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The EBV-HIV interrelationship and the value of EBV-DNA analysis

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Följ inte de gamle i spåren utan sök vad de sökte.

Matsuo Basho (1644-1694)

Abstract

Epstein-Barr virus (EBV) infects the vast majority of humans and resides latently in B-cells. This virus carries genes that can induce and sustain mature B cell growth. EBV is associated with a wide range of B-cell lymphomas including Burkitt lymphoma and non Hodgkin lymphoma (NHL) in human immunodeficiency virus 1 (HIV-1) infected patients. Latent EBV infection in B lymphocytes is a risk factor for B-cell lymphomas in conditions of combined antigen stimulation and immunosuppression as with Burkitts lymphoma in malaria endemic African regions and non Hodgkin lymphoma in HIV-1 infected patients. In the era of modern combination antiretroviral therapy (cART) there has been an impressive reduction of Acquired Immunodeficiency syndrome (AIDS)-related opportunistic infections and lymphomas, although patients still suffer an increased risk for NHL. This work is based on EBV-DNA load measurement in blood as a tool to analyse EBV-host relationship in HIV-1 infection.

In general HIV-1 infected individuals have a higher EBV-DNA load and symptomatic HIV-1 infected even higher. Individual variables, immunological factors and treatments as cART affect this pattern. In one of our studies we identified one group and one risk factor that influenced EBV-DNA load. HIV-1 infected individuals with a history of a symptomatic primary infection in combination with induced immune stimulation by therapeutic vaccination/adjuvant showed an increased load. Without the vaccination/adjuvant stimuli this group did not show the same increase. HIV-1 infected patients with a history of a symptomatic primary infection might therefore be at risk for developing NHL. Therapeutic vaccination/adjuvant increases the EBV-DNA load and we regard this immunomodulation as a risk factor. Different pattern of EBV-host restoration by cART was seen in a long term follow of patients with increased EBV-DNA load after vaccination. The EBV-host relation seems to be reconditioned by successful cART treatment, measured by the CD4+ cell count returning to normal levels, with some reservation for the functional restoration, together with remaining undetectable HIV-1 RNA. For individuals with unsuccessful cART treatment the distinct decrease of EBV-DNA could not be seen. In a patient treated for EBV positive plasmablastic lymphoma we observed a sharp increase of EBV-DNA load before clinical signs of recurrence.

Measurement of EBV-DNA load is valuable in monitoring disease progression in HIV-1 infected patients. After cART treatment the dynamics of EBV-DNA load reveal if the antiviral treatment is suboptimal, even if breakthroughs detected as HIV RNA peaks are missed. When an EBV positive tumour is treated successfully EBV-DNA monitoring can be of importance to observe early signs of relapse. Monitoring EBV-DNA load during therapeutic vaccination studies seems highly motivated. In conclusion EBV-DNA load analysis is a useful additional instrument to monitor different groups of HIV-1 patients with increased risk for lymphoma development.

Populärvetenskaplig sammanfattning

Körtelfebervirus, Epstein-Barr virus (EBV), finns i kroppen hos nästan alla människor. De flesta märker varken sin primära infektion eller att viruset livslångt finns i kroppen. EBV kan i vissa situationer bidra till eller till och med orsaka cancer. Detta gäller speciellt när vårt immunförsvar är försvagat, och därför inte kan skydda oss lika bra.mot infektioner

Humant immunbristvirus (HIV) -1 har orsakat den största dödliga epidemin i modern tid. HIV-1 attackerar främst de celler i vår kropp som utgör skydd mot infektioner. En HIV-1 infektion leder utan behandling till att ett immunbristtillstånd uppkommer med allvarliga och svårbehandlade infektionssjukdomar som följd. Det påverkade immunförsvaret innebär även att EBV:s relation till sin värd blir förändrad. Personer med HIV-1 och EBV löper därför en mycket större risk än patienter utan HIV-1 att utveckla cancer, till exempel non Hodgkins lymfom.

I denna avhandling har vi studerat samspelet mellan människa och EBV hos patienter med HIV-1 infektion. Detta har genomförts genom att analysera mängden EBV infekterade blodceller hos HIV-1 infekterade patienter. Generellt innebär HIV-1 infektion en ökning av EBV i blodceller med de högsta nivåerna vid AIDS.

Vi undersökte under en tidsperiod HIV-1 infekterade individer som erhöll en modern effektiv behandling mot sin sjukdom. Vi kunde se tre olika mönster: En grupp återskapade med behandlingen sitt immunförsvar och kunde därmed normalisera de förhöjda EBV nivåerna. En mellangrupp var inte lika framgångsrik och lyckades inte förbättra EBV nivåerna trots en relativt god kontroll av sin HIV-1 infektion. Den tredje gruppen slutligen uppvisade kvarstående höga EBV nivåer och samtidigt en ofullständig kontroll av sin HIV-1 infektion. Vi undersökte även två grupper av HIV-1 infekterade patienter med olika mönster i sin sjukdom. En grupp var HIV-1 infekterade med mycket lång sjukdomsperiod och utan påverkan på immunförsvaret, dvs ett stabilt immunförsvar. Den andra gruppen var patienter med tydliga symtom vid primär HIV-1 infektion, möjligen beroende på ett överaktivt immunförsvar. Vi ser dessa båda grupper som olika i sin immunförsvarshantering av HIV-1 infektionen och därmed möjligen även olika när det gäller att kunna kontrollera EBV. Vid undersökning av patienter som erhöll behandling av sin HIV-1 infektion med hjälp av ett framtaget vaccin mot del av HIV-1 kunde vi med vår analys visa på intressanta skillnader. Patienter med tydliga symtom vid primär HIV-1 infektion fick kraftigt ökade EBV nivåer, oberoende av om de erhöll vaccin eller placebo (s.k adjuvans). Patienter utan symptom vid primär HIV-1 infektion hade inte samma ökning av EBV nivåer efter vaccination. De med längre sjukdomstid utan immunförsvarspåverkan hade EBV nivåer nästan i nivå med icke HIV-1 infekterade. Det faktum att HIV-1 infektion generellt innebär en ökning av EBV, att denna ökning förstärks av symtom vid primär infektion samt att vaccination och placebo ytterligare försämrar kontrollen av EBV och ökar nivåer i blod speglar en tilltagande störning av balansen mellan människa och ett virus, EBV. Vi har slutligen följt en HIV-1 infekterad patients behandling av ett EBV-innehållande lymfom. Hos denna patient kunde behandlingseffekt med cellgifter speglas i låga EBV nivåer men vid återkom av patientens cancer noterades en kraftig ökning av EBV innan sjukdomen givit symtom.

Vi kan med våra EBV analyser hos HIV-1 infekterade patienter, notera en ökad information om kvalitativa förändringar i immunförsvaret vilket kompletterar tidigare använda mått på patientens immunförsvarsläge. Genom EBV undersökningar på grupper av HIV-1 infekterade patienter med större risk för förhöjda EBV nivåer och därmed ökad risk för lymfomutveckling, ges möjligheter till en tidigare diagnos och sannolikt förbättrad behandling. Vid uppkommet EBV innehållande lymfom ger EBV undersökning ett mått på behandlingseffekt och även ett mått på återfall i sjukdomen. Vi kan även notera att en ursprunglig mer personalkrävande EBV analysmetod inte är sämre än en mer kostsam modern metod och att den av oss använda ursprungliga metoden skulle därmed kunna vara användbar i länder med begränsade hälsoekonomiska resurser.

Original papers

This thesis is based on following papers, which will be referred to in the text by their roman numerals.

I Anna M.C. Friis, Katarina Gyllensten, Anna Aleman, Ingemar Ernberg, and Börje Åkerlund, The Effect of Antiretroviral Combination Treatment on Epstein-Barr Virus (EBV) Genome Load in HIV-Infected Patients. Viruses 2010;2: 867–879

II Anna M.C. Friis, Börje Åkerlund, Katarina Gyllensten, Anna Aleman, Ingemar Ernberg. Host-Epstein-Barr virus relationship affected by immunostimulation in HIV-infected patients representing distinct progressor profile groups. Scand J Infect Dis 2012 May;44(5):388-92.

III Anna M.C. Friis, Börje Åkerlund, Katarina Gyllensten, Anna Aleman, Eric Sandström, Göran Bratt, Ingemar Ernberg. Epstein-Barr virus genome load is increased by therapeutic vaccination in HIV-l carriers, and further enhanced after a history of symptomatic primary infection. Submitted

IV Anna M.C. Friis, Birger Christensson, Katarina Gyllensten, Anna Aleman, Jie-Zhi Zou, Börje Åkerlund, Ingemar Ernberg. EBV-DNA analysis in blood predicts disease progression in a rare case of plasmablastic lymphoma with effusion. Manuscript

Content

Abbreviations

AIDS	Acquired immunodeficiency syndrome
BL	Burkitt's lymphoma
BMT	Bone marrow transplant
BZLF1	BamHI Z EBV replication activator 1
cART	Combination antiretroviral therapy
CNS	Central nervous system
CTL	Cytotoxic T-lymphocyte response
EBER	EBV-encoded small nuclear RNAs
EBNA	Epstein-Barr nuclear antigen
EBV	Epstein-Barr virus
ePCR	End-point dilution PCR
GC	Germinal centre
HIV-1	Human immunodeficiency virus type 1
HD	Hodgkin's lymphoma
HLP	Oral hairy leukoplakia
IM	Infectious mononucleosis
KS	Kaposi's sarcomas
LCL	Lymphoblastoid cell lines
LMP	Latent Membrane Protein
LTNP	Long term non progressors
MHC	Major histocompatibility complex
NHL	non Hodgkin's lymphoma
NPC	Nasopharyngeal cancer
PCNSL	Primary central nervous system lymphoma
PBL	Plasmablastic lymphoma
PCR	Polymerase chain reaction
PEL	Primary effusion lymphoma
PHI	Primary symptomatic HIV-1 infection
PTLD	Post transplant lymphoproliferative disease
qPCR	Real time quantitative PCR
RS	Reed-Sternberg cells
sqPCR	Semiquantitative PCR
VCA	Virus capsid antigen

Background

Epstein-Barr virus

General

Epstein-Barr virus (EBV) is one of the most widespread viruses pathogenic to humans. Around 95% of the adults worldwide carry EBV as a lifelong asymptomatic latent infection [1]. The vast majority of individuals infected by EBV never show apparent signs of disease. In the western world half of the children get the infection without noticing any symptoms during their first decade of life [1]. The major route of infection is through transmission of saliva [2, 3].

EBV and Human Herpes virus 8 (HHV8) are the only herpes viruses consistently associated with human malignancies. EBV is often considered as a tumour virus based on the fact that the EB-virus is strongly associated with a range of malignancies, notably nasopharyngeal carcinoma (NPC) and Burkitt's lymphoma (BL) (Table 1) [4-6]. Considering that the virus is found in most adults in the world and the relative low prevalence of EBV associated tumours the direct causative role of EBV without cofactors could be argued. The risk of an EBV infected cell becoming malignant can be estimated to be in the order of less than one per 10^{11}-10^{12} infected cells. The other tumour associated herpes virus HHV8 is found to be associated with Kaposi's sarcoma (KS) and Primary effusion lymphoma (PEL) in Human immunodeficiency virus type 1 (HIV-1) infection [7].

Table 1. Tumours and diseases where EBV has frequently or occasionally been detected, indicting the affected cell and latency type.

Disease	EBV positive cell type	Latency
Burkitt's lymphoma		I
Primary effusion lymphoma (PEL; also HHV8 positive)	B-lymphocytes	I
Plasmablastic lymphoma (PBL)	B-lymphocytes	I
Nasopharyngeal carcinoma (NPC)	Undifferentiated epithelial tumour cells	I/II°
Gastric carcinoma	Lymphoepithelioma-like tumour cells	I/II
T and NK lymphomas		I/II
Non-Hodgkin's lymphoma		I/III
Angioimmunoblastic lymphadenopathy with dysproteinaemia	B and T cell immunoblasts	II
CLL	Chronic lymphocytic leukaemia	II
Hodgkin's lymphoma	Reed-Sternberg cells	II
Lethal midline granuloma	Medium/large T/NK cells	II
Lung carcinoma	Non-small cell	II
Salivary gland carcinoma	Lymphoepithelioma like tumour cells	II
Post-transplant lymphomas and similar lesions	B immunoblast	III
AIDS-related lymphoma	Polymorphic immunoblasts	III
Infectious mononucleosis	B lymphocytes	III
Testicular tumour	Seminoma, embryonal carcinoma	III
Polyclonal lymphoproliferative lesions		III
Primary CNS lymphomas	Polymorphic immunoblasts	III
Leiomyosarcoma	Smooth muscle	variant*
Oral hairy leukoplakia	Epithelial cells	Lytic

° Expression of BARF1

* Variant of latency only EBNA1 and EBNA2.

The Virus

The British surgeon Denis Burkitt working in Uganda observed a jaw-localised lymphoma he had never seen before. He investigated the prevalence of the lymphoma also in surrounding countries and suggested an infectious aetiology [8]. Based on these findings the Epstein-Barr virus was discovered in the sixties in the lymphoma tissue by Sir Anthony Epstein, Bert Achong and Yvonne Barr.

The virus is the fourth of eight human viruses of the Herpesviridae family, besides Herpes Simplex- I and II, Cytomegalo-, Herpes Zoster-, and Human Herpes viruses 6, 7 and 8. The genome is a linear double-stranded DNA strand molecule, 172 kilo-bases in length (Fig. 1). The virus code for some 80 major genes out of which 60 have been characterised to some extent. The capsid is icosahedral and enclosed by an envelope. After entry into the target cell, the genome circularises to form an episome. EBV DNA may occasionally be integrated into cellular DNA. The replication in latent infection occurs once per cell cycle during S phase.

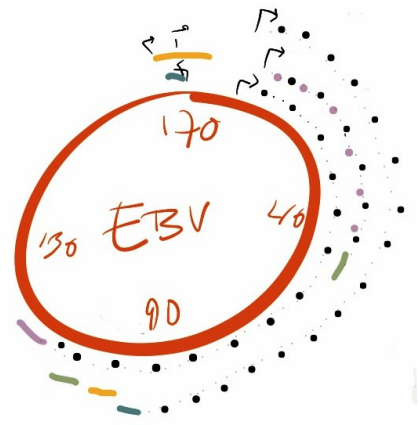

Figure 1: Schematic representation of the EBV genome. Episomal form of EBV. The black dotted lines represent the RNA transcripts identified in EBV growth transformed cells. Arrows indicate promoters.

The primary infection occurs in infiltrating B lymphocytes or mucosal epithelial cells in the naso- or oropharyngela mucosa. After the establishment of the infection the viral episomes persist in memory B cells and around 1 in 10^{5-6} cells is EBV infected [9, 10]. Under special circumstances, the virus may infect T cells or natural killer (NK) cells, and possibly also monocytes [1, 11, 12].

The receptor for C3d (CR2 or CD21) a subcomponent of complement factor 3, is known to serve as the receptor on cell membranes for EBV viral receptor glycoprotein (gp) 320/220 and thereby allow EBV to enter the cell [13]. CR2 is expressed on the B cell surface. It has also been found on thymocytes, and rare EBV infection of thymus cells is reported, as well as EBV-positive T-cell lymphomas [14, 15]. On the contrary infection of epithelial cells is not well characterised but it has been shown in vitro that a virus particle bound to a resting B cell can infect epithelial cells more easily [16]. The envelope protein gp42 mediating membrane fusion by binding to MHC class II molecules has been suggested as an explanation of the "switched" tropism where EBV virions produced in epithelial cells infects B cells and vice versa [17].

EBV carrying cells could always be detected in the B cell compartment in healthy EBV infected humans. In contrast, the productive infection occurs in the oropharyngeal epithelium, and in other mucous membranes [18, 19]. It is possible to detect replicating EB virus in the saliva, throughout the entire life in 10-60% of healthy EBV seropositive individuals [3]. The detection was earlier done by cord blood transformation, and later on by polymerase chain reaction (PCR) assays. It has also been suggested that the virus could replicate in the parotid gland and in the urogenital tract [20-23]. EBV DNA, mRNA from lytic genes, and infectious virions has been reported in oropharyngeal epithelial cells during the terminal stages of host cell differentiation within buccal fluid [20].

The reservoir of latent infection is most likely localised in circulating CD27+ memory B-lymphocytes 24. The latent infection could be eradicated by conditioning irradiation followed by bone marrow transplantation (BMT) from an EBV negative donor. The eradication may be

enhanced by some graft-versus-host reactivity or administration of cytotoxic drugs [25]. The persistence of latent virus in B cells is independent of virion production shown by prolonged treatment with acyclovir, which efficiently blocks the productive cycle, but does not significantly affect the number of virus-carrying B cells in the blood [26]. Even the combination of acyclovir and steroids does not affect the number of virus carrying B-cells [27]. Maintenance of the infected B-cell pool therefore normally seems to be independent of continuous reinfection. In an infected individual the dominant strain remains for a long period of time [28].

Gene products

The gene products can be grouped into four categories. These are: latent genes, immediate early genes, early genes and late genes. The 12 major latent genes have been studied in detail (Table 2), while the recently described microRNAs are less well characterised. Most proteins needed for virus DNA replication during latent infection are of host origin, including the DNA polymerase.

During the lytic EBV cycle more than 70 proteins are expressed. Initiation of lytic DNA replication (oriLyt) is different from the episomal virus replication initiated in oriP [29]. The initiation of lytic viral DNA replication depends on the viral DNA polymerase. The viral gene products expressed during the lytic cycle are classified in three groups immediate-early, early and late proteins, according to their relation in time to the viral DNA synthesis. BZLF1 (BamHI Z EBV replication activator), the protein also named as ZEBRA, is an immediate early gene [30]. The ZEBRA-protein triggers alone the disruption of viral latency and is likely to function as a transcriptional transactivator but can also have a down regulating effect on TNF1α thereby avoiding apoptosis [30, 31]. One EBV gene, BHRF1, encodes for a protein with significant co-linear sequence similarity with the proto-oncogene bcl-2 [32, 33]. The BHRF1 gene product also inhibits, as bcl-2 does, apoptotic cell death. However, BHRF1 is expressed only early in the lytic replication cycle and is thereby not expressed in latently infected cells where the antiapoptotic effect could have a real impact on a malignant outcome [34, 35]. The antiapoptotic effect is transient as the lytic infection ends with cell death [36].

Latency

Historically three types of latency patterns has been described, each with a specific pattern of gene expression (Table 3). But more recently variants of expression patterns have been found [1]. One variant is called type 0 latency and is found in memory B cells where no viral proteins are expressed besides the EBV-encoded small nuclear RNAs (EBERs). The type of EBV latency varies between different tumours (Table 1).

Table 3. Gene products in different latencies

	EBNA-1	**EBNA-2, 3, 4, 5, 6**	**LMP1, LMP2**	**EBERs**	**BARTs**	**Promotor for EBNA1**	**Normal type of cell**
I	+	-	-	+	+	QP	dividing memory B cells
II	+	-	+	+	+	QP	in vivo in T cells, memory cells when GC passage
III	+	+	+	+	+	Wp, Cp	proliferating B cells, LCLsT

Epstein-Barr virus variation

Initially EBV infection was characterised using serological methods and virus subtypes were originally identified by such methods. Later on characterisation of virus variants is built on restriction fragment length polymorphism, immunoblotting and sequencing. Generally more variants are found in saliva than in blood as the production of virions takes place in the mucosa

Table 2. Gene products

Product	Physical properties B95-8 prototype strain	Function, examples	Immunologic findings and tumour capabilities
RNA products			
EBER	2 different products. Approx. length is 170-180 nucleotides. *In vivo* associated with a cell nucleus localised protein [37, 38].		
miRNA	Small, 19-24 nucleotides, non coding, 44 identified [39, 40].	Induce cellular miRNAs. Regulate cellular genes.	Indirectly increases onconprotein bcl-6, c-myc.
Proteins			
EBNA1	41 amino acids, large internal repeat gly-ala residuals. Lack of MHC Class I restriction [41]. Varies between 67-97 kD [42].	Maintenance of EBV episomes [43]. prevents virus rom proteosomal degradation [44]. Transactivatet genes [45].	Cause tumours in nude mice, clone in soft agar [46]. Inhibits EBNA1 expression in BL *in vivo* [47]. Induces apoptosis *in vivo* thereby eradication of tumour cells [47]. Destabilises p53 [44]
EBNA2	Phospho-protein. Varies between 85-97 kD. Two subtypes, type 1 and 2, 47% variation	Required for immortalisation. Transactivates viral genes [48]. Transactivates several cellular genes as CD21, CD23, LFAs, c-myc. Inhibits apoptosis mediated by Nur-77 [49]. Down regulates DNA synthesis [50].	
EBNA3 (-3A)	Varies between 140-158 kD [42]. Two subtypes, type 1 and 2, 16% variation.	Essential for transformation [51, 52]. Associates with RBP-Jκand thereby down regulates expression of c-myc [53].	
EBNA4 (-3B)	CTL against HLA-A11 restricted. Two subtypes, type 1 and 2, 20% variation.	Essential for transformation [51, 52].	Induce bcl-2 expression *in vitro* [54].
EBNA5 (-LP)	Spaced size ladder 30-130 kD [55]. Two subtypes, type 1 and 2, 28% variation.	Essential for transformation [51, 52]. Affect expression of B-lymphocyte gene, a mediator of cell growth or differentiation.	
EBNA6	Composed of 22 and 44 amino acid segments. Varies between 150-183 kD. Differ in sequence between type 1 and 2 [56].	Induces CD21 [57]	Inactivate retinoblastoma (Rb) overcome G1 phase arrest [58]
LMP1	Membrane protein. Varies between. 57-66 kD. 3 domains. Large number HLA class I restricted CTL epitopes [59].	Induces gene expression changes mimics B cell activation. Maintenance infection and virus production [50]. Up regulation cytoskeletal protein synthesis, especially vimentin [60]. Induce DNA synthesis [50]. Immunosuppressive effect [61].	Oncogenic potential as member of the tumour necrosis factor receptor superfamily. Upregulating expression anti-apoptotic genes bcl-2, Mcl-1, A20 [62]. Activate PI3-K 63. Activates NF-κβ thereby angiogenesis and invasivenessIn mouse experiments predispose lymphomagenesis [64]
LMP2	Two variants LMP2A and LMP2B. Membrane protein.	LMP2A: Interacts with kinases and affects signal transduction pathway Notch [65]. Stops the cells leaving latency. Enhances the efficiency of malignant transformation. Maintain viral latency [66]. Binds to proteins involved in cell signalling [67]	Indirectly activates PI3-K as LMP1 and thereby also show oncogenic properties [68].
BARF0	279 amino acids	Can up regulate LMP1 expression via interaction with Notch [69].	
BARF1	221 amino acids	Early lytic protein. Receptor for colony stimulation factor 1 [70].	Putative transforming protein [71].

and in the occasional developed oral hairy leukoplakias (HLP) on the border of the tongue. Infections by several strains and increased replication that can occur in immunosuppressed individuals accelerate the genetic variation [72]. Variation can be detected at the genetic, protein, and immunological level. Strain variations has not been investigated properly in healthy EBV-carriers.

The two major EBV virus subtypes, type 1 and 2 with a worldwide distribution, has a difference in the sequence for Epstein-Barr nuclear antigen (EBNA)2, -3, -4 and -6 but also slightly in the EBER region. The two types have a different geographic distribution where type 1 is found globally, while type 2 is predominantly found in Central Africa, Alaska and Papua New Guinea. In contrast to immunocompetent persons, in HIV-1 seropositive individuals co-infection with both types is common [73, 74]. In these studies, of mostly HIV-1 infected homosexual males multiple EBV variants have been identified and isolated. The homosexual lifestyle might result in both a higher exposure to different EBV variants and co-infection with other viruses, which both could have an impact on EBV host balance.

EBV protein variation has been a valuable tool for the studies of virus epidemiology. Different research groups have used the molecular weight variation of the EBNA polypeptides in an immunoblot method to distinguish variants of these EBV sub-types [25, 75-77]. Subsequent studies have shown that healthy EBV carriers have one predominant strain but occasionally more than ten variants could be identified. In contrast to the immunocompetent individual, more than half of the immunosuppressed persons show multiple variants [73, 78-80].

Most of the latent proteins have multiple Major Histocompatibility Complex (MHC) Class I cytotoxic T lymphocyte (CTL) epitopes [81, 82]. Some of them are specific for EBV type 1 or 2, other exist in both types. One HLA-A11 epitope found in EBNA4 can be abolished by a point mutation. Frequency of this point mutation in a particular area is strongly correlated with the HLA-A11 frequency in the same area [83, 84]. In Papua New Guinea an EBV variant with amino acid substitutions within HLA A11-, B35- and B8-restricted CTL epitopes has been described. As a consequence of this none of the epitopes could be recognised by CTLs. Distribution of HLA in different Papua New Guinea populations did not correlate with the distribution of amino acid substitutions [85]. Therefore this observation suggest that EBV variants can arise not only due to immune selection.

Human immunodeficiency virus 1

The virus and natural infection

The first appearance of HIV-1 infection was documented as cases of Acquired Immunodeficiency syndrome (AIDS) reported in the USA in May 1981 [86]. Previously healthy young homosexuals did show a complex disease-picture with several uncommon immunodeficiency related diseases caused by virus, fungi and bacteria. Soon after this observation the same diseases were also found among intra-venous drug abusers (IVDUs), Haitians and haemophiliacs. The virus, HIV-1, was identified in France and US, 1983 respectively 1984 [87, 88]. The epidemic thereafter spread beyond control around the globe. Currently more than 34 million people worldwide live with the infection (WHO[a]). In Sweden there was about 7800 people living with the disease in 2011 (SMI[b]) an accumulating number due to a decreased death rate nowadays.

HIV-1 is included in the genus Lentivirus in the family Retroviridae, and infects mainly CD4 expressing T-helper lymphocytes. Besides the primary receptor, CD4, the virus also uses co-

a www.who.int/mediacentre/factsheets/fs360/en/index.html 2012-05-25
b smi.se/statistik/hivinfektion/?t=com&p=20049 2012-05-30

receptors as CCR5 and CXCR4 for entrance [89]. Other cell types such as dendritic cells and macrophages are also target cells to become infected [90, 91]. The RNA genome is relatively small, approximately 9 kb, and encodes for 14 proteins including 3 structural proteins, 2 envelope proteins, 6 accessory proteins, and 3 enzymes [92]. The viral envelope consists of a lipid bi-layer and two glycoproteins, env; gp120 and gp41. Glycoprotein 120 binds primarily to the cellular CD4 receptor with a high affinity, and can thereafter enter the cell. The glycoprotein 120 can also bind to chemokine receptors, mannose-binding C-type lectin receptors, and the homing integrin $\alpha 4\beta 7$ thereby potentially perturbing key players in the immune response such as T- and B lymphocytes, monocytes, macrophages, and dendritic cells [93]. The lack of fidelity and proofreading of the reverse transcriptase lead to a high mutation rate and opens up for virus escape from the immune responses as well as development of drug-resistance.

Predominantly HIV-1 is transmitted by the sexual route or by parenteral transmission as for IVDUs, but mother to infant transmission is also an important route. The transmission risk varies due several factors e.g. concurrent infections and epithelial integrity [94]. Virus replication is continuously observed after primary infection. HIV-1 has a high turnover time with a life cycle time and a generation time[c] of 1.2 days [95, 96]. In an infected individual as much as 10^{10} virus particles can be produced in one day [96]. After the eclipse period of 10 days HIV-1 RNA can be detected. The viral load peaks normally in 20 to 30 days. During this time seroconversion takes place and usually a fairly constant viral load is established until progression to the pre AIDS state.

The asymptomatic chronic infection lasts for years with almost no malignancies except from sporadic cases of Hodgkin lymphoma (HD) and no opportunistic infections. The earliest signs of opportunistic infections are oral candidiasis, herpes zoster infections and EBV related HLP. When CD4+ cell level decreases below 200 x 10^6/L more severe manifestations flourish and the infected individual may get cerebral toxoplasmosis, atypical mycobacterial infections, and systemic cytomegalovirus infection manifested in blood, central nervous system (CNS), retina and the gut. This conditions is designated AIDS. The most rapid progression from asymptomatic infection to AIDS occurs in 1 to 2 years whereas the majority of the infected individuals are asymptomatic for decades.

Symptomatic primary HIV-1 infection

When the first cases of acute HIV-1 infection were reported in the middle of the 1980s the symptoms were quite similar to infectious mononucleosis (IM), later on the symptoms were found to be more divergent but also rather common [97]. As many as 50% to 70% newly HIV-1 infected individuals are believed to have symptoms upon their primary HIV-1 infection [98]. Laboratory blood findings are lymphopenia, reduced number of CD4+ cells and usually an increase in activated CD8+ cells [99].

The observed clinical symptoms are unspecific including fever, pharyngitis with ulcers, headache, arthralgias, myalgias, malaise, and weight loss and also a nonpruritc maculopapular rash [100, 101]. The weight loss and mucocutaneous ulceration is used to distinguish HIV-1 infections from other viral infections [100]. The interval between infection and onset of symptoms is reported to be 5 to 29 days but most commonly two weeks [99]. The symptoms last for two to six weeks, with a median value of two to almost three weeks [102, 103]. The lengths do vary in different reports probably due to different definitions of recovery.

In IVDUs the reported incidence of primary symptomatic infection (PHI) is lower, but this might be biased since patients in this group do not normally seek hospital care when they get ill

c Defined as release of virions until infection of another cell.

[99]. An explanation for the higher incidence in the homosexual group could be due to that this group is more routinely tested and scrutinised and an illness caused by another virus could be reported as PHI. This could be compared to the side affects reported by placebo in pharmaceutical trials i.e. if you look you will find. Notable, in a study of Pehrson et. al. where they compared disease progression and death in IVDU and homosexual males, they found a significant longer survival time in the IVDU group [104]. This finding indicates that transmission route plays a role in the disease development.

For individuals with symptomatic PHI the progression towards AIDS and/or a CD4+ cell count below 200 x 10^6 was faster than for individuals with asymptomatic PHI [105]. Today the recommendation in Sweden is that a patient with symptomatic PHI should early initiate combination antiretroviral treatment (cART) [106].

Elite controller & long-term asymptomatic HIV-1 infection

The duration of asymptomatic HIV-1 infection varies to a large extent between individuals [107]. In the beginning of the 1990s several studies of individuals with a long-term (10-15 years) asymptomatic HIV-1 infection (LTNP) were published. LTNP vary in frequency between 8% to 23% in different cohorts [108 23%, 109, 110]. The variation is caused by non specified definition of this group with different length of monitored infection time, follow up time as well as CD4+ cell count. With a longer follow up period the fraction of LTNP is estimated to be about 5% and with tightened criteria for the CD4+ cell decline the number decreases to 1-3% [111].

The HIV-1 load in peripheral blood is another way to identify the LTNP group [112]. This group distinguishes itself with a low viral load and high CD4+ cell count irrespectively of the long infection time [113-115]. Elite controllers constitute an even smaller group and besides the above mentioned characteristics they have undetectable HIV-1 RNA values. But analysis of T-cell activation shows that even this group is affected by the infection [116]. The combination of low HIV-1 viral load, efficient virus-specific immune responses, and/or some degree of attenuation of the virus has been found in LTNP [113, 117]. The LTNP group is heterogeneous, and today no explanatory factor, genetic or immunologic has been identified, even though there are numbers of candidates. One candidate is a lower proportion of CD38 expressing CD8+ cells [118], another a stronger antibody response to six different HIV-1-related proteins and a third better differentiated HIV-1 specific CD8+ cells [108, 119]. In LTNP children the frequency of CD4+ cells positive for CD38 is higher and frequency of DR+ cells lower than in non LTNP [120]. The numbers of HIV-1 memory CTL precursor cells as well as CTL effector cells are found at a high level in this group compared to improved immune status by cART where the patients have low level of CTL effector cells [121].

Antiretroviral therapy - how it started and where we are today

For some decades now antiretroviral treatment has been given to prevent virus replication in the HIV-1 infected individuals. Around 1985 nucleoside analogues became available such as AZT and later DDI. Initially these drugs administered as monotherapies seemed successful but soon drug resistant strains appeared. New drugs were developed that targeted the reverse transcriptase enzymes directly. But similar to the nucleoside analogues drug resistance could develop when these drugs were used as monotherapies. Combination therapy trials were initiated and immediately showed promising results. The combination therapy was named highly active antiretroviral treatment - cART.

Today about 25 different drug substances exist from four classes. A combination of two reverse transcriptase inhibitors with one HIV-1 protease inhibitor in combination with or

replaced by a non-nucleoside reverse transcriptase inhibitor is the actual treatment regimen. The same strategy is now applied on other chronic viral infections such as HBV and HCV. Combination ART was introduced around 1996 in the western world and has an impressive effect on morbidity as well as on mortality [122]. The treatment regimen substantially decreases the plasma HIV-1 RNA levels and CD8+ cell count, and increases CD4+ cell count [123, 124]. It also reconditions the lymphocyte population [123]. Most patients with cART treatment will get undetectable HIV-1 RNA levels in 4-6 months. Even in patients with advanced stages of the disease, an improvement of the immune status could be observed. Combination ART can also induce a recovery of CD4+ cell reactivity and the receptor repertoire is reported to improve after 6 months of therapy [125]. Irrespectively of how early treatment is initiated there is still a noticeable ongoing immune activation, see "The immune response in HIV-1 infection".

In Europe the death rates has declined 80% with cART from 1995 until the beginning of 1998 when Mocroft et. al. summarised data [126]. In parallel with the introduction of cART the incidence of several lymphomas has decreased in treated individuals compared to untreated (see chapter "EBV in HIV environment"). Today HIV-1 infected people with cART die of other non-AIDS related causes such as cardiovascular illness, malignancies and liver related complications [127].

The optimal time to initiate therapy is under debate, and have been so for many years. The concern about a treatment is the possible long-term drug toxicity that will affect the life long adherence. The patients compliance must be optimal not to risk development of viral mutations, that in turn will cause drug resistance.

Diseases caused by Epstein-Barr virus

Infectious mononucleosis

The primary EBV infection could occur after the disappearance of maternal antibodies [128]. In childhood and adolescence the EBV infection is a clinical disease with non specific symptoms. The primary infection may cause a benign lymphoproliferative disease named IM, in some adolescent or adult individuals and occasionally also in children [129, 130]. The incubation time is 35 to 40 day long. Suggested explanations of the more frequent symptomatic disease in young adults is that this age group has a mature immuneresponse and another is the larger initial dose from kisses in this age group [1]. The latter fact reveals the background of the trivial name of the disease: "kissing disease". The primary site of clinical infection is likely to be in the oropharynx [129, 131]. The symptoms of the disease correlates to CD8+ cell lymphocytosis and the released proinflammatory cytokines rather than to the high level of virus shedding [132, 133].

Malignancies

Malignancies associated with EBV were for long thought to be of only B cell or epithelial origin. However, tumours with other original cell-types have recently been shown to be EBV-associated and the list of EBV associated malignancies is growing. As earlier mentioned, the virus is designated a tumour virus. This could be challenged due to the high incidence of EBV in humans and the low incidence of EBV associated malignancies. The virus associated malignancies must be considered as rare events which depend on one or several cofactors. One example of an identified cofactor is malaria in endemic BL.

Undifferentiated NPC, PTLD and endemic BL show the strongest EBV association known today [4, 5, 134, 135]. Depending on geographic areas and histological variants there is different degree of EBV association. A significant portion of pleomorphic T-cell lymphomas as well as the Reed-Sternberg (RS) cells present in HD lesions, non Hodgkin's lymphoma (NHL),

peripheral T-cell lymphomas, lethal midline granulomas and also smooth muscle tumours are EBV positive, further presented below [136].

The origin of the B cell lymphomas are the different cell types and latency program that create a divergent mosaic of different possibility for tumour development. BL seems to be originated from c-myc expressing germinal centre (GC) generating lymphoblast that are stuck in proliferative state while HD arise from cells blocked at the GCs due to mutations. PTLD and NHL could be a consequence when cells incapable of differentiation out of cell cycle gets infected, i.e. naïve B-cells [137, 138]. In immunosuppressive patients, lymphocytes that should be destroyed in the GC are rescued in the absence of cytotoxic T cells thereby giving rise to lymphoproliferative diseases. While the origin of EBV in NPC and gastric carcinomas could be viruses released from plasma cells [139]. BL, HL, nasal T- and Natural killer (NK)-cell lymphomas, gastric lymphoma, and NPC all have a long latency period indicating a complex multistep pathogenesis.

Non Hodgkin lymphoma

NHL consists of a variety of different malignancies originating from lymphocytes. These tumours can develop either in a circulating form, within organised lymphoid tissues or in tissue from other sites, or even exist as a solid tumour. The REAL classification (Revised European-American Classification of Lymphoid Neoplasm) is used today to distinguish the different lymphoma types. In short the tumours are divided into two groups depending if the origin is B cell or T/NK-cell. Secondly the tumour is classified based on if it is a precursor or peripheral neoplasm. The peripheral neoplasm group is then further divided into subgroups.

Worldwide, NHL is estimated to account for 2.5% of all cancers [140]. Throughout the world the incidence varies, being highest in United States and lowest in Southeast Asia, India and sub-Saharan Africa. A ubiquitous steady increased incidence has been noted until 2009[d], but yet not explained 141.

B cell non Hodgkin lymphoma

In Europeans 13% has detectable EBV DNA in their NHL B-cells. PCNSL are nearly always of B cell origin and also here the EBV presence is low. Gastrointestinal tract lymphoma is rare in immunocompetent individuals and the frequency of EBV involvement is low [142]. A newly identified category is diffuse large B-cell lymphoma (DLBCL) in elderly. In the upper airway and digestive tract EBV has been detected in T/NK-cell lymphoma but seldom in B-cell tumours [143, 144].

T- and NK-cell non Hodgkin lymphoma

In healthy individuals EBV infection of T cells are rarely found. Occasionally non-B-lymphocytes lymphomas can develop from EBV infected α/β T cells, γ/δ T cells, and NK cells [145, 146]. EBV positive T cells are also reported in peripheral T-cell lymphomas and some of the cases do also have a chronic EBV-associated illness [147, 148]. In a Japanese study, EBV was found in as many as a quarter of NHL T-cell lymphoma cases [149]. It has been shown that Southeast Asia has more such cases. EBV positive lymphomas are found to be aggressive with poor response to chemotherapy, and a short survival time [150]. The EBV positive cases showed an ongoing apoptotic process, but on the other hand also a proliferative activity.

T-cell lymphoma with location to sinus is most strongly associated with EBV. B-cell lymphomas with the same localisation is much less frequent EBV positive. Tumours positive for CD54, suggested to be of NK cell origin, were EBV positive while CD54 negative cases were more

d http://seer.cancer.gov/csr/1975_2009_pops09/results_merged/sect_19_nhl.pdf 2012-06-21

often found to be EBV negative [85, 151]. Another condition where EBV is highly prevalent is lymphomatoid granulomatosis that reassembles sinusoidal lymphomas [152].

Burkitt lymphoma

BL is a poorly differentiated malignant lymphoma of NHL type and predominantly with extranodal locations. The BL tumour consists of memory B cells which are poor antigen presenters and in addition the only EBV protein expressed is the immunosilent EBNA1, peptide transporters are down regulated and occasionally even MHC class I. BL could be divided in an endemic and a sporadic form. In the holoendemic malaria affected equatorial Africa where BL is endemic among children EBV genome is found in almost all tumours, while only in 15-85% of the sporadic tumours [153]. Endemic BL is most often located in the jaw of the affected young children. Abdominal involvement is found in about half of the EBV positive cases while in sporadic cases the frequency is much higher [154]. CNS involvement is found more often in endemic cases while bone marrow involvement is more often seen in sporadic cases [155].

The abnormally high number of circulating EBV-infected B-cells together with the antigen stimulating effect malaria confers on the immune system have been proposed to be cooperators in the development of endemic BL. The c-myc translocation seen in all tumours might be the result of these cooperating factors. This translocation will connect the c-myc gene to the proximal end of either the light or heavy chain in the immunoglobulin locus. Hence the oncogene c-myc gene will be deregulated and continuously expressed.

Nasopharyngeal carcinoma

NPC is a tumour localised to the nasopharynx, it is of epithelial origin and frequently found in Southern China. This cancer is present with intermediate incidence in Southeast Asia and natives of the Arctic region, Northern Africa and Middle East. In Western countries NPC is a rare malignancy, with an incidence of less than 1 per 100,000 individuals per year, and comprise only about 0.25% of all cancer types [156]. In comparison to the high-risk areas in China where the annual incidence is about 25-50 cases per 100,000 inhabitants. Men are 2-3 times more often affected than women. A multifactorial aetiology with e.g. ethnic, genetic and environmental factors are suggested to explain the increased incidence. Nonkeratinizing NPC has the strongest virus association of all virus-associated tumours with 100% EBV positivity in human beings [1].

Lymphoepithelioma carcinomas and adenocarcinomas

Epithelial neoplasms of undifferentiated nasopharyngeal type located in salivary gland or stomach are always EBV positive. In gastric adenocarcinoma EBV has a prevalence of only 10% [157]. EBV positive adenocarcinoma shows a better prognosis with less chance of metastatic spread suggested to be due to a CD8+ cell infiltrate. Rarely these neoplasms can also be located in other places: lung, thymus, and pancreas. The virus associated frequency for the other variants is correlated to ethnicity. EBV association of lung and salivary gland tumours is restricted to humans living in or originated from Greenland and Asia. Lung biopsies from Asian patients were EBV positive, while biopsies from Western world patients were found to be negative [158, 159]. EBV association of salivary lymphoepithelioma carcinomas does also seem to have a geographic distribution pattern. The tumour is more often seen in Eskimos and Chinese people than in others. Still, the tumour is rare among them. The association of EBV to gastric and thymus Lymphoepithelioma-like carcinomas is on the other hand independent of ethnicity [160].

Hodgkin's lymphoma

HD is found worldwide and is the second most common malignant lymphoma in the developed world. EBV associated nodular sclerosis is a variant of HD that has an age relation and the incidence peaks between 15 and 34 years of age and a second peak in older adults [161, 162]. In developing countries the pattern is similar but on a lower level.

The presence of EBV is related to a less favourable host response towards HD in elderly patients [163]. Individuals with an EBV positive tumour seem to have markers of diminished cellular immunity and an abnormal EBV antibody response with elevated anti virus capsid antigen (VCA) titers [164]. Interestingly a recent history of IM increases the risk of HD [165].

In HD the conspicuous few RS cells with malignant character are embedded in non malignant cell infiltrate. Based on the nature of the latter cells HD is classified in three different histotypes with different EBV percentages. In developing countries HD is EBV positive in majority of all cases, irrespectively of histotype [166].

Post-transplant lymphoproliferative disorder

Iatrogenic immunosuppression after organ or BM transplant may result in immunoblastic lymphomas, in uncontrolled lymphoproliferation and/or EBV-positive B-cell lymphomas such as polyclonal hyperplasia, polymorphic B-cell lymphomas, extra nodal B-cell lymphomas, generally involving the CNS and the gastrointestinal tract but also rarely of T cell origin lymphoma [167, 168]. Post transplant lymphomas has some similarities with HIV related lymphoma but they develop in different locations. While hepatocellular involvement is more common for PTLD, gastrointestinal lesions are predominant in HIV-1 infection [169]. All early diagnosed cases of PTLD of B cell origin are EBV positive but in the later diagnosed cases there could be EBV negative examples [170, 171]. T cell cases are time to time positive for EBV [172]. EBV is both necessary and sufficient to induce tumour growth in an immunocompromised host. The proof is the short latency period for this disease.

The determining risk factor for PTLD is the intensity of T cell suppression and EBV is a key player for the development [134, 135]. Most PTLD occur during the establishment of the new bone marrow, which takes place during the three first months and up to one year after transplantation. During this time the number of EBV specific CD8+ cells are limited. In patients receiving BMT from allogenic donors the frequency is less than 1% but rises dramatically among patients receiving T-cell depleted grafts [134]. In these cases EBV-specific T cells are undetectable, even in the presence of Epstein-Barr viremia [173]. A recipient who is EBV seronegative has 20 times higher incidence of PTLD and this could be the explanation why children are at higher risk [174]. By infusion of donor-derived EBV-specific T lymphocytes PTLD as well as lymphomas could be avoided or even cured in the recipient [175-177].

Early onset PTLD-lesions resemble IM tonsillar B-cell population in the EBV gene expression pattern [170, 171]. Other correspond more to the pattern of a naïve or a memory B cell [171, 178]. In further development of PTLD one can occasionally bee similar as in the early ones. In these one can also see centroblastic cells of GC origin representing atypical survivors that have escaped apoptosis 170, 171, 178. Many of these late cases show mutations and occasionally sign of defect mis-match repair [134, 179].

EBV in the HIV-1 environment

Non treated HIV-1 patients have a 60- to 1000-fold increase of NHLs such as BL, DLBCL with centroblastic features and DLBCL with immunoblastic features [180-183]. NHL is also an AIDS defining criteria. The increased risk is due to immunological and virological factors.

Some of the lymphomas can be seen in immunocompetent individuals as well, but others are specific for HIV-1 infected patients.

One of the factors increasing the lymphoma risk is the defective T-cell immunity seen in patients with AIDS or AIDS related disorders that results in an abnormally high number of circulating EBV-infected B cells in the blood. By indirect methods the number of EBV infected cells was shown to be 1.8 per 10^6 circulating B cells in HIV-1 seronegative individuals compared to 13.1 and 20.7 for individuals with AIDS and AIDS related disorders [184-186]. Moreover, the activity of memory T lymphocytes decreases during development of symptoms and the activity of natural killer cells is also decreased [185, 187, 188]. EBV CTL response is shown to decline while EBV-DNA load is increased and this might lead to an increased risk for development of EBV related diseases as NHL [189]. In patients with EBV associated NHL, EBV-CTL precursor decreased before the development of EBV lymphoma, and an increase of the EBV-DNA load was also found several month before diagnosis [190].

The increased antigenic stimulation in HIV-1 infected individuals will result in an increased production of T helper 2 (Th2) cell cytokines. These cytokines, among other factors, stimulate directly B-cell proliferation. The resulting persistent increase of B-cells may contribute to the increased risk of B-cell malignancies observed. As much as 19% of non sufficiently treated HIV-1 patients will develop an NHL during their infection and together with undiagnosed post mortem cases the accumulated incidence has been as high as 35%, where the majority of the diagnose was PCNSL [191]. The localisation is often extranodal or in the CNS. Approximately 60% of the lymphomas are large B-cell lymphomas, about 30% are BL and the rest are of T cell or non-B, non-T cell origin [192, 193]. After introduction of cART the pattern has changed, see chapter "Lymphomas and other tumours".

In HIV-1 positive patients, signs of a persistent reactivation of EBV-infected B cells can be demonstrated, resulting in an increased immunoglobulin production [184]. Nevertheless reactivation is a normally occurring phenomenon that can be seen transiently also in immunocompetent individuals [194]. Measurement of high EBV-DNA load detected at a single time point is not always a sign of reactivation. Reactivation is characterised by five observations: a consistent elevation of antibody titres against EBV antigens (particularly VCA), increased titres of EBV shedding in saliva, an increased lymphoproliferative ability of B cells in the peripheral blood, increased number of circulating EBV positive cells, as mentioned above, and finally evidence of BZLF1 expression [184, 185, 195-197]. In HIV-1 infected patients not only the antibody titres are increased, but also the spectrum of different antibodies is changed. All the mentioned factors taken together and yet unknown genetic and viral influences on the immune system may be the explanation of the more frequent lymphomas in this patient group.

Lymphadenopathy syndrome

EBV-infected cells were found in lymph nodes in 70% of the patients with HIV-1 related lymphadenopathy syndrome (LAS). Different LAS variants have different frequency of EBV but in general more presence of EBV is seen in HIV-1-unrelated LAS [198]. LAS with follicular hyperplasia can be EBV positive in 75% of the cases while LAS with follicular involution is EBV positive in all cases.

Oral hairy leukoplakia

HLP is a wart like lesion associated with a chronic productive infection of EBV in epithelial-cells typically found on the lateral part of the tongue. HLP was used in the pre-cART era as a predictive marker for the development of AIDS.

White plaques can also be observed in HIV-1-seronegative transplanted immunocompromised patients especially with a history of rejection episodes [199]. However, EBV is not found in HLP of HIV-1 seronegative persons [200].

Table 4. EBV associated malignancies and other diseases in HIV-1 positive patients

Malignancy/Disorder	Localisation	EBV prevalence	
		Immunodeficient HIV-1 patients	Immunocompetent patients
T-cell lymphoma (e.g. anaplastic, peripheral, nasal, midline granulomas)		Yes	yes
HD		>50%	19-50%
HD, nodular sclerosis form		100%	24%
PTLD and similar lesions		Post-transplant >90%	>90%
Non Hodgkin lymphomas			
Anorectal lymphoma	Anorectal	About 93% of gastrointestinal lymph.[a]	non of 3-6% gastrointestinal lymph.
Body cavity based lymphoma	Abdomen	Almost 100%[b]	
Burkitts Lymphoma	Lymph-nodes	30-40%	Endemic 100% Sporadic 15-85%
Immunoblastic lymphomas	Systemic	Almost 100%	
Immunoblastic plasmocytoid malignant B-cell lymphoma		yes[c]	
Primary central nervous system lymphomas	Central nervous system	66-100%	15%
Primary cerebral lymphoma	Cerebral	Yes	no
Smooth-muscle tumours[d,]	Muscles	yes	yes
Non-malignant disorders			
Chronic lymphocytic interstitial pneumonia[d,]	Interstitial	yes	
Lymphadenopathy syndrome		70%[e]	40%

[a] Homosexual males
[b] also HHV8 in the lymphoma (see below)
[c] One case reported
[d] In children
[e] Different variants of LAS have different degree of incidence

Lymphomas and other tumours

The risk of developing malignant B-cell lymphomas with atypical localisation is increased in HIV-1 infected individuals. Lymphomas of other origin than from B cells are rarely seen and EBV infection is likely to play an important role in the pathogenesis of several B-cell malignancies, such as BL, DLBCL, and PTLD in this group [201]. Generally half of the malignant B-cell lymphomas are associated with EBV [202]. The lymphomas could, for example, be located in the brain, oral cavity or in the gastro-intestinal tract but can also have a more uncommon localisation. Some of the lymphoma types are more often infected with EBV while others more rarely. Two major mechanisms appear to be involved in the development of NHL, they are loss of immunoregulatory control of EBV and chronic B-cell activation due to the HIV-1 induced immune dysfunction. AIDS-NHL has some special characteristics with recurrent multiple chromosomal alteration that can cause the B cell hyperactivation besides the processes driven by EBV oncogenes [203]. The pool of EBV positive cells are expanded and the virus can induce B-cell activation, either directly through its viral genes or indirectly by inducing cellular genes, [204].

After introduction of cART the incidence of several malignancies has decreased substantially such as Kaposi's Sarcoma, PCNSL, some types of NHL and PEL, while for HD a decrease is not obvious [205-208]. Other NHLs as BL and DBLCL has not decreased substantially with cART [209]. The suppression of HIV-1 infection must be complete as insufficient suppression does not reduce the risk of NHL [210].

Non-Hodgkin lymphoma

The chronic stimulation of B-cell and loss of immune regulation are known to be risk factors for NHL in HIV-1 patients [211]. NHLs found in HIV-1 infected individuals are heterogeneous both histologically and clinically. The lymphomas are often clinically aggressive and are frequently found in normally rarely affected locations. Morphologically, most HIV-1 related NHL could be divided into two groups: diffuse large cell lymphoma including large non-cleaved cell lymphoma, large cell immunoblastic lymphoma plasmacytoid with a predominant population of immunoblasts, and CD30-positive anaplastic large B cell lymphoma or small non-cleaved cell lymphoma as BL and Burkitt like lymphoma. In the first group 30-40% were EBV positive while in the latter EBV positivity was found in a more variable extent, 5-40% [212]. BL is rather common and accounts for about 30% of the AIDS lymphomas. NHL could have systemic localisation or CNS localisation as commonly found. Most of the NHL are clinically aggressive B-cell lymphomas, exhibiting immunoblastic, large-cell morphology, or of Burkitt-type.

In patients with an intact immune function BL has an advantage with its immunosilent appearance due to restricted EBV gene expression and MHC down regulation. Also the pattern of c-myc proto-oncogene translocation is different from what is seen in endemic cases. On the other hand in individuals with severe immunosuppression immunoblastic lymphomas are more often found. They almost always contain EBV, have a broader EBV gene expression pattern, including expression of latent membrane protein (LMP) type 1, while only one-quarter display c-myc rearrangements, and few of them have p53 mutations [213]. As described in the chapter "Gene products" viral proteins can transactivate cellular oncogenes. This effect might be enhanced in is immunosuppressed patients.

At the time of AIDS diagnosis, only 9% of the lymphomas are found in CNS, but after the AIDS diagnosis the number of CNS lymphomas rise to 38% of the NHL [214]. A major part of the HIV-1 related PCNSL's were found to be associated with EBV [215, 216]. The origin of the EBV positive malignant cells is still not known. Infiltrating B-cells are found in the brain of HIV-1 infected patients, but they were not infected by EBV [217]. The low CD4+ cell count and a long duration of HIV-1 infection increase the risk for developing a malignancy [218].

Also in the cerebral compartment there are differences between HIV-1 infected and uninfected individuals. In HIV-1 seronegative patients only a minority of PCNSL cases are EBV positive and the tumour type is also uncommon in this population with only 1.6% of lymphoma cases [219, 220]. The PCNS lymphomas are of B cell origin and mainly monoclonal [221].

In HIV-1 positive homosexual men with gastrointestinal lymphomas the frequency of anorectal NHL is much higher, 26% compared to the non-HIV-1 infected population, 3 to 6% [142, 222, 223]. Also the incidence of gastrointestinal manifestations is somewhat increased in the HIV-1 infected group 20% compared to 9% in the non-infected group [142]. The histological type is also different in the two groups, the HIV-1 related are of high grade, predominantly of immunoblastic and polymorphous types while the HIV-1 unrelated only two of the four lymphomas investigated is of high grade histotype [142]. Ioachim et al. has suggested male homosexuality as a risk factor for gastrointestinal NHL [142]. The majority of these lymphomas

are large B cell variants of high-grade malignancy, while low-grade subtypes and T-cell lymphomas are rare.

Peripheral effusion lymphoma and plasmabalstic lymphoma

Some types of lymphomas are rarely seen in HIV-1 negative cases, but occur in HIV-positives: PEL and plasmablastic lymphoma (PBL). These lymphomas represent distinct subgroups because of their unusual clinical picture, immunophenotype, and molecular genetic characteristics [224, 225]. EBV is occasionally present in PEL but always present in PBL. The PEL is positive for the KS associated Herpes virus, HHV8, while PBL only occasionally is. The role of EBV in the tumour pathogenesis is uncertain.

Hodgkins lymphoma

Cases of HD are more common in HIV-1 infected individuals than in immunocompetent persons and the appearance of the tumour is also different both regarding biological as well as clinical manifestations [226, 227]. The appearance in HIV-1 infections is aggressive, with a high frequency of unfavourable histotypes for example mixed cellularity [227]. EBV prevalence varies between different subtypes of HD but is higher than in HIV-1 negative cases. For example nodular sclerosis HD in HIV-1 infected patients is always EBV positive, while HIV-1 unrelated cases are only EBV positive to a low extent [228]. In HIV-1 unrelated cases RS cells are surrounded by CD40L T cells, this is not seen in HIV-1 positive cases, possible due to the T cell depletion or because LMP1 expressed by EBV plays the same role [229]. The EBV gene and EBV protein positive RS cells are probably the explanation of the aggressive appearance of the cases in HIV-1 positive patients. The immunodeficiency plays a role as transplanted individuals also have a higher frequency of EBV positive cases and a skewed pattern of histotypes [230].

Other lymphomas

The presence of EBV is higher in anaplastic large cell lymphomas in HIV-1 infected patients and expression of EBV genes are detected in 71% of the cases, while in HIV-1 seronegative only in 21% [231]. The increased frequency could be explained by a different origin of the tumour, or relation to another variant of anaplastic, diffuse large cell B-cell lymphoma compared to HIV-1 seronegative cases. The presence of EBV in the systemic lymphoma cases increases the risk for CNS involvement 10-fold [232].

EBV is also found in a majority of systemic immunoblastic-rich/large cell AIDS related lymphomas, but only a minority of monomorphic centroblastic lymphomas [233, 234]. One case of immunoblastic plasmocytoid malignant B-cell lymphoma was reported to be EBV positive. The patient in this case was both HIV-1 and HTLV-I infected [235].

Small-non-cleaved-cell lymphomas almost always show c-myc rearrangement and p53 inactivation but are EBV infected in a lower extent, 30%. On the contrary, in diffuse large-cell lymphoma the major part is EBV positive and less than half of these cases have rearrangements and translocations [236]. In immunoblastic lymphomas however the oncogenic c-myc rearrangement could be found in coexistence with EBV.

EBV-DNA and expression of EBV latent gene products are also detected in (anaplastic) T-cell lymphomas [237, 238]. EBV positive T-cell lymphomas can also be seen in HIV-1 negative individuals, see chapter "T- and NK-cell non Hodgkin lymphoma".

Leiomyosarcomas and leiomyomas

EBV is found in smooth-muscle tumours (leiomyosarcomas and leiomyomas) in children with AIDS [239, 240]. Leiomysarcoma is the second most frequent tumour disease in HIV-1 infected

children after NHL and it is not found at all in adults [241]. Immunocompetent children can rarely get leiomysarcoma, but these cases are EBV negative. Otherwise the spectrum of cancer in HIV-1 infected children is the same as in adults.

Other complications in children

Chronic lymphocytic interstitial pneumonia is characterised by the presence of polyclonal lesions found in HIV-1 infected children. This form of pneumonia is often associated with EBV replication [242].

Table 5. Changes of EBV-associated parameters in HIV-1 positive patients

Changed spectrum and level of antibody titres reactive against EBV antigens
Increased numbers of circulating EBV positive B cells
Increased EBV titres in saliva
BZLF1 expression

EBV load

EBV viral load has been measured for some decades now. Earlier studies were based on outgrowth after limiting dilution, which is an indirect method [243]. Later on more direct approaches utilising end-point dilution PCR, competitive PCR, sqPCR, and qPCR has been used to study EBV-DNA load in many types of patients. Blood, serum, lymphoblastoid cells and cerebrospinal fluid are materials used for EBV quantification by PCR technique. There is a huge variation in the definition of normal EBV-DNA load as well as increased value that could indicate e.g. PTLD. Variation in the results could also be explained by the use of different calibrators. On top of that different matrises are used – some researchers use whole blood, others total DNA and there are different ways to report results e.g in relation to weight, volume or number. Therefore it is a need for standardisation with systematic comparisons.

Today EBV-DNA load analysis is today most common in immunosuppressed individuals. In all reports individuals with immunosuppression at a low level has a persisting low amount of virus. Organ transplant and BMT patients were the first patient groups to be investigated. Their risk for developing a LPTD could be followed by measuring EBV-DNA load [244-246]. The second group to be investigated is HIV-1 infected patients with and without symptoms or tumours.

Also in other important diseases quantitative PCR for EBV-DNA analysis can be a useful tool, e.g. detection of manifest EBV positive tumours as NPC [247, 248]. Measurement of EBV-DNA load in plasma from NPC patients can be used as a sensitive instrument to monitor therapy response and also tumour recurrence [248-250]. Moreover the concept of EBV-DNA load is also useful in T- and NK-cell lymphomas, and for the discrimination between EBV positive and negative HL [251, 252].

Individuals with diagnosed PTLD might have as much as 100,000 times higher EBV-DNA load than healthy individuals [253, 254]. Consequently quantification of EBV-DNA load can be utilised to detect high-risk patients before onset of clinical symptoms but the EBV-DNA load is not always predictable as cases with PTLD without an increased load have been observed [176, 255-257]. The increase in genomes is not caused by increased number of genomes per cell, but by increased number of infected B-cells as also seen in other conditions, see below [258]. The greater part of the viral load in peripheral blood in patients with PTLD is believed to be related to immortalised cells and not a lytic infection [259]. When frequent measurements are made transient increases could be seen without PTLD while the observation of longer periods of constant increased values are prognostic for PTLD [256, 260, 261]. Interestingly in acute rejection event the

EBV-DNA load increases but not to the same level, and thereby rejection events could be discriminated from PTDL [262].

In contrast to measurements of cell free virus in serum, plasma or saliva, monitoring B-cell associated EBV may reflect viral load related to cell population. The fragmented genome found in plasma is thought to predominately originate from dying cells and could thereby bias quantification. On the other hand measurement of non-cell bound EBV could be of value in diseases where production of virions takes place as in IM. PCR detection of EBV DNA in serum plasma and saliva can be affected by occurrence of inhibitors found in hypoalbuminemic patients so the PCR measurement in these patients have a limited clinical value [263].

EBV load in HIV-1 infected patients

In contrast to the stable EBV load set-point[e] established in the immunocompetent host the balance in HIV-1 infected patients between EBV and the host is disturbed. Early work in HIV-1 infected individuals showed similarity to findings in immunocompromised transplant recipients. Measured values overlap the range found in healthy individuals but is generally higher [80, 190, 264]. Later on quantitative assays have been used and better information has been retrieved.

As described above outgrowth methods was earlier used and resulted in abnormally high number of EBV-infected B cells in blood in HIV-1 infected individuals [184]. Using PCR methods in a study of homosexual men showed a stable four-fold increase of EBV-DNA load after HIV-1 seroconversion [265]. The increase can be caused by EBV reactivation or by cell division. The set point of EBV-DNA load is individual and there is no relation found between the EBV-DNA load and the cellular response in HIV-1 immunocompromised patients, but there is a relation to the degree of B cell exhaustion [266, 267]. In one study EBV-DNA load was not predictive for lymphomas in general while it was so in another [268, 269]. This finding could be due to the smaller group studied in the former study. Other studies show that the EBV-DNA load in serum increases a couple of months before diagnosis of NHL [270]. A low EBV-DNA load in an already malignant state does not reflect what have happened before the malignant transformation some years ago i.e. a peak could have appeared years ago, therefore longitudinal studies are recommended.

In one study 17 EBER-positive AIDS lymphomas were scrutinised, and shortly before the lymphoma diagnosis the EBV-DNA load in blood increased, whereafter initiation of lymphoma treatment resulted in a decreased level of EBV DNA [270]. As in transplanted individuals a successful treatment is followed by rapidly decreasing EBV-DNA load. Tumours localised in CNS do not result in increased EBV-DNA load in blood, as released virus or viral antigens probably could not pass the blood-brain barrier therefore the cerebral spine fluid has to be analysed instead [271].

In studies with HIV-1 treated patients studies one concludes that cART does not restore the host EBV balance effectively [272, 273]. This is in some contrast to our findings where we could observe a positive effects of cART on the EBV-DNA load but it is of great importance to distinguish between successful cART compared to antiviral treatment in general. Repeated peaks of EBV-DNA load to high values should most likely be considered as a manifestation of an insufficient treatment result, as we do not see this phenomenon in treated patients with a complete immune reconstitution (paper I).

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e A set point is a level of virus in an individual, here EBV-DNA load, that persist for a long time

Immune defence system

The immune system has the capability to protect the body from foreign cells and invaders The system can discriminate foreign agents from self and is also able to protect the body from new unknown agents. This adaptive immune system has an almost unlimited capacity to identify evolving and new pathogens. The hugh variability allow development of immunological weapons toward self. Therefore it has to be a selection system where cells with self specificity are eradicated. All in all development of new variants of recognition and elimination of self recognising immune cells results in a complicated and strictly regulated self controlled system, both positive and negative. This system can also occasionally end up in endless loops of stimulation by self with anergy and exhaustion as the ultimate result. If the elimination processes is not successful the immune system could target the own body resulting in autoimmune diseases e.g. rheumatoid arthritis. It may also react to strongly to common non-infectious agents in the surrounding as in allergic diseases. Finally the immune system has a memory, that effectively spare us from getting infected again by virus and bacteria and this is the function used when a preventive vaccination is performed.

There are two arms of the immune system: 1) the innate with NK cells, macrophages, monocytes, neutrophiles and dendridic cells as actors, 2) the adaptive system with the T and B cells as the key actors. Both systems are needed and have counteractive and specific roles in defeating any infection.

1) The innate immune system is fast and controls the early phase of the infection at the same time as it activates the adaptive immune system. Due to germline encoded receptors that recognise pathogen specific structures the innate immune system can recognise intruders and capture them. The foreign particle is transported to lymphoid organs and is presented for cells from the adaptive immune system. Ingestion and processing pathogens induces release of cytokines and chemokines. Thereby an inflammatory response is induced attracting more immune cells as well as preventing the a spread of the pathogen. Viruses are targeted effectively by this system.

2) The second defence line with B and T cells as key players is initiated after some days or a couple of weeks. The T cells circulate in blood and peripheral lymphoid organs after production in the bone marrow and maturation in the thymus. After meeting a matching alien in the lymphoid organ the cell develop into effector or memory cells. Depending on if the T cell is CD4+ or CD8+, different MHC classes can present the antigen and different factors are produced by the cell. A subset of CD4+ cells can activate B cells. Bacterias are targeted effectively by the adaptive immune system.

B cells also circulate in blood and peripheral lymphoid organs after production in the bone marrow but mature in secondary lymphoid organs as spleen and lymph-nodes. The variation and specificity towards different and new agents are generated and every B cell has a unique region where an antigen can bind the complementary determining region. When a match is found between an antigen and a specific B cell, the B cell present the antigen to a pre GC cell whereafter antibody producing plasmacells can be formed. Affinity gets sharpened in the GC reaction and the B cell proliferate and differentiate into plasma or memory cells. The plasma cells produces high numbers of secreted antibodies. The unique antigen binding region is amplified to be used for antigen elimination. The memory cell is a sort of dormant cell that can be awake in the future when a similar agent infects the body. An optimisation process occurs which improve specificity during repeated rounds of proliferation.

The immune response to EBV

Cellular and humoral immune responses play a crucial role in limiting the spread of virus throughout the body (fig. 2). Cytotoxic T cells in peripheral blood from EBV-seropositive healthy people have been shown to inhibit growth and differentiation of autologous B cells activated by EBV in culture [274, 275]. The mechanisms involved in controlling the virus infection may be several, including natural killing, lymphokine-activated killing, antibody-dependent cytotoxicity, complement-dependent cytotoxicity and macrophage response, and in particular EBV-specific T-cell response. Cytotoxic T-cells react against MHC class I-associated peptides from the nuclear proteins EBNA2-6 and the latent membrane proteins (LMP1 and LMP 2) expressed on the membrane of lymphoblastoid EBV transformed B-cells. The immunogenicity of viral proteins varies, for example LMP2 is more immunogenic than LMP1 [276]. As much as one specific cytotoxic T cell precursor per 1,000 to 10,000 circulating T cells persists throughout life after the primary infection and controls the latent infection [277]. These level of precursors are remarkably stable over several years. Their high frequency and stability suggest that immunogenic EBV-infected cells are continuously being generated and provide a frequent restimulation of the T-cell system.

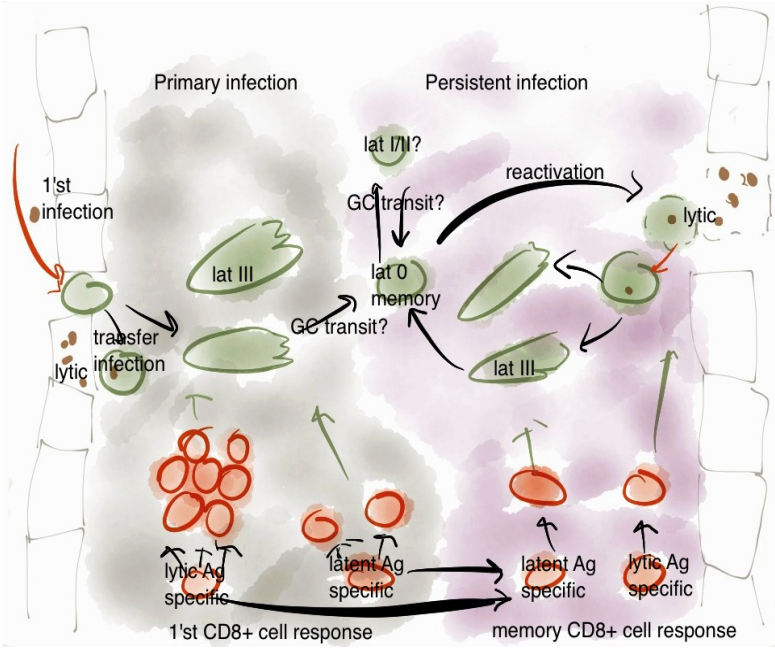


Figure 2. The virus-cell interaction in primary, persistent and secondary EBV infection and epithelial, B-, and T-cells, grey, green respectively red. Red arrow- free virus movement, brown dots is virus. Black arrow-cell deviation and movement. Bluish arrow-cytolytic effector function.

EBV shows different patterns of protein expression i.e. latency programs in different environments. In the GC latency II is expressed. This is followed by proliferation and differentiation ending up in memory B cell, now in latency 0. The infected circulating B cell cannot be detected by the immune system as no viral proteins are presented to the surface. When memory B cells divide latency phase I program is activated.

So called atypical lymphocytes, which are predominantly of T cell origin, appear as specific indicators of IM. The failure of T cell-mediated control of the infection is likely to be caused by their low specificity to viral antigens, and the predominant lack of HLA-restriction of the antigens. The cellular response during the acute phase of primary infection is dominated by CD8+ cells, but also by a presence of CD56+ NK cells [278]. The levels of CD4+ cells on the other hand remains stable with a small number latent and lytic antigen specific CD4+ cells [279]. The CD4+ cells modify the infection to provide more time for the adaptive immune response to develop. Another mechanism to counteract the infection is up-regulation of HLA class I expression [280].

During the primary infection, IgM antibodies, directed towards VCA and heterophilic antibodies are present. In the acute phase antibodies with neutralising capacity are often complement-dependent and predominantly of IgM type directed to gp350. During the several

month long recovery after IM, IgG antibodies to VCA, early antigens, and membrane antigen appear and IgM antibodies disappear. Of these, antibodies directed against the membrane antigen complex gp350 can neutralise EBV particles [131]. Cells with a lymphoblastoid cell line-like expression of EBNAs and LMPs could be detected in reactive lymph nodes in IM patients. A CTL response directed to multiple epitopes seems most efficient in resolving the infection, a phenomenon also manifested in other infections [281, 282]. Nonspecific B-cell activation is noted with increased levels of IgM, IgG and IgA and could either be driven by the EBV infection of B-cells or be the result of an abnormal T-cell helper activity [129, 283, 284]. The EBV load is directly correlated to the frequency of EBV-specific CD4+ cell response indicating that this response is antigen driven [285]. T-cell repertoire perturbations, absence of increased levels of blood mononuclear cells, and absence of a CTL response will result in an asymptomatic infection even though the viral load is high [286]. The immune response in itself is therefore thought to explain the symptoms in IM.

In healthy EBV carriers gp350 neutralising, EBNA1 and VCA antibodies are found [129, 287]. The anti-gp350 can bind to the surface of a cell undergoing a lytic infection as well as virions and therefore one function of these antibodies is to control reactivation of the latent infection. Specific CD8+ cells also might control reactivation. Most individuals with clinically latent infection have CD4+ memory T-cells directed to one or more of EBNA1, EBNA2 and EBNA6 and less frequently directed to EBNA3 and the LMPs. EBNA2-6, LMP1 and the LMP2s serve as targets for specific cytotoxic T-cells [288-290]. EBNA1 is targeted by MHC class II- but not by MHC class I restricted CTLs [291].

The immune response to EBV in HIV-1 infected patients

EBV is affected by the HIV-1 induced immunodeficiency with expansion of EBV activated cells as a consequence of the loss of immunoregulation by the EBV specific CD8+ cells, CD4+ cell depletion, and the HIV-1 associated chronic activation of B-cells [204, 292]. Such chronic B cell stimulation can be manifested clinically as increased EBV-DNA load together with a generalised lymphoadenopathy[f] [267]. EBV-DNA load has also been shown to inversely correlate to a decrease in EBV cellular immunity indicating a state of exhaustion [189]. A parallel to this situation comes from studies on BMT patients. In these patients high EBV-DNA load is counteracted by activated CD8+ cells in order to avoid development of PTLD 293. The EBNA1 specific response decline slowly but could be restored in HIV-1 infection by cART [265]. In the end EBV load increases as no EBV specific CD4+ cells are left to counteract the increased EBV activity [189, 190, 268]. In some patients with cART the memory B cell pool is not well restored and in those patients with persistent increase of EBV-DNA load an uncommon immature or transitional-like cell harbouring EBV is still present [292].

The immune response in HIV-1 infection

HIV-1 infection is incurable at present and ones established the infection will persist. Every non treated patient will suffer from a progressive destruction of the immune system. Some patients have the possibility to control the infection up to 25 years, so called elite controllers, others develop AIDS shortly after seroconversion. During the early stage of HIV-1 infection more than half of the memory CD4+ cells are lost, the rest of the cells are consumed by the virus in a faster pace subsequently which finally results in an almost eradicated immune system [294].

f Swelling or abnormal enlargement of the lymph nodes

The innate immune response is the first defence line in HIV-1 infection. Toll-like receptors on dendritic cells are activated and inflammatory cytokines are released. These cells can be infected by HIV-1 as well which might be an explanation for their impaired function 295, 296. NK cells are also recruited and their numbers increase substantially and correlate to viremia decline. In the long run they develop anergy resulting in an accumulation of nonfunctional cells [297].

In the second line of defence the adaptive humoral response produce the antibodies 1-2 weeks after primary infection. Some antibodies have neutralising capabilities but sequence variability is high and escape mutants soon appear [298]. The lack of an early and sufficient concentration of neutralising antibodies will however initially manage to influence the virus load but they do not affect the progress of the disease. Most of these antibodies are nonprotective as they are directed towards virion debris [299, 300]. The adaptive cellular response react as well with increasing levels of the CD8+ cells, which suppress the viremia [301]. The CD8+ cells target more than a dozen HIV-1 peptides [302]. Strong HIV-1–specific CD4+ cell responses have been associated with better control of viral replication and as a consequence viral escape from their targeted epitopes has been observed [303, 304]. Most of the CD4+ cells are directed against the Gag protein.

Even though the immune system is activated its function is suboptimal resulting in an altered humoral immunity as well as a chronic activation of the immune system [305-307]. Already during the primary infection this impaired function with an activation of B-lymphocyte is seen. About one-half of the GC in terminal ileum is lost affecting the capability of the immune system [308]. The T-cell activation is revealed by three changes: 1) expression of activation markers on both CD4+ and CD8+ cells; 2) high turn-over of lymphocytes; 3) increased levels of inflammatory cytokines in the plasma where most of them are produced by T helper cells [307, 309, 310]. Characteristics for the B cells activation are: hypergammaglobulinaemia; increased polyclonal B-cell activation; increased cell turnover; increased expression of activation markers e.g. CD38; an increase in the differentiation of B cells to plasmablasts; increased production of autoantibodies; loss of memory B cells; and an increase in the frequency of B-cell malignancies (fig. 3) [306]. A possible consequence of chronic antigenic stimulation as well as lack of CD4+ cells might be an exhaustion of CD8+ cells by preventing them from normal progression to memory cells [311, 312].

The activation of B-cells also results in a decreasing number of memory B cells [313]. The high serum level of IL-7 prevent B cell maturation [306]. In addition there are also several B-cell subpopulations formed in abnormal amounts. These include immature transitional B cells, exhausted B cells, activated mature B cells and plasmablasts. Moreover a population of functionally exhausted HIV-1 specific B cells are found that might contribute to the insufficient antibody response towards the virus [306].

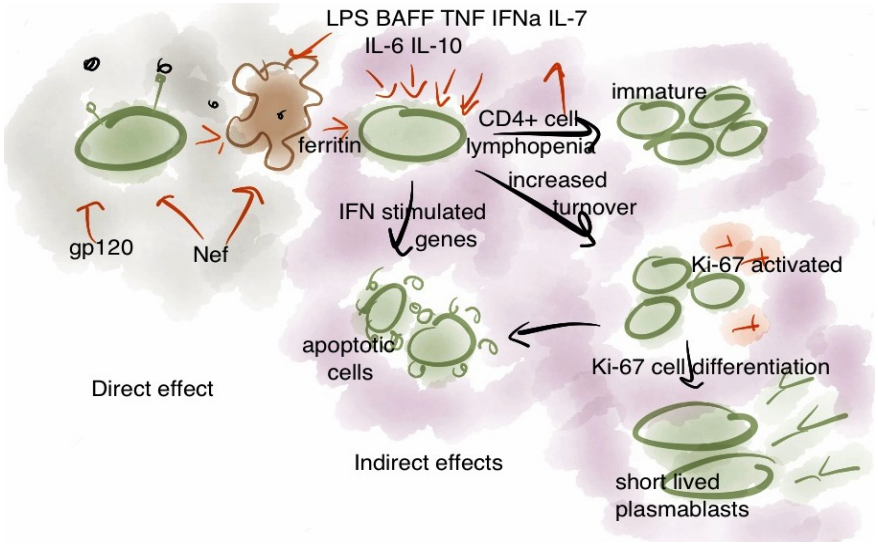
These findings are suggested to be a consequence of the ongoing HIV-1 replication, lymphopenia[g] and microbial translocation[h] [306, 314]. Another explanation is the presence of many cytokines that can directly or indirectly trigger the activation [306]. All B cell changes mentioned above might also affect the EBV carrying subset of B cells, which in turn will increase EBV activity and EBV load.

After the introduction of cART some B cell abnormalities return to normal e.g. the HIV-1 induced expression of activation markers. An increased level of memory B cells could be seen and is due to less B cells prone to cell death. Indirectly the CD4+ cell- increase induces a decrease in transitional B cells. But functional B cell abnormalities are still present, particularly among the memory B cells [306]. The possibility of memory B cells to be restored is depending

g I.e. lymphocyte count is low

h Bacterial lipopolysaccarides that pass from the intestinal lumen into the circulation

Figure 3. Direct and indirect effects of HIV replication on B cells.
Direct effects on B cells: Binding of complement- bound HIV virions (black) to B cells (bluish), which can enhance virus dissemination and increase B-cell depletion by apoptosis and also induce B cells to secrete inflammatory cytokines. In addition, secreted Nef can diffuse into B cells and suppress B-cell class switch recombination. HIV-infected macrophages (brown) release factors, that stimulate B cells.



Indirect effects: HIV-induced immune-cell activation and CD4+ cell depletion. Increased serum levels of cytokines, increased B-cell immaturity, decreased responses to antigen and increased apoptosis. Various systemic mediators of immune-cell activation.

on the stage of HIV-1 disease when cART is initiated. Even though the levels of memory B cells increases to normal level, the restoration could be incomplete as the capacity to induce B-cell response is limited. The latter may be due to the persistent loss of naïve CD4+ cells or the expression of T-cell activation markers reflecting a skewed CD4+ cell pool with more differentiated and activated T cell phenotype in combination with a dysfunction (see also chapter "Immune status and vaccination") [315-317].

HIV-1 infection creates a thriving milieu for continuous virus survival and development of disease. The treatment with cART improves this situation although not completely.

Immune status and vaccination

Several examples of successful vaccination programs is seen worldwide today. Some of them have even eradicated life-threatening diseases e.g. smallpox. Attempts to develop vaccines for infections like EBV to protect against EBV induced malignancies has been presented. Prophylactic vaccination is preferable but also therapeutic vaccines might improve the illness and decrease the risk of transmission of the virus. The difficulties with preventive and therapeutic vaccination in HIV-1 infection are reflected by vaccination trials that has been ongoing for more than a decade. So far none of the vaccine trials have generated broad neutralising antibodies [318]. The tremendous variability of the virus with the glycogen shedding of antigens makes it difficult to create broad neutralising antibodies [300, 318, 319]. Still many questions remain. Another possible way suggested to solve the dilemma of neutralising antibodies could be a NK and T-cell based vaccine [320].

The adjuvant alum ($KAl(SO_4)_2$) was earlier used to increase the humoral response in many subunit vaccines such as tetanus and diphtheria. With alum a slow releases of the active ingredients and also an inflammatory response is induced by antigen presenting cells and finally alum induce ureic acid formation that has the capability to recruit and activate dendritic cells [321].

There are a few publications about the effect on the immune system after vaccination of immunocompetent and HIV-1 infected immunocompromised individuals. Hepatitis B virus vaccine could reduce the allogenic reactivity of the T cells in immunocompetent individuals [322]. Herpes virus reactivation is seen in individuals receiving either hepatitis A, influenza, or rabies

vaccination [323]. In HIV-1 infected patients Stanley et al showed that after administration of a tetanus booster, HIV-1 RNA load increased and one could also isolate HIV-1 more easily from these individuals [324]. Transient HIV-1 RNA increases have also been reported after influenza and pneumococcal vaccination [325, 326]. In cART treated individuals this vaccination effect is absent [327].

In HIV-1 infection the function of the immune system is distorted and any vaccination might show unusual and unexpected reactions. A polysaccharide pneumococcal vaccine, which is a T-cell-independent immunogen, resulted in a decreased memory B-cell response, supporting that HIV infection is also associated with intrinsic B-cell memory defects [305]. This loss of responsiveness to pneumococcal antigen was not reversed by cART. Booster injection for influenza did show a compromised B cell response [328]. In spite of cART induced immune reconstitution, revaccination and primary vaccination does not boost or induce immunity, in line with a malfunctional immune system, see chapter "The immune response in HIV-1 infection" [329].

Effects on the immune system of repeated vaccination in immunocompromised or immunocompetent individuals have not been investigated. Only in one Nordic HIV-1 vaccination study with repeated infusions of glycoprotein 160 (gp160) to improve the immunologic response a modest improvement of the CD4+ cell count was observed [330]. A recent analysis of this patient material did show an increase of a CD4+ memory cells (CD3+, CD4+, CD45RA-, CCR7+) [331]. We find a clear influence of a rgp160 vaccine/adjuvant on the EBV host balance with increased EBV DNA values especially among patients with a history of PHI (Paper II and III).

The specific aims were:

- to explore the effect of cART on the reconstitution of host EBV balance as measured by EBV-DNA load

- to explore the host control of latent EBV-infection as measured by EBV-DNA load in specific subgroups of HIV-1 infected patients

- to explore the effect of therapeutic vaccination on EBV-DNA load in HIV-1 patients

- to explore the utility of EBV-DNA analysis in follow-up of a patient with an EBV-associated lymphoma

Summary of the present investigation

EBV host balance and cART outcome (paper I)

The host EBV balance was examined in a longitudinal study with EBV-DNA load in a small cohort of HIV-1 infected individuals. All of them where infected during the era before introduction of combination antiretroviral treatment (cART). Some of them had developed signs of immunodeficiency as shown by a significant decrease in their CD4+ cell count. After the introduction of cART we could see three patterns of EBV-host interactions in relation to CD4 cell counts and HIV-RNA control. One pattern showed an improved control of EBV infection with decreasing or stabilised low EBV-DNA values. This finding was accompanied by a continuous increase in CD4+ cell count until values were normalised with constantly undetectable HIV-1 RNA. The second pattern also showed improvement towards normalisation of the CD4+ cell count, but HIV-1 RNA was detected occasionally in blood. In these individuals the host control of EBV seems to be not fully recovered and there is a variable level of EBV-DNA load maintained at a higher level. The third pattern did show only a small increase in CD4+ cell count and HIV-1 RNA almost constantly detectable. This group retained high levels of EBV-DNA load.

These results suggest that the host-EBV balance seems more difficult to regain than reconstitution of HIV-1 infection. For the former to happen the CD4+ cell count has to normalise together with a recovered T-cell function and a constantly suppressed the HIV-1 replication. This is also reflected in the natural course of the HIV-1 infection during which EBV related NHL can appear before the occurrence of HIV related severe complications.

EBV host balance and immunologic distinct groups (paper II)

EBV DNA-levels were examined in blood from patients representing two distinct progressor profile groups. One group was long term asymptomatic patients or elite controllers. These individuals did not get treatment for a long period of time and in spite of that, they maintained a high CD4+ cell count. We believe that this group has a close to optimal control of the HIV-1 induced effects on the immune system. These patients are likely to have less unbalanced cell populations and a lower inflammatory activity affecting the B cell population. The second group examined were patients with a history of symptomatic primary HIV-1 infection (PHI). This group has been associated with more rapid progression to AIDS compared to patients with asymptomatic seroconversion. The PHI patients might have a predisposition to increased inflammatory reactions with unbalanced cell populations affecting the B cell compartment.

We found that patients with a history of PHI had increased EBV-DNA load compared to the elite controllers. This was more prominent in the findings in paper III when these two immunologically distinct groups were vaccinated.

EBV host balance and therapeutic vaccination (paper III)

In therapeutic vaccine trials using recombinant glycoprotein-160 vaccine HIV-1 infected patients are repeatedly and regularly exposed to vaccine or only to the adjuvant (placebo). Such trials are performed in order to get an improved immune reaction towards and better control of the HIV-1 infection.

We studied a group of patients included in two therapeutic vaccine trials receiving vaccine or only the adjuvant (placebo). HIV-1 infected individuals usually show an increase of EBV-DNA load, higher in those with a more advanced disease. However the patients included in the vaccine trials showed an even higher increase irrespectively if they got vaccine or only the

adjuvant (placebo). A subgroup of the vaccinees who had a documented symptomatic PHI showed an even stronger increase in EBV-DNA load. Thus we suggest that there may be an additive effect of the vaccination and the earlier symptomatic PHI on the EBV-host relation. It was remarkable that the increase was as substantial and significant in the placebo vaccinated patients, pointing to an effect of the vaccine induced immunestimulation by the adjuvant, rather than by the specific component of the vaccine. This finding strongly suggests that different (constitutional?) host responses to HIV-1 infection can be reflected by the EBV host balance.

EBV-DNA load and relapse in EBV-associated lymphomas (paper IV)

Untreated HIV-1 infected persons may occasionally develop peripheral effusion lymphoma (PEL) or the histologically related plasmablastic lymphoma (PBL). The former is associated with human herpesvirus 8 and usually with EBV, the latter only with EBV.

We have studied a case of EBV-positive lymphoma in an AIDS patient, initially diagnosed as PEL but finally as PBL with pleural and intra-abdominal effusions. One year prior to lymphoma presentation the patient received cART and responded well with undetectable HIV-RNA and increased CD4+ cell count. The patient had a history of Kaposi sarcoma and was human herpesvirus 8 seropositive. Biopsies from the later occurring lymphoma, and three cell lines derived from the tumour cell effusions on different occasions, were all EBV-positive but human herpesvirus 8 negative We found a noticeable decline of EBV-DNA load during the remission of the lymphoma following CHOP-therapy. Preceding or at the time of recurrence EBV-DNA load increased dramatically.

We suggest that EBV-DNA load can be an important tool in monitoring the effect of lymphoma treatment. Its value in estimating the risk of EBV-associated lymphoma in HIV-1 infected patients with pronounced immunosuppression should be further evaluated.

Concluding discussion

My thesis addresses the interrelationship between latent EBV infection and the immune suppression generated by HIV-1 infection. The long term effects of HIV-1 on the immune system may activate the EBV-infection in B lymphocytes as an early step in the lymphomapathogenesis. A well established example of the shift of the EBV host balance is seen in transplant patients where PTLD, caused by a combination of immune stimulation and medical immunosuppression, constitutes a transition state towards lymphoma. The interaction between EBV and its human host is a complicated balance with paradoxical features: 1) EBV is a very immunogenic virus which however can persist throughout life in immnocompetent hosts, and 2) EBV is a highly oncogenic virus, but this EBV tumorigenesis predominantly takes place in immunocompromised individuals.

The strong immunogenicity results in an efficient eradication of cells expressing EBV proteins or products thereof on cell membranes as well as of circulating EB-virions. The latent reservoir of EBV in the infected B cells can only be maintained as an immunosilent latency, latency-programs 0 or I. In a situation of immunosuppression together with cofactors such as malaria, the control of the latent virus infection may be disturbed, and occasionally shifts in latency to proliferative and antiapoptotic latency III. In latency III the B cell gets activated into lymphoblasts and an expansion of EBV-positive B cells take place. The increased number of EBV carrying cells together with proliferative latency III program are most likely the cornerstones in the EBV induced risk for malignant transformation. The strong impact of the host control is demonstrated by the low number of EBV associated lymphomas in immunocompetent individuals without exposure to cofactors. However, in individuals with disregulation of the immune system this picture changes.

The impaired control of EBV in HIV-1 infected individuals correlates to a substantial increase in the risk of EBV related malignancies. Lymphomas may even develop before the CD4+ cell counts is substantially decreased, suggesting a functional T-cell impairment. Transplant patients also show an increased risk of EBV associated complications due to the shift in EBV host balance with PTLD as one common consequence. This risk is diminished by reduced immunosuppressive therapy after which PTLD can remit.

The introduction of effective combination antiretroviral treatment (cART) has dramatically improved the prognosis for HIV-1 infected patients. Previously quite frequent in AIDS patients, EBV-positive primary central nervous system lymphomas (PCNSL) have virtually disappeared with efficient cART, while the risk for HD and DLBCL/BL remain [205-208]. The fact that some EBV related diseases disappear while others remain after the introduction of cART points to differences in the impact of the host control of EBV and related pathogenesis of the different types of lymphomas. PCNSL occurs in patients with severe immunodeficiency and their disappearance correlates to the generally improved immune status. Still prevalent lymphomas like DLBCL/BL might depend on a constant immune stimulation from inflammation affecting the B cell population and the EBV-infected cells, with increased risk mediators of tumorigenesis.

All patients in our study are recruited from two Swedish HIV-cohorts with extremely well documented histories of illness. Necessary information could be retrieved from their first contact after HIV-1 infection up to our analysis time. We could only recruit small patient groups to our studies which often preclude decisive statistical analysis and makes conclusions less definite. This limitation makes it e.g. difficult to judge if an outlier is an outlier or if he or she belongs to a separate group. Confounding factors may also be difficult to eliminate, e.g.

HIV-1 negative controls lack beside HIV infection also other possible co-factors affecting the EBV-host balance such as intravenous drug abuse and other virus infections with influence their immune status.

In this thesis a semiquantitative EBV-DNA PCR was used. The method has strengths in being both sensitive and not affected by quenching inhibitors that can appear in blood samples of severely sick individuals. As enriched B-cells were used in all analysis the results are not influenced by relative changes of the B cell pool in the total amount of lymphocytes. The method utilises analysis of parallel samples. A weakness of the method is that the extra processing step to enrich B cells may lead to loss of cells, only really a problem if the cells are few (e.g. in limited samples from children or shortly after transplantation, when reconstitution has not yet occurred). This separation step can vary between different patients. In some severely ill HIV-1 infected patients the B cells are quite fragile and this may reduce the yield.

The original methods, end point dilution polymerase chain reaction (ePCR) and sqPCR, demands more personnel but simpler and cheaper equipment. The more recently developed qPCR requires less manual handling but more expensive instrumentation and reagents. qPCR is more suitable for high trough-put analysis due to higher degree of automation including the quantification step. Although qPCR has become the method of choice in most PCR-based vital diagnostics in the Western world, sqPCR and ePCR can detect EBV DNA in some samples where qPCR fail to do so, due to quenching inhibitors. The methods are otherwise comparable regarding specificity and sensitivity.

Most of the HIV-1 infected individuals treated with cART normalises their CD4+ cell number, even though it may take years to do so. This quantitative normalisation does not necessarily include restoration of functional defects. Studies of the CD4+ cell population has linked the failure to improve T-cell functions to individuals who had developed substantial immunodeficiency before the initiation of cART. The proportion between naïve and memory CD4+ cells is skewed with very few naïve cells in relation to memory cells similar to immune senescence in patients with a CD4+ cell nadir below 200 x 10^6/L [317]. This functional defect together with a chronic inflammation e.g. bacterial intestinal translocation will interfere with the B-cell homeostasis [314, 317]. In the lymphoid tissue fibrosis and altered chemokine and cytokine levels could be observed that may affect EBV-infected cells during lymph node passage.

Traditionally the treatment outcome of cART is monitored by CD4+ cell count and HIV-1 RNA copy number. An improved and finally normalised CD4+ value is one of the corner stones for evaluation, the other is persistent undetectable HIV-1 RNA values.

Our results show that EBV host balance is more difficult to reconstitute than HIV-1 control.

Other immunological factors may also affect the relation between EBV and the host. This is suggested by our study of patients with history of symptomatic PHI. When a non-specific immune stimulating factor like the adjuvant of the therapeutic vaccine is added, the EBV-host balance is disturbed resulting in a substantial increase of the EBV-DNA load (paper II). Symptomatic PHI patients might be a HIV-1 infected group with a need for early initiation of antiretroviral treatment. All HIV-1 seropositive individuals included in the therapeutic vaccine trial seem to have a disturbance in the EBV host balance as they all show at least some increase of EBV-DNA load compared to individuals not included in the trials. This finding is irrespective if they got the adjuvant only or the vaccine. Repeated distribution of vaccine/adjuvant provides an immune activating effect in the HIV-1 infected patients. It might be considered one cause of a proinflammatory state with adverse effects on the EBV host balance, even preceding increased risk for malignant development.

Future considerations

EBV-DNA load is today used to screen patients at risk for post transplant lymhoproliferative disease (PTLD) and can be used for risk assessment of nasopharyngeal carcinoma (NPC) in high risk areas [247, 248, 256, 257].

The relation between HIV-1 and EBV together the possible risk of NHL development is currently a global problem. This is more pronounced in developing countries. There is a need in those countries to monitor patients at risk for NHL to avoid treatment delays and reduce risk of NHL development.

Based on our current understanding of the EBV host balance and our findings of increased EBV-DNA load in different HIV-1 infected patient groups we propose that EBV-DNA load would be a valuable instrument to monitor qualitative restoration of the immune system. The EBV-DNA load can be analysed using different variants of PCR-technology: ePCR, sqPCR or qPCR. In countries with limited resources and low costs for technical staff, the more manpower demanding methods ePCR and sqPCR offers advantages to qPCR as the latter is more expensive both in machine investments and reagents. Usage of technically simpler methods will unleash resources for the society and has clear advantages in developing countries from a health-economic perspective.

EBV DNA load can also be of value to monitor disturbances caused by an external immunomodulating treatment like therapeutic vaccination. To further improve the health for HIV-1 seropositive individuals with cART additional facts points to the need of complementary treatments to improve the function of the immune system. This is another upcoming issue where we believe that a qualitative evaluation tool as EBV DNA might add information in the evaluation of patients immune status. Another identified group which so far has evoked little attention are so called late testers, patients with a long period of unknown HIV-1 infection and with a pronounced immunodeficiency at diagnosis. In these patients EBV-DNA load monitoring might be used to estimate lymphoma risk, as increased lymphoma risk might prevail in these patients and a lymphoma may persist a long time after treatment has been initiated. Finally, EBV-DNA load is an important measure to monitor treated EBV-positive lymphoma patients at risk of relapse. Future studies are needed to establish cut off values, test intervals and simple methodological strategies. EBV-DNA load monitoring has a definite clinical value in different patient groups.

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ai – joining, unifying, combining, fit

ki – spirit, energy, mood, morale

do – way, path

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