• Basic Research •

Effects of Epstein-Barr virus on host gene expression in Burkitt's lymphoma cell lines

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[Abstract] **Background and Objective**: Epstein-Barr virus (EBV) is present in Burkitt's lymphoma (BL) cells in a latent form, showing a highly restricted pattern of gene expression with few tumor cells undergoing viral lytic replication. BL cell lines can be induced to enter the viral lytic cycle and initiate replication by stimulating surface immunoglobulin molecules. During this process many EBV genes are expressed that have the potential to influence host gene expression. We aimed to identify host genes that are regulated by EBV in BL cells and those that are regulated following ligation of surface IgG. **Methods**: The differentially expressed genes in EBV-positive Akata cells and EBV-negative AK31 cells were detected by microarray. **Results**: A total of 91 human genes were differentially expressed between Akata and AK31 cells and 198 were differentially expressed when cells were stimulated to enter lytic replication. The differential expression of one gene, myd88, was correlated with disrupted TLR9 signaling. **Conclusions**: EBV down-regulates most of the genes regulated by surface Ig cross-linking in the early stages of lytic cycle activation. These include genes involved in cell survival, signal transduction, transcription control and the immune response that may mediate EBV transformation of B-lymphocytes and others such as HDAC4 that may affect virus replication.

Key words: Epstein-Barr virus, Burkitt's lymphoma, replication, microarray, TLR9, HDAC4

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Epstein-Barr virus (EBV) is a gamma-herpes virus present in more than 90% of the worlds population as a lifelong persistent infection. The virus is associated with a number of neoplastic diseases, which include Burkitt's lymphoma (BL),[1] undifferentiated nasopharyngeal carcinoma (NPC),[2] Hodgkins disease,[3] gastric carcinoma,[4] and lymphoproliferative disease in immunocompromised individuals.[5] Following initial infection of B lymphocytes, EBV enters a latent non-productive state expressing few viral genes. Several of these latency-associated genes contribute to the growth activation and immortalization of B cells observed following infection and are expressed in tumor cells;[6] these are prime candidates to mediate the oncogenic phenotype of the cells. Reactivation of EBV from latency occurs sporadically in vivo, resulting in expression of the majority of EBV genes and replication

of the virus (reviewed in reference 7). The possibility that EBV modulates the host environment during this period, in order to optimize conditions for viral replication, has been proposed and is exemplified by the ability of EBV to modify the effects of interferon-γ (IFN-γ) signaling by regulating expression of the receptor.[8]

During both EBV latency and EBV lytic cycle, transcription factors encoded by EBV are expressed and have the potential to alter the pattern of host gene expression. Indeed, the lytic cycle gene BZLF1 is known to be able to regulate the expression of several specific host genes,[9-12] as can the latency genes EBNA2 and EBNA3C.[13,14]

Here we have taken a systematic approach to identify host genes regulated by EBV during viral latency and following stimulation of viral replication. A cell line, Akata, derived from BL,[15] is used as an example of a transformed cell containing EBV genomes and expressing the group I pattern of latency genes [EBNA1 (EBV nuclear antigen 1), EBERs (EBV-encoded RNAs), LMP2A (latent membrane protein 2A) and the BamHI A transcripts].[16] Sub-clones from this cell line that have lost the EBV genome have a reduced transformation phenotype;[17] they have lost the ability to grow in a semi-solid medium and they are more prone to undergo apoptosis.[17] Upon re-infection with EBV these clones regain the original phenotype.[16,18] Furthermore, Akata cells express surface immunoglobulins (IgG) and can be readily stimulated to enter into the viral replicative cycle by cross-linking the surface IgG.[19] EBV-positive and -negative sub-clones of Akata cells therefore provide a system in which to identify host genes regulated by EBV during viral latency and during viral replication.

Material and Methods

Cell culture. Akata is an EBV-positive Group I Burkitt's lymphoma cell line,[15] AK31 is an EBV-negative sub-clone derived from Akata cells.[20] Cells were maintained in RPMI-1640 culture medium (Invitrogen). All culture media were supplemented with 10% (v/v) fetal calf serum (FCS), 2 mmol/L L-glutamine, 100 U/mL penicillin and 10 μg/mL streptomycin (all from Invitrogen) unless otherwise stated. Cells were incubated in a humidified atmosphere at 37°C supplemented with 5% CO_2. For stimulation of surface IgG, cells were re-suspended at a density of 2×10^6/mL and rabbit anti-human IgG was added to a final volume of 0.5% (v/v) for 6 h. For microarray expression analysis, experiments were undertaken with three biological replicates; other experiments were carried out with at least two biological replicates.

Where indicated, Akata and AK31 cells were concentrated by centrifugation (1300 rpm for 5 min) and re-suspended to 2×10^6 cells/mL in culture medium. Rabbit anti-human IgG (DakoCytomation) was added to cells (0.5% v/v), which were incubated at 37°C for 6 h. Following incubation, cells were harvested by centrifugation (1300 rpm for 5 min), washed twice with PBS (Invitrogen), and re-suspended in PBS at the indicated concentration.

Where indicated, cells were concentrated by centrifugation and resuspended in culture medium at a concentration of 2×10^6 cells/mL. Cells were seeded in flat-bottom 24-well plates, 1 mL/well and stimulated with 0.6, 6 and 30 μg/mL of CpG or GpC at 0, 4 and 18 h. For each stimulus, assays were set up in triplicate. After 48 h, cells were stained for surface CD86 expression and assayed by flow cytometry. We used the CpG-containing oligonucleotides (CpG) which mimic bacterial and viral genomic DNA,[21-23] to act as ligands for TLR9 and induce signal transduction. In B-lymphocytes, this results in B-cell activation and increased expression of cell-surface antigens including CD86.[24,25] The oligonucleotides used were CpG, 5-TCGTCGTTCCCCCCCCCCCC-3, and GpC, 5-TGCTGCTTCCCCCCCCCCCC-3.

Fixation of cells for flow cytometry analysis. Cells were harvested by centrifugation (1300 rpm for 5 min) and washed twice with PBS. The cell pellets were fixed in PBS / 2% (w/v) paraformaldehyde (for TLR9 detection) or not (for CD86 and surface IgG detection) at a concentration of 1×10^7 cells/mL. CD86 and IgG were detected using FITC-conjugated monoclonal antibodies (Dako Cytomation) or

isotype controls. TLR9 was detected using a rabbit polyclonal antibody (Oncogene) and a rabbit anti-mouse FITC-conjugated control (Dako Cytomation).

RNA preparation and analysis. RNA was prepared from cells that had been washed with PBS using an RNA easy kit (Qiagen). The quality of the RNA was analyzed using a RNA 6000 Nano assay chip (Agilent) on the 2100 Bioanalyser (Agilent).

Double-stranded cDNA synthesis for GeneChip analysis. First strand cDNA was synthesized from 10 μg of total RNA using 100 pmols T7- (T)24 primer, and incubated at 65-70°C for 10 min. cDNA was generated using Superscript II reverse transcriptase (Invitrogen). Following the incubation, the reactions were cleaned using the GeneChip sample cleanup module (Affymetrix). Each double-stranded cDNA was transcribed with T7 polymerase (Affymetrix) at 37°C for 5 h, then cleaned using the GeneChip sample cleanup module (Affymetrix).

Fragmentation of cRNA and preparation of GeneChip hybridization sample. Biotin-labeled cRNA was mixed with fragmentation buffer (Affymetrix) and incubated at 95°C for 35 min. Control oligonucleotide B2 and eukaryotic hybridization controls (both from Affymetrix) were heated at 65°C for 5 min and added to the hybridization mix (Affymetrix), then hybridized to a GeneChip array according to the manufacturers instructions (Affymetrix). Total RNA was prepared from exponentially growing cultures of the cells and biotin-labeled cRNA was transcribed from the total RNA. The cRNA was then processed to probe human expression arrays in triplicate (HG-U95Av2 GeneChip). The expression profiles from each set of biological replicates were compared and differential expression was identified. Selection criteria for differentially regulated genes were that there should be either (i) greater than 100% change in gene expression with a significance value of less than 0.05 or (ii) 50% change in gene expression with a significance value of less than 0.01.

Results

Identification of host genes regulated by group I EBV gene expression and lytic cycle reactivation. In order to identify host genes regulated by the type I latency pattern of EBV gene expression, the profiles of gene expression in EBV-positive Akata cells and their EBV-negative counterpart, AK31 cells, were compared. In addition, since both Akata and AK31 cells express surface IgG to a similar level (data not shown), comparisons were also made with these cells following cross-linking of surface IgG in order to identify genes specifically regulated in response to an inducer of viral lytic cycle. At this point, the immediate early genes BZLF1 and BRLF1 were expressed, but viral genome replication had not yet occurred.

Genes regulated by group I EBV gene expression. Forty-three genes were up-regulated and 48 were down-regulated in Akata cells when compared with those in AK31 cells. Those genes that were specifically up-regulated by EBV are shown in Table 1. Of these genes, HDAC4 and ORC2L had previously been shown to have roles for EBV.

Altered signal transduction through myd88. Expression of myd88 RNA was down-regulated by three folds in EBV-positive Akata cells when compared with that in EBV-negative AK31 cells (Table 2). Myd88 is a component of several TLR-signal transduction pathways and fluorescent activated cell sorting revealed that TLR9 was expressed at almost equivalent levels in both cell lines (Fig. 1), thus providing a route to question the relevance of the regulation of myd88 for signal transduction in these cells.

In order to evaluate whether the observed down-regulation of myd88 expression observed in Akata cells is sufficient to impact on TLR9 signal transduction, we stimulated Akata and AK31 cells with either an CpG-containing oligonucleotide or an oligonucleotide containing a GpC motif as a control. Analysis of cell surface expression revealed that the basal level of CD86 expression was 70% higher in AK31 cells than in Akata cells

(Fig. 2). Furthermore, stimulation with CpG-containing oligonucleotides induced CD86 expression by a further 60% over basal levels in AK31 cells in a dose-dependent manner. In contrast, no significant induction of CD86 was observed in Akata cells.

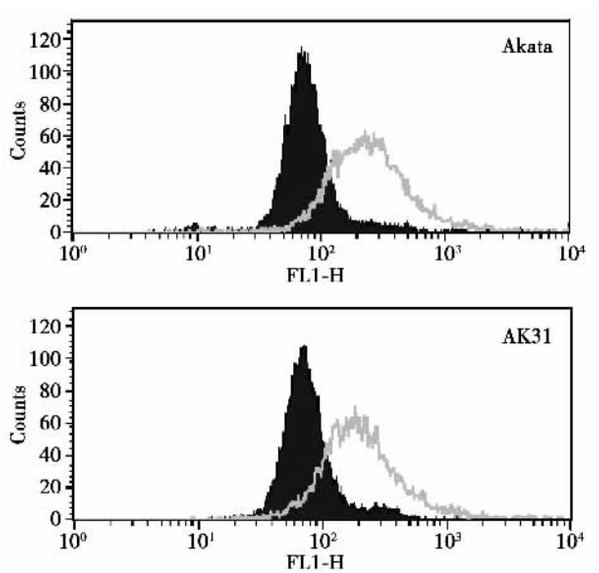

Figure 1 Myd88 and TLR9 expression in Epstein-Barr virus (EBV)-positive and EBV-negative cells detected by flow cytometry

The solid histogram represents the antibody control and the open histogram the TLR9 signal.

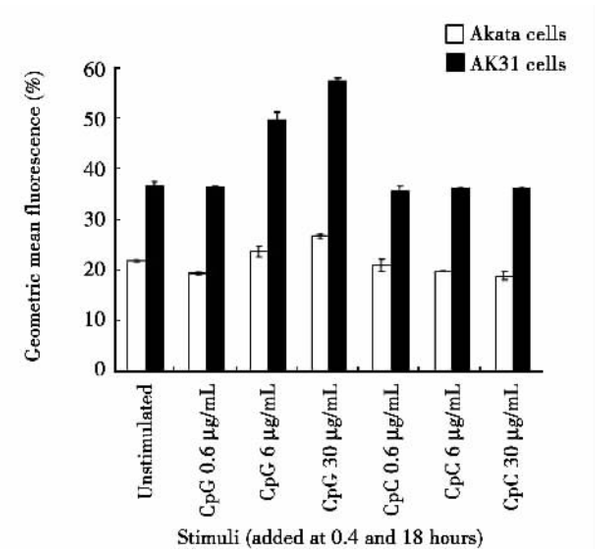

Figure 2 Expression of CD86 on Akata and AK31 cells after 48-hour stimulation of CpG or GpC detected by flow cytometry

IgG induced host gene expression blocked by EBV. IA sub-set of genes that are co-regulated following surface IgG stimulation in both cell lines were identified, including 62 genes up-regulated and 29 down-regulated (supplementary information, Table 1). In addition, we identified a further sub-set of genes that were regulated in AK31 cells, including 64 genes up-regulated and 27 down-regulated, in response to surface IgG signaling, while not regulated in Akata cells (supplementary information, Table 2). We also identified 16 genes that were regulated uniquely in Akata cells in response to surface IgG stimulation. The regulation of these genes was clearly augmented by the presence of the EBV genome and these genes were either candidates for regulation in response to the early stages of EBV lytic cycle activation or genes whose regulation by surface IgG stimulation was enhanced by EBV latency genes (Table 3). Among these genes, three transcription factors (Lyl1, TCF7 and RELB) and an ATP helicase (RECQL4) might play direct roles in the control of gene expression and viral replication.

Discussion

In this study, 289 host genes that are differentially regulated by EBV during viral latency and the initial phase of lytic cycle activation were identified.

EBV establishes a lifelong infection maintaining viral replication and undergoing occasional spontaneous reactivation in the face of a competent immune system; it is therefore not surprising that many of the cellular genes down-regulated in Akata cells are components of the innate or adaptive immune response. Genes from several stages of the IFN response are down-regulated, including the signaling molecule and transcriptional activator Stat1, the transcriptional activator ISGF3G and several anti-viral IFN-stimulated genes such as the

Table 1 Host genes up-regulated in Epstein-Barr virus (EBV)-positive Akata cells when compared with those in EBV-negative AK31 cells

GenBank gene symbol	Name	Change in expression
Intracellular signaling genes		
CCBP2	Chemokine-binding protein 2	Absent to present
CD80	CD80 antigen (CD28 antigen ligand 1, B7-1 antigen)	2.5 fold, *P*=0.003
HA-1	Minor histocompatibility antigen HA-1	Absent to present
LRP8	Low density lipoprotein receptor-related protein 8, apolipoprotein e receptor	Absent to present
RIT1	Ras-like without CAAX 1	Absent to present
PDE4B	Phosphodiesterase 4B, cAMP-specific (phosphodiesterase E4 dunce homologue, Drosophila)	2.1 fold, *P*=0.026
PLCG1	Phospholipase C, gamma 1	Absent to present
TFE3	Transcription factor binding to IGHM enhancer 3	Absent to present
EBV-associated genes		
HDAC4	Histone deacetylase 4	Absent to present
ORC2L	Origin recognition complex, subunit 2-like (yeast)	2.3 fold, *P*=0.034
Pro-survival genes		
BCL2L2	BCL2-like 2	Absent to present
CYP26A1	Cytochrome P450, family 26, subfamily A, polypeptide 1	1.6 fold, *P*=0.008
DNAJB4	DNAJ (Hsp40) homolog, subfamily B, member 4	Absent to present
MGMT	O-6-methylguanine-DNA methyltransferase	Absent to present
NISCH	Nischarin	Absent to present
PRKAB1	Protein kinase, AMP-activated, beta 1 non-catalytic subunit	2.2 fold, *P*=0.002
Genes with other functions		
ACAT2	Acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase)	2.0 fold, *P*=0.027
ALDH3B2	Aldehyde dehydrogenase 3 family, member B2	Absent to present
ATP10B	ATPase, Class V, type 10B	Absent to present
AUTS2	Autism susceptibility candidate 2	2.0 fold, *P*=0.011
BRPF1	Bromodomain and PHD finger containing, 1	Absent to present
CRHR2	Corticotropin releasing hormone receptor 2	Absent to present
FADS1	Fatty acid desaturase 1	2.3 fold, *P*=0.017
LAMP2	Lysosomal-associated membrane protein 2	2.2 fold, *P*=0.004
P4HA2	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide II	Absent to present
PKP4	Plakophilin 4	Absent to present
PYGB	Phosphorylase, glycogen; brain	2.1 fold, *P*=0.003
RHBDL1	Rhomboid, veinlet-like 1 (Drosophila)	Absent to present
SEPHS1	Selenophosphate synthetase 1	2.1 fold, *P*=0.005
SLC20A2	Solute carrier family 20 (phosphate transporter), member 2	Absent to present
SPINK2	Serine protease inhibitor, Kazal type 2 (acrosin-trypsin inhibitor)	2.1 fold, *P*=0.042
STARD3	START domain containing 3	Absent to present
SULT1A1,SULT1A2,SULT1A3	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1/2/3	2.6 fold, *P*=0.030
THOC1	THO complex 1	Absent to present
UBE2E3	Ubiquitin-conjugating enzyme E2E 3 (UBC4/5 homolog, yeast)	Absent to present

2-5-oligoadenylate synthetases (OAS 1 and 2). Regulation of these genes may explain the observed resistance to IFN-α -induced apoptosis reported in EBV-positive Akata cells compared with their EBV-negative sub-clones.[26,27] The ability of Akata cells to resist IFN-α -mediated apoptosis has been mapped to viral polymerase III-transcribed EBER RNA molecules, although their precise mode of action remains to be determined.[18,26] In EBV latency, HDAC4 is

Table 2 Host genes down-regulated in EBV-positive Akata cells when compared with those in EBV-negative AK31 cells

GenBank gene symbol	Name	Change in expression
Anti-proliferative genes		
NFKBIE	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	Present to absent
TUSC3	Tumor suppressor candidate 3	Present to absent
Immune response genes		
AIF1	Allograft inflammatory factor 1	Present to absent
IFIT1	Interferon-induced protein with tetratricopeptide repeats 1	Present to absent
IFIT3	Interferon-induced protein with tetratricopeptide repeats 3	Present to absent
IFI35	Interferon-induced protein 35	Present to absent
MX2	Myxovirus (influenza virus) resistance 2 (mouse)	Present to absent
OAS2	2′-5′-oligoadenylate synthetase 2, 69/71 kDa	2.0 fold, P=0.038
RSAD2	Radical S-adenosyl methionine domain containing 2	Present to absent
Cell surface receptor signal transduction genes		
BTK	Bruton agammaglobulinemia tyrosine kinase	2.0 fold, P=0.040
IL27RA	Interleukin-27 receptor, alpha	Present to absent
MAP3K5	Mitogen-activated protein kinase kinase kinase 5	Present to absent
MYD88	Myeloid differentiation primary response gene (88)	2.1 fold, P=0.007
SH3BP5	SH3-domain binding protein 5 (BTK-associated)	Present to absent
STAT1	Signal transducer and activator of transcription 1, 91 kDa	1.6 fold, P=0.017
Transcriptional regulatory genes		
CREB3L2	cAMP responsive element binding protein 3-like 2	Present to absent
ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	Present to absent
REA	Repressor of estrogen receptor activity	1.7 fold, P=0.002
RUNX1T1	Runt-related transcription factor 1; translocated to, 1 (cyclin D-related)	Present to absent
SMAD3	SMAD, mothers against DPP homolog 3 (Drosophila)	Present to absent
Genes with other functions		
C2orf3	Chromosome 2 open reading frame 3	Present to absent
CHN2	Chimerin (chimaerin) 2	Present to absent
CLPP	ClpP caseinolytic protease, ATP-dependent, proteolytic subunit homolog (*E. coli*)	Present to absent
CMAH	Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMP-N-acetylneuraminate monooxygenase)	Present to absent
CSTB	Cystatin B (stefin B)	2.4 fold, P=0.005
CYP4F12	Cytochrome P450, family 4, subfamily F, polypeptide 12	Present to absent
DHRS2	Dehydrogenase/reductase (SDR family) member 2	22.1 fold, P=0.049
DMD	Dystrophin (muscular dystrophy, Duchenne and Becker types)	Present to absent
EPPB9	B9 protein	Present to absent
FBXL7	F-box and leucine-rich repeat protein 7	Present to absent
HUWE1	HECT, UBA and WWE domain containing 1	1.6 fold, P=0.003
IDS	Iduronate 2-sulfatase (Hunter syndrome)	Present to absent
LAMC1	Laminin, gamma 1 (formerly LAMB2)	Present to absent
LRMP	Lymphoid-restricted membrane protein	Present to absent
P2RY10	Purinergic receptor P2Y, G-protein coupled, 10	Present to absent
PALM2-AKAP2	PALM2-AKAP2 protein	3.1 fold, P=0.006
SCRN1	Secernin 1	1.8 fold, P=0.005
SNRPD2	Small nuclear ribonucleoprotein D2 polypeptide 16.5kDa	1.6 fold, P<0.001
TNNI1	Troponin I, skeletal, slow	Present to absent
XRCC4	X-ray repair complementing defective repair in Chinese hamster cells 4	Present to absent

Table 3 Host genes regulated uniquely in EBV-positive Akata cells as compared with those in AK31 cells after IgG stimulation

GenBank gene symbol	Name	Change in expression
Up-regulated genes		
EVI2A	Ecotropic viral integration site 2A	Absent to present
FABP6	Fatty acid binding protein 6, ileal (gastrotropin)	Absent to present
LYL1	Lymphoblastic leukaemia derived sequence 1	2.1 fold, P=0.004
NCALD	Neurocalcin delta	Absent to present
PLEK	Pleckstrin	2.4 fold, P=0.003
RECQL4	RecQ protein-like 4	Absent to present
RELB	v-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (avian)	1.9 fold, P=0.003
RNF5	Ring finger protein 5	2.3 fold, P=0.002
SFTPD	Surfactant, pulmonary-associated protein D	Absent to present
SGK	Serum/glucocorticoid regulated kinase	2.5 fold, $P < 0.001$
Down-regulated genes		
CRYZ	Crystallin, zeta (quinone reductase)	2.4 fold, P=0.005
GPR30	G protein-coupled receptor 30	2.2 fold, P=0.004
NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa	Absent to present
PDLIM5	PDZ and LIM domain 5	1.6 fold, P=0.008
SEPT6	Septin 6	Absent to present
TCF7	Transcription factor 7 (T-cell specific, HMG-box)	2.4 fold, P=0.003

recruited to the BZLF1 promoter by MEF2 and inhibits expression of BZLF1.[28] The identification of HDAC4, as a gene up-regulated in Akata cells, suggests that EBV plays a role in up-regulating its expression to repress the BZLF1 promoter and thereby promote latency. ORC2L encodes a subunit of the origin recognition complex [29] and ORC2L protein specifically associates with and is necessary for EBV genome replication through the EBV latency origin of replication (OriP).[30] The transcriptional up-regulation of ORC2L by EBV will therefore aid replication of the viral episome in latently infected cells. It is interesting to note that a large number of the genes down-regulated by EBV (Table 2) are known IFN response genes, or have roles in signal transduction or transcriptional regulation.

This study revealed that basal level expression of CD86 was 70% higher in AK31 cells than in Akata cells, suggesting that signal transduction through TLR9 is reduced in the EBV-positive cells. This taken together with the discovery that stimulation with CpG-containing oligonucleotides induced CD86 expression yet further in EBV-negative cells, but not in EBV-positive cells led us to conclude that the reduced expression of myd88 in EBV-positive Akata cells correlates with decreased basal expression of a TLR9 receptor downstream target and abrogates the ability to further stimulate expression.

It was expected that altered patterns of host gene expression would occur following stimulation of surface IgG, and this response would be relatively conserved in Akata and AK31 cells. In addition, it was anticipated that regulation of host genes would also be detected as a result of the activation of the early stages of the viral lytic cycle in Akata cells. However, we were surprised by the extent of different patterns of gene expression following surface IgG stimulation in the EBV-negative and -positive Akata cells, which suggests that the presence of EBV results in an impaired BCR response in these cells.

Two previous studies of genes regulated following stimulation of Akata cells documented changes in EBV gene expression, with the unexpected discovery that genes normally expressed only in latency III are activated late during viral replication (12 h after stimulation).[31, 32] Analysis of host gene expression[31] revealed complex changes in gene expression in both

EBV-positive and -negative cells over the 48-hour time span investigated. For example, the gene RGS16 is transiently up-regulated in EBV-positive cells 6-12 h after stimulation, but its regulation in EBV-negative cells appears to be cyclicalL it is first down-regulated at 4 h, then transiently up-regulated before showing down-regulation again at 24 h after stimulation. In this study, we investigated single time point of regulation at 6 h after stimulation to focus on the early events of lytic cycle reactivation. Our major conclusion is that 6 h after stimulation much of the gene regulation in response to IgG ligation is blocked in EBV-positive cells. In contrast, Yuan et al.[31] reported that more genes are regulated in EBV-positive cells than in EBV-negative ones when considering the entire 48-hour period after stimulation. We consider that the differing conclusions as to the extent of gene regulation are dictated by the time frame under investigation. The striking differences in gene expression seen in response to stimulation that we highlight at the early time point may have been overlooked due to the sheer scale of change observed over the 48-hour period. Nonetheless, both studies identify three host genes (PLEK, EVI2A and SGK) in common that are regulated early after stimulation of EBV-positive cells.

Many studies have now compared host gene expression in a variety of EBV-positive and -negative cell lines and tumors.[13,31-59] A complication to this type of analysis is that tumors may be contaminated with activated EBV-positive B cells, or some cells may be undergoing viral lytic cycle. For example, Yuan et al.[31] reported that 5% of the unstimulated Akata cells in their study were undergoing viral lytic cycle, making it difficult to assign changes in gene expression to either EBV latency or replication.[31] Nonetheless, a meta-analysis of studies on gene expression changes observed between EBV-positive PEL and NPC has identified some common regulatory mechanisms.[60]

The majority of the expression regulation in our study was about two-fold. This raises the question of how relevant small changes in gene expression are to the cell. The demonstration here that a 50% reduction in the expression of Myd88 correlates with reduced function of a Myd88-linked TLR9 signal transduction pathway suggests that these small changes in gene expression can lead to significant effects on cells. The identification of host genes regulated by EBV both during group I latency in BL cells and following IgG stimulation will contribute to a better understanding of the optimization of the host environment by EBV.

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