Chapter

Human Herpetic Viruses and Immune Profiles

Marwa Mohammed Ali Jassim,
Majid Mohammed Mahmood and Murtada Hafedh Hussein

Abstract

Herpesviruses are large, spherical, enveloped viral particles with linear double-stranded DNA genome. Herpesvirus virion consists of an icosahedral capsid containing viral DNA, surrounded by a protein layer called tegument, and enclosed by an envelope consisting of a lipid bilayer with various glycoproteins. Herpesviruses persist lifelong in their hosts after primary infection by establishing a latent infection interrupted recurrently by reactivations. The Herpesviridae family is divided into three subfamilies; α-herpesviruses, β-herpesviruses, and γ-herpesviruses based on the genome organization, sequence homology, and biological properties. There are eight human herpes viruses: Herpes simplex virus type 1 and 2 (HSV-1, –2) and-Varicella-zoster virus (VZV), which belong to the α-herpesvirus subfamily; Human cytomegalovirus (HCMV), and Human herpesvirus type 6 and 7 (HHV-6,HHV-7), which belong to the β-herpesvirus subfamily; and Epstein–Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) or Human herpesvirus 8 (HHV-8), which belong to the γ-herpesvirus subfamily. Within this chapter, we summarize the current knowledge about EBV and CMV, regarding their genome organization, structural characteristics, mehanisms of latency, types of infections, mechanisms of immune escape and prevention. Epstein–Barr Virus (EBV) genome encodes over 100 proteins, of which only (30) proteins are well characterized, including the proteins expressed during latent infection and lytic cycle proteins. Based on major variation in the EBNA-2 gene sequence, two types of EBV are recognized, EBV type 1 and 2. Epstein–Barr virus types occur worldwide and differ in their geographic distribution depending on the type of virus. **EBV** spreads most commonly through bodily fluids, especially saliva. However, EBV can also spread through blood, blood transfusions, and organ transplantations. The EBV is associated with many malignant diseases such as lymphomas, carcinomas, and also more benign such as infectious mononucleosis, chronic active infection. The EBV has also been suggested as a trigger/cofactor for some autoimmune diseases. Overall, 1–1.5% of the cancer burden worldwide is estimated to be attributable to EBV The latently infected human cancer cells express the most powerful monogenic proteins, LMP-1 and LMP-2(Latent Membrane Protein-1,-2), as well as Epstein–Barr Nuclear Antigens (EBNA) and two small RNAs called Epstein–Barr Encoded Small RNAs (EBERs). The EBV can evade the immune system by its gene products that interfering with both innate and adaptive immunity, these include EBV-encoded proteins as well as small noncoding RNAs with immune-evasive properties. Currently no vaccine is available, although there are few candidates under evaluation. Human cytomegalovirus (HCMV) is a ubiquitous beta herpesvirus type 5 with seroprevalence ranges between 60 to 100% in developing countries. CMV is spread from one person to another, usually by direct

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and prolonged contact with bodily fluids, mainly saliva, but it can be transmitted by genital secretions, blood transfusion and organ transplantation. In addition, CMV can be transmitted vertically from mother to child. CMV infection can result in severe disease for babies, people who receive solid organ transplants or bone marrow/stem cell transplants and people with severe immune suppression such as advanced human immunodeficiency virus (HIV) infection. The HCMV has several mechanisms of immune system evasion. It interferes with the initiation of adaptive immune responses, as well as prevent CD8+ and CD4+ T cell recognition interfering with the normal cellular MHC Class I and MHC Class II processing and presentation pathways. Challenges in developing a vaccine include adeptness of CMV in evading the immune system. Though several vaccine candidates are under investigation.

Keywords: human cytomegalovirus, Epstein-Barr virus, mononucleosis, transplantation, immune evasion, oncogenesis

1. Introduction

1.1 The Herpesviridae family

Herpesviridae is a large family of double-stranded DNA viruses, which is included in the recently classified order Herpesvirales. This family can be further classified into three distinct subfamilies: *Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae*, according to their biology and DNA genomic sequence [1]. The *Alphaherpesvirinae* subfamily includes five distinct genera, *Simplexvirus* and *Varicellovirus* are most important members causes infection to human. The members of Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) infect almost 85% of the world human population and cause orolabial.herpes and genital herpes; other members of the family include varicella-zoster virus (VZV), which is responsible for chicken-pox and shingles. The *Betaherpesvirinae* subfamily includes four genera, the most important members that infect the human are Human *cytomegalovirus* also known as human herpesvirus 5 (HCMV or HHV-5) and Human herpesviruses 6 and 7 (HHV-6 and HHV-7) [2].

The *Gammaherpesvirinae* subfamily is composed of four distinct genera, *Lymphocryptovirus* (LCV) and *Rhadinovirus* (RDV)infect the human, Taxonomically, the oncogenic Epstein–Barr virus (EBV) is also designated as human herpesvirus 4 (HHV-4) belongs to the genus lymphocryptovirus (LCV) and it is the only human pathogen of this genus. The RDV Kaposi's sarcoma-associated virus (KSHV, also known as HHV-8), another oncogenic herpesvirus, is the only known human RDV [3]. The *Gammaherpesviruses* may promote oncogenic effects and also contribute to the development of malignancies but this is a rare outcome [2]. Altogether herpesviruses can establish latent infection within specific tissues, with immune surveillance evasion. The human herpesviruses and their diseases are summarized in (**Table 1**).

1.2 Epstein–Barr Virus (EBV)

EBV is ubiquitous virus, with a seroprevalence of more than 90% of the adult population worldwide. It was first identified in 1964 by Anthony Epstein's group in a cell line from a Burkitt's lymphoma biopsy [4, 5]. The EBV has also been identified as a B lymphotropic oncogenic virus owing to its capacity to convert resting B lymphocytes in vitro, inducing continuous dissemination of infected B cells and producing lymphoblastic cell lines (LCLs) [6]. This discovery was central to the identification of EBV as the first nominee human tumor virus. Subsequently,

Subfamily	Genus	Species	Disease
Alphaherpesvirinae	*Simplexvirus*	*Human alphaherpesvirus* 1 (Herpes simplex virus 1) *Human alphaherpesvirus* 2 (Herpes simplex virus 2)	Acute Herpetic gingivostomatitis, Keratitis, Conjunctivitis, Encephalitis, Dermal whitlow, Herpes labialis Herpes genitalis
	Varicellovirus	*Human alphaherpesvirus* 3 (*Varicella Zoster* virus)	Chickenpox/ shingles
Betaherpesvirinae	*Cytomegalovirus*	*Human betaherpesvirus* 5 (Human cytomegalovirus)	Congenital abnormalities
	Roseolovirus	*Human betaherpesvirus* 7 (Human herpesvirus 7)	Febrile illnesses
		Human betaherpesvirus 6A, 6B (Human herpesvirus 6A, 6B)	Infant rash Exanthem subitum
Gammaherpesvirinae	*Lymphocryptovirus*	*Human gammaherpesvirus* 4 (Epstein–Barr Virus)	Infectious mononucleosis (Glandular fever), Burkitt's lymphoma, Hodgkin's lymphoma, Nasopharyngeal carcinoma, Oral hairy leukoplakia
	Rhadinovirus	*Human gammaherpesvirus* 8 (Kaposi's sarcoma-associated virus or Human herpesvirus 8)	Kaposi's sarcoma

Table 1.
Taxonomy of Human Herpesviruses [3].

EBV was correlated with a variety of clinical malignancies, including Hodgkin's Lymphoma (HL), post-transplant lymphoproliferative disease (PTLD) and X-linked lymphoproliferative disease (XLPD). The potential to invade other cell types other than B lymphocyte, such as T, natural killer (NK) and epithelial cells, has led to the association of EBV with other malignancies: peripheral T cell, nasal T or NK cell lymphomas, gastric and nasopharyngeal carcinomas (NPC) [2, 7]. However, infection with EBV induces contagious mononucleosis during or after adolescence [8]. Even though EBV exhibits a strong growth transforming capacity, that asymptomatically infects up to 95% of the human population, whereas it is perfectly immune-controlled [2].

1.2.1 The EBV Virion and Genome organization

The virus is 122–180 nm in diameter. Epstein–Barr virion contains a linear, double-stranded DNA genome wrapped on an icosahedral capsid, approximately (100–110) nm in diameter, containing 162 capsomeres with a pore running down the long axis. The protein tegument with viral and cellular proteins including actin, tubulin, and cofilin separates the nucleocapsid from the lipid envelope that coats the virus and contains numerous viral glycoproteins (GP) spikes such as gp350/220, gp42, GH, GB and gp150 on the outer surface. These glycoproteins play an important role in cell tropism and recognition of receptors [8, 9] as shown in the **Figure 1**.

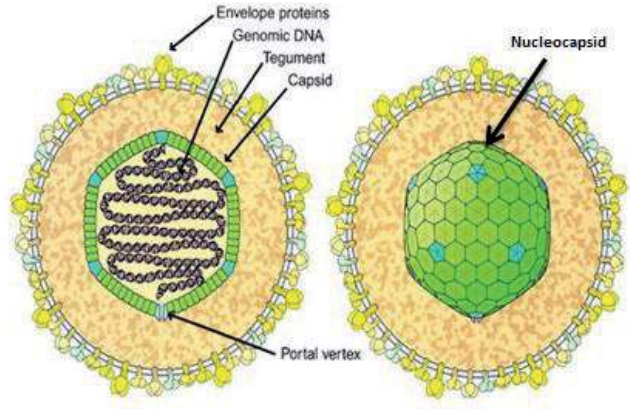

Figure 1.
The structure of EBV viral particle.

The double-stranded DNA (172Kb) linear genome encodes more than 100 proteins as well as non-coding functional RNAs (EBER RNAs, BART miRNAs, and BHRF1 miRNAs). There are some similar tandem terminal repeats (TR) of 0.5 kb at each terminal of the genome [10] and other internal direct repeats of 3 kb (IR) including the latency promoter (Wp) and the special short unique sequence domains (US)and UL (long). The US and UL sequences comprise nearly all of the genome encoding capacity [11] as shown in **Figure 2**. The EBV genome is classified as C genome, which is linear in a virus particle, but distributed as an episome in the nucleus of infected cells; circulating occurs by terminal repeat units (TRs) following B cell infection with EBV [11]. The first cloned and sequenced EBV strain was typing 1 EBV: B95.8, this strain was obtained from an infectious mononucleosis patient's. Sequencing was based on previously generated EcoRI and BamHI restriction fragments (**Figure 3**). B95.8 strain is commonly used in labs around the world; however, a 13.6 kb portion of its genome is incomplete. Subsequently, the missing fragment was sequenced from the Raji strain and a revised EBV consensus genome was released several years later [12].

1.2.2 The EBV classification

Two major types of EBV, type 1 and 2, have been described in humans based on major variations in EBNA-2 gene sequence [11]. Type 1 is dominant throughout most of the world, but the two types are equally prevalent in Africa. The EBNA-2 is the most variable locus in the EBV genome which is characterized by 70% identity at the level of nucleotide sequence whereas only 56% similarity at the amino acid level between these two types (3). In addition, the variation between type 1 and type 2 is also linked to the sequence variation in the viral latent genes EBNA-3A, EBNA-3B, EBNA-3C and EBNA-LP [13].

1.2.3 Epstein Barr virus life cycle

1.2.3.1 Cell attachment and viral entry

The initial attachment of EBV is mainly regulated by the association between its envelope protein (gp350/220) and the cellular complement component receptor 2 (CR2/CD21) protein located on the B cell surface. This association activates Cluster of differentiation (CD21) receptor aggregation in the plasma membrane and also a tyrosine kinase signal transduction through CD19 that contributes to Nuclear factor-kappa B (NF- kB) activation and cell cycle entry [14].

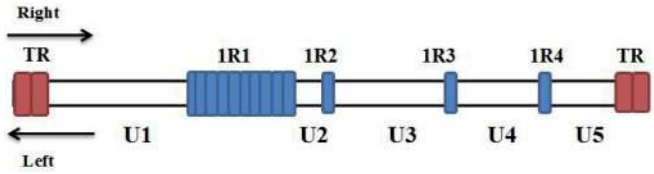

Figure 2.
Linear Organization of the EBV genome.

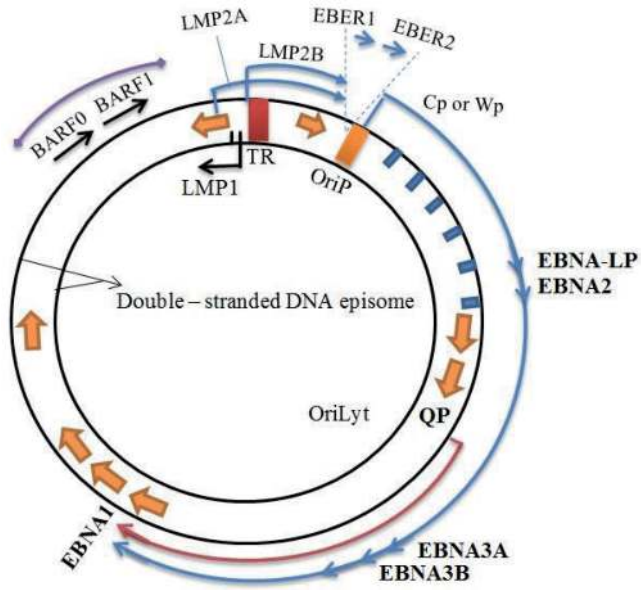

Figure 3.
Circular Organization of the EBV genome.

The attachment of the second viral glycoprotein gp42 to the human leukocyte antigen class II receptor (HLA class II) activates the viral envelope fusion with the membrane of the cell and the viral entrance in a cycle that relies on the glycoprotein complex GH/GL and also on GB [15]. The GH/GL complex is supposed to serve as a receptor that activates GB-mediated fusion after gp42 binding to HLA Class II molecules. Thereafter, virion nucleocapsids are released into the cytoplasm and transported to nuclear pores on microtubules [13]. As a result, the viral linear genome is transferred to the nucleus of B lymphocyte and the viral genome is then retained in the nucleus as a covalently locked extrachromosomal episome [16].

For epithelial cells, as there are no CD21 or HLA class II molecules on their surface, the entrance of EBV does not involve gp350/220 and gp42. Viral BMRF2 protein can mediates interaction with cellular β1 integrins [14]. The fusion of viral envelope is activated by the attachment of the viral gH/gL complex to 5–007vβ6/8 integrins, which is confirmed by the effectiveness of infection in virions missing gp350/220 glycoproteins. The EBV virion expresses three- gH / gL/ gp42 and two- gH/gL glycoprotein complexes that grant the capacity to invade either B cells or epithelial cells [13].

The virus is endocytosed into a low pH vesicle where fusion occurs after the interplay of EBV glycoprotein gp350 and receptor type 2 (CR2). Glycoprotein gp42 is bound directly to GH and transforms dimeric GHGL in a trimeric gHgLgp42, modifying the conformation of gp42 to cause its attachment to the human leukocyte antigen (HLA) class II molecule. It will allow the central fusion machine to support effective B cell infection. Besides, GH can bind cellular components [15].

The epithelial cells do not constitutively express HLA class II, which makes gp42 useless in the process of fusion. The interaction of dimeric GHGL complexes with integrins, however, replaces the cell fusion caused by the interaction between gp42 and HLA class II. The use of dimeric GHGL complexes to cause epithelial cell fusion and gHgLgp42 trimeric complexes to contribute to B cell fusion was expected that the virus would trigger B cells and epithelial cells to alter the viral tropism: The gp42 spike in epithelial viral particles makes it 100 times more infectious than the virus produced from B-cells. The opposite is not so dramatic: the B-virus is five times more contagious for the epithelial cell than the epithelial virus [17]. After binding to the primary B cell, most virions do not internalize with the epithelial cell and the infection can be significantly increased by co-culturing with EBV negative B cells. Such virions stay on the surface of the cell B and can then be passed via the formation of the intracellular synapse to CR2-negative epithelial cells. This transfer technique involves the interaction between gp350-CR2 and GH and GB viral glycoproteins. This mechanism has been suggested to allow EBV to enter both lymphoid and epithelial cells simultaneously [18].

1.2.3.2 EBV Lytic Infection

The lytic infection is characterized by the active release of new contagious virus particles, either infecting new human hosts or infecting other naive B cells in the same host. The lytic cycle is divided into three stages: Immediate-early (IE), Early (E), and Late (L). The expression of immediate early BZLF1 and BRLF1 genes included in the activation of the lytic process is activated by signal transduction by the B cell receptor (BCR) [15]. The BZLF1 is a viral transactivator protein responsible for activating the production of lytic genes and the repression of latent genes, resulting in cells' death and the release of contagious virons. The signal transduction of BCR initiates BRLF1 development and also improves its production allowing the transition from latency to lytic cycle [14]. The BZLF1 protein is a bZIP-specific transcription factor close to c-FOS and C/EBP. The BZFL1 and BRLF1 motivate functions of early genes, such as viral DNA polymerase (BALF5) and thymidine kinase, to initialize viral DNA replication from the lytic origin of replication (OriLyt) in tandem with other direct and early gene products [13]. Late lytic genes encode viral structural proteins, including tegument proteins, glycoproteins, and BcLF1 main capsid proteins. Newly synthesized viral DNAs are packed into nucleocapsids in the nucleus of the cell, which moves across the nuclear membrane to the cytoplasm, creating vesicles carrying virons with an envelope. The vesicles fuse with the plasma cell membrane and the virus particles exocytose [19].

1.2.3.3 Latency

Herpesviruses are distinguished by their ability to establish and sustain a latent infection in their hosts. Latent EBV expresses its genes in one of three latency systems: Latency I, II, or III variations in either of these systems assist in the development of a distinct series of viral RNAs and proteins [20] **Table 2** and **Figure 4**. This chronic infection is characterized by inhibition of viral replication and viral dormancy, and immune evasion in the host. The EBV determines latency in the B cell pool which is the long-term reservoir for the virus in vivo. Naive B cells infected with EBV in the Waldeyer ring proliferate as activated B bursts, which are close to antigen-activated B lymphocytes in terms of the structure and morphology of their cell surface [24].

Opposite to the lytic infection, replication of the viral genome in latent infection occurs through host DNA polymerase and from a separate source, Orig of

EBV latent protein	Function related to B-cell lymphomagenesis
EBNA1	Regulation of viral DNA replication and transcription of many viral and cellular genes; It facilitates p53 disintegration and thus promotes tumorigenesis
EBNA2	One of the main viral transcription factors; In combination with EBNALP, EBNA2 regulates the transcription of many of viral and cellular genes; Fundamental for B cell transformation
EBNALP	EBNA2-mediated transcription activator, both for viral and cellular genes; Bypassing the innate immune response of cells; Fundamental for B cell transformation
EBNA3A	Together with EBNA3C, it suppresses the genetic transcription of BIM, p14, p15, p16 and p18 through epigenetic regulation; Prevents differentiation of B cells into plasma; Essential for B cell transformation
EBAN3B	Virus-encoded tumor suppressor protein
EBNA3C	Together with EBNA3A, it suppresses the genetic transcription of BIM, p14, p15, p16 and p18 through epigenetic regulation; Assists G1-S and G2-M transformations of the cell cycle; Ubiquitin-proteasome pathway; Supresses p53-, E3F1- and Bim-mediated apoptosis; Activates autophagy necessary for B cell transformation
LMP1	Functionally mimics CD40 signaling pathway; one of the major transcriptional regulators; Mainly activates NF-kB, JAK/STAT, ERK MAPK, IRF and Wn't signaling pathways; Induces BCL-2 and a20 expression to prevent apoptosis; Essential for B cell transformation
LMP2A	Functionally mimics BCR signaling pathway; prevents apoptosis; EBV latency regulation
LMP2B	Regulates LMP2A functions
EBERs	Most of the non-coding viral RNA is found in all forms of latency programs; Affects the innate immune response and gene expression; Inhibits apoptosis dependent on activated dsRNA dependent protein kinase (PKR)
miRNAs	Transcribed from BART and BHRF1 loci; maintains latently infected B cells through blocking cellular apoptosis

Table 2.
Impact of latent antigens in EBV on B-cell transformation and subsequent development of lymphoma [21–23].

replication. During latent infection, the viral genome is present as a closed circular, extrachromosomal plasmid or episome. The viral DNA is wrapped with host histone molecules and replicates steadily once throughout the cell cycle together with the host genome [25], this enabled EBV infected B blasts during proliferation to express all latent EBV genes which are known as latency III or growth-program that play important role in cell activation and proliferation.

This is achieved by the expression of two viral latent membrane proteins (LMPs), LMP-1, and LMP-2A, which constitute a functional homolog of the CD40 receptor in B lymphocytes and often mimic the constitutively active BCR, respectively [26]. B cell migrates to nearby primary follicles to form germ centers and the viral transcription system switches to latency II or a default system to enable the B cells to differentiate into memory B cells, Latency II is characterized by the expression of LMPs and EBNA-1 protein.. In the absence of antigen-mediated signals, LMPs are necessary to provide cell survival signals needed to prevent apoptosis of latently infected B cells [26].

Epstein Barr-virus nuclear antigen-1(EBNA-1) protein is important for EBV DNA replication and for preservation of viral genome in the cells [20]. The memory B cells lately infected reach peripheral circulation and represent viral persistence reservoir; [27]. Such latently infected memory B cells with EBV are distinguished by a silence of the expression of viral protein in a program called latency 0 or latency-program which is intended to permit immune evasion and therefore lifelong

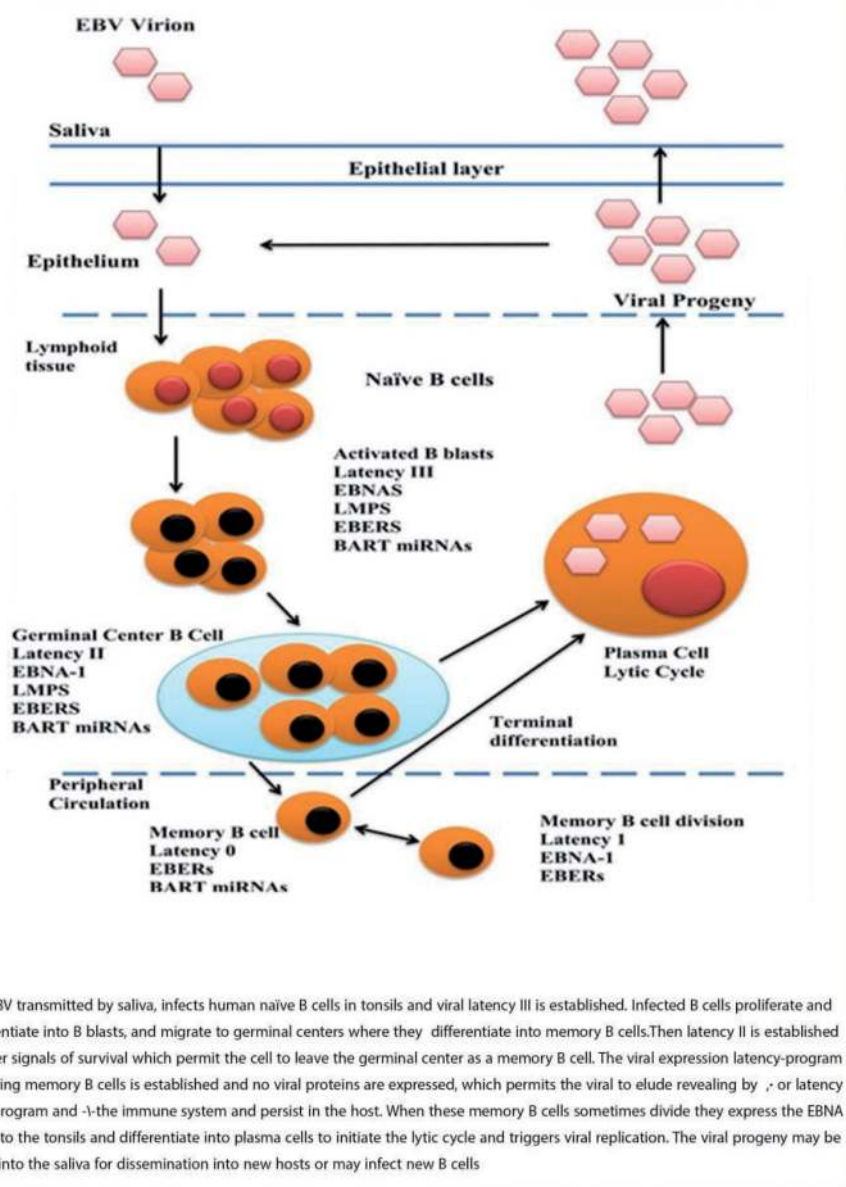

Figure 4.
Model for the establishment of EBV persistent latent infection.

persistence on the host. The expression EBNA-1 is enabled and allows the division of the viral genome in the cells carrying the virus. This is known as the transcription program Latency I or EBNA-1 only program [26]. From peripheral circulation, latently infected memory B cells migrate into oropharynx and tonsils and then differentiate into plasma antibody-producing cells. Reactivation of the virus is triggered and infectious viruses are created as they bear the virus. Therefore, these viral particles will infect additional hosts with new naive B cells [27].

1.2.4 Transmission

The oral route is the primary route of the EBV transmission commonly through bodily fluids, especially saliva [28]. However, it has been reported that EBV infection can also be transmitted after the transfusion of a large volume of fresh blood [29]. Although EBV has been detected in cervical secretions of 8% -28%, of

women, it is still controversy on whether EBV is transmitted through sexual contact [30]. Possible spread via organ transplantation can occur which is of particular concern in association with subsequent infection by EBV [25]. Transmission by milk is also a possible route, but is a non-significant mode of EBV transmission [31].

1.2.5 EBV epidemiology

Epstein–Barr virus types occur worldwide, but they differ in their geographic distribution. For instance, Type 1 is prevalent in population from Europe, America, China, and South Asia, while Type 2 is less prevalent in these populations and is more observed in African and Papua New Guinean populations. Over 90–100% of adults have been infected with EBV, and the infection is most commonly affecting those patients aged 2 to 4 years and those aged 15 years. Epstein–Barr virus causes approximately 90% of the cases of infectious mononucleosis, which is commonly seen in both the community and the hospital setting. Among infants and young children who are primarily infected with EBV, in Africa, and where Burkitt lymphoma is common, 50% of them are infected with this virus before their 1 year of age. About 70% of cases of PTLD are associated with Epstein–Barr virus (EBV), especially in cases that occur early after transplantation [32]. Recent studies from Kenya reported a striking overlap between increased incidence of malaria transmission and Burkitt lymphoma [33]. Furthermore, various studies have demonstrated the presence of 8: 14 translocation in both the endemic African Burkitt lymphoma and in the non-endemic tumor type (Europe, America, and Japan).

1.2.6 EBV clinical features

The EBV is associated with many malignant diseases such as lymphomas, carcinomas, and also more benign such as infectious mononucleosis, chronic active infection. The EBV has also been suggested as a trigger/cofactor for some autoimmune diseases. Overall, 1–1.5% of the cancer burden worldwide is estimated to be attributable to EBV [3].

1.2.6.1 Primary EBV infection

The primary EBV infections of infants and children are often asymptomatic or have nonspecific symptoms, but infections of adolescents and adults frequently result in infectious mononucleosis (IM). Around 80% of infected adults mostly experience symptoms, including sore throat, cervical lymphadenopathy, weakness, upper respiratory infection, headache, reduced appetite, fever, and myalgia (muscle aches). It is characterized by a large number of lymphocytes, mainly CD8 + T-cells, which, as opposed to healthy individuals, can reach five to ten times more numbers in the blood. The causes of this expansion of T-cells in IM are not clear, but factors such as failure of natural immune control by natural killer (NK) cell, memory CD8+ T cells of memory of EBV or genetic background have been suggested [34].

The severity of symptoms in primary EBV infection is associated with age and immune system of the patients. The complications of the disease include splenomegaly, and/or chronic hepatitis, pneumonia and lymphadenitis. Less common are complications, such as hemolytic/aplastic anemia, myocarditis, Guillain–Barré syndrome, encephalitis, and meningitis [35].

Chronic active EBV infection is a rare disorder characterized by the presence of severe illness of more than six months' duration, high virus-specific antibody titers and organ disease with the demonstration of EBV antigens or EBV DNA in tissue [35].

1.2.6.2 EBV reactivation and EBV associated diseases

The reactivation of latent EBV infection has been shown to occur following impairment of the cellular immune response which is important in the long-term control of persistent EBV infection. Chronic uncontrolled EBV reactivation may result in the development of carcinoma. The followings are diseases and cancers associated with EBV infection [2, 36–39]:

1. X-linked Lymphoproliferative Syndrome-XLP (Duncan's disease). It is an inherited disease of males, characterized by an uncontrolled increase in the number of cytotoxic T and NK lymphocytes where their activity is directed against normal cells of various organs. Subsequent studies showed that the disease has a variety of phenotypes after primary EBV infection, such as fatal infectious mononucleosis, hypogammaglobulinemia, and malignant lymphoma.

2. Post-transplant Lymphoproliferative Disorders (PTLD). They are a heterogeneous group of diseases characterized by uncontrolled proliferation of B cells (90%), T cells (9%), and NK cells (0.5%). 90% of PTLD lymphomas are EBV positive and they are typically limited to the lymph nodes. PTDL often occurs as a complication of both solid organ transplant (SOT) and hematologic stem cell transplant (HSCT) patients.

3. Leukoplakia Hairy Mouth. It is a benign, mild proliferative change in epithelial cells of the tongue and the nasopharynx. It is caused by EBV and occur usually in persons who are immunocompromised, most frequently seen in those infected with HIV.

4. Burkitt's Lymphoma (BL). It is a rapidly growing malignant tumor composed of large D-type lymphoblasts and mainly affects facial bones, mandibles, and jaws. EBV-associated BL has an incidence of 5–10/100 000 children and accounts for up to 74% of childhood malignancies in the African equatorial belt.

5. Hodgkin's Lymphoma (HL). It is a proliferative disease of the lymphatic system with a peak incidence in those between 20–30 years and after 50 years of their age, the risk of developing HL is four times higher in those infected with EBV as compared to the general population.

6. Nasopharyngeal Carcinomas (NPC). It is the most common cancer originating in the nasopharynx, most commonly in the postero-lateral nasopharynx or pharyngeal recess which can extend to the base of the skull, palate, nasal cavity, or oropharynx [40]. Most NPC is associated with EBV infection, mainly in middle-aged patients and is more common in men than women [41, 42]. The International Agency for Research on Cancer (IARC) classified the EBV virus into the first group of carcinogens due to this association [43].

7. Sinonasal Carcinoma.

About 90% of head and neck cancers are squamous cell carcinoma (SCC), they originate from the mucosal lining that, causes tumor development in the nasal cavity and mouth, nasopharynx, larynx, esophagus and paranasal sinuses [44]. The International Agency for Research on Cancer (IARC) estimated that 16% of total

new cancers, as well as 20% of deaths caused by cancers worldwide, were due to infections with EBV [2, 7]. Sinonasal carcinoma is a rare tumor comprised of about 1% of all cancers and 3% of all head and neck cancers [44, 45].

1.2.6.3 C. Autoimmune diseases.

EBV has variously been linked with a number of autoimmune diseases including multiple sclerosis (MS), systemic lupus erythematosus (SLE), Sjögren's syndrome (SS) and rheumatoid arthritis (RA). The EBV links with these diseases include raised titers of EBV antibodies, decreased T cell response to EBV and elevated EBV viral load. It has been suggested that EBV triggers the activation state of the immune system by inducing the development of pro-inflammatory mediators, which may play a role in autoimmune pathogenesis [46].

1.2.7 EBV oncogenesis and association of latency type

Oncogenesis is a cytological, genetic, and cellular transformation process that results in malignant tumors. Discovery of viral oncogenes and the discovery that they are derived from cellular genes called protooncogenes led to the understanding that c-onc genes have roles in different tumor types. The activation of viral oncogenes requires genetic changes in cellular protooncogenesby 3 genetic mechanisms: (a) Mutation (b) Gene amplification (c) Chromosome rearrangements. These mechanisms result in either an increase in protooncogene expression or a change in protooncogene structure [47]. The EBV-mediated B-cell change is associated with a global improvement in viral and cellular expression of genes. The biologic characteristics of the virus were instantly fascinating, as it was shown that cell lines could be determined from samples of Burkitt's lymphoma (BL) and could propagate a virus that could strike primary B cells with EBV and turn them into immortalized cell lines [48]. This study of molecular phenotype led to the discovery of viral proteins that are necessary for latent infection and needed for cell transformation [49]. The mechanisms of EBV oncogenesis include:

1.2.7.1 Alteration of host cellular signaling pathways

EBV encoding oncogenes induce the changes in the host cellular signaling pathways that control proliferation, differentiation, cell death, genomic integrity, and recognition by the immune system.

LMP1, LMP2A, and LMP2B, latent membrane proteins are generated of the common viral locus with converging and interfering primary transcripts [50]. The LMP1 is one of the main EBV-encoded oncoproteins and it is a constitutively active mimic of cellular CD40 receptor. It is critically important for the EBV-induced B-cell transformation via the activation of NF-κB, c-Jun N-terminal kinase (JNK), and p38 cascades [21]. LMP1 also regulates cellular apoptosis by triggering the NF-κB pathway by increasing the antiapoptotic expression of Bcl2 via IRAK1 and TRAF6 whereIRAK1 is necessary for both p38 and p65/RelA phosphorylation [50]. Also, LMP1-stimulated proapoptotic polycomb complex protein (Bmi-1) is further been recruiting by EBNA3C for the transcriptional funnel of other genes. LMP2A acts as a functional homolog of the B-cell receptor (BCR) and thus promotes the survival of B-cells. Likewise, it is essential for the growth transformation of germinal center-derived B-cells which are BCR negative [21]. LMP2B negatively regulates LMP2A functions and transition from latent to lytic activation by depleting LMP2A-mediated BCR cross-linking and restoring Ca2+ mobilization [51].

EBNA1 is important for the DNA replication and maintenance of the viral latent genome. It binds to the viral episomal replication origin (OriP) and simultaneously to the host cell hromosomes that enable viral genome duplication during each cell cycle [22]. Through promotor selection, combined with comprehensive epigenetic control, EBNA1 can organize the shift between different latency programs, and EBNA1 can produce transcripts for different cells and help improve the control of telomeres on cell chromosomes [52]. The p53 and Mdm2 affected by the EBNA1 binding with ubiquitin-specified protease 7 (USP7), contributes to antiapoptotic activity control, likely by promoting survivin expression levels [23, 53].

EBNA2 and EBNALPare the first latent genes expressed following B-cell infection. EBNA2 is the main viral transcription factor responsible for activation of the expression of the entire repertoire of latent transcripts along with several host genes, utilizing cell transcription factors, RBP-J and EBF1 [22]. At the same time, EBNALP supports transcriptional regulation by EBNA2 via blocking off the activity of NCoR and RBP-J [52]. The EBNA2 contributes most strongly to the proliferation of B-cells through the activation of about 300 cell genes, such as the transcription of MYC and RUNX3 [52].

EBNA3 protein family consisting of EBNA3A, -3B, and -3C are transcription factors that precisely regulate host gene transcription and the proliferation of B-cells, particularly in the immunosuppression environment. Also, EBNA3B knockout virus-induced tumors demonstrated a lack of T-cell infiltrate and related CXCL10 chemokine activation [53]. In comparison, EBNA3A and EBNA3C cooperate as predominant viral oncoproteins by controlling the transcription of the cellular gene. This phenomenon is also true for EBNA3A [54].

The EBNA3A and EBNA3C have been demonstrated to react with a long list of cellular proteins and transcription factors involved in the regulation of multiple cell signaling pathways [55]. Interactive partners for EBNA3C involve transcription factors, chromatin modulators (both histone deacetylase and histone acetylase enzymes), cell-cycle proteins including G1-S and G2-M transitions, metastases suppressors, post-translational modifiers, E3-ubiquitin ligase, ubiquitin-specific proteases, unfolded protein response (UPR) regulators, cell tumor suppressors, and oncoproteins [56]. The EBNA3C has been shown to form a complex with Chk2 and thus manipulates the G2/M step of the cell cycle [54]. Overall, the B-cell transformation and B-cell lymphoma are directly affected by the EBNA3 protectors by targeting main cell signaling cascades including cell cycle, apoptosis, and autophagy [56].

- Noncoding viral transcripts.

A variety of noncoding RNAs (ncRNAs) in EBV infected B cells can be expressed, known as the EBV-encoded non-polyadenylated RNAs (EBER1 and EBER2) and numerous miRNAs [57]. Such ncRNAs are not necessary for the transformation of B-cells, but they are associated with immune evasion, and demonstrated in various forms of latency systems. In addition, EBER in situ hybridization is the most reliable and sensitive method to detect EBV infection in tissues of various EBV-related malignancies. EBER expression promotes the growth of B cells by blocking of PKR phosphorylation and inhibition of translational initiation factor eIF-2a and alpha-interferon (IFN-α)-induced apoptosis [56]. EBER can interact with ribosomal protein L22 that regulates protein translation, gene expression and PKR dependent apoptosis [58]. The EBER2 directly recruits PAX5 for the control of LMP2A expression, which has been verified by the usage of the EBER2 mutant virus with lower LMP2A expression [58]. EBV encodes more than 40 mature miRNAs, which are encoded at 2 different loci in the EBV genome:

BHRF1 locus (BHRF1 miRNAs) and BART locus (BART miRNAs). The expression of various EBV miRNA is different among different cells. Viral miRNAs can either target other EBV transcripts or transcripts of host cells. BHRF1 miRNAs exhibit expression that is restricted to latency 3 whereas the BART miRNAs are expressed in all latency types. The expression of BHRF1 miRNAs in infected B lymphocytes, target multiple tumor suppressor proteins such as PTEN and P27KIP1 for the B-cell transformation. Viral miRNAs also inhibit the expression of several tumor suppressor genes, including DICE1, PUMA, PTEN and BCL2L11 to promote the survival of an epithelial cell [59].

Table 2 and **Figure 5** explain the key latent transcription mechanisms of EBV.

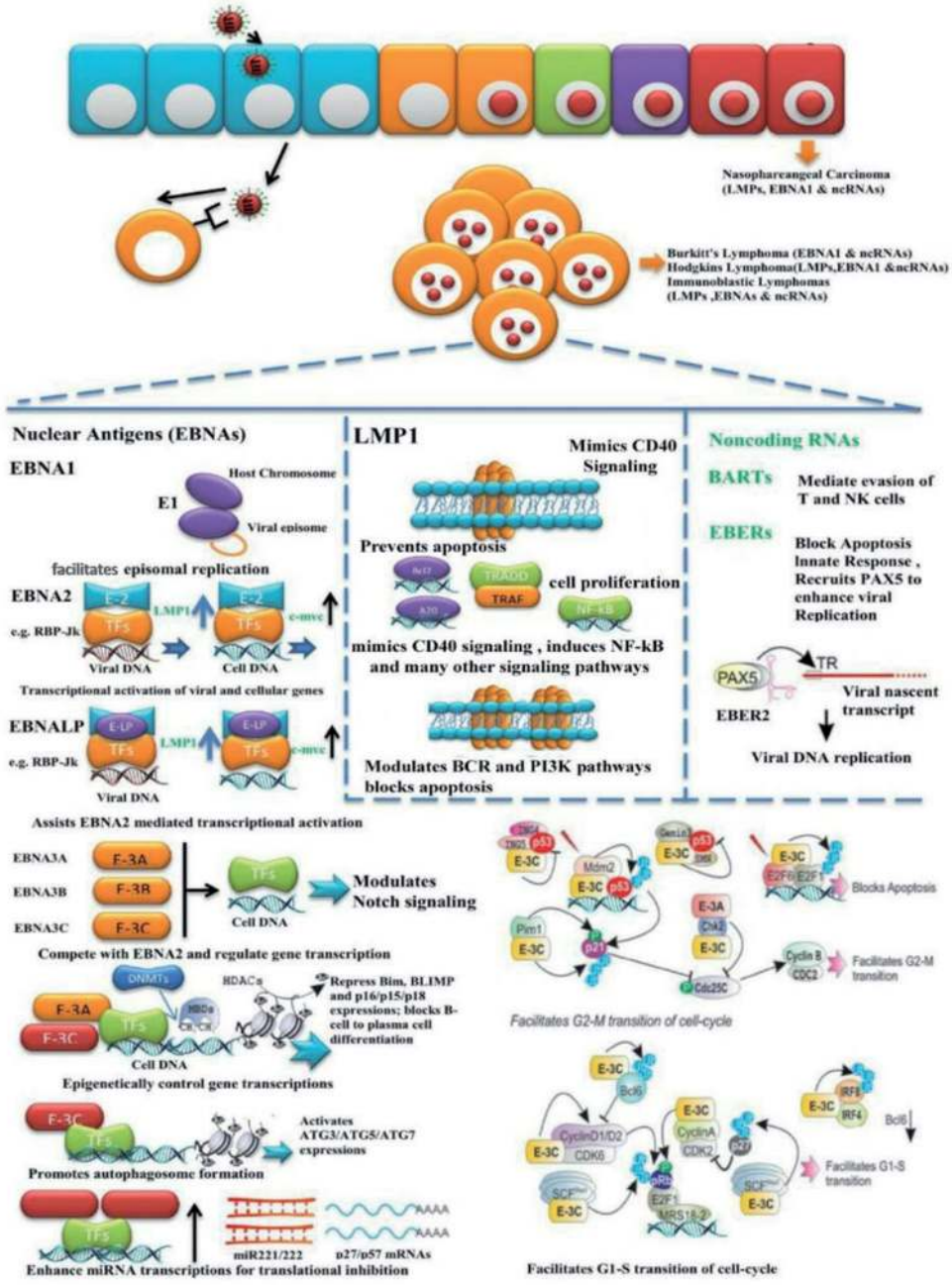

Figure 5.
Special features of EBV latent transcripts during B-cell transformation associated in the development of B-cell lymphoma.

1.2.7.2 Suppression, escape, and modulation of host immunity

Multiple genes have been reported to suppress antigen presentation. EBNA1 contains a Gly/Ala repeat sequence, through which proteasomal degradation and antigen presentation of the protein is impaired, while BNLF2A targets the transporter associated with antigen processing and blocks antigen presentation. BGLF5 represses HLA class I synthesis, whereas BILF1 downregulates cell surface expression of the molecule. It is highly likely that at least LMP1 and LMP2A, the viral functional mimic of CD40 and BCR, have tactfully evolved to modify those processes in the germinal center, and thus, these EBV gene products can deregulate the immune system for survival [60]. See section 1.1.8.

1.2.7.3 Genetic or epigenetic background/alteration of the host genome

Virally-induced epigenetic alterations of the host genome are evident in EBV-associated cancers, which are the result of genetic mutations, changes involving DNA methylation and chromatin structure that in turn alter the expression of growth promoting or suppressing genes. Enhanced Ig-Myc translocation has been demonstrated in Burkitt lymphoma [34, 35] and silencing of tumor suppressor genes (e.g. p16INK4A) in many EBV-positive cancers. Several reports have demonstrated that the predisposition of individual HLA allele significantly affects the morbidity of EBV-positive proliferative disorders, particularly in NPC and Hodgkin lymphoma [37–42]. LMP1 seems to induce genetic/epigenetic alterations by DNA hypermethylation and chromatin modifications [51].

1.2.8 Immune evasion mechanisms by Epstein–Barr virus

In response to primary EBV infection, both innate and adaptive antiviral responses have been activated. Despite the very effective immune response, the virus is not cleared.. A lifelong, latent infection is established within the memory B-cell and EBV genomes are propagated during the division of the transformed, latently infected B cells. During this period, broad range of EBV early gene products interfere with immune response which helps the virus to persist and to reactivate [60].

1.2.8.1 Evasion of innate immunity

Various pattern recognition receptors (PRRs) including cell surfaces and endosomal Toll- receptors (TLRs) and cytoplasmic DNA and RNA sensors are capable of detecting EBV particles. The EBV is identified by Toll-like receptors (TLRs) on the cell surface and in endosomes [61]. Virus-derived or virus-induced components can be detected by RNA and DNA sensors as well as by inflammasomes within the cytosol. TLRs, RNA and DNA sensors trigger a cascade of intracellular signaling events that enable the activation of the interferon regulatory factors (IRFs) and NF-κB. As a result, activated gene transcriptions induce the production of cytokines and type I interferon (IFN I). Different levels of these PRR signals are attacked by latent and/or lytic EBV proteins or EBV miRNA as recently seen [62, 63].

1.2.8.1.1 Reduction of Toll-like receptor expression

EBV can inhibit the synthesis of cellular proteins in infected cells through global mRNA destabilization. This mechanism is via the EBV DNase (alkaline

exonuclease) BGLF5 regulated by the supplementary BGLF 5 RNase feature, which is expressed during the active period of infection and uses the same catalytic position as DNase action, but the substrate-bindings site is only partly shared by DNA and RNA substrates [62].

1.2.8.1.2 Modulation of IRF signaling and Type I interferon production

A significant number of lytic EBV proteins interact with host IRFs, which are the transcription factors that stimulate the synthesis of type IFN. The immediate-early EBV transactivator BZLF1, BRLF1 and tegument protein LF2 interact with IRF7 andIRF3, and inhibit its transcriptional activity on IFNα4 and IFNβ promoters resulting in the suppression of antiviral state induction. In addition, EBV protein kinase BGLF4 phosphorylates and inhibits IRF3 transcriptional activity, thus decreasing IFNβ expression [64].

1.2.8.1.3 Interference with NF-κB and inflammatory pathways

The EBV infection is linked to the decreased NF-κB--dependent gene expression. The expression of viral BZLF1 and cellular NF-κB is reciprocally inhibited. The higher levels of the NF-κB in the absence of BZLF1 instigate EBV latency, while increased expression of the BZLF1 after the induction of the lytic cycle overwhelms the minimal amount of the NF-κB [65].

1.2.8.1.4 Interference with innate effector molecules

Several EBV gene products have an impact on the function of effector molecules of innate immunity. The host cytokine colony-stimulating factor 1 (CSF-1) activates the differentiation of the macrophage, and the secretion of the IFN-α, EBV encodes the soluble form of the CSF-1 receptor BARF1, which neutralizes the effects of the host CSF, leading to the reduction of the IFN-secretion of EBV infected mononuclear cells. EBV BZLF1 counteracts intrinsic effector molecules in a variety of ways [62]. First, BZLF1 decreases TNF5–007 and IFNΔ receptors to minimize cellular susceptibility to these cytokines; second, BZLF1 induces SOCS3-signaling cytokine suppressor, which inhibits JAK/STAT signaling and thus promotes IFN-responsiveness Type I state; Third, BZLF1 triggers TGFβ immunosuppressive cytokine expression and disrupts the development of Promyelocytic leukemia bodies (PML-bodies) that may have antiviral activity [66].

1.2.8.2 Evasion of adaptive immunity

The EBV compromises the activation of both CD8 + and CD4 + T cells by interfering in different stages of HLA Class I and Class II antigen presentation pathways, especially during the productive phase of infection [67].

1.2.8.2.1 Evasion of CD8+ T Lymphocytes

The EBV encodes at least three proteins that independently interfere with antigen presentation through deregulation of the surface expression of HLA I in many ways to prevent EBV-specific (memory) T cell recognition [68]. BGLF5 induces degradation of HLA I-encoding mRNA and reduces the presence of peptide at the cell surface which inhibits T-cell recognition. Ut has been suggested that BNLF2adeplete peptides from the ER (HLA I loading compartment) and inhibits the importation of peptides by the antigen- transporter (TAP). BILF1, encoding a

constitutively active G protein-coupled receptors (GPCR) which reduce the transportation of HLA I from the trans-Golgi network. In addition, cell surface turnover is increased and subsequently i degradation by lysosomal proteases. These proteins are expressed during the replicative process of EBV and function in tandem with the prevention of CD8 + T cells being recognized [67].

1.2.8.2.2 Evasion of CD4+ T Lymphocytes

The EBV has adopted several strategies for immune evasion that interfere with CD4 + T-cell immunity. The EBV receptor Gp42 can bind to the B-cell HLA class II molecules. The HLA Class II/peptide complex relationship blocks T-cell receptor (TCR)—class II interactions and prevents CD4 + T cell activation. Besides, protein GP42/gH/gLdecreases the HLA II cell surface expression by the HLA II mRNA degradation. Inhibiting the activities of CIITA promoters and, as a result, lowering the HLA II surface levels, EBV also encodes a viral IL-10 homolog (BCRF1) that has been identified as impairing the IFNβ signal. The IL-10 is an anti-inflammatory cytokine that can inhibit CD4 + priming and effector functions and modulates them; BCRF1 was suggested to inhibit CD4 + T-cell antiviral response similar to IL-10 [69].

1.2.8.2.3 Immune evasion during latency

The EBV severely restricts latent infection viral protein expression to prevent host immune recognition. Different latency forms represent different stages from primary B cell infection to the transformation of the growth. Thus, in latency III cells, EBNA1inhibits its translation and proteasomal degradation. This strategy ensures adequate levels of EBNA1 to preserve the viral genome while decreasing the turnover of proteins to minimize the appearance of viral antigens to CD8 + T cells. LMP1 and 2 mediate NF-κB activation and decrease the TLR9 surface expression and accelerate the turnover of IFN receptors, resulting in a decrease in the incidence of IFN receptors. During latency II, the expression is limited to EBNA1 and LMP1, and 2. Latency I only contains an expression of EBNA1, and latency 0 occurs without any expression of EBV protein [70]. The EBV encodes different types of non-coding RNAs, including two EBV-encoded small RNAs (EBERs) that inhibit PKR activity and miRNAs that de-regulate T-cell attracting CXCL-11 chemokines and de-regulates T-bet and IFNΔ transcriptional regulator [71].

1.2.9 EBV prevention and vaccine

In the vast majority of individuals, EBV is a harmless passenger, controlled easily by immune defenses, but in some individuals, EBV drives a broad range of diseases that can cause significant morbidity and mortality.

A vaccine is currently unavailable. A prophylactic vaccine which prevents acute disease, the most beneficial using the humoral immune approach, vaccines expressing the major viral envelop protein, gp350 have been developed. Most recently live recombinant vaccinia vaccine expressing gp350 protected two- thirds of the vaccinated infants [20]. as Also, therapeutic vaccines are investigated. These vaccines are based on direct peptide immunization approaches. The use of immunodominant HLA Class I and II epitopes of LMP1, LMP2, and EBNA1 may induce a strong and sustained T-cell response, which was demonstrated with some success primarily in reactivating CD4+ and CD8+ cell in vitro [72, 73].

The use of antiviral therapy in EBV infection is limited. Antiviral therapy can be used as preemptive therapy of PTLD in EBV- organ transplant recipients. These

agents can block EBV replication in donor B-cells and infection of recipient B-cells. Prophylactic intravenous ganciclovir after liver transplantation lead to decreasing of PTLD incidence, which may be due to a reduction in the number of latently-infected B-lymphocytes [32].

1.3 Human Cytomegalovirus (HCMV)

Human Cytomegalovirus (HCMV) or human herpesvirus 5 (HHV-5) [74], is ubiquitous in human populations and was first isolated and cultivated in 1956 [56]. The HCMV derives its name from the Greek cyto-, "cell", and -megalo-, "large", because of the enlargement of virus infected cells, (cytomegaly), [75]. Human cytomegalovirus is a leading - cause of congenital viral infections with numerous consequences such as birth defects including intrauterine growth restriction, still-birth, low birth weight, preterm birth, microcephaly, neurodevelopmental delay, cerebral palsy, hematological disorders, pneumonitis, blindness, and sensorineural hearing loss [76]. HCMV infection is typically clinically silent in immunocompetent hosts, with few mild symptoms like fever, myalgia andcervical lymphadenopathy. Individuals with weakened immunity – neonates, diagnosed with HIV/AIDS, and those on long-term immunosuppressive treatments, such as transplant recipients – HCMV infection often results in clinically severe diseases. The worst outcomes, including mortality and long-term morbidity, are with congenital infection [56].

1.3.1 The virion and genome organization

A complete virus particle consists of a DNA core with a protein coat and external envelopes representing the extracellular infective form of a virus called virion [77]. The average size of viral particle varies between 200–300 nanometers and has linear double-stranded (235-kb) DNA genome which is enclosed by an icosahedral capsid. The capsid's diameter is (110–130 nm) and made of 162 capsomeres (12 pentons and 150 hexons).. HCMV has three kinds of capsids: A capsid (only capsid shell), B capsid (capsid shell and assembled proteins), and C capsid (a mature capsid containing the viral genome). These three capsids represented in three various stages of capsid maturation that takes place in the nucleus of infected cells capsid is composed of at least 5 proteins, i- Major Capsid Protein (UL86), ii- Minor Capsid Protein (UL85), iii- Smallest Capsid Protein (SCP, UL48–49), iv- Assembly protein (Fragments of UL80) and v- Minor Capsid Binding Protein (MCP, UL46) [78].

The widest layer inside the virion structure is the tegument layer that closely surrounds the capsid; anchoring the envelope to the tégumented capsid is believed to be essential. The layer of tegument is composed of several proteins like pp65/ppUL83, pp71/ppUL82, pp150/pUL32, and pp28/pUL99 which play main roles during the entry of virus (un-coating), intracellular capsid transportation and assembly [79]. The tegument is covered by a lipid bilayer called the envelope that keeps the entire virion intact. It interacts with the host cell membrane in target cells and thus plays a significant role in the attachment and entry of viruses. Lipid bilayer envelope is derived from cellular ER or endosomal membranes and associated with 23 viral glycoproteins. The viral glycoproteins gpUL55 (gB), gpUL73 (gN), gpUL74 (gO), gpUL75 (gH), UL100 (gM), gpUL115 (gL) and the pentameric complex consist of gL, gH and UL128–131, are known to play crucial roles in viral entry, cell-to-cell spread and virion maturation [80] (**Figure 6**).

Among herpesviruses, HCMV has the largest genome consisting of a linear dsDNA ranging from 220 to 240 kbp and comprising up to 250 opening reading frames (ORFs) [81]. The herpesvirus genomes are categorized into A-F sections depending on the organization of the genome segments (**Figure 7**). The genome of

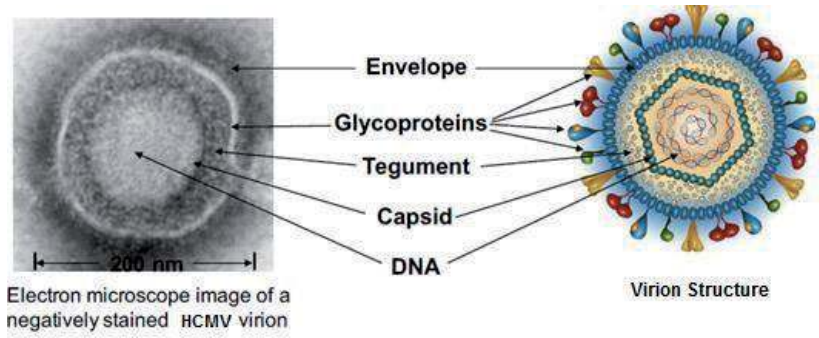

Figure 6.
Structure of an HCMV Virion.

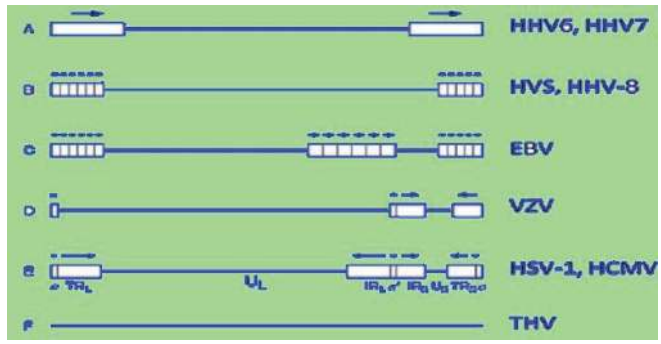

Figure 7.
The six classes of herpesvirus genomes.

HCMV is classified as an E genome. The sequencing analysis of the HCMV genome has shown that it has a very complex structure. Generally, the genome is organized into two parts: The single long regions (UL) and unique small regions (US) flanked by terminal repeats (TR) and internal repeats (IR), UL-area ORFs and US-region ORFs are classified according to their location [82] (**Figure 8**). More than 70 viral proteins have been identified from the purified virions [83]. Only 50 proteins are important for viral replication, while the vast majority of HCMV proteins are involved in host immunomodulation via their interference with cellular signals [84]. HCMV encodes for at least 4 long polyad-encoded RNAs and 26 microRNAs which have major functions during host-virus and virus replication interactions [85].

1.3.2 Life cycle of human Cytomegalovirus

1.3.2.1 Entry

Cytomegalovirus virus (CMV) enters host cells through membrane fusion as shown in **Figure 9**. The viral entry involves the binding of viral glycoproteins on the surface of the viral lipid envelope and the specific receptors on the external membrane of the host cell [86]. This initial interaction makes the cell susceptible to further interactions that fuse the membranes and eventually disassemble and release the viral genomic DNA into the host cell. Many tegument proteins are thought to mediate the delivery of the DNA-containing nucleocapsid to the nuclear pore complex and the release of the viral DNA into the nucleus [87].

HCMV-gB mediates attachment to cells via binding to cellular receptors that include heparan sulfate proteoglycan, integrins, and epidermal growth factor receptor (EGFR) to promote the entrance process [9]. The heparan sulfate proteoglycan

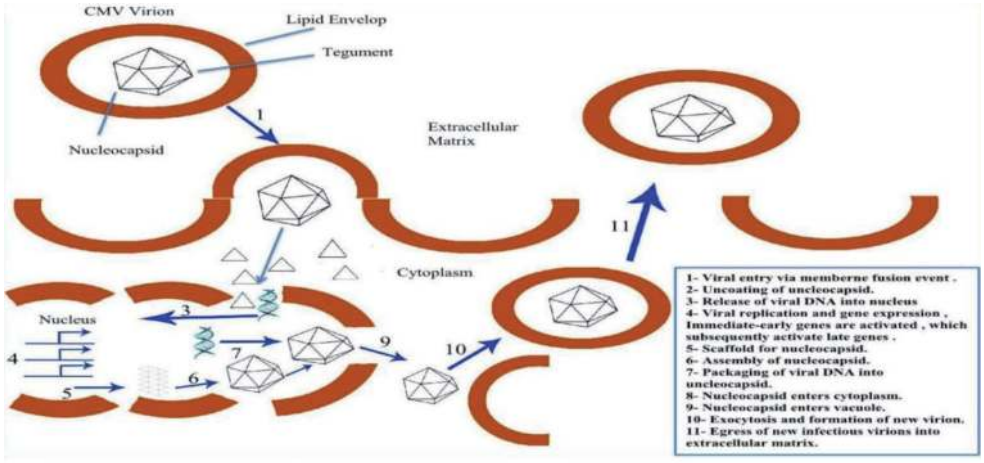

Figure 8.
Illustration of the CMV life cycle from viral entry to egress of new infectious virions.

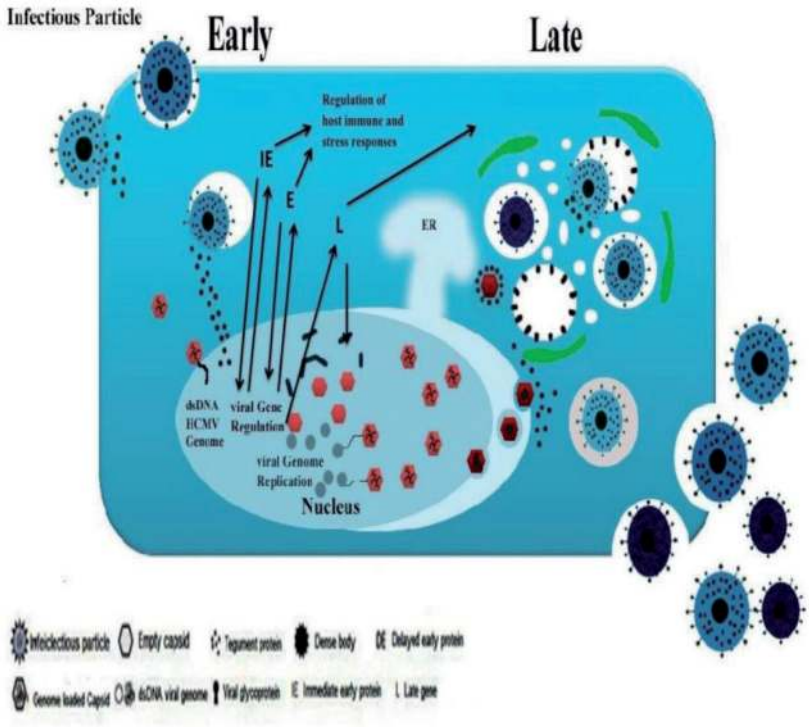

Figure 9.
Overview of the human cytomegalovirus life cycle.

molecule permits the HCMV particle to attach closer to the cell membrane where viral glycoproteins bind to more specific receptors. This is mediated by gB and gH or their complexes, and many other viral protein complexes may also mediate the process of entry; a homodimer of gB known as gC-I, and a heterodimer composed of gM and gN which form gC-II and a heterotrimer of gH, gL and gO which form the gC-III complex. HCMV has two different routes of entry in different cell types [88]. Virus entry in fibroblasts is mediated by viral envelope glycoprotein complexes gH/gL-gB and gH/gL/gO via direct fusion at the plasma membrane. Aminopeptidase N (CD13) and Annexin-II may also serve as receptors to promote entry at the plasma membrane. The entry of CMV into other relevant cell types, such as endothelial

cells, follows an endocytic route, clathrin is the major constituent of coated vesicles and plays an important role in the endocytic entry of viruses [89]. HCMV has a wide cell tropism and can infect different cell types [34], such as neuronal cells in the brain and retina [90], fibroblasts, epithelial and endothelial cells (EC) in lung and gastrointestinal tract [91], hepatocytes in the liver [90] and peripheral blood mononuclear lymphocytes (PBML) [92]. Infected monocytes can release infectious viruses into target organs through tissue-macrophage differentiation [93]. Efficient infection of EC of blood vessel may lead to separation of these cells and hematogenic dissemination may, therefore, be initiated [94]. Fibroblasts, on the other hand, are likely to contribute to the efficient development of HCMV and will help the establishment of the primary infection. HCMV can also infect various kinds of cells in vitro. However, the replication of viruses varies between different cell types. In fibroblasts, smooth muscle cells, endothelial cells, and epithelial cells, HCMV inducesproductive infection, while in poorly differentiated cells, such as myeloid-linear progenitor cells, viral replication is limited [91]. Fibroblasts are the most commonly used cells for HCMV cultivation in the laboratory because HCMV binds to fibroblasts with the efficiency of 2500–3000 particles per cell [94].

HCMV strains show broad variations in the relative pentamer and trimer rates of viral glycoproteins incorporated into virions, which correspond to the cell tropism differences between strains [93]. Many HCMV genes are capable of influencing viral cell tropism at the entry stage and most likely function by composite effects of gH/gL complexes.

1.3.2.2 Lytic replication

HCMV infection of the cell leads to an active replication with the production of new viral particles that are released by exocytosis of the infected cells, known as the lytic phase [95]. The replication of viral DNA is dependent on the expression of certain viral proteins [96]. Gene expression of viral DNA takes place in three stages: immediate early genes (IE) expression, followed by early genes (E) and late genes (L) expressions [95]. The IE gene products act as transcription factors as well as trans-activators to regulate expression of the E and L genes. Two predominant nuclear phosphoproteins, IE1-p72 (Immediate Early 1 – Protein 72KD) and IE2-p86 (Immediate Early 2- Protein 86KD) have the key roles., IE1-p72 is expressed from the UL123 ORF during the IE phase of replication cycle. IE1-p72 transactivates the promoters of numerous HCMV early genes including gene products that facilitate the replication process. It also interacts with the p107 protein through a domain at the N-terminus of IE1-p72 and increase the p107- mediated repression of E2F promoters leading to the inhibition of p107- mediated growth suppression [97]. Therefore, it appears that IE1-p72 can induce E2F activity. The expression of IE1-p72 can promote S phase entry only in cells lacking p53 or p21 [96]. Controversial to that, IE1- p72 expression causes wild-type cells to arrest, most likely in G1 due to increased levels of p53 protein, which results in a p53-dependent induction of p21 expression and subsequent growth arrest [96, 97]. IE2-p86 is expressed from the UL122 ORF during the IE phase of the replication cycle and is essential for HCMV replication [98]. The protein IE2-p86 specifically interacts with pRb through more than one domain and induce pRb mediated repression of E2F promoters. The IE2-p86 disruption of pRb-E2F complexes enable E2Fs transactivation of its target genes [99]. However, IE2-p86 induces cells to enter S phase, an effect that could be attributed in part to IE2-p86 transactivation of the cyclin E promoter and induction of E2F activity [100].

Phosphoprotein 65 (pp65) is a tegument phosphoprotein that exhibits kinase activity. This protein may affect the activity of a specific subset of cytotoxic

T –Lymphocytes (CTLs) by modification of IE1- p72, possibly through its phosphorylation and may interfere with its processing and/or degradation [82].

Following peak expression of IE regulatory proteins, early genes (E genes) become transcriptionally active (**Figure 9**). These proteins regulate replication process of HCMV DNA, such as a DNA-polymerase (UL54) and DNA primase (UL70) which sustain an efficient production of new viral progeny [89]. The L proteins, which are mainly structural components, are essential for virion assembly and egress. HCMV genome contains a cis-acting lytic origin of DNA replication (OriLyt) element to initiate bidirectional DNA replication (theta form of replication), followed by a rolling circle mode of replicationof viral DNA molecules and their incorporation into new virus particles [80]. The entire replication cycle for HCMV takes approximately 72 h and the mature new virions infect the new cells either by their release from the infected cells or by the spread via cell-to-cell mechanisms [80]. During latency, only selected IE gene transcription and translation of viral proteins occur and when conditions are favorable, the virus may be reactivated into lytic replication and new viral progeny occurs [101].

1.3.2.3 Virus assembly

The newly synthetized DNA is inserted into an immature B capsid after the precapsid assembly stage and becomes a fully mature C capsid. This DNA packed capsid, egresses through the nuclear membrane from the nucleus, through an envelope and de-envelopment cycle [102]. The mechanism of assembly of tegument proteins is still unclear. It has been suggested that tegument proteins are added to nucleocapsids sequentially starting in the nucleus and continuing in the cytoplasm, which provide stability during nucleocapsid translocation from the nucleus to the cytoplasm.. The final envelopment of tegumented particle occurs at ER/endosomal membranes. By transporting Rab3 secretory vesicles, mature particles are released by fusion of the vacuole with the plasma membrane and shed out by exocytosis [103] (**Figure 9**).

1.3.2.4 Latency and reactivation

The establishment of latency is one of the major biological characteristics of herpesviruses. Primary HCMV infection is often asymptomatic in a healthy person (immune-competent host) and leads to latent and recurrent infection [104].

The MIE gene acts as a transactivator for transcribing the majority of encoded HCMV genes and is necessary for the virus replication and the lytic process of infection. During latency, cellular factors transcripts control the MIE promoter. These factors also suppress the chromatin around the MIE gene, which prevents the lytic cycle stage and is a part of preserving the latent stage of the infection [105].

People who have had an organ or bone marrow transplant and those with AIDS can develop serious illness caused by CMV. Typically, latent virus from a previous infection (the primary CMV infection may have occurred many years earlier) becomes active again because the person's immune system is compromised [106].

To establish viral latency and maintenance, the latency- gene product UL138 is required. HCMV-LUNA and UL138 are generated during HCMV latency and lytic infection and activate CD4 + T cells, resulting in the development of IL-10 and IFN-π; one with immunosuppressive effects and one with immune-activating effects that facilitate the replication and reactivation of latent HCMV. The produced IFN-β can promote macrophage differentiation, which can lead to reactivation and enhanced HCMV replication. UL138 also downgrades protein-1, MRP, which can lead to lower exports of C4 cell leukotriene, preventing DCs from entering lymph nodes and impairing an HCMV-specific immune response [105].

The expression of the UL 111a gene, which encodes a functional IL-10 homolog with strong immunosuppressive effects, also offers HCMV strategy in viral latency to suppress the immune system. Through the latent infection process, UL111a undergoes alternative splicing, which results in the expression of a latency related transcript cmvIL-10, and the production of a protein that mimics the function of human immunosuppressive cytokine IL-10. This favors the infected cells not to be recognized by the immune system and to avoid clearance. Also, US28 and UL144 are expressed during latency redirect the immune response or block the immune recognition [107].

Latent HCMV infection also modulate the cell expression of MHC molecules class I in order to evade the immune response. At least four proteins encoded by (US) region of HCMV genome involved in inhibition of MHC class I expression, either by directly acting on MHC class I moleculeor acting on MHC class I-associated proteins, such as TAP (transporter associated with antigen processing) and tapas in [101]. These loci of HCMV DNA encodes several distinct IE proteins, pUL36, pUL37, and pUL37_1, which appear to stop engagement of the apoptotic signals associated with the tumor necrosis factor (TNF) family of receptors including Fas. As a result, the activation of Fas-associated death domain (FADD) is inhibited, which prevents activation of procaspase-8 FLICE (FADD-like interleukin-1 beta-converting enzyme) and ultimately prevent the active caspase-8 to cause the subsequent activation of downstream effector caspases and prevent apoptosis of the cells [107, 108].

1.3.3 Transmission

The spreading of HCMV from one person to another primarily occurs through infected bodily secretions such as saliva, blood, tears, milk, and urine [109]. Close or intimate person to person contact usually is necessary for viral transmission. Accordingly, sexual transmission has been implicated in the spread of CMV. Seropositivity is higher among persons with multiple sexual partners and histories of sexually transmitted diseases [110].

Cytomegalovirus (CMV) may be transmited from mother to infant before, during or after birth. During pregnancy, vertical CMV transmission occurs via either passage of virions from maternal blood to the fetus and subsequent infection of the placenta or via the entry of infected maternal leukocytes, endometrial, or cervical cells into the fetal circulation. The infection ascending from the genital tract may also be possible. During birth, the infection of neonate occurs via the contact with infected maternal genital secretions. After birth, breastfeeding is the most frequent route of CMV transmission to the neonate [111]. Cytomegalovirus transmission also occurs naturally after receipt of an organ containing latent virus where these transplant recipients (who are undergoing immunosuppressive treatment) are at risk of contracting the disease [112].

1.3.4 CMV clinical features

1.3.4.1 CMV infections in the immunocompetent host

Cytomegalovirus (CMV) infection is common among patients of all age groups, but it has traditionally been considered as a problem in neonatal and immunosupressed patients. Cytomegalovirus infection in immunocompetent patients is usually asymptomatic or subclinical. Symptomatic disease usually results in mononucleosis-like syndrome. The symptoms are similar to classic mononucleosis, caused by the Epstein–Barr Virus. However, the mononucleosis syndrome

associated with CMV typically lacks signs of enlarged cervical lymph nodes and splenomegaly [113]. Rarely, CMV infection in immunocompetent persons is associated with severe diseases such as enteritis, thrombotic disease, hemolytic anemia, thrombocytopenia, encephalitis, Guillain–Barré syndrome and myocarditis. Guillain–Barré syndrome is immune-mediated peripheral neuropathy characterized by neuromuscular paralysis. The first case of Guillain-Barré Syndrome associated with cytomegalovirus (CMV) infection was reported in a renal transplant recipient in 1970 [114].

1.3.4.2 CMV infections in the immunocompromised patients

CMV infection is one of the most important infectious complications of solid-organ transplantation and is responsible for serious, life-threatening diseases in patients infected with human immunodeficiency virus (HIV) and other immunodeficiencies [115]. CMV disease manifests in the vast majority of transplant recipients as a viral syndrome that includes fever, malaise, myalgia, or headache (sometimes called *CMV syndrome*) or more severe organ-specific diseases such as pneumonitis, gastrointestinal lesions, hepatitis, retinitis, pancreatitis, myocarditis, and rarely, encephalitis or peripheral neuropathy. In solid organ transplant (SOT) recipients, primary HCMV infection has been consistently linked with dysfunction of the transplanted organ. In HIV-infected patients, retinitis is the single most common manifestation of CMV disease, accounting for 85% of all cases [116]. In developing countries, CMV retinitis is still the most frequent cause of visual loss in HIV-infected patients [115].

1.3.4.3 Congenital infection and diseases

Congenital infection refers to a condition where cytomegalovirus is transmitted in the prenatal period. Worldwide, approximately 1 in 100 to 500 babies are born with congenital CMV. Approximately 1 in 3000 will show symptoms and 1 in 7000 will die [117]. Congenital HCMV infection occurs after primary infection (or reactivation) during pregnancy. Congenital infections are less common in poorer communities with high seropositivity of, women in childbearing age.. In industrialized countries, up to 8% of HCMV seronegative mothers acquire primary HCMV infection during pregnancy, of which roughly 50% will transmit CMV to the fetus [118]. Between 10–15% of infected fetuses are born with the symptoms of congenital CMV disease, [119] which may include pneumonia, gastrointestinal, retinal, and neurological diseases [120].

1.3.5 Epidemiology

Human CMV is an ancient virus that is ubiquitous in human populations, reaching a prevalence of 100% in Africa and Asia, and approximately 80% in Europe and the USA, depending on socioeconomic status [121]. Seroprevalence rates of HCMV vary depending on age (higher rates are observed among older persons), geography (higher rates in developing countries), and socioeconomic status (higher rates in economically depressed regions) **Figure 10** [122].

A comparison of literature estimates shows that congenital CMV-related disabilities are as common among newborns and children as other better known diseases such as Down syndrome, fetal alcohol syndrome, or spina bifida [123]. Of the approximately 30,000 United States babies born with an infection with CMV every year almost 20 percent are born with or experience permanent sequelae such as hearing loss, eyesight loss, brain damage or cognitive impairment.

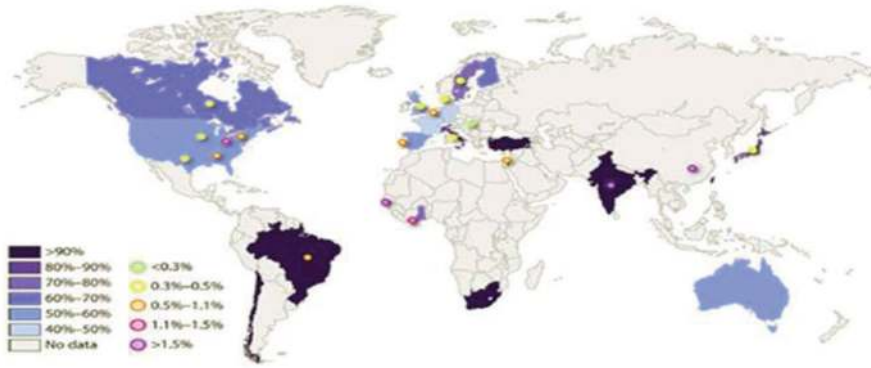

Figure 10.
Globally, rates of CMV seroprevalence in women of reproductive age and the incidence of congenital CMV infection. Studies have been performed in Australia, Belgium, Brazil, Canada, Chile, England, Finland, France, Germany, Ghana, India, Israel, Italy, Japan, South Africa, Spain, Sweden, Taiwan, Turkey, and the USA.

The contribution of congenital CMV infection to childhood hearing loss is of particular importance, with approximately 20% of moderate to profound bilateral sensorineural hearing impairment occurring due to congenital CMV infection [124]. Approximately 90% of newborns with congenital infection have no symptoms at birth; If symptoms are present, they are often nonspecific [125]. When disabilities such as hearing loss appear, often months or years later, it is usually too late to make a retrospective diagnosis that identifies congenital CMV infection as the cause. For an individual woman, the greatest risk of having a baby with congenital infection comes from the mother's primary infection during pregnancy [126]. Consequently, babies of women who are CMV negative prior to pregnancy are particularly vulnerable to poor outcomes if the mother becomes infected during pregnancy [127].

In a nationally representative survey it has been stated that between 30% and 50% of United States women under 45 years of age are seronegative for CMV and that as many as a half a million US women of childbearing age experience primary CMV infections every year [128].

1.3.6 Immune evasion by Cytomegalovirus

HCMV persistence is correlated with the interaction between the immune response of the host and the virus evasion mechanisms, where HCMV interferes with both adaptive immune responses and immune effectors. A variety of evasion strategies have been developed by HCMV to prevent selected dendritic cell functions. The differentiation of CMV infected monocyte into macrophages and CD1 a-positive Dendritic cells (DCs) is inhibited, which does not require viral replication [129].

1.3.6.1 Evasion of Innate immunity

The innate immune system is essential in driving an efficacious acquired immune response. This includes the induction of type I interferons, activation of professional antigen presenting cells, and recruitment and activation of NK cells which themselves promote more efficient activation of antigen presenting cells and T cells. The binding and entry of HCMV into the cell initiates several pathways leading to the upregulation of NFkB and interferon regulatory factor 3 (IRF3) which can ultimately lead to the production of type I interferons and certain

inflammatory cytokines. This innate cellular response to the initial stages of infection is mediated by Toll like receptor 2 (TLR2) signaling, which has been shown to recognize the viral surface glycoproteins gB and gH [130].

1.3.6.1.1 Natural Killer (NK) cells and immune evasion mechanisms

The importance of NK in the innate immune response to HCMV is suggested by the extensive studies of mechanism of HCMV prevention of the activation of NK cells. NK cells are inhibited by signals sent via inhibitory receptors that interact with class I MHC molecules on the surface of target cell. Low surface levels of Class I MHC on HCMV-infected cells may also reduce the inhibitory signaling of NK. This could make the infected cells susceptible to NK cell cytotoxicity [131]. Two mechanisms describing HCMV-mediated inhibitory receptor signaling have been reported. Firstly, HCMV uses the host HLA-E pathway to inhibit NK cells via the CD94/NKG2 heterodimeric inhibitory receptor by promoting cell surface HLA-E expression. Viral UL40 protein contains a nonomeric peptide which binds HLA-E promoting its cell surface expression. Secondly, HCMV expresses a viral homologue of cellular MHC Class I, UL18. UL18 is trafficked to the cell surface where it binds to the inhibitory NK cell receptor, LILRB1 (LIR-1) with higher affinity than MHC Class I inhibiting LILRB1+ NK cell activation. HCMV encodes five genes controlling NK cell activation and cytotoxicity by the provision of inhibitory signals and suppression of activating signals. The pp65 tegument protein (UL83) dissociates CD3_ signaling from NKp30, whilst intracellular retention of CD155 and CD112 by UL141 prevents activation of NK cells via receptors CD226 and CD96, the remaining viral proteins interfere with a major receptor on all human NK cells (NKG2D) that mediated NK cell activation [132].

1.3.6.2 Evasion of adaptive immunity

Primary infection of HCMV provokes the production of antibodies specific for many HCMV proteins including structural tegument proteins such as (pp65 and pp150), envelope glycoproteins predominantly (gB and gH) as well as non-structural proteins such as the Immediate Early 1 protein (IE1, UL123).

1.3.6.2.1 CD8+ T cell responses and MHC Class I downregulation

In human bone marrow transplantation studies where HCMV infection can cause significant morbidity, it was evident that there was a strong correlation between recovery of the CD8+ T cell population and protection from CMV disease. HCMV employs several mechanisms to interfere with the normal cellular MHC Class I processing and presentation pathways to prevent CD8+ T cell recognition. HCMV encodes at least four related glycoproteins, each with a unique mechanism to prevent antigen presentation. HCMV viral genes US2 and US11 degrade newly synthesized MHC class I heavy chains, US3 retains MHC-I in the endoplasmic reticulum by interfering with chaperone-controlled peptide loading and US6 inhibits the translocation of viral and host peptides across the endoplasmic reticulum membrane by the dedicated peptide transporter TAP (transporter associated with antigen processing) [133]. The action of these genes may not completely protect cells from CD8+ T cell recognition dependent on the T cell-antigen specificity. Whereas HCMV-infected cells expressing US2–11 prevent any presentation of IE antigen to human T cells, pp65 peptides were still presented. CMV-specific T cells are also marked by the lack of expression of the costimulatory receptors CD27 and CD28, which are otherwise commonly expressed on naïve T cells [134].

1.3.6.2.2 CD4+ T cell responses and MHC Class II downregulation

During primary infection, HCMV specific CD4+ T cells can be detected 7 days after the detection of HCMV DNA in peripheral blood in response to same ORFs asCD8 + T cells and pp65 and IE genes gB and gH. These cells produce T helper type 1 (Th1) cytokines IFNγand TNF. A large number of HCMV- encoded gene products target the MHC class I antigen presentation pathway in an effort to avoid recognition by CD8+ T cell [134].

Human cytomegalovirus also avoids the CD4+ T cell response by several ways. Disrupting IFN- induced, upregulation of MHC class II molecules to the cell surfaces by preventing the expression of Janus kinase 1 and suppression of Class II transactivator mRNA. The virally-encoded gene product of US2 also suppress MHC class II presentation to CD4+ T cells by redirecting the HLA-DR-and HLA-DM-chains to the cytosol where they are degraded. HCMV re-programs human hematopoietic progenitor cells (HPCs) into immune-suppressive monocytes that express IL-10 in a process requiring US28. Recently a truncated transcript to UL111A, a viral homolog of the immunomodulatory cytokine IL-10, which is expressed during latency (cmvLA IL-10) has been shown to downregulate expression of MHC class I and class II molecules, inhibit both proliferation of mononuclear cells and the production of inflammatory cytokines [135].

1.3.7 CMV prevention and vaccines

The lack of specific and effective treatments for HCMV infection has highlighted the need to understand HCMV host-cell interactions, including viral entry and host immune responses against this virus [136]. The HCMV vaccine is designed to be used to prevent infection or to prevent its re-activation in those infected already [137]. To eliminate and eradicate congenital HCMV infection, vaccination would be a priority. Several researchers have attempted to develop vaccines against HCMV, such as live attenuated vaccines, recombinant chimeric vaccines, and subunit vaccines such as glycoprotein B and tegument protein pp65 vaccines [138]. However, none of these vaccines exhibited effective protection, as well as, and to this day, no licensed HCMV vaccine is available.

Strategies to reduce Congenital CMV disease burden may be implemented at different stages, and include prevention of maternal infection, prevention of mother-to-child transmission, early detection and intervention by neonatal screening, and neonatal antiviral therapy. The principal sources of exposure for women of childbearing age are sexual contacts and children secreting the virus [136]. Pregnant women also should be advised to avoid close contact with individuals likely to shed CMV such as adults with symptoms consistent with mononucleosis and toddlers attending group day care. Preemptive therapy is an approach in which patients are monitored for early replication (ie, viremia by polymerase chain reaction [PCR]), and the antiviral drug is administered only when CMV replication is detected to prevent its progression to higher-grade viremia and CMV disease. Small, noncomparative studies have shown lower rates of CMV disease (especially delayed-onset disease) with preemptive compared with the use of antiviral prophylaxis in CMV-seronegative liver transplant recipients with seropositive donors [139].

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Author details

Marwa Mohammed Ali Jassim[1], Majid Mohammed Mahmood[2*]
and Murtada Hafedh Hussein[3]

1 Studies and Planning Department, University of Baghdad, Baghdad, Iraq

2 College of Science, Mustansiriyah University, Baghdad, Iraq

3 College of Science, Thi-Qar University, Thi-Qar, Iraq

*Address all correspondence to: majidmahmood93@yahoo.com

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