC which after IL-6 stimulation is able to contribute to immunoglobulin production in addition to dominant EBV-negative IgA producing PB/PC.

PBMC were stimulated with 10 ng/mL IL-6 for 7 days and thereafter stained using fluorophore-labeled antibodies specific to CD19, CD27, CD38, CD138, surface immunoglobulin (sIg) and intracellular immunoglobulin (iIg), and hybridized with EBV DNA probe specific to EBV-encoded small RNAs. Cells were analyzed by flow cytometry. EBER^{pos} cells are in red, EBER^{neg} cells are in black. Data are means \pm SD. Multiple groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

Our results showed a substantial discrepancies in migratory behavior of Epstein-Barr virus infected B cells in peripheral blood. Using multiparametric flow cytometry when intracellular and extracellular antigens are detected

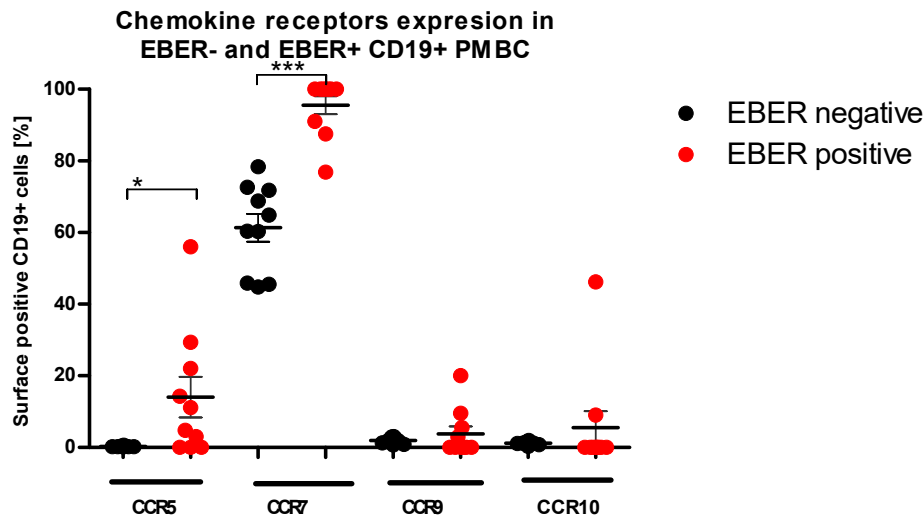


Fig. 5. The expression of chemokine receptors on B cell relative to EBV infection.

together with intracellular EBV-encoded small RNAs we are able to analyze properties of individual B cells subpopulation. Beside maturation and isotype distribution in EBV-positive and -negative B cells we could analyze other characteristics such as integrin and chemokine receptors expression as a marker of trafficking destiny.

All previously published analyses of EBV-infected cells were based on pool of cells isolated by specific phenotype (naïve cells, memory cells, plasmablast, or immunoglobulin isotype (IgA⁺, IgG⁺, IgM⁺) followed by analysis of EBV nucleic acid copies within cells or after cell stimulation in the culture supernatants²¹⁻²³. Other often used approach focuses on describing of particular changes in cells that have been immortalized by EBV (ref.^{24,25}). Different chemokine receptors as well as integrins expression were described^{26,27}.

Another very frequently used approach for EBV detection uses fixed embedded tissues, that are immunohistochemically characterized using *in situ* hybridization with EBV-specific PNA probes²⁸⁻³⁰. Nevertheless, none of such methods enables a broad and specific characteristic of individual EBV-infected cells at various maturation stage, expressing various migratory molecules and various antibody isotype intracellularly or on their surface in one assay.

Our results describe the characteristics of peripheral blood EBV-infected cells in healthy individuals. We show that EBV-positive B cells are predominantly IgM⁺ (and less IgG⁺) memory B cells or IgA⁺ (and less IgM⁺) plasmablasts/plasma cells and minority of naïve B cells. These results correspond with the high percentage of IgM⁺ B cells in peripheral blood. Nevertheless, applied on percentage of each sIg⁺ population, it could be summarized, that EBV preferentially infects sIgA⁺ B cells (Fig. 2C).

Moreover our results show that the trafficking characteristics of EBER^{pos} B cells are distinct from EBER^{neg} B cells. Maximal differences were detected for $\alpha 4\beta 1$ fol-

lowed by $\alpha 4\beta 7$ and CCR7 and weakest differences were detected for CCR5. Although EBER^{neg} B cells from peripheral blood are mainly naïve B cells targeted to peripheral lymph nodes by $\alpha 4\beta 7$, L-selectin and partially by CCR7 (Fig. 4A, Fig. 5), EBER^{pos} B cells are mainly of memory and PB/PC subtypes, expressing less frequently $\alpha 4\beta 7$, but, in absolute majority of EBER⁺ cell CCR7. Thus, it could be hypothesized that one of dominant destination for EBER⁺ cells are peripheral lymph nodes including spleen³¹⁻³⁵, in other words, it could be hypothesized that EBV infection of B cells actively narrows their migration through body compartments. The only EBER^{pos} B cell population which is not reduced in $\alpha 4\beta 7$ expression are surface IgA⁺ cells (Fig. 4B) indicating that EBER⁺ IgA⁺ cells could reach more easily peripheral lymph nodes and spleen than their healthy counterparts.

The EBER^{pos} PB/PC subpopulations expressing $\alpha 4\beta 7$, CCR9, and CCR10 are expected migratory cells destined to places of antibody secretion, such as gut lamina propria or nasal cavity. In contrast to healthy B cells of which almost none are CCR9 and CCR10 positive (Fig. 5), at least some EBER^{pos} B cells express CCR9 and CCR10 which could indicate that EBV infection enhances migration to gut or nasal mucosa. EBER^{pos} IgG⁺ B cells express $\alpha 4\beta 7$ and 10% also $\alpha 4\beta 1$, of which the second would target them to bone marrow^{36,37}.

The differences in EBER^{pos} B cells and EBER^{neg} B cells in maturation stage, homing-migration molecules and immunoglobulin isotype expression in peripheral blood of healthy individuals clearly shows that infection of B cells by EBV has dramatic effect on their function. Although generally EBV-infected B cells represent only minor subpopulation of total B cell pool, due to their atypical behavior, they could contribute to development of immune abnormalities such as autoimmune diseases or some tumors, as was proposed shortly after description of EBV biology^{23,38}.

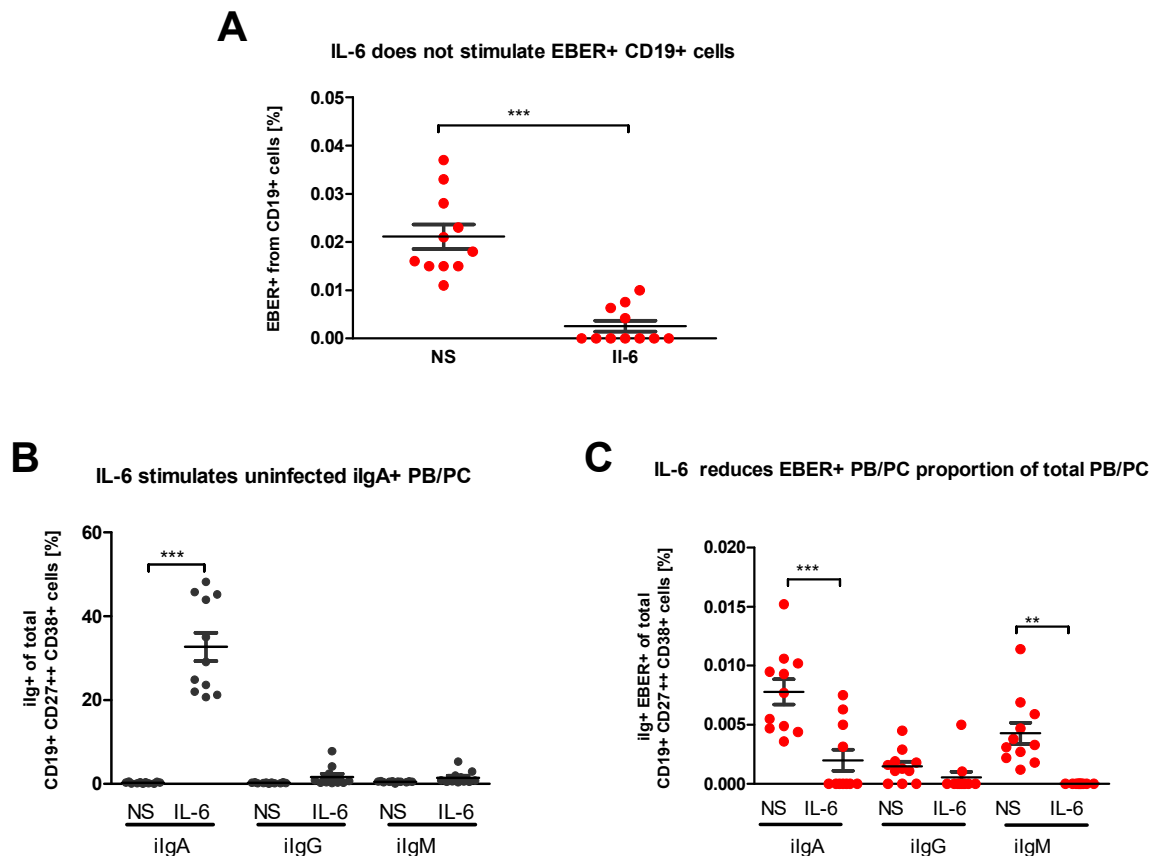


Fig. 6. Peripheral blood B cells response to IL-6.

CONCLUSION

Using multi-parameter flow cytometry analysis it is possible to measure simultaneously several characteristics on individual cell including surface receptor, intracellular DNA or RNA molecules, and intracellular protein expression.

We found that the trafficking characteristics of EBV-infected B cells are distinct for EBV-negative B cells with most dominant differences detected for $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrins and CCR5 and CCR7 chemokine receptors. EBV-infected cells are predominantly memory IgM⁺ B cells or plasmablasts/plasma cells positive for IgA and less cells positive for IgM. In comparison to uninfected B cells, EBV-positive B cells contain smaller population of cells expressing $\alpha 4\beta 7$ and almost no cells expressing $\alpha 4\beta 1$. EBV-infected B cells contained significantly higher proportion of CCR5⁺ and CCR7⁺ cells in comparison to EBV-negative cells. *In vitro* exposure of blood mononuclear cells to pro-inflammatory cytokine IL-6 reduces substantially the population of EBV-positive B cell of IgM⁺ and IgG⁺ subsets.

Although EBV-infected B cells represent only a minor subpopulation of all B cells in peripheral blood, their atypical functions could contribute in predisposed person to development of diseases of autoimmune or malignant nature.

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Author contributions: JM, MR: designed the study; KZ, MR: wrote the manuscript; KZ: performed the flow cytometry analyses; PK, KK: processed the patient's blood specimens; JZ, KM, KV: collected patient's clinical data and collected the blood samples; PH, HTH: interpreted experimental data.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

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