**EXPRESSION PROFILE OF NUCLEAR RECEPTORS UPON EBSTEIN — BARR VIRUS INDUCED B CELL TRANSFORMATION**

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**Background:** Infection of human B cells with Epstein — Barr virus (EBV) induces metabolic activation, morphological transformation, cell proliferation and eventual immortalization. Aim: To identify the nuclear receptors, which are the cellular interaction partners of EBV proteins, that will help to elucidate the mechanism of B cell transformation. Methods: We have compared the nuclear receptor profile in the naïve and EBV-transformed B-lymphocytes, using TaqMan LDA microfluidic card technology. Results: Out of 48 nuclear receptor, 17 showed differential expression at the mRNA level. The expression of 5 genes was elevated in EBV-transformed cells, whereas 12 genes were down-regulated in lymphoblastoid cells (LCLs). 7 genes were studied at the protein level; 2 genes were up regulated (Nr2F2 and RARA) and 4 genes were down regulated (ERB, NUR77, PPARG, and VDR) in LCLs. Conclusion: The nuclear receptor profiling on EBV infected B cells showed alterations of nuclear receptors expression at both mRNA and protein levels compared with non infected peripheral blood cells. Further analysis on a possible role of each nuclear receptor in EBV induced cell transformation should be performed.

**Key Words:** EBV, cell transformation, nuclear receptors, expression pattern, microarrays.

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Infection of human B cells with Epstein — Barr virus (EBV) induces metabolic activation, morphological transformation, cell proliferation and eventual immortalization [1, 2]. Six of the 9 EBV-encoded proteins (EBNA -1, -2, -3, -5, -6, and LMP-1) are necessary for the efficient transformation [3]. During transformation no genetic aberrations were detected. However, signal transduction pathways were ultimately changed — either blocked, either activated. For example, LMP1 activates TNFα/CD40 downstream signaling pathways that can stimulate cell growth and survival through activation of NFκB, jun and p38/map kinase. LMP2A activates constitutively B-cell receptor (BCR) (reviewed in [1]). Latency III genes expression leads to the change of gene expression profile. EBNA-2 activates and regulates the transcription of Notch and PU.1 responsive promoters of the cellular genes due to a binding to RBP-Jk. A more detailed description of some cellular pathways and, moreover, nuclear receptors that may be implicated in the EBV-induced B-cell transformation, are reviewed in [4].

Anyway, many questions about the mechanism of B cell transformation into lymphoblasts are unanswered yet. The identification of the cellular partners of EBV proteins and determination of the intervening cellular pathways will help to elucidate the mechanism of B cell transformation. The aim of the present paper was to compare the nuclear receptor profile in the naïve and EBV-transformed B-lymphocytes (freshly infected B cells and long-term cultured LCLs).

**MATERIALS AND METHODS**

**Western blotting.** We prepared whole cell lysates using NP40 lysis buffer (1% NP40, 150 mM NaCl, 50mM Tris, pH = 8) with a protease inhibitor cocktail (Sigma-Aldrich, St Louis, MO, USA). Lysates were cleared by centrifugation. Proteins were separated using the sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE). After transfer the membrane was probed with the specific antibodies: mouse monoclonal anti-actin (Sigma-Aldrich), anti-Nr2F2 (Abnova Corp., Taipei, Taiwan), anti-RARA (Abnova Corp.), anti-VDR (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), and polyclonal mouse serum against PPARG (Abnova Corp.); rabbit polyclonal serum against NUR77 (Santa Cruz Biotechnology Inc.). Secondary antibodies (anti-rabbit and anti-mouse IgG Horseradish-conjugated) were purchased from GE Healthcare Bio-Sciences AB, Uppsala, Sweden.

**Low density array.** TaqMan LDA microfluidic card technology from Applied Biosystems (Foster City, CA, USA) allows the simultaneous assay of mRNA gene expression of up to 384 targets on a single card. The LDA used in this study was custom designed to consist of 48 TaqMan Gene Expression Assays (Applied Biosystems) per loading port (48 genes × 5 samples run three times for statistical significance). Each reaction well contained all reagents specific for a given assay. Each target assay consisted of a forward primer and a reverse primer.

**Cell culture, immunostaining and imaging.** All cells were cultured at 37 °C, in Iscove’s medium containing 10% fetal bovine serum and appropriate

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**Abbreviations used:** EBV — Epstein — Barr Virus; EBNA — EBV-encoded nuclear antigen; LCL — lymphoblastoid cell line; LMP — latent membrane protein; PBC — peripheral blood cells; TBC — tonsil B cells.
antibiotics. Tonsil B cells (TBC) were isolated from human tonsils obtained from routine tonsillectomy (Karolinska Hospital, Stockholm). The tonsils were cut into the fragments and passed through a metal mesh. Peripheral blood B cells were isolated from buffy coat blood (Karolinska Hospital) on Lymphoprep gradients. An ethical permission was received for both procedures of B cell isolation. Two subsequent rounds of E-rosetting removed the T-cells. The B95.8 EBV strain was used for B cell infection. Control B cells were activated by anti-CD40 mouse monoclonal antibody (Nordic Biosite AB, Täby, Sweden, 1 μg/ml) and IL4 (ImmunoTools GmbH, Friesoythe, Germany) 25 ng/ml for 48 h. Prior to immunostaining experiments, the cells were spun on glass slides, using Cytosin centrifuge. Immunostaining and digital image capturing was performed as described earlier [5]. Briefly, cells on slides after cytospin were fixed in a 1:1 mixture of cold methanol and acetone (−20 °C). After rehydration in phosphate buffer saline, cells were stained with antibodies. Hoechst 33258 (Sigma-Aldrich) was added at a concentration of 0.4 μg/ml to the secondary antibody for DNA staining when necessary. The images were captured using DAS microscope Leitz DM RB with a dual mode cooled charged coupled device (CCD) camera C4880 (Hamamatsu, Japan) or Zeiss LSM 510 laser scanning confocal microscope with ORCA-ER CCD camera (Hamamatsu).

RESULTS
Nuclear receptor expression profile on the microfluidic cards. We have run Nuclear Receptor profiling in freshly EBV-infected B cells (48 h) and lymphoblastoid cell lines (LCLs) versus primary B cells. We have used two different RNA samples of B cells: peripheral blood B cells (PBC) and TBC, and three different RNA samples of EBV-infected cells: 48 h post infection (EBV 48 h), and LCL that were cultured for 2 month and 1.5 year. The set of 48 nuclear receptors and GAPD were on the cards. Among them 12 receptors were not expressed, and 33 nuclear receptors and GAPD were captured using DAS microscope Leitz DM RB (CCD) camera C4880 (Hamamatsu, Japan) or Zeiss LSM 510 laser scanning confocal microscope with ORCA-ER CCD camera (Hamamatsu).

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<tr>
<th>No.</th>
<th>Name of the gene</th>
<th>Accession number, OMIM link</th>
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| 1   | PPAR-gamma, Peroxisome proliferator-activated receptor gamma; PPARG | NP_617925*
| 2   | ER-alpha, Estrogen receptor alpha; ER; ESR1; ER1 | NP_000116*
| 3   | ER-beta, Estrogen receptor beta; ER2 | NP_00116*
| 4   | Nr1H3, Nuclear receptor subfamily 1, group H, member 2; Liver X receptor alpha; LRX | NP_005684*
| 5   | Nr2F1, Nuclear receptor subfamily 2, group F, member 1; Transcription factor COUP1; TFCOUP1 | NP_005645*
| 6   | Nr3C1, Nuclear receptor subfamily 3, group C, member 1; Glucocorticoid receptor; GCCR | NP_001108087*
| 7   | Nr4A1, Nuclear receptor subfamily 4, group A, member 1; NAK1; Nuclear hormone receptor TR3; TR3; NUR 77 (homolog of mouse NUR77) | NP_139139*
| 8   | RARB, Retinoid acid receptor beta | NP_000956*
| 9   | RORC, RAR-related orphan receptor gamma | NP_000505*
| 10  | RXRB, Retinoid X receptor beta | NP_602943*
| 11  | THR2, Thyroid hormone receptor beta | NP_000452*
| 12  | VDR, Vitamin D3 receptor | NP_000367*

**Genes downregulated in EBV-infected B cells.** Eleven genes were downregulated in LCLs compared with primary B cells (Fig. 2, a, b). We have run Western blotting for the 5 of them: ER-α and -β (Estrogen receptor -α (NP_000116) and -β (Q92731)), Nr4A1 (Nur77, NP_775180), PPARG (peroxisome proliferator-activated receptor γ, NP_619725), and VDR (vitamin D receptor, NP_000367). ER-α protein was not detected by Western blotting. ER-β protein level was very low in primary B cells and LCLs. Nur77 and PPARG protein levels in LCLs do not differ much from the protein level in the primary B cells (Fig. 2, c). Different trends in mRNA and protein levels (compare Fig. 2, a, c) could be due to the protein stability. However, VDR protein expression (Fig. 2, d) followed a pattern of mRNA expression (compare Fig. 2, a, d). Moreover, after a brief increase, all 11 receptors were expressed at lower level in LCLs, compared with primary B cells.

We have to mention, that not only the level of expression, but also a cellular distribution of the nuclear receptors were changed after EBV infection. For
example, in primary TBC the PPARG was expressed as small cytoplasmic patches (Fig. 3, a). Upon activation, pattern of expression of PPARG was changed. Upon LPS (lipopolysaccharide) stimulation of B cells PPARG showed perinuclear localization, which could also be found in the patches (Fig. 3, b). In B cells stimulated by anti-CD40 and IL-4, PPARG expressed not only in patches, but also at the membrane of the joined cells (Fig. 3, c). Upon EBV-infection PPARG was observed in the cellular membrane, and great portion of protein was distributed in both, a nucleus and the cytoplasm (Fig. 3, d). We may hypothesize that PPARG would function differently depending on distribution pattern in cells. However, this should be further elucidated.

**DISCUSSION**

EBV-encoded proteins expressed in latently infected B cells are known to interact with a cellular signaling pathways to establish latency and ensure the growth of...
The nuclear receptor profiling on EBV infected B cells showed alterations of nuclear receptors expression at both mRNA and protein levels compared with non infected peripheral blood cells. In most of the cases, the mRNA levels observed via LDA are strongly corroborated by expression at protein level. Further analysis on a possible role of each nuclear receptor in EBV induced cell transformation should be carried out.

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REFERENCES


