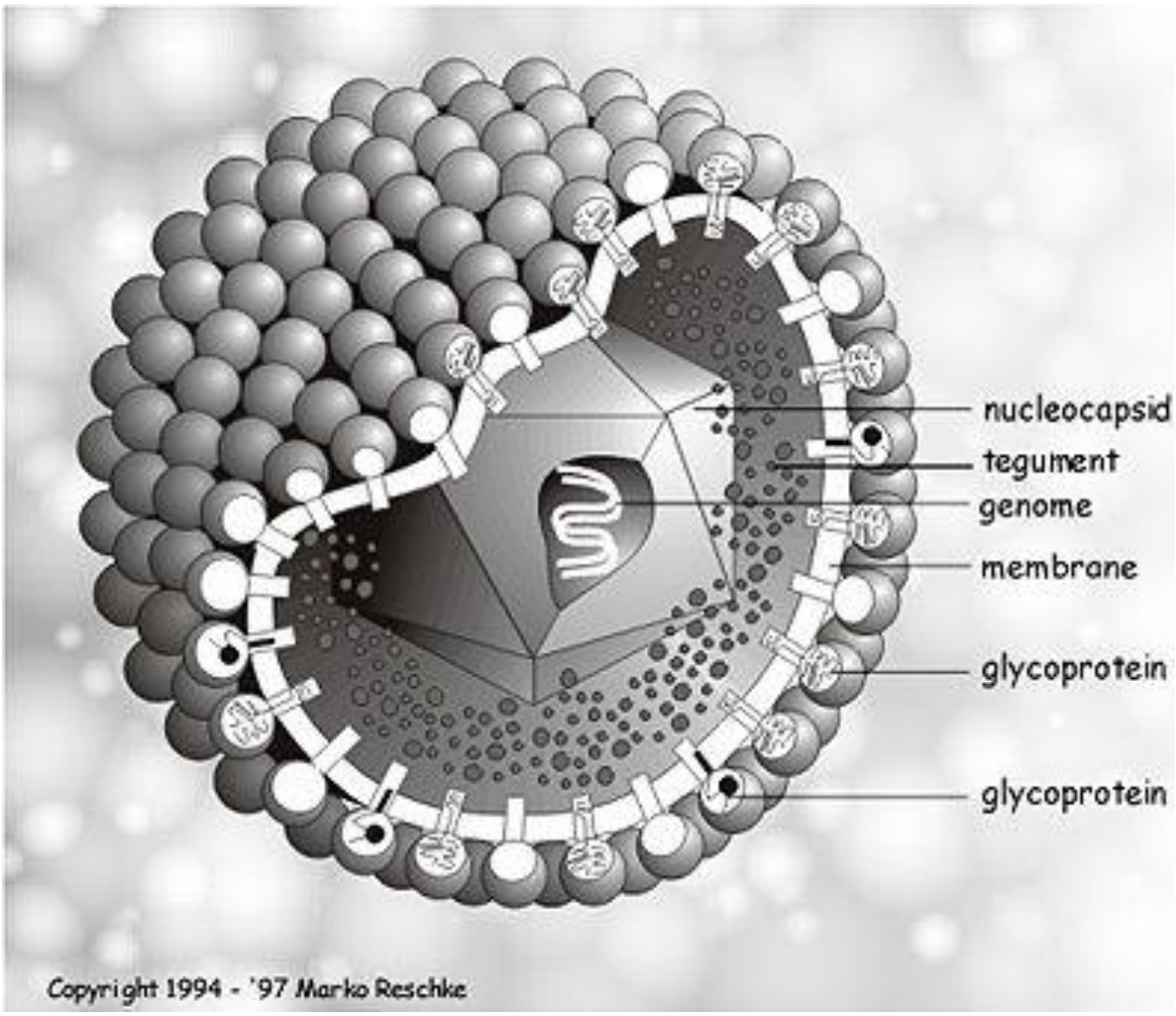
Immune evasion by human herpesviruses and its effect on vaccine development.



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Abstract

In our lifetime we will encounter at least one if not several human herpesviruses. These viruses elicit a strong immune response upon primary infection. However, this immune response is unable to clear the virus due to its immune evasive properties. Instead, it drives the virus into a latent state. In healthy individuals these viruses are considered fairly harmless, whereas in immunocompromised individuals they are associated with the onset of serious diseases. These complications and the discomfort which can be associated with infection are strong motivators for vaccine development. By reviewing the clinically most relevant herpesviruses a general pattern emerged concerning the problems encountered during vaccine development. For instance, some human herpesviruses are able to avoid immune detection even when a good memory response is present. Therefore, vaccine development for these viruses appears to be nearly impossible. Additionally, individuals encounter some herpesviruses before their first birthday which complicates vaccination against a primary infection. Aside from vaccinating against primary infections, another strategy would be to vaccinate against complications associated with reactivation or immune deficiency. This field is even more challenging, it seems unlikely one can boost a deficient immune system. In this thesis an overview of the immune response, the immune evasion properties and the status of vaccine development is provided for the clinically most relevant human herpesviruses.

Introduction

The over 120 members of the mammalian *Herpesviridae* family are divided into three sub-families, the *Alpha-*, *Beta-* and *Gamma-herpesvirinae* [2-4]. At first, this distinction was based on biological criteria, though nowadays sequence analysis is used [2, 5]. To date, a total of 8 human herpesviruses have been identified [2, 3, 6, 7]. It is thought that the viruses seen today are the product of millions of years of co-evolution with their hosts [7]. Sequence analysis indicates they have derived from a common ancestor that lived approximately 400 million of years ago [8].

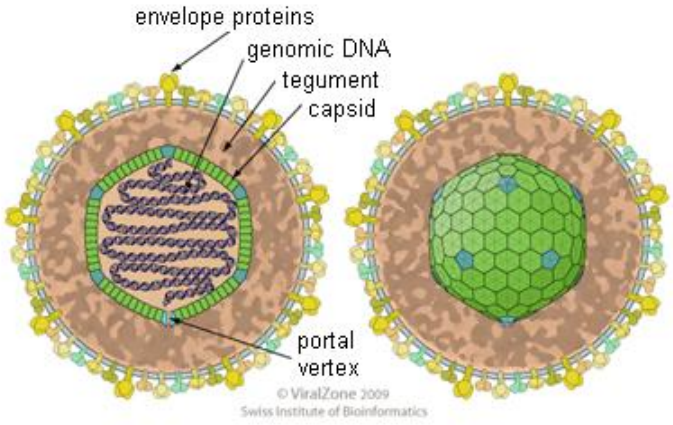

Figure 1. The general make up of a herpesvirus virion.
A herpesvirus virion consist of several structures namely the envelope coated with glycoproteins, the tegument, the capsid and a copy of dsDNA [115].

Herpesviruses are enveloped, icosahedral, dsDNA viruses (Fig. 1). The different layers of the viral particle (virion) all have their own function, allowing efficient transmission from one cell to the next, or from one host to another [3].
The envelope is a lipid bilayer that provides protection from environmental components, such as proteolytic enzymes (Fig. 1) [3]. Additionally, the envelope contains glycoproteins which promote attachment and/ or cell entry. For instance, glycoprotein gB and the dimer gH/gL play an important in cell entry of all human herpesviruses, [9, 10].
Underneath the envelope lies the tegument, which consists out of multiple proteins, some cellular and some viral RNA [6, 11]. The tegument allows readily synthesized proteins to enter the cell with the viral genome. Thus, the virus is able to manipulate the cell immediately after entry [8]. There are two types of proteins present in the tegument, those with a structural function, like HSV-1 encoded US11 which is involved in envelopment and transport, and those that alter the cellular response to infection such as HSV-1 encoded Vhs, a host shutoff factor [3, 11, 12].
Beneath the tegument there is an icosahedrally shaped nucleocapside that carries the viral genome [3]. The size of the nucleocapside limits the packaging size of the genome, consequently the linear dsDNA genome of herpes viruses is packaged “naked” (free from chromatin proteins) into the nucleocapside [6].
The genome, which varies in size between ~125kbp to ~235kbp for human herpesviruses, has approximately 40 genes that are conserved for all alpha-, beta- and gamma-herpesviruses. These genes are involved in virion production, DNA replication and nucleotide metabolism (table 1) [8]. At subfamily level even more genes are conserved. For instance, in the *Alphaherpesvirinae* subfamily the gene encoding gD and certain genes involved in latency are conserved.
Genes unique for each virus are frequently involved in immune evasion for example, several viruses have independently acquired IL-10 homologs thereby allowing them to interfere in MHC surface expression on lymphocytes for example [8, 13].

Latent and lytic infections

Herpesviruses enter their target cell either through membrane fusion or receptor mediated endocytosis. Once in the cell, the nucleocapside will be transported to the nuclear membrane where the genome will pass through a nuclear membrane pore. Herpesviruses have the unique and characteristic property to induce a lytic or a latent infection. Because of their ability to enter a latent phase they are able to induce life long infections.

Lytic infection: in this phase new virions are produced which will eventually lead to cell lysis. First, the immediate early genes are transcribed. These genes are generally transcription factors which regulate the transcription of early and late genes. Second, the early genes, involved in DNA replication are transcribed. Lastly, the late genes are transcribed. These can be divided into two types, those augmented by DNA synthesis and those transcribed after DNA synthesis. They mainly encode for the structural proteins as the nucleocapside proteins and glycoproteins [8].

Latent infection: in this phase the DNA has circularized and transcription is reduced to a minimum. These latent genomes still have the capacity to replicate and cause disease upon reactivation. The trigger for reactivation and the involved molecular pathways are largely unknown, additionally they could differ per virus [8].

The RNA formed in the latent phase can remain RNA as observed for HSV-1 which can create up to 100,000 copies of latency associated transcripts (LATs) in the nucleus [5]. In contrast, the RNA produced by the latent EBV genome is translated into proteins which play a role in growth transformation and maintaining latency [13-15].

Thus, patients infected with a herpesvirus either have a latent infection, a lytic infection but are asymptomatic or a lytic infection which results in disease [8].

Table 1. Genes conserved in alpha-, beta- and gamma-herpesviruses [8].

Function	HSV homolog
Gene Regulation	
Multifunctional regulatory protein	UL54
Nucleotide Metabolism	
Ribonucleotide reductase, large subunit	UL39
Uracil DNA glycosylase	UL2
dUTPase	UL50
DNA Replication	
Helicase/primase complex (3 subunits)	UL5, UL8, UL52
DNA polymerase	UL30
ssDNA binding	UL29
DNA polymerase processivity factor	UL42
Virion	
Maturation	
Alkaline exonuclease	UL12
Genome cleavage/ packing	UL28, UL32, UL33
Terminase/ packaging	UL15a
DNA packaging	UL25
Scaffold protease	UL26
Scaffold	UL26.5
Capsid nuclear egress	UL31, UL34
Virion	UL16
Capsid	
Major capsid protein	UL19
Minor capsid protein, portal protein	UL6
Capsid triplex	UL18, UL38
Hexon tips	UL35
Tegument	
Large tegument protein	UL36
Tegument protein	UL7
Protein kinase	UL13
Myristoylated	UL11, UL14, UL17, UL37, UL51
Envelope	
Glycoprotein B	UL27
Glycoprotein H	UL22
Glycoprotein L	UL1
Glycoprotein M	UL10
Glycoprotein N	UL49.5
Other	
Cell-to-cell fusion	UL24

Human herpesviruses

Members of the human herpesviruses are found in all three subfamilies and although these viruses share common features, the cell tropism and disease outcome vary greatly between the different viruses [6].

The *Alphaherpesvirinae* sub-family consists of two lineages, the α1 lineage or *Simplexvirus* genus to which Herpes simplex virus-1 and -2 belong and the α2 lineage or *Varicellovirus* genus to which Varicella Zoster virus belongs (Fig. 2). The alpha viruses are characterized by latency in the sensory neurons, a relatively short replication cycle and efficient destruction of infected cells [6, 16].

Human Cytomegalovirus, Human herpesvirus-6 and -7 belong to the B*etaherpesvirinae* sub-family which are characterized by a long replication cycle and a slow progression in an *in vitro* culture (Fig. 2) [8].

The *Gammaherpesvirinae* sub-family contains the members Epstein Barr virus and Human herpesvirus 8 (Fig. 2) [6]. Remarkably, the gamma herpesviruses are strongly linked to neoplastic diseases probably due to their ability to alter cellular pathways involved in apoptosis, cell growth, antiviral responses and immune surveillance [7].

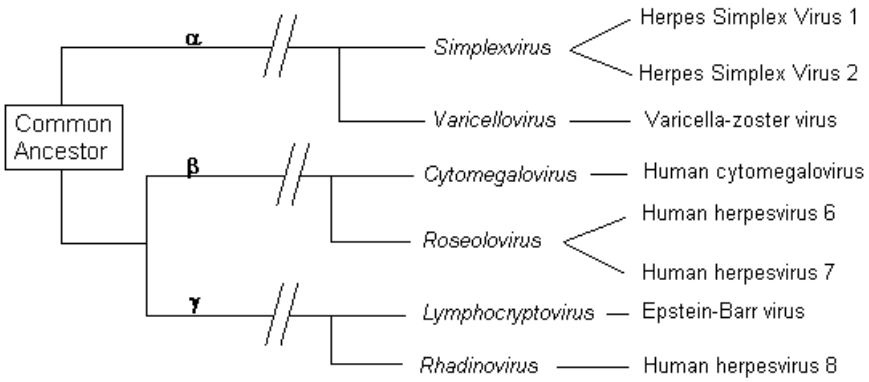

Figure 2. The polygenetic tree of human herpes viruses [2].

Herpes simplex virus-1 (*HSV-1*) is one of the two herpes simplex viruses that infect humans. HSV-1 is known as herpes labialis and gives rise to orofacial infections. A primary infection with HSV-1 will lead to primary hepetic gingivostomatitis (PHGS), which for most immunocompetent individuals is asymptomatic. When it is a symptomatic infection fever, malaise, oral and/ or perioral vesicles are observed [17]. From the site of infection the virus moves up the nerves to the trigeminal ganglion where a latent infection is established [12, 17]. Latency is probably induced by the interaction of the hosts immune system with the virus [17].

Recrudescence's (symptomatic viral reactivation) can be triggered by stress, physical trauma, exposure to UV etc. The symptoms will be lesions or blisters mainly around the junction of the mucosa to skin (mucocutaneous junction) [5, 12, 17].

In immunocompromised hosts HSV-1 can cause frequent and persistent HSV reactivations which can spread to keratinized and non-keratinized tissues [17]. Additionally, more severe conditions can be caused by HSV especially in immunocompromised patients such as herpes encephalitis and neonatal herpes [17].

Globally the prevalence of HSV-1 is high, transmission occurs through oral contact like kissing, sharing a glass or a toothbrush during reoccurrences (asymptomatic viral reactivation) or recrudescence's [17].

Herpes simplex virus-2 (*HSV-2*) is known as herpes genitalis and is spread mainly by sexual contact. After infection of the genital mucosa, HSV-2 moves up the nerves to the sacral ganglion where a latent infection is established [9]. Both HSV-1 and -2 can spread from cell- to-cell and are thereby invisible to the neutralizing antibodies. Cell mediated immune responses are thus necessary to control the infection. Unfortunately, this immune response also contributes to the symptoms. Clinical manifestations associated with HSV-2 infection can vary from unspecific symptoms to painful lesions in the genital area [18]. Immunocompromised individuals can suffer from life threatening diseases like meningitis and encephalitis [12, 19, 20]. Moreover, during labour women can infect their child resulting in the life threatening condition of neonatal herpes [12].
Globally the prevalence of HSV-2 varies per country. An European cross-sectional study from 1989 to 2000 found an age-standardized prevalence that varied from 4% in England to 24% in Bulgaria. In contrast to the age adjusted prevalence in sub-Saharan Africa which varies between 10% and 80% [21]. Cross-over infections where HSV-2 induces herpes labialis or HSV-1 that induces herpes genitalis are possible [17].

Variciella zoster virus (*VZV*) primary infections cause varicella (chicken pox, itching skin lesions). In addition to the skin, the virus can infect the upper respiratory tract, regional lymph nodes, peripheral blood mononuclear cells (PBMCs) and the sensory nerves that innervate the skin [5]. From the nerves the virus moves to the sensory ganglia where they can induce latency [3]. In immunocompromised individuals a VZV infection results in a more abundant rash. Additionally, VZV can disseminate in these patients to other organs causing among others retinitis and hepatitis [22].
Upon reactivation the pathology will be limited to one dermatome (a piece of skin innervated by a single sensory nerve) and is known as herpes zoster or shingles.
VZV infection is very common, in Europe, Australia, Asia and South America, 95 to a 100% of the population is VZV seropositive at the age of 30. In contrast, in tropical regions as many as 50% of the adolescents over 18 have not had a primary VZV infection [22, 23].
The virus is transmitted in, a symptomatic patient, through respiratory secretions and the fluid from the skin lesions [24]. Asymptomatic shedding of VZV is a very rare event [5].
A common complication in healthy individuals is the development of postherpetic neuralgia (PHN), pain that persists for over 4 weeks after the development of the rash. Whether it is caused by direct viral damage to the nerve tissue or damage from immunopathological events as a consequence of infection remains unknown so far [25].

Human Cytomegalovirus (*HCMV*), infects approximately 60% of the population in the United States. Transmission occurs through blood, tissue and other bodily fluids [3, 11, 26].
In most immunocompetent individuals, the primary infection is asymptomatic and relapses do not occur. However, in immune incompetent patients HCMV will cause symptomatic primary infections and relapses. In AIDS patients common HCMV symptoms include retinitis, enterocolitis and esophagitis, while organ recipients suffer from general illness (fever, malaise and leucopenia), pneumonitis and enterocolitis. [11]. Another high risk group are unborn children of which the mother suffers from a primary infection or a relapse. In both cases symptoms as deafness, jaundice, hepatosplenomegaly, seizures and lethargy can occur [11].
HCMV is an opportunistic virus with a huge cell tropism, for example HCMV can infect endothelial cells, hepatocytes, smooth muscle cells, mucosal tissue, connective tissue and nerves.

Even nonpermissive cells, which do not allow viral replication, play a role in spreading the virus through the body [26].
HCMV will give rise to a lifelong infection due to the induction of latency in a cell type presumably derived from the myeloid lineage [3, 11].

Human herpes virus-6 (*HHV-6*) consists of two variants, A and B, which differ in epidemiology, biology and disease association despite their overall nucleotide sequence similarity (90%) [16, 27]. Primary infection causes exanthema subitum, a rash also known as roseola. Recrudescence's can occur in immunocompromised individuals, which have been linked to many diseases such as multiple sclerosis and lymphoma, although the significance of these associations remains to be determined [16, 27, 28].
In vivo HHV-6 has a broad cell tropism, it has been isolated from PBMCs, salivary glands, liver, lymph node, brain tissue and endothelial cells but is found predominantly in $CD4^+$ T cells [16, 28]. Additionally, latency can also be induced in many cell types such as, brain tissue, salivary glands, monocytes and early bone marrow progenitor cells [27].
In general, people get infected before the age of 1, most likely by transmission through saliva[16].

Human herpes virus-7 (*HHV-7*) is related to HHV 6 in biological terms. The clinical manifestations of a primary infection are unknown, though in some patients HHV 7 has been linked to exanthema subitum [28]. The virus was first isolated from $CD4^+$ T cells in 1990 and like HHV 6 it appears to infect children at a young age [28].

Epstein Barr virus (*EBV*) primary infections vary in severity, dependent on the age one contracts the virus. Young children will have a clinically unremarkable primary infection while many adolescents or adults will suffer from infectious mononucleosis (IM). IM is a self-limiting lymphoproliferative disease with symptoms as a sore throat, fever, splenomegaly and adenopathy [29, 30]. The T cells required to control the infection are involved in creating the symptoms. Furthermore, EBV can cause more severe diseases in both immunocompetent as immunocompromised patients. In immunocompetent individuals EBV has been associated with Burkitts Lymphoma (BL), Hodgkins Lymphoma (HL) and nasopharyngeal carcinoma whereas in immunocompromised patients post-transplantation lymphoproliferative disorder (PTLDs, almost all are lethal clonal B cell proliferations), BL, HL, oral hairy leukoplakia [29, 30].
EBV is transmitted through salivary excretions and can replicate in the oropharynx epithelial cells [3, 29-31]. Additionally, EBV can infect B cells by adhering to the CD21 and MHC-II. Other cells, like the oral epithelial cells, can also be infected but far less efficient [15]. Latency is established in B cells [29].

Human herpes virus 8 (*HHV-8*) also known as Kaposi's sarcoma-associated herpesvirus (KSHV) is strongly associated with Kaposi's sarcoma (KS) and rare B cell lymphomas. KS is characterized by red, brown or purple colored lesions that occur cutaneously, mucosally or viscerally and is also associated with HIV [7]. B cell lymphomas associated with HHV 8 are primary effusion lymphoma (PEL), an unique form of NHL and plasmablastic Multicentric Castelman's disease (MCD) [7].
The virus can be transmitted either through sexual contact or most likely through saliva [32]. The route of transmission appears to be dependent on the prevalence of the virus in the population.

In low prevalence zones, like Western Europe and the United States, transmission occurs mainly through sexual contact, especially homosexual contact. In the high prevalence zones, like Africa and the Mediterranean Basin infection often occurs during childhood through the exchange of saliva [32].

The immune system

Physical barriers (e.g. skin), chemical barriers (e.g. lysozymes) and a cellular barrier (e.g. macrophages) form the first line of defense, known as the innate immune system. The cellular barrier consists of several cell types, namely macrophages, dendritic cells (DCs), granulocytes and natural killer cells (NK) (Table 2) [3, 33]. The second line of defense is the adaptive immune system that consists of a cellular and humoral component. Antibodies (Abs) secreted by plasma B cells form the humoral component of the adaptive immune system whereas the cellular components consists out of T cells. In a viral infection, Abs aid in the prevention of viral spreading through our body while cell mediated immunity is necessary for the eradication of virally infected cells.

The T cells can be divided into two groups; the $CD4^+$ T helper (Th) cells and the $CD8^+$ cytotoxic T (Tc) cells. For both groups Ag presentation is essential for activation. $CD4^+$ T cells recognize antigens (Ags) in complex with MHC II molecules, while $CD8^+$ T cells recognize Ags in complex with MHC I molecules. Nearly all nucleated cells express MHC I, thus are able to activate $CD8^+$ Tc cells. In contrast, only certain cell types are able to express MHC II. These cells, known as professional antigen presenting cells (APCs), are B cells, macrophages and dendritic cells (DCs) [14]. Besides the APCs some other cell types can be stimulated to express MHC II. These cells are known as nonprofessional APCs and are for instance the intestinal epithelial cells [34].
To complicate things even further, the $CD4^+$ Th cells can be divided into two subgroups; the Th_1 cells which elicit a cell mediated immune response and Th_2 cells which elicit a humoral response (Table 3) [35]. Whether a $CD4^+$ T_H cell becomes a type 1 or 2 helper cell is decided by the cytokine response of the innate immune system to the pathogen and the costimulatory molecules upregulated on the APCs [35].
In summary, our immune system consists of many different components which all interact with each other to form a solid immune response against pathogens.

Table 2. The main cellular components and their characteristics of the innate immune system [14].

Neutrophil	Macrophage	Dendritic cell	Natural killer cell
Phagocytosis	Phagocytosis	Ag presentation	Lysis of viral-infected cells
Reactive oxygen and nitrogen species	Ag presentation	Costimulatory signals	Interferon
Antimicrobial peptides	Cytokines	Interferon	Activation macrophage
	Reactive oxygen and nitrogen species	Cytokines	
	Inflammatory mediators	Reactive oxygen and nitrogen species	
	Complement proteins		

The neutrophils represent the granulocytes and other less common members of this group the basophils and eosinophils. Additionally the monocytes are not mentioned but these have many similar functions as macrophages.

Table 3. An overview of the T cells of the adaptive immune system [35].

	Cell-mediated immunity		Humoral immunity
Typical pathogens	Vaccinia virus Influenza virus	M. tuberculosis P. carinii	Polio virus S. aureus
Location	Cytosol	Macrophage vesicles	Extracellular fluid
Effector T cell	Cytotoxic $CD8^+$ T cell	T_{H1} cell	T_{H1} & T_{H2} cell
Ag recognition	MHC I complex on infected cell	MCH II complex on infected macrophage	MHC II complex on Ag specific B cell
Effector actions	Killing of infected cell	Activation of infected macrophage	Activation of B cell T_{H1}: opsonizing Ab (IgG) T_{H2} : neutralizing Ab (IgM, IgA, IgE)

Infections with herpesvirus also elicit $CD4^+$ T lymphocytes which mediate cytotoxicity [22].

For a virus it is essential to find a equilibrium within their host. Killing the host is not desired since they depend on them to survive. On the other hand, viruses do not want to be eliminated by their hosts immune system. Herpesviruses are masters of immune evasion and since Ag presentation plays a huge role in the activation of a solid immune response, herpesviruses have devised mechanisms that counteract nearly all steps involved in this process. For a better understanding of these immune evasive properties the general mechanism of MHC I and MHC II Ag presentation will be discussed in detail below.

Antigen presentation

In general MHC I presents antigens obtained from proteins present in the cytosol of the cell. Proteins that are damaged, misfolded or simply ready for replacement are ubiquinated which is a signal for the proteasome to degrade the protein to small peptides [14, 33]. These peptides are either further processed to amino acids (AA) or transported through TAP (transporter associated with antigen processing) into the endoplasmatic reticulum (ER). TAP is a membrane spanning heterodimer located in the membrane of the ER. It is a key protein in loading the MHC I complex with peptide. TAP consists of TAP1 and 2. Both belong to the ATP binding cassette family and consist of two domains, a transmembrane domain and a cytosolic nucleotide binding domain. The ATP binding cassette family allows ATP dependent transport of peptides (in this case) or AA, sugars and/ or ions by other members [14].

TAP is part of the peptide loading complex (PLC) and transports peptides of approximately 8 to 16 AA preferably with hydrophobic or basic AA at the C terminus from the cytosol into the ER. Aminopeptidase like ERAP present in the ER lumen can trim the peptides transported by TAP to the appropriate length for MHC I binding or process peptides shorter than 9AA into AA [14, 33]. Peptides optimal for MHC I binding are 9 AA long with a hydrophobic or basic C terminus.

Polysomes on the ER are linked to Sec61 complexes, so, while the α chain and β_2 microglobulin, that form the MHC I molecule, are produced they are translocated directly into the ER [36]. However, to form a mature MHC I molecule the aid of several molecular chaperones is required. First, calnexin, a membrane bound protein, promotes the correct folding of the α chain. After β_2 microglobulin binds to the α chain, calnexin dissociates. Subsequently, calreticulin and tapasin (TAP associated protein) associate with the immature MHC I. Tapasin will also bind to TAP, thereby bringing the immature MHC I in close proximity to the peptides entering the ER (Fig. 3).

An additional protein, ERp57 will interact with the complex by forming a sulfide bond with tapasin and a non covalent bond with calreticulin. This entire complex is known as the PLC and allows efficient loading of peptides onto the MHC I molecule (Fig. 3). When the appropriate peptide binds into the peptide binding groove of MHC I, the mature MHC I dissociates from the complex and traffics to the cell membrane [14].

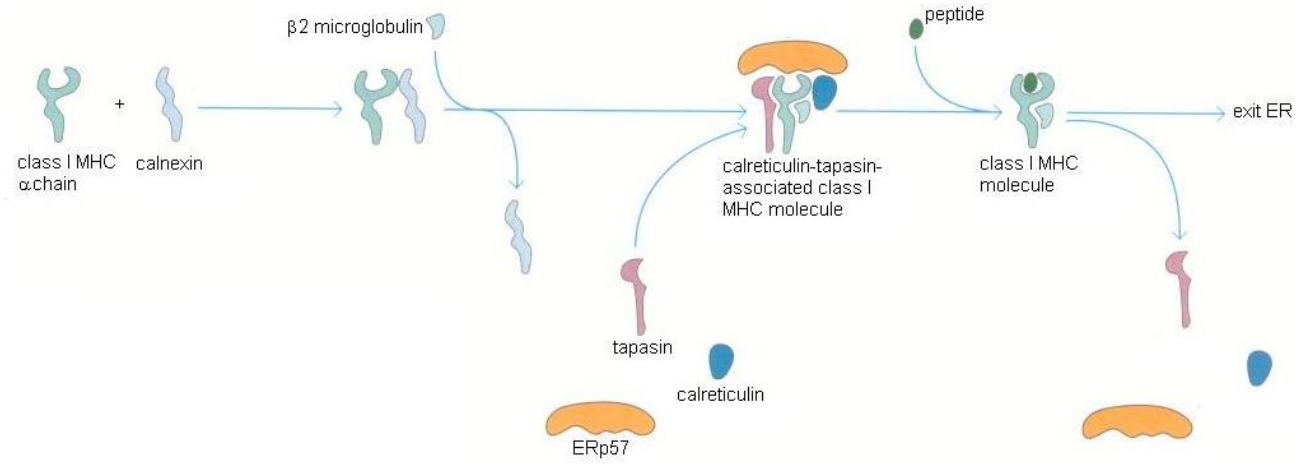

Figure 3. MHC I maturation in the ER [14].
Calnexin promotes the correct folding of the α chain and dissociates after binding of β_2 microglobulin. Subsequently, calreticulin and tapasin associate with the immature MHC I. Tapasin will also bind to TAP, thereby bringing the immature MHC I in close proximity to the peptides entering the ER. Additional ERp57 will interact with the complex by forming a sulfide bond with tapasin and a non covalent bond with calreticulin. This entire complex, known as the PLC, is involved in efficiently loading peptides onto the MHC I molecule. When the appropriate peptide binds into the peptide binding groove of MHC I, the mature MHC I dissociates from the PLC and traffics to the cell membrane [14].

In general, MHC II molecules present antigens taken up from the environment through the endocytic pathway. Peptides taken up by endocytosis are contained in early endosomes, which will evolve to late endosomes and eventually to lysosomes. In the consecutive compartments the pH decreases, consequently the proteolytic activity increases causing the peptides to be degraded.

Polysomes on the ER produce the α and β chain that form MHC II. A chaperone known as the invariant (Ii) chain appears to be involved in the folding and transport of MHC II. From the ER MHC II is transported via the golgi system to MIIC, the MHC class II containing compartment which has characteristics of a late endosome [3, 34, 37]. The increasing proteolitic activity in MIIC causes the invariant chain to be degraded gradually. Eventually, only CLIP (class II associated invariant chain), the part associated to the peptide binding groove, remains. The exchange of CLIP with an actual peptide is mediated by HLA-DM, a non classical MHC II molecule [3, 34, 37]. This process is regulated by another non classical MHC II molecule, HLA-DO, which binds to HLA-DM thereby decreasing its efficiency (Fig. 4).

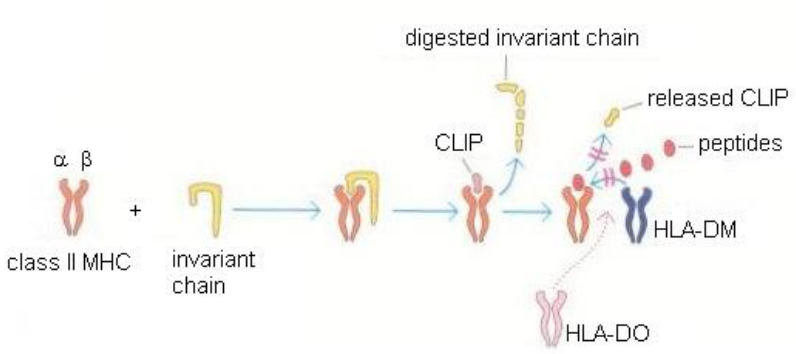

Figure 4. MHC II maturation [14]. The invariant chain adheres to immature MHC II and is involved in the correct folding. Furthermore, the Ii chain facilitates transport of MHC II from the ER via the golgi system to MIIC [3, 34, 37]. The increasing proteolitic activity gradually degrades the Ii chain until only CLIP is left. Subsequently, HLA-DM facilitates the exchange of CLIP with a peptide. A process regulated by HLA-DO, which decreases HLA-DMs efficiency by adhering to it [3, 34, 37].

Vaccination strategies

Herpesviruses give rise to life long infections and some are associated with or cause severe and sometimes even lethal disease. Vaccination would provide a solution to these problems. Nowadays there are several strategies for prophylactic vaccination.

There are two main types of vaccination, passive and active. With passive vaccination the components necessary to fight the infection are introduced into the body. Therefore, the patient will not induce an immune response, thus no memory will be built up [14]. In active vaccination the pathogen or part of it is introduced into the body, what results in the generation of an immune response. Memory will be built up thus when encountering the actual pathogen one is protected [14].

When active vaccination is desired, there are several designs. Live attenuated vaccines are pathogens which are no longer capable of causing disease but have retained the ability to grow in the host. The advantages of these vaccines are that they provide a strong immune response due to prolonged exposure of the immune system to several epitopes. Therefore a single immunization is often enough for life long protection. A major disadvantage is the possibility that the pathogen mutates and becomes virulent again. Another concern is the fact that sometimes complications seen in the natural disease are also induced by the vaccine [14].

An alternative is vaccination with inactivated or death pathogens. Important is to realize that the structure of the epitopes need to be conserved in the dead or inactivated pathogen for them to be able to elicit an useful immune response. These vaccines provoke a good Ab response though are less effective in inducing cell mediated immunity. In general they are safer in use than attenuated vaccines though here the danger lies in incorrect inactivation which leads to infection [14].

An even safer alternative are the subunit vaccines in which only specific, purified macromolecules are introduced in our body. For example viral glycoproteins form good candidates.

In DNA vaccines plasmid DNA molecules encoding for a certain antigenic protein are injected in muscle tissue. This way the protein is produced in a natural setting, with the correct 3D structure and elicits a strong humoral and cellular response. Lastly, an attenuated virus which is genetically modified so it contains genes encoding proteins of another pathogen can be used for immunization.

Unfortunately it is not that simple to design vaccines for herpesviruses. As mentioned above, herpesviruses have developed many mechanism to avoid immune detection and modulate the immune response. For instance, HCMV and EBV are able to secrete an IL-10 homolog.

When viral IL-10 (vIL-10) is secreted into the DC-T cell synapse, T_{H0} cells are stimulated to differentiate into T_{H2} cells which are not very helpful in a viral infection. Additionally, when vIL-10 is produced outside of the synapse, regulatory T cells can be produced which downregulate the immune response [34]. In the following chapters the immune response, the immune evasion strategies and the status of vaccine development of each of the clinically most relevant human herpesviruses are discussed in more detail.

Herpes Simplex Virus 1 and 2

HSV can not penetrate the intact stratum corneum (Fig. 5). Thus, infection occurs at skin lesions or at the oral or genital mucosal regions, which lack a stratum corneum [38]. HSV enters cells using its surface expressed glycoproteins. First, glycoproteins gB and gC adhere to their receptor, a heparin sulfate proteoglycan. Next, gD adheres to its receptor, nectin 1, after which the viral envelope fuses with the plasma membrane of the cell. Subsequently, the capsid containing the viral genome is released into the cell [5, 39].

Replication takes place in epidermal cells and results in the release of numerous cytokines including IFN-α, -γ, Il-6, -12 and β-chemokines [38, 40].

The β-chemokines recruit monocytes and T cells into the infected area. $CD4^+$ T lymphocytes are the first T cells to infiltrate these areas [41]. Under the influence of IL-12 and IFN-γ the Th cells differentiate into Th_1 cells. Subsequently, the Th_1 cells act as an important IFN-γ source which restores MHC I expression in infected cells, blocked by the viral protein ICP47 (see immune evasion). Additionally, IFN-γ induces MHC II expression on keratinocytes making them a target for the $CD4^+$ T cells. Cytotoxic effects of $CD4^+$ T cells have been observed against cells presenting Ags derived from the HSV-2 tegument proteins VP16 and 22 in the context of MHC class II [41, 42]. Besides IFN-γ production and cytotoxic effects, $CD4^+$ T cells also secrete lymphokines, inhibit of viral growth, support humoral and $CD8^+$ responses and can have cytotoxic effects [42]. Epitopes targeted by $CD4^+$ T cells are surface glycoproteins gD and gB whereas the $CD8^+$ T cells often target ICP27, an early protein [38]. The upregulation of surface expressed MCH I molecules allow the $CD8^+$ T cells to infiltrate the infected areas. There they will recognize and eliminate the infected cells [38, 41]. Infiltration of cytotoxic $CD8^+$ T cells in the infected skin correlated with virus eradication from these sites [38, 43].

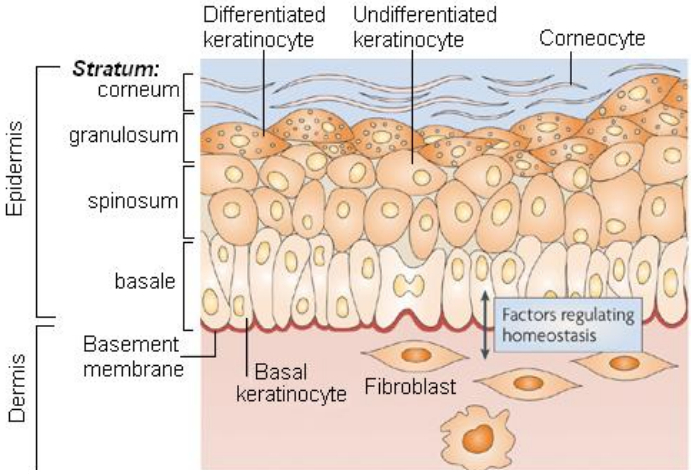

Figure 5 . An overview of the human skin layers [116].

Once the immune system controlled the primary infection, HSV remains in our body in a latent state. As mentioned earlier, reactivation of HSV can be triggered by stress, menstruation, UV irradiation etc. [5]. During HSV latency gene expression is regulated very tightly, only latency associated transcript (LAT) is transcribed. It has been suggested that LAT is involved in the establishment and maintenance of latency [5]. Other factors involved in the establishment of latency are both the innate and adaptive immune response. Research indicated that SCID (severe combined immune deficiency) mice, which lack the adaptive immune response, could only partially control HSV-1 infection in the sensory neural ganglia. When αβ T cells were administered to these SCID mice their ability to control HSV-1 was fully restored [44].

Further research demonstrated the presence of $CD4^+$ and $CD8^+$ T cells in ganglia containing infected neurons, indicating that they play a role in controlling HSV infection. $CD8^+$ T cells specific for HSV structural proteins lay opposite of the latently infected neurons and secrete IFN-γ, thereby preventing reactivation. Experiments have indicated that IFN-γ might function through inhibition of the immediate early protein ICP0 [38, 44]. This is described in more detail by Khanna and colleagues [44]. The role of the $CD4^+$ T cells, also found in the latently infected ganglia, is unknown at this moment [44].

Recently, research on HSV infections has focused on the details of the immune response elicited. One of these studies highlighted the importance of TLR3 on $CD8\alpha^+$ DCs in priming a $CD8^+$ T cell response to HSV-1 [45]. The absence of TLR3 led to a reduced T cell response which resulted in impaired viral control [45].
Another study investigated the importance of cross-presentation (presentation of exogenous Ags on MHC I molecules) by DCs [39]. This study also indicated that the $CD8\alpha^+$ DCs subset is the main contributor to the activation of naïve virus specific cytotoxic T lymphocytes (CTLs). Interestingly, this subset is good in cross-presentation [39]. Jirmo and colleagues provided evidence that although cross presentation amplifies the T cell response it is not absolutely necessary for a response [39].

The information regarding the antibody response elicited during primary infection with HSV is dated, but suggests that the presence of neutralizing Abs prevents the virus from spreading when administered 24h postinfection. This was shown in a mouse model [46].
There is no indication of ADCC and although Abs are present, this does not prevent the virus from being reactivated. Abs are unable to prevent the virus from spreading by a cell-to-cell strategy which is found in tissue monolayers [46].

Immune evasion

Certain essential components of the human immune system can be targeted by HSV, allowing the virus to hide from the immune system and persist in our bodies. Interfering in Ag presentation is a popular mechanism to evade the immune system. HSV encodes for several proteins which interfere in this process. One of them is the plasma membrane associated TAP inhibitor ICP47. Its cytosolic domain, competitively binds the peptide binding domain of TAP. However, the binding affinity of ICP47 for TAP is higher than the binding affinity of other peptides. Consequently, once ICP47 adhered to TAP it will not release it anymore [47]. ATP binding is not influenced by ICP47 although the hydrolysis of ATP stimulated by peptide binding is inhibited [47, 48]. Another study suggested that ICP47 induces a conformational change which might destabilize and inactivate the transporter [49].

Trgovcich and colleagues showed that cells infected with HSV-1 had a reduced number of MHC II molecules on their membrane compared to mock transfected cells [34, 50]. This was confirmed by Temme and his colleagues who indicated that the surface expressed glycoprotein gB is the cause. They observed that gB associated with the MHC II haplotype DR after the degradation of the Ii chain, thus in a post golgi compartment [51]. Once DR is associated to gB, it will no longer be expressed on the cell surface. Instead, the vesicles in which gB-DR are located display exosomal markers such as CD63, indicating that gB interferes with the trafficking of DR. Exosomes are endosomal derived vesicles located in the MBV and released in the extracellular space once a MBV fuses with the plasma membrane [52].

Previous research indicated that ubiquitination of proteins is involved in trafficking of vesicles to multi vesicular bodies (MBVs). In agreement with this, Temme and colleagues observed that gB was extensively ubiquitinated which probably provided the signal to traffic the gB-DR containing vesicles to the MVB [51].

In glioblastoma cells *vhs* and ICP34.5 encoded by HSV-1 are able to reduce the number of MHC II molecules on the cell surface. *Vhs* encoded by the UL41 gene, is a host shutoff protein that degrades viral and host mRNA probably including the MHC I and II mRNAs[34, 50, 53]. The mechanism behind the decreased surface expression of MHC II by ICP34.5 is still under debate [50]. The ICP34.5 protein has various other effects, among others it has been suggested to play a role in autophagy, inhibiting protein synthesis and determining the host range [54-56].

Aside from modulating Ag presentation, IFN responses are modulated. Humans encode three types of IFN; type I consisting of IFN-α, -β, -ε, -κ and –ω, type II consisting of IFN-γ and type III consisting of IFN-λ_{1-3}. Type I and III IFN are produced by many cell types whereas type II is only produced by NK and T cells [57]. Normally, when a host cell senses the presence of a virus, the IFN pathway is activated. This pathway consists of three phases. The first one is the sensitization phase in which the pattern recognition receptors of the innate immune system sense the presence of the virus and induce low levels of IFN-β. In the second phase, known as the inductive phase, the secreted IFN-β adheres to its receptor and signals through the JAK and STAT pathway. This results in the production of IFN-α and a number of interferon stimulated genes (ISGs). During the last phase, the amplification phase, IFN-α and -β induce the transcription of many ISGs which limit the spread and replication of the virus [57]. An example of an ISG, is the protein kinase R (PKR). This protein will phosphorylate the α subunit of eukaryotic initiation factor 2 (eIF-2), thereby inhibiting protein synthesis [57]. In Figure 9 (p36) an overview is provided of IFN type I activation in a normal infection.
However, HSV encodes for several proteins that will interfere with this pathway. ICP0 is one of those proteins ,though instead of blocking the pathway at a single step, this protein targets multiple steps. Among others, ICP0 can inhibit trafficking of IRF3 to the nucleus while mediating its degradation, inhibit IRF7 mediated induction of ISGs and interferes with STAT-1 signaling. [57]. Another protein known to interfere with the type I IFN pathway is ICP34.5. The N terminus of this protein is able to form a complex with TBK-1, the kinase that activates IRF3 and 7, and inactivates it [57]. A last example of type I IFN pathway interference is protein US11. This protein has, among others, the ability to inhibit PKR thereby allowing protein synthesis to continue [57].

Additional proteins with a known immune evasive function can be found in table 4 .

Table 4. An overview of the immune evasion proteins encoded by HSV [5, 57].

Viral protein	Mechanism
Ag presentation	
Vhs	Digest host mRNAs (reduces MHC I and II surface expression)
ICP47	Inhibits TAP
ICP34.5	Reduces MHC II surface expression [50]
gB	Traffics MHC II to the exosomes instead of cell surface [51]
IFN production	
ICP0	Inhibits IRF-3 and -7mediated ISG production Antagonizes STAT-1 activation Degrades PML Interferes with RNase L-independent rRNA degradation
ICP27	Inhibits IRF3 and STAT-1 activation Inhibits eIF-2α phosphorylation
ICP34.5	Interacts with TBK thus prevents IRF3 phophorylation Inhibits eIF-2α phosphorylation and promotes dephosphorylarion of phosphorylated eIF-2α
US11	Interacts with PKR and prevents eIF-2α production
Vhs	Interferes in ISG transcription Inhibits STAT-1 phosphorylation Disrupts STAT-1, -2, p48 complex Prevents eIF-2α phosphorylation
US3	Modulates TLR3 transcript levels Posttranscriptional modification of type II IFN receptor resulting in inhibition of ISG induction
Other	
US3	Inhibits TCR dependent effecter function of CTLs [58]
gC	Accelerates C3b decay of the alternative pathway [59]
gE	Binds the Fc domain of IgG Blocks C1q binding and ADCC [59]

Vaccine

HSV-1 infects 60 to 80% of the world population while HSV-2 infection differs per country and varies between 4% and 80% [21, 38]. There are a few indications for vaccine development. First, HSV-2 appears to enhance the change of acquiring HIV-1 by a 2 or 3 fold [38]. Second, HSV-1 is causing increasingly more genital herpes, particularly in adolescents [18, 38]. Lastly, life threatening diseases are associated with HSV infection in newborns and immunocompromised individuals [12].

It has been observed that, preexisting immunity against HSV-1 will reduce the severity of a HSV-2 infection which might implicate that one vaccine can be used to vaccinate against both viruses [60].

A good prophylactic vaccine would induce a solid immune response that protects the susceptible population from infection. Nevertheless, an imperfect vaccine could also be beneficial [61]. Especially when this vaccine would reduce the virus' infectivity or frequency of shedding. Such a vaccine would result in a decreased incidence of infection. On the other hand, vaccines which only have a moderate effect on the acquisition of the virus would be far less effective [61].

Creating a strong $CD8^+$ T cell response is an important property of both therapeutic and preventive vaccines [39]. In order to be able to induce a solid immune response, detailed knowledge of the mechanisms inducing the immune response upon infection is necessary [39].

Thus far, there is no vaccine for HSV-1 or -2. However, recently a recent study Brans and colleagues looks promising. They tested whether the life attenuated CJ9-gD vaccine was able to protect guinea pigs from primary and recurrent infection of wildtype HSV-2. The use of a life replication deficient virus has risks, especially in latently infected individuals. The major risk is that the virus regains replication competence or reactivates the latent wildtype virus [18]. To minimize these risk a new type of vaccine was developed. The CJ9-gD vaccine strain was unable to replicate and inhibited viral DNA replication of wildtype virus upon coinfection. Additionally, its UL9 gene, encoding for an immediate early protein, is replaced by the late gene encoding gD. Thereby, gD is expressed during the early phases and stimulates a strong immune response. In mice this vaccine elicited long lasting humoral and cellular immune responses against both HSV-1 and -2 [62]. However, whether the vaccine also prevents recrudescence's is unknown since spontaneous reactivation of HSV rarely occurs in mice. In guinea pigs, HSV can spontaneously reactivate like in humans, however there are no reagents available to fully characterize the immune response elicited by guinea pigs thus mice are still often used [18, 38].
The vaccinated animals had a similar Ab response after challenge compared to mock vaccinated animals, although virus replication was significantly reduced. The vaccine also protected against lesions. The guinea pigs that did develop lesions had fewer and less severe lesions than the mock immunized animals. In addition, the number of HSV copies found in the ganglia was greatly reduced in vaccinated animals and no recurrent viral shedding was observed up to 30 days after recovery from the challenge with wildtype HSV-2 [18]. Thus, this vaccine appears to be very promising. However, previous vaccine candidates, like the subunit vaccine gD2/AS04 appeared promising in guinea pigs though during the phase III clinical trail it provided approximately 73% efficacy against HSV-2 infection in HSV seronegative women, not in men [60]. This suggests that the long coevolution between HSV and man limits the power of animal models in predicting the abilities of a vaccine [63].

A remarkable feature of HSV infection is that some people appear to be resistant to infection or disease. These people are immune seronegative (IS), have no clinical or virological evidence indicating infection though do posses HSV specific T cell responses [19]. Research indicates that the T cell repertoire of these individuals target different epitopes than T cells of HSV-2 infected individuals. The T cells of IS individuals mainly target epitopes of immediate early proteins of HSV-2. How these altered T cell responses offer protection is not clear yet. Future research on these individuals may lead to new ideas regarding vaccination against HSV-2 [19].

Varicella zoster virus

Different to other herpesviruses, VZV spreads through the respiratory route. It will infect the mucosa of the upper respiratory tract, most likely by adhering to a heparin sulfate proteoglycan and subsequently a lower affinity receptor before cell entry [22]. From the respiratory tract the virus spreads to the regional lymph nodes, liver and reticuloendothelial cells. Once in the regional lymph nodes, $CD4^+$ and $CD8^+$ T lymphocytes become infected and spread the virus throughout the body to the skin [22].
The early immune response of the host to VZV is non specific and consists of NK cells and IFN-α producing T lymphocytes [22, 24]. These responses limit the replication and spreading of the virus through our body. IFN-α is a cytokine known to induce resistance to viral infections, inhibits cell proliferation and regulates MHC I expression [14]. The protective effect of IFN-α was established by the reduced severity of varicella disease in immunocompromised children when IFN-α was administered [24]. A product of NK cells involved in VZV infection is granulysin. Granulysin acts to overcome the inhibited apoptosis in VZV infected cells [24]. Whether VZV can evade these initial antiviral host responses is not known yet.

The onset of specific T cell responses is essential for clearing acute varicella, prevention of reinfection and reactivation. A delayed onset of VZV specific T cell responses, can lead to persistent viremia and life threatening dissemination [22, 24].
During the primary infection, $CD4^+$ Th_0 cells are predominantly stimulated to become VZV specific Th_1 cells. These cells produce high amounts of IFN-γ which induces among others MHC II expression on unprofessional APCs, such as epithelial cells. Other cytokines produced by Th_1 cells are IL-2, IL-10 and IL-12. Additionally, $CD4^+$ Th_1 cells can induce MHC II restricted cytotoxicity on infected cells [24]. Similarly, VZV specific cytotoxic $CD8^+$ T cells, induced during primary infection, can elicit MHC I restricted cytotoxicity on infected cells [22]. Targets for cytotoxic $CD4^+$ or $CD8^+$ T cells, are cells expressing gE, gI, gC or IE62 in MHC II or I context, respectively [22].
A primary infection also elicits a strong humoral response including IgG, IgM and IgA Abs. These Abs are directed against multiple viral proteins, varying from surface expressed glycoproteins to viral enzymes [22, 24]. In the case of a VZV infection, the Abs are either neutralizing or induce antibody mediated cellular toxicity. However, despite these effects, the role of Abs in protection against a primary VZV infection is thought to be small due to several reasons. First, the onset of these Abs is slow, in healthy individuals they appear 3 days after the onset of symptoms. Second, children with agammaglobulinemia, which are unable to produce Abs, undergo a normal uncomplicated varicella disease course [22]. Moreover, the administration of VZV specific immunoglobulins to immunocompromised children after the appearance of skin lesions, did not effect the disease course [22, 24]. On the other hand, when VZV specific IgG Abs were administered to immunocompromised patients within 72h of exposure, the infectivity and replication of VZV was limited [22]. Thus, Abs might play a role in preventing reinfection and recrudescence's but are induced to slow to play a role in primary infection.

Although healthy latently infected individuals are protected against reinfection, exposure to the virus boosts the T cell responses and Ab titers [24]. Subclinical reactivation of varicella may also contribute to the maintained immune response, though this is difficult to prove.

A study performed by Wilson et al. (described in [22]) provides evidence supporting this suggestion. Wilson and colleagues were able to detect VZV reactivation, using PCR, in stem cell transplant patients with no clinical signs of herpes zoster [22].
Despite a solid, maintained immune response, reactivation causing herpes zoster can occur. The reactivation will boost the immune response against VZV, making a second episode of herpes zoster are rare event [5, 22].

Immune evasion

Since reactivation of VZV is possible, VZV must have immune evasive properties, allowing them to evade the specific $CD4^+$ and $CD8^+$ T cells and reach the skin [24]. Other indications, describing the need for VZV immune evasion, are during the inoculation period and latency. During inoculation, immune evasion is necessary for the virus to be able to infect dermal cells, while during latency, established in both satellite cells and neurons of the dorsal root ganglia, MHC I expression in the satellite cells must be controlled [5, 24, 64].
On the other hand, if VZV would induce a lethal infection, the virus can not establish latency and cause recrudescence's which is not beneficial for the virus. Hence it is thought that the immune evasion observed during acute varicella is partial or transient [24].

As part of the immune evasion, VZV can decrease surface expression of MHC I in $CD8^+$, $CD4^+$ and $CD4^+/8^+$ T cells. Experiments with phosphonoacetic acid (PAA), an inhibitor of viral DNA replication, indicated that the protein or proteins responsible for the decrease in MHC I surface expression are immediate early or early proteins [24]. VZV does not encode for homologs of known MHC I interfering molecules [24].
Thus, to test whether immediate early or early genes play a role in MHC I downregulation, several genes were cloned into a plasmid with which cells were transfected. This experiment indicated that the early gene encoded by ORF66 is able to downregulate MHC I. In contrast, immediate early genes ORF4, ORF61, ORF62, ORF63 and early genes ORF10 and ORF47 are unable to alter the expression of MHC I on the surface of human foreskin fibroblasts (HFF) [64].
The specific mechanism by which ORF66 works is not clear yet. It is known that the kinase domain of this protein is necessary for the downregulation of surface expressed MCH I. This indicates that phosphorylation is of importance. Currently there are two hypothesis, the first suggests that TAP is phoporylated by ORF66 thereby hindering peptide transport (though peptides and ATP can still bind). Alternatively, ORF66 can phophorylate the cytoplasmic tail of MHC I, which usually occurs at the cell surface and late or recycling endosomes. Thus, phophorylation of MHC I tails in the pre-golgi-complex could result in deviated trafficking [65]. Since the MHC I molecules are not retained in the ER but are localized in the Golgi compartment of VZV-infected cells [64], the latter hypothesis is the preferred one. To prevent NK cell-mediated lysis, viruses can encode for MHC I homologs or like HIV only downregulate certain MHC I alleles while leaving the expression of others intact. The latter appears to be the mechanism by which VZV avoids the immune system [24].
Although ORF66 is the only known mechanism of MHC I downregulation by VZV, studies with VZV lacking ORF66 also indicated a decreased surface expression of MHC I. Thus, indicating VZV encodes for multiple mechanisms that interfere with MHC I expression [65].
Furthermore, ORF66 appears to be involved in more processes, it also inhibits apoptosis of T lymphocytes by downregulating caspase 3 [65]. The prolonged survival of infected T lymphocytes might contribute to the spread of the virus to the skin [65]. Additionally, ORF66 might play an

important role in latency, suppressing nuclear translocation of VZVs transcriptional transactivators and preventing Ag presentation [65].

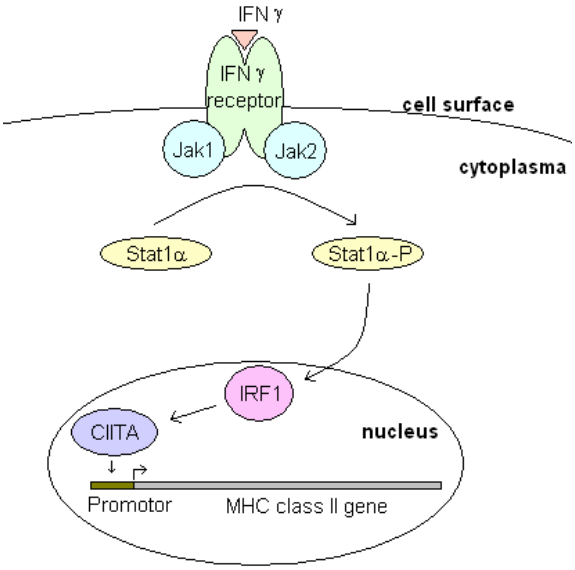

Figure 6. IFN-γ induced MHC II upregulation [1]. This figure shows the normal pathway of IFN-γ induced MHC II upregulation. However, when cells are infected with VZV, the expression of Jak2 and Stat1α is decreased thereby inhibiting their downstream effects.

Besides MHC I interference, VZV can also inhibit the induction by MHC II expression on unprofessional APC by IFN-γ (produced by VZV specific $CD4^+$ Th_1 cells). The normal pathway by which IFN-γ can induce MHC II expression is through the Jak/Stat pathway as shown in Figure 6. In VZV infected cells Jak2 and Stat1α protein synthesis is reduced thereby inhibiting IFN-γ signaling and thus its downstream effect of MHC II induction [1, 24].
However, cells exposed to IFN-γ prior to infection have normal levels of MHC II on their surface. VZV infection can not reverse IFN-γ induced MHC II expression. Thus, IFN-γ producing $CD4^+$ Th_1 cells prime cells and make them more vulnerable for the immune system upon infection. In this manner spreading of the virus is limited [1].

It is thought that VZV has more mechanisms to evade the immune system though most are not or ill defined so far. Other potential immunomodulators are ORF17, a virion host shutoff protein ortholog, which can induce mRNA degradation [65]. The gE/gI complex on VZV infected cells can act as Fc receptor which might block the anti viral effect of the VZV specific Abs. Downregulation of IFN-α in infected epidermal cells might also contribute to immune evasion although the neighboring cells can create an IFN-α response large enough to prevent cell-to-cell spread [5]. Thus, additional research is required to gain a better insight in the immune evasive properties of VZV and how the immune system protects infected individuals from reinfection upon exposure to new VZV virions. (

Vaccine

Varicella zoster infection is hard to prevent because individuals are contagious 24 to 28h before onset of symptoms [22]. Fortunately, there are several treatments to decrease disease severity and there is even a vaccine against primary infections and recrudescence's.
As mentioned above, the administration of VZV specific IgG Abs within 72h of exposure can decrease the severity of disease.
This treatment is recommended for immunocompromised individuals, who are in close contact with a patient suffering from herpes zoster [22]. Important to realize is that this treatment is not effective against primary infection after the onset of symptoms and does not protect against recrudescence's [22].
After the onset of symptoms, antiviral agents such as acyclovir can control the severity of varicella and herpes zoster. These drugs can not prevent reactivation, which was proven in bone marrow transplant patients. These patients were given antiviral drugs after transplantation, though instead of

decreasing the incidence of herpes zoster the onset was just postponed until the treatment was stopped [22].
The third strategy in the fight against VZV is a life attenuated vaccine (VZV Oka strain) developed in the 1970s by Takahashi et al. [22]. Since 1995, a similar vaccine became available on the American market, known as Varivax. This vaccine was tested in over 7,000 children and 1,600 healthy, susceptible adults. None of them showed any clinical symptoms after vaccination, even if the vaccine contained over 9,000 PFU of infectious virus per dose [22]. Additionally, the vaccine elicited cellular immunity and Abs. The formed cytotoxic T cells were able to lyse VZV expressing cells like those formed during a natural infection. Moreover, the amount of cytotoxic T cells was similar in vaccinated individuals and those who had a natural infection [22]. Persistence of T-lymphocytes against VZV has been documented for up to 6 years in healthy children given the vaccine [22].
Next, the vaccine was tested on children who were in remission of leukemia for over a year with an absolute lymphocyte count over 700. In order to prevent disease after household exposure to VZV [22]. In these immunocompromised children it was harder to induce a persistent cell mediated immune response. These studies indicated the importance of cell mediated immunity, since the children with the lowest cell mediated immune response had recrudescence's of the vaccine virus. Thus, different from most vaccines, the VZV vaccine strain is able to induce latency. However, the frequency of herpes zoster in vaccinated high risk children was lower than in leukemic children with a latent wildtype VZV infection [22].

Although the vaccine has positive effects it is not licensed for general use in immunocompromised children because approximately 50% of the leukemic children developed a rash limited to the site of inoculation or spread over the entire body. This rash did allow viral transmission to other susceptible individuals while the transmission of the vaccine is otherwise far lower than that of wildtype VZV [22]. Another strategy to protect immunocompromised children from household exposure, is the vaccination of the susceptible household contacts [22].

Besides the immunocompromised individuals the elderly also have an increased risk of developing herpes zoster. The approximated change of 25 to 35% to develop herpes zoster, represents older and/ or immunocompromised individuals [22, 23]. In elderly individuals the cell mediated immunity (CMI) declines therefore recrudescence are thought to occur when the protective immunity falls below a certain threshold required to maintain latency [5]. Since exposure to the virus boosts the immune response, vaccination against recrudescence's has been developed [23]. Additionally, patients who became immunocompromised, due to disease or medication, also have an increased risk to develop herpes zoster [23].
The difficulties and costs in treating herpes zoster and its most common complication, post herpetic neuralgia form a strong argument for vaccine development [23]. Zostavax® is a live attenuated VZV vaccine that is able to boost the VZV specific cell mediated immune responses (CMI). It thereby reduces the incidence of herpes zoster with 51%, the incidence of post herpetic neuralgia with 67% and when an individual does develop herpes zoster despite vaccination, the severity of disease is less [23]. The boosted VZV specific CMI responses were persistent in the 6 year follow up period.

Alternatively, the safety of the vaccine implicates that this strain would also form a good vector in immunotherapy against other pathogens [22].

Human Cytomegalovirus

HCMV elicits a strong immune response in all arms of the immune system [11]. Primarily, HCMV infects APCs like blood monocytes and even though no viral replication occurs in monocytes, latency can be established [66].
Monocytes can differentiate into macrophages and DCs. There are two main types of DCs; the myeloid DCs (mDCs) and plasmatoid DCs (pDCs). The mDCs regulate Th_1 and Th_2 responses of the adaptive immune system by modulating IL-12 secretion. In contrast, the pDCs, though very similar in Ag presentation, trafficking and maturation, secrete type I interferons (IFNs) in response to a virus infection. Consequently, pDCs are essential for innate immunity and only influence the adaptive immune system through downstream effects on B- and T-cell responses [66].
Once monocytes differentiate viral replication becomes possible. Therefore, it is thought that reactivation of latent HCMV infections is associated with differentiation of latently infected monocytes [66]. HCMV will influence several functions of these APCs as reviewed in Varani et al. [66].

Infection with HCMV results in the generation of many Abs directed against gB. Evidence suggests that these Abs limit the dissemination and severity of disease [11]. Besides the humoral response, HCMV also elicits a strong cellular response which is the predominant mechanism for controlling HCMV replication. Loss of $CD8^+$ T cells in the mouse or rhesus macaque model showed an increase in CMV reactivation. Additionally, the presence of high levels of IE1 specific $CD8^+$ T cells in heart and lung transplant patients was correlated with protection of HCMV disease [11]. Furthermore, a study by Dunn and colleagues suggested that in healthy seropositive adults HCMV frequently restimulated the HCMV specific $CD4^+$ and $CD8^+$ T cells, an indication of subclinical HCMV reactivation [67]. Another observation supporting this suggestion is the variable size of HCMV memory $CD8^+$ T cell pool. Normally, in a viral infection the memory $CD8^+$ T cell pool increases during the acute phase which is followed by a contraction when the infection is cleared [11]. However, even during a latent HCMV infection the memory $CD8^+$ T cell pool is expanding and contracting similar as in the acute phase (though at lower levels) thus hinting toward subclinical reactivation [11, 67].
Aside from the role of $CD8^+$ T cells, the $CD4^+$ level might also be associated with disease outcome. Studies have indicated that $CD4^+$ cells can play a role in cytotoxic killing of virus-infected cells. For instance, from healthy HCMV seropositive individuals it has been possible to isolate gB-specific cytotoxic $CD4^+$ T cells [11].
During primary HCMV infection a massive immune response is elicited, even when the virus is no longer lytically active, over 10% of the circulating memory T cell population is HCMV specific [68]. It is unknown what causes these viruses to elicit such a large immune response. Moreover, the effect of this immune dominancy on the immune response to other pathogens is largely unknown [11]. So far, HCMV infection has been associated with lower success rate of influenza vaccination and it appears to act as a cofactor for disease progression towards AIDS [11].

Immune evasion

Even though HCMV elicits a huge immune response, HCMV encodes for multiple immune evasion proteins (Table 5). Interfering with Ag presentation by downregulating MHC I and MHC II is an important strategy to facilitate immune evasion. HCMV encodes for 6 gene products in the US2-11 region, which are all able to interfere with MHC I Ag presentation [34, 69].

The first protein discussed here is US2. US2 adheres to the newly synthesized MHC I β chains as they enter the ER through the Sec61 complex. Subsequently, US2 mediates retrograde transport of the MHC I β chain via the Sec61 complex. Once in the cytosol, N-glycanase will deglycosylate the MHC I β chain after which it is degraded by the proteasome. Furthermore, in the ER, US2 can recruit already folded MHC I β chains to the Sec61 complex and mediate their transport to the cytosol, followed by deglycosylation and proteasomal degradation [36]. Thus, US2 downregulates MHC I expression by inducing rapid proteasomal degradation of the MHC I β chains.
The second protein interfering with MHC I Ag presentation is US3. US3 is a type 1 membrane protein, produced in the immediate early phase of infection. There are several hypotheses on the mechanism US3 uses to reduces MHC I Ag presentation [49, 70-72]. In the first hypothesis, US3 adheres to tapasin, thereby interfering with the ability of tapasin to optimize peptide loading [71]. This probably relies on the induction of a conformational change that deactivates tapasins catalytic site [71]. This is consistent with the observed downregulation of tapasin-dependent MHC I molecules [71]. In the second hypothesis, the ER retention motif of US3 was proposed to function synergistically or additively with the first mechanism [71]. On the other hand, without this domain US3 still inhibits surface expression of MHC I [71]. Lastly, Park and colleagues identified an additional component of the peptide loading complex, protein disulfide isomerase (PDI). PDI stabilizes the MHC I peptide binding groove which is essential for optimal peptide selection [72]. US3 was found to associate with PDI, resulting in its degradation and thus impaired MHC I Ag presentation [72].
The third protein is US6. This transmembrane protein is located in the ER membrane. The luminal domain of US6 adheres to TAP and induces a conformational change, resulting in the inability of TAP to bind ATP. Hence, without ATP binding and hydrolysis, peptide transport is abolished [49, 69, 73-75].
Similar to US2, the fourth protein US11 induces rapid proteosomal degradation of newly formed MHC I β chains. However, US11 accomplishes MHC I degradation by interacting with both newly formed MHC I molecule and derlin-1, a molecule that extracts misfolded proteins from the ER membrane [68, 69].
Aside from these four MHC I Ag presentation inhibitors, HCMV encodes for two other proteins, US8 and 10, which interact with MHC I molecules. However, these proteins are less efficient as they neither influence cell surface levels of MHC I molecules nor alter Ag presentation to T cells [69]. US8 binds to free MHC I β chains in the ER. However, this interaction does not appear to influence the maturation of MHC I. The function of this interaction is still unknown. Additionally, US8 is mainly localized outside the ER suggesting that this viral protein has other functions as well [76]. The last protein, US10 associates with MCH I heavy chains and delays their maturation. However, MHC I maturation or surface expression is not prevented by US10 [77, 78].

There are several hypotheses why HCMV has so many proteins interfering with MHC I expression. At first, all these proteins impair MHC I Ag presentation via a different mechanism, which compensates for their inability to completely inhibit all haplotypes and/ or peptides. For instance, US6 inhibits peptide translocation by TAP but can not inhibit MHC I binding to peptides formed in the ER itself [73, 74]. Secondly, these genes are expressed at different time points. Thus, several proteins are required to inhibit MHC I expression during the entire lytic cycle [73, 74]. More specifically, US3 inhibits the translocation of MHC I at immediate early times. Next, US2 and US11 are expressed, with a maximal concentration 24h post infection.

Lastly, US6 is expressed with a maximal concentration 72h post infection, corresponding with the minimal time it takes for HCMV to complete its lytic lifecycle [70, 73, 74]. Thirdly, the need for HCMV to suppress MHC I Ag presentation in a wide variety of cells could be a reason for the expression of multiple immune evasion proteins [73, 74].
Downregulation of MHC I seems a good method to avoid detection by cytotoxic T cells. However, cells without MHC I on their surface are a target for NK cell-mediated lysis. To avoid this, the virus also encodes for MHC I homologs that function as a decoy [11]. Table 5 shows some more immune evasive molecules produced by HCMV.

In addition to interfering with MHC I Ag presentation, US2 and US3 also inhibit MHC II Ag presentation. US2 is able to rapidly induce degradation of the HLA-DM α chain, thereby interfering with peptide loading [34]. Additionally, it targets the MHC II complex itself for proteasomal degradation [11].
US3 interferes with MHC II Ag presentation by stably adhering to newly synthesized MHC II complexes and thereby prevents binding of the Ii chain. Without the Ii chain, MHC II can no longer traffic to the MIIC compartment [34, 69].

Table 5. More HCMV immune evasion proteins [11].

HCMV protein	Function
gpTRL11	A viral Fc receptor to block ADCC
gpUL16	Binds nonclassical MHC proteins to avoid NK cell activation
gpUL18	LIR1 ligand (interferes with NK recognition), HLA-G ligand
gpUL27	Chemokines receptor
gpUL33	Orphan chemokines receptor, might be important for viral dissemination
gpUL40	Ligand for HLA-E (interferes with NK recognition)
gpUL78	Orphan chemokines receptor, might be important for viral dissemination
gpUL83	Inhibits proteosomal processing of IE-1 (UL83 = pp65)
gpUL111a	IL10 homolog (inhibits lymphocyte proliferation and MHCI and II expression)
gpUL118	IgG Fc receptor
gpUL144	TNF receptor
gpUL146	IL8 homolog (chemotaxis of neutrophils)
gpUL28	Chemokines receptor (cleaves CC chemokines and might help in viral dissemination)

ADCC is antigen dependent cellular cytotoxicity.

Vaccination

Nearly half of the population in the Western hemisphere and nearly everyone elsewhere is infected with HCMV [79]. Although HCMV infection is asymptomatic and without relapses in immunocompetent individuals, immune incompetent and congenitally infected individuals will display a variety of diseases. Congenitally infected infants can suffer from permanent damage such as sensorineural hearing loss (SNHL), blindness and mental disorders [79, 80]. The economic burden of caring for these children was approximately 1.9 billion dollar annually in the early 1990s in the US [80]. Additionally, HCMV seronegative patients who receive a graft from a HCMV seropositive donor are at risk of developing serious, even life-threatening diseases [79].

Vaccination would provide a solution to these problems. Several groups in the population would benefit from vaccination:

- Infants or toddlers, this would also benefit the mothers who can not be infected by their children any more. Additionally, when these children have children no congenital infection can occur.
- Adolescents, since the chance of HCMV infection increases with the onset of sexual activity and exposure to children (especially those attending daycare).
- Transplant patients, since after transplantation their immune system is suppressed and HCMV can cause disease, especially when the donor was HCMV infected and the recipient was HCMV seronegative [80].

There are clear indications of humoral and cellular defense against HCMV disease. For instance, the number of congenitally infected children from mothers suffering a HCMV relapse during pregnancy is lower compared to those suffering from a primary infection [80].
Indications for cellular defense are the fact that T cell transfer of a HLA-matched seropositive donors to bone marrow transplant recipients reduced the incidence of severe HCMV disease [79]. Also, kidney transplant patients who received a monoclonal Ab against $CD3^+$ lymphocytes have more frequent and severe HCMV infections [79].

The biggest obstacle in research on HCMV is the very narrow host tropism, they only infect humans. Mouse CMV lacks a lot of the immune evasive proteins seen in HCMV thus does not provide a good model. The best animal model would be the chimpanzees but due to ethical difficulties the commonly used animal model for HCMV are Rhesus macaques [69]. In RhCMV the region Rh182-189 encodes for functional homologs of the human CMV US2-11 proteins [69]. More specifically Rh182, 184, 185 and 189 are the ortologs of US2, 3, 6 and 11, respectively [69].

Several clinical trails of both HCMV live attenuated vaccines and subunit vaccines have already been performed, though, thus far non have reached the market.
One of these vaccines is the life attenuated HCMV strain named Towne. This vaccine could elicit neutralizing Abs, $CD4^+$ and $CD8^+$ T cells but was unable to protect renal transplant patients from acquiring an infection after transplantation [80]. Moreover, a study on women at child baring age indicated that the vaccine was also unable to protect HCMV seronegative mothers from acquiring infection of their infected children [80, 81].
One of the main concerns with life attenuated vaccines is the fear for persistent latent infections despite the fact no evidence of this was ever reported according to Schleiss (2008) [80]. Therefore subunit vaccines are preferred and several membrane proteins are considered as a target for these vaccines. One of these targets is gB, since every HCMV seropositive individual contains Abs against this glycoprotein. Another target is pp65 which elicits a strong $CD8^+$ T cell response in naturally infected individuals. Berencsi and colleagues showed that a carnarypox virus could be used as a vector expressing HCMV pp65 and elicit a strong $CD8^+$ CTL and Ab response [79]. Unfortunately, the study did not provide direct evidence of a protective effect caused by these CTLs. Although they claim this vaccine is a promising candidate for HCMV immunization, HCMV has the ability to infect a host several times (superinfection) and establish a life long infection each time even though healthy individuals develop an immune response involving Abs, $CD4^+$ and $CD8^+$ T cell after the first infection [68, 79]. More HCMV vaccine trails are reviewed in Schleiss [80].

Research on rhesus maquaces infected with RhCMV indicated that MHC I evasion facilitated by genes in the US2-11 region are essential in the initial phases of a superinfection. In contrast, a primary CMV infection results in life long infection even when the MHC I evasion genes are missing, suggesting that this primary infection is not controlled by $CD8^+$ cytotoxic T cells in the initial phase [68].
The ability to evade pre-existing immunity makes HCMV a difficult target for vaccine development [68]. It is even questionable whether prophylactic vaccination is possible.

On the other hand, the ability of HCMV to superinfect hosts with pre-existing immunity and establish life long infections make them a good candidate for immunotherapy. In the search of a HIV vaccine Hansen and colleagues used RhCMV as a vector to provide a maintain a robust Simian immunodeficiency virus (SIV) specific $CD4^+$ and $CD8^+$ effector memory T cell (T_{EM}) response [82]. Previous vaccination strategies led to central memory T (T_{CM}) cells which develop too slow to prevent primary SIV infections. However, SIV is transmitted via the mucosa where effector memory T cells (T_{EM}) are dominant. Thus Hansen's hypothesis was that SIV specific T_{EM} cells would lower the viral load and thus reduce spreading and disease progression. The results looked promising, it appears that T_{EM} biased T cell immunity is able to reduce progressive systemic disease after mucosal challenge with SIV. Thus, suggesting that HIV vaccines with a T_{EM} component may protect against sexual transmission of HIV [82].

Epstein Barr virus

Over 90% of the human adult population in the world carries the Epstein Barr virus. In general, EBV is harmless, although severe life threatening malignancies are associated with EBV infection in immunocompromised individuals [31].
Primary EBV infections elicit a strong cellular immune response against both lytic and latent antigens. The induced $CD8^+$ and $CD4^+$ T cells are of vital importance to resolve primary infection and induce a persistent latency [15, 31]. When an individual is infected during infancy, the infection usually passes asymptomatically. However, when an individual is infected during adolescence, the infection can cause infectious mononucleosis (IM) [83]. The difference in disease course is thought to be caused by the more severe immune response adolescents elicit upon exposure to EBV.

During primary infection the epithelial cells of the oropharynx are lytically infected. From there the virus spreads to the lymphoid tissue where B cells are latently infected [15]. B-cell entry is mediated by the attachment of envelope protein gp350/220 to complement receptor CD21, followed by binding of gp42 to MHC II [13].
Currently, there are two hypothesis regarding B cell infection. The first hypothesis suggests that naïve B cells are infected which subsequently differentiate into memory B cells by passing through a germinal center (Fig. 7). The second hypothesis claims that the memory B cells are directly infected (Fig. 7). However, both hypothesis do not fully explain the observed changes of the tonsilar B cells in patients with IM. The infected B cells in these patients localize to the extrafollicular areas, not to the germinal centra as suggested by the first hypothesis. On the other hand, the second hypothesis fails to explain the disappearance of infected naïve B cells [15].

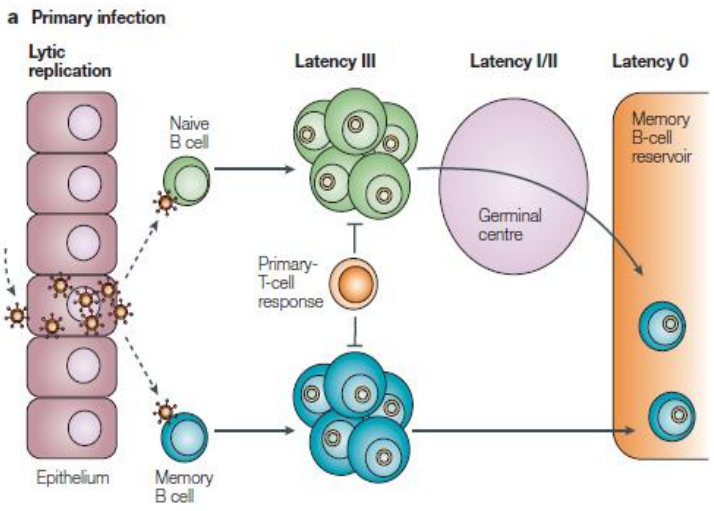

Figure 7. Hypothetical course of primary infection [15].
During primary infection the epithelial cells of the oropharynx are lytically infected. From there either the naïve B cells, which will subsequently differentiate into memory cells, or the memory B cells are infected. It is thought that B cells are initially infected with a latency type III infection. Subsequently, the primary T cell response controls this infection and drive the virus to a more quiescent state known as latency type I or II. Over time, the virus is even further suppressed and persists in a latency type I or 0 [15].

Unlike other human herpesviruses, EBV can induce several types of latency resulting in a very complicated infection. The major difference between the latency types is the amount of genes brought to expression. In latency type 0 no viral proteins are expressed whereas during latency type I EBNA1 is present. [84]. EBNA1 is essential for maintaining the viral genome in dividing cells [84]. It starts viral DNA replication from the latent origin of replication and thereby promotes segregation of the latent EBV genome when cell division takes place [13]. Without this protein the viral genome would be diluted and eventually is lost during successive cell divisions [85]. In latency type II even more proteins are expressed, besides EBNA1, LMP1 and 2 are brought to expression. LMP1 consists of three domains; a short N-terminal cytoplasmic tail, six transmembrane domains and a long cytoplasmic C-terminal tail with a CTAR1 and CTAR2 domain. These CTAR domains are able to constitutively activate the CD40 signaling pathway which normally provides the costimulatory signal for B cell activation and differentiation [14, 15].
LMP2 has 12 transmembrane domains and a long C-terminal tail with 8 tyrosine residues. These tyrosine residues, are involved in the activation of several signaling pathways that mimic B cell receptor (BCR) activation (described in more detail by Young et al. [15]). Thus, B cells infected with latency type II EBV are able to differentiate without the presence of external stimuli. Lastly, in latency type III, EBNA2, EBNA-LP, EBNA3A, B and C as well as BHRF1 are translated on top of the proteins expressed during latency type II. The nuclear proteins EBNA2 and EBNA-LP, are involved in activation of gene transcription, while the EBNA3 protein family counteracts these effects [15]. Furthermore, BHRF1 is a homolog of the anti-apoptotic protein Bcl-2 [15].
To complicate EBV latency even further, non coding RNA transcripts are produced during all types of latency. Part of these transcripts (the miRNAs known as BARTs) can silence mRNA by adhering to their complementary mRNA sequences (Fig.8) [84]. Figure 8 provides an overview of the different latency associated genes, RNAs and their genomic localization.

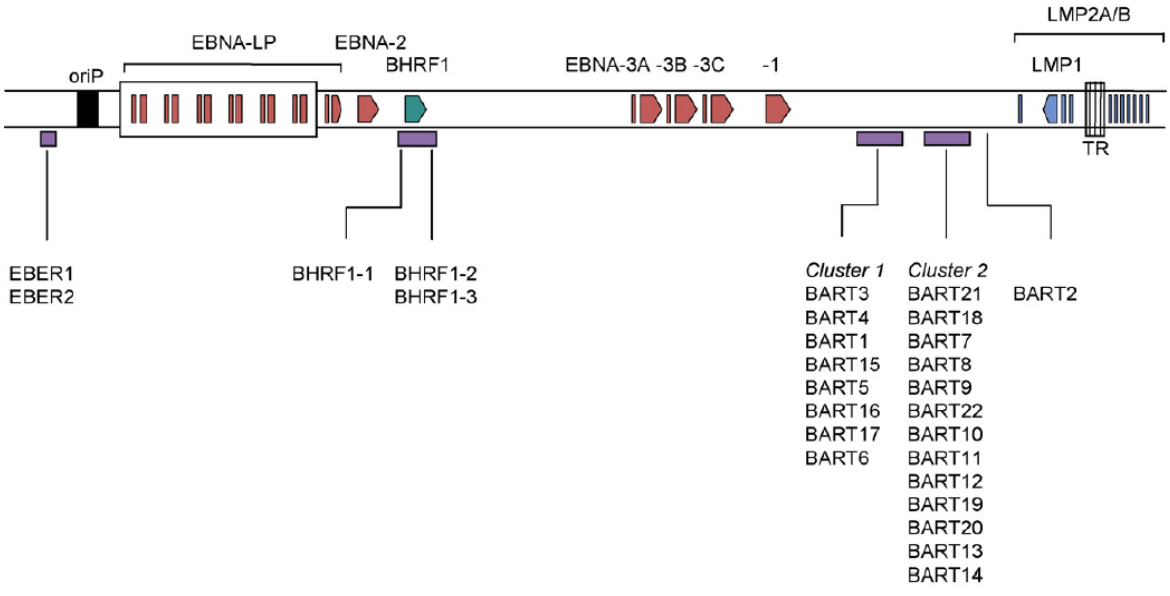

Figure 8. An overview of the genes and RNAs expressed during EBV latency [84].
There are two types of RNAs transcribed during latency. Namely, BamH1 A rightward transcripts (BART) and EBER, RNA polymerase III transcribed small non polyadenylated RNAs. BARTs are miRNAs which silence mRNAs with a complementary sequence. The function of EBER is unknown [84].

It is thought that B cells are initially infected with a latency type III infection. The primary T cell response controls this infection and drives the virus to a more quiescent state known as latency type I or II. Over time, the virus is even further suppressed and persists in a latency type I or 0 (Fig. 7) [15]. In immunocompromised individuals, the lack of a good T cell response allow latency type III infections to persist. Phenotypically the EBV growth transformed B cells (latency type III) seen *in vivo* are similar to the lymphoblastoid cell lines (LCL). Therefore, these cell lines provide a good *in vitro* cell model [31, 84].

Immune evasion

EBV has many immune evasive properties which are mainly expressed during the lytic lifecycle. Hence, most of the during primary infection induced $CD8^+$ T cells are specific for immediate early and early genes. Remarkably low amounts of T cells target late antigens, implying good immune evasion at this stage [31]. A previously discussed mechanism of $CD8^+$ T cell evasion is the inhibition of TAP. This results in deficient peptide transport to the ER thus inhibiting MCH I maturation and expression at the cell surface.

EBV encodes for the TAP inhibitor BNLF2a, which is expressed during the lytic lifecycle [13]. BNLF2a is a small, 60 AA protein, with a hydrophobic C terminal tail that most likely anchors BNLF2a into the ER membrane. Its cytosolic N-terminus can then associate with TAP and prevent both peptide and ATP binding to TAP [13, 31, 86].

BNLF2a is maximally expressed 6h after onset of the lytic cycle. After 24h, the concentration of BNLF2a has declined to almost undetectable amounts although the gene transcripts only declined modestly during this time [13, 31]. The mechanism responsible for the transient expression of BNLF2a is unknown. Consistent with the presence of BNLF2a, the presentation of late antigens remain unaltered in cells infected with a BNLF2a knock out virus, indicating that the antigen presentation of late proteins is controlled by different mechanisms [31].

Another MHC I inhibitor encoded by EBV is BILF1. This viral protein resembles a constitutively activated G protein coupled receptor. In the plasma membrane, BILF1 adheres to mature MHC I complexes and significantly decreases their half life [13, 31, 87]. Surprisingly, BILF1 almost completely colocalizes with CXCR4, a cellular chemokine receptor also capable of downregulating MHC I expression at the cell surface. However, unlike CXCR4, BILF1 does not adhere to β2-microglobulin, does not induce MHC I heavy chain ubiquitination and does not rely on signaling [13]. Furthermore, a study by Zuo and colleagues indicated that BILF1 is also able to associate with immature MHC I in the ER. Accordingly, this indicates the possibility that MHC I is degraded before reaching the cell surface [31, 87]. Thus, at this point the exact working mechanism of BILF1 is unknown and requires additional research.

A third MHC I evasion protein encoded by EBV is BGLF5. This early gene was defined as a DNase (alkaline exonuclease) enzyme. Only recently, it became clear that BGLF5 also functions as a host shutoff protein, capable of downregulating among others MHC I and MHC II [13, 31]. Aside from its DNase activity, BGLF5 has an intrinsic 5'- 3' RNase activity which selects for the breakdown of mRNA [31]. Apparently, the catalytic site for DNase activity also allows RNase activity. This suggestion is based on the inability to create a mutant in which the DNase or RNase activity was lost. Furthermore, when the aspartate on position 203 (located in the DNase catalytic site) is substituted for a serine, the RNase activity is lost [31].

Although BGLF5 targets mRNA, it can not distinct between viral and host mRNA [13]. Hence, it is thought there is an equilibrium between the BGLF5-mediated mRNA turnover and synthesis.

This would enable some viral mRNA to escape degradation and consequently allow the lytic cycle to be completed [13].
The last MHC I evasion protein is the during latency produced EBNA1 protein. Unlike the other proteins which interfere with MHC I, EBNA1 prevents its own degradation by the proteasome. In doing so, EBNA1 prevents its antigens from being presented on MHC I. The domain responsible for escaping proteasomal degradation consists of glycine-alanine repeats (GAr domain) [88]. When this domain is deleted from the protein, EBNA1 will be presented to $CD8^+$ T cells which subsequently to kill the latently infected cells [13]. An additional function of the GAr domain is limiting expression of EBNA1 itself. Although the exact molecular mechanism is unknown at this time the hypothesis is that the secondary mRNA structure of the GAr domain is responsible for this [13, 89]. Namely, the genetic code of the GAr domain is extremely purine rich. When the code is altered, so it contains more pyrimidine bases which still encode for the same AA, the stability of EBNA1 mRNA increases. This will enhance EBNA1 protein synthesis and will thereby increase presentation of EBNA1-encoded antigens [13, 90].

MHC II evasion is less well described, although infected B cells express less MCH II on their surface than uninfected cells, clearly indicating the existence of MHC II downregulating proteins. One of the proteins involved in MHC II interference is BZLF2-encoded gp42. Gp42 is located on the cell surface and forms a complex with gL and gH. This complex is able to sterically hinder the interactions between $CD4^+$ TCR and MHC II [13, 31, 34]. Another EBV encoded protein able to influence MHC II is BZLF1. BZLF1 is an immediate early gene encoding for a transcription factor that initiates the lytic lifecycle. Additionally, this gene interferes with IFN-γ signaling by reducing the IFN-γ receptor α chain mRNA levels [13]. As shown in Figure 6 (p21), IFN-γ induces MHC II upregulation in several cell types. Additionally, BZLF1 is able to interfere with many other processes. For instance, it inhibits LMP-1 induced upregulation of MHC I, it inhibits transactivation of IRF-7 and NF-κB subunit p65 (central players in the innate immune response) etc. [13].

Aside from altering antigen presentation, EBV has many more mechanisms to alter or evade the immune system. One of these mechanisms is the production of a IL-10 homolog.
Human IL-10 has both immunosuppressive and immunostimulatory properties dependent on the microenvironment and the targeted cell type. BCRF1 encodes for a IL-10 homolog which only exerts the immunosuppressive properties. This difference, between human IL-10 (hIL-10) and viral IL-10 (vIL-10) is mediated by a single AA substitution. If the isoleucine at position 87 (hIL-10) is changed into an alanine (vIL-10), the ability of IL-10 to stimulate thymocytes and proliferate mastcells is remarkably reduced, while its immunosuppressive properties remain unmodified [13, 91].
Additionally, vIL-10 plays an important role when EBV infects and replicates in monocytes and macrophages. vIL-10 is able to prevent IFN-γ mediated upregulation of MHC II, ICAM-1 and co-stimulatory molecules CD80 and CD86 required for T cell activation [92]. Furthermore, vIL-10 can inhibit non infected monocytes and macrophages from producing IL-1α, IL-1β, IL-6 and TNF-α, thus contributing to a reduced pro-inflammatory response to the infected cells. Furthermore, vIL-10 can inhibit $CD4^+$ T cell responses on infected monocytes. [13].

Vaccine

For vaccination one can think of several approaches; vaccination against primary infection, vaccination against EBV complications associated with primary infection or complications associated with reactivation.

The main complication of primary infection is IM, which occurs in 30-40% of the adolescents with a primary infection [31, 83, 93]. In 2007 a phase II clinical trial of a recombinant gp350 vaccine which was supposed to protect against IM was finished [93]. Gp350 is a glycoprotein located on the viral envelope. It adheres to the CD21 receptor of the B cell and thus helps in the invasion of B cells. The goal of this vaccine strategy was to decrease the incidence of IM, which should be achieved after 3 vaccinations. After the third vaccination, the gp350 Ab titer was higher than one elicited during a natural infection. The vaccine was well tolerated, none of the patients stopped the treatment due to side effects [93]. More importantly, the elevated Ab titer appears to offer protection against IM. Additionally, an insignificant decrease in EBV infection was seen in the vaccinated group. A larger study should determine whether this is due to the vaccine. In addition, the effect of the vaccine on other complications associated with EBV is unknown so far [93]. Furthermore, additional research is necessary to determine the duration of protection, the mechanism of protection etc. However, this study is a promising start for vaccine development against IM [93].

Another complication of EBV infection after transplantation is post transplantation lymphoma disease (PTLDs). In 2009 a phase I clinical trial with the gp350 vaccine was finished. Here the goal was to determine the vaccines tolerability and immunogenicity in EBV seronegative children with chronic kidney disease waiting for transplantation [94]. The outcome of this trial was less successful than hoped. The vaccine was immunogenic, although the vaccination strategy was unlikely to have influenced posttransplant EBV infections or PTLD development. However, this study was conducted on a very small group of children and the observation that EBV seropositive children typically have a lower EBV peak after transplantation than EBV naïve children provides hope for the future. A larger phase II trail should proof the principal, that pretransplant vaccination for EBV naïve children reduces the posttransplant EBV burden [94].

Another approach to treat or prevent malignancies associated with an immunocompromised status is immunotherapy. Table 6 provides an overview of the malignancies associated with EBV, the types of latency often found in these diseases and the cellular origin of the tumor. From this table it becomes clear that namely PTLD, AIDS associated primary central nervous system lymphoma (PCNSL) and diffuse large cell lymphoma immunoblastic (DLCL-IB) are caused by EBV, whereas for BL and HL other cellular mutations are required.
In 1998 Rooney and colleagues described how the administration of donor derived polycolonal EBV specific $CD4^+$ and $CD8^+$ T cells, could prevent the development of PTLD in children who received a HLA-mismatched bone marrow transplant. Of the 39 patients none developed PTLD in contrast to the control group in which 11.5% (7/ 61) developed PTLD. Furthermore, 2 patients who developed PTLD after transplantation fully recovered after treatment with these CTLs [95].
Since then, more studies on immunotherapies have conducted including the administration of LMP2A loaded DCs to elicit a solid $CD8^+$ T cell response [96]. Furthermore, Feng and colleagues indicated that gemcitabine and doxorubicin in combination with ganciclovir provide a more effective treatment to lymphoproliferative disease than chemotherapy alone.

Gemcitabine and doxorubicin are able to induce lytic infections in EBV transformed B cells (latency III), thereby inducing the enzymes necessary to convert ganciclovir to its active component [97]. These studies all provide evidence that immunotherapy is a better approach than vaccination, to tackle complications associated with a deficient immune system.

Table 6. EBV associated malignancies.

Lymphoma	EBV association (%)	EBV latency	Cellular origin of lymphoma
Burkitt lymphoma	95-100% endemic 20-30% sporadic	Type I	GC B cells
Hodgkins lymphoma	40% Western world 90% children in Central America	Type II	Pre-apoptotic GC B cells
Post-transplant lymphoma	80%	Type III (possibly I or II)	(pre-apoptotic) GC B cells
AIDS associated B cell lymphoma	100% PCNSL		
	30-50% BL		
	90-100% PEL		
	30% DLCL-CB	Type I (CB)	GC or post GC B cells
	90% DLCL-IB	Type III (IB	GC or post GC B cells

PCNSL; primary central nervous system lymphoma, BL; burkitt lymphoma, PEL; primary effusion lymphoma, DLCL-CB; diffuse large cell lymphoma centroblastic, DLCL-IB; diffuse large cell lymphoma immunoblastic, GC; germinal center. Adapted from: [98].

Kaposi Sarcoma-associated herpesvirus

KSHV is the causative agent of Kaposi Sarcoma (KS), primary effusion lymphoma (PEL) and plasmablastic multicentric Castelman's disease (MCD) [85, 99-101]. Despite the importance of KSHV in human disease, the immune response elicited by this virus is poorly characterized [99-101]. That cytotoxic T lymphocytes (CTLs) play an important role in suppressing KSHV is clear from the onset of disease in immunosuppressed patients [100]. A study by Lambert and colleagues indicated that $CD8^+$ T cell responses differed between patients that drove the virus into a latent state and those who developed KS [100].
Recently, several studies have been performed to determine the $CD8^+$ T cell responses to certain Ags. In summary, these studies concluded that both latent and lytic Ags of KSHV are recognized by $CD8^+$ T cells. The antigens known thus far to elicit a T cell response are derived from the latently expressed ORF57 and K12 and the lytically expressed ORF73, gB, ORF6, 61 and 65 [99, 100].
Aside from the importance of $CD8^+$ T cells, it is known that KSHV is able to elicit Abs which significantly reduce the infectivity of KHSV. On the other hand, seroconversion is a predisposition for KS development [102].
The role of $CD4^+$ T cells remains largely undefined. From the point of view concerning immune evasion a clear preference for the induction of $CD4^+$ Th_2 cells is observed.

Immune evasion

Approximately 25% of the KSHV genome encodes for proteins involved in immune evasion or modulation. Although such a large percentage of the genome is involved, general themes can be identified, among others interfering with antigen presentation, skewing the immune system and blocking IFN type I pathway [85].

As for all human herpes viruses, KSHV interferes with antigen presentation. It encodes for two immediate early proteins, MIR (modulator of immune recognition) 1 and 2, which are prototypes of a new E3 ubiquitin ligase family. These proteins are present in the plasma membrane, where their transmembrane domain interacts with MHC I. Their N termini, located in the cytosol, form Zn fingers. With this domain MIR recruits E2 ubiquitin conjugates which will ubiquitinate the MHC I molecule, resulting in its endocytosis, followed by proteasomal degradation [85].
Although both proteins have similar working mechanisms, they target different proteins. MIR1 is able to downregulate the MHC I haplotypes A, B, C and E while MIR2 only downregulates the MHC I haplotypes A and B. Additionally, both are able to downregulate surface expression of CD1d, which is involved in the presentation of lipid and glycolipid antigens. Furthermore, MIR2, unlike MIR1, is able to downregulate the costimulatory molecule CD86 and the adhesion molecule ICAM1, both involved in the activation of $CD4^+$ T cells [32, 85].

Aside from evading the immune system, KSHV also skews the immune system towards a Th_2 response, while a Th_1 response is more efficient in clearing viral infections. The viral chemokines vCCL1, 2 and 3 aid in driving the immune system to a Th_2 response. vCCL1 and 3 are agonists for CC-chemokine receptor (CCR) 8 and CCR4, respectively. These receptors are found on Th_2 and regulatory T (Tregs) cells, indicating that these cells will migrate toward the infected area [85, 103-105].

vCCL2 functions as a Th_2 chemoattractant and is a Th_1 antagonist by inhibiting the signaling of many chemokines receptors [85, 106]. Another protein also involved in skewing the immune system is viral IL-6 (vIL-6). This IL-6 homolog promotes CCL2 production which in turn attracts monocytes and promotes Th_2 development [85]. Yet another protein involved in skewing the immune system is KSHV encoded CD200. The receptor for vCD200 is CD200R, which is mainly expressed by myeloid- and T-cells. Interaction of vCD200 with its receptor results in an inhibitory effect on myeloid activation and reduces Th_1 cell associated cytokine production *in vitro [85]*.
When leukocytes isolated from the KS lesions were analyzed, they were found to express the Th_2 associated cytokine receptors CCR3 and 8. Furthermore, when the T cells were analyzed a predominant Th_2 cell associated cytokine profile was found [85, 107]. Thus, skewing the immune system appears to be of real importance for the development of KSHV-associated diseases. Whether the immune system is skewed to the same degree in healthy KSHV seropositive individuals remains to be determined [85].

Another KSHV immune evasion strategy is blocking IFN type I mediated responses. Normally, when the presence of a pathogen is sensed by the cell it activates the IFN type I pathway (Fig. 9). However, when a cell is infected with KSHV, several proteins will interfere with this pathway (Fig. 9). For instance, ORF45 interacts with IFN regulatory factor (IRF) 7, thereby preventing it from being phosphorylated. Consequently, unphosphorylated IRF7 is unable to traffic to the nucleus and thus fails to transcribe IFN-α and –γ [85].
Another KSHV-encoded protein, RTA (replication and transcription activator), also interacts with IRF7. This protein promotes ubiquitination of IRF7, followed by its proteasomal degradation. However, RTA might not mediate the destruction of IRF7 for the purpose of immune evasion. Research indicated that IRF7 adheres to the promoter of ORF57, thereby inhibiting its transcription. RTA also adheres to the promoter of ORF57 in order to activate the lytic lifecycle. Thus, RTA, also known as the latent-to-lytic switch, plays an essential role in viral replication [85].
In addition to these two proteins, virally encoded IRF1, 2 and 3 interfere at different sites of the IFN type I pathway (Fig. 9). However, despite all these strategies, KSHV remains sensitive for IFN-α [85].

Another important group of modulating proteins are those enabling transformation of healthy cells into cancer cells. LANA (latency associated nuclear antigen) is one of these proteins. Like EBNA1 encoded by EBV, LANA is essential for maintaining the viral genome in dividing cells. Research indicated that LANA and EBNA1 have a similar working mechanism which is discussed by Coscoy [85]. However, LANA is also able to interfere with several cellular pathways. For instance, LANA can adhere to p53, thereby inhibiting p53-mediated activity and apoptosis. Additionally, LANA inhibits the tumor suppressor protein Rb (retinoblastoma), resulting in cell cycle progression past the G1/S checkpoint. Furthermore, LANA has the ability to upregulate telomerase reverse transcriptase. All these changes aim towards immortalizing the infected host cell [7].

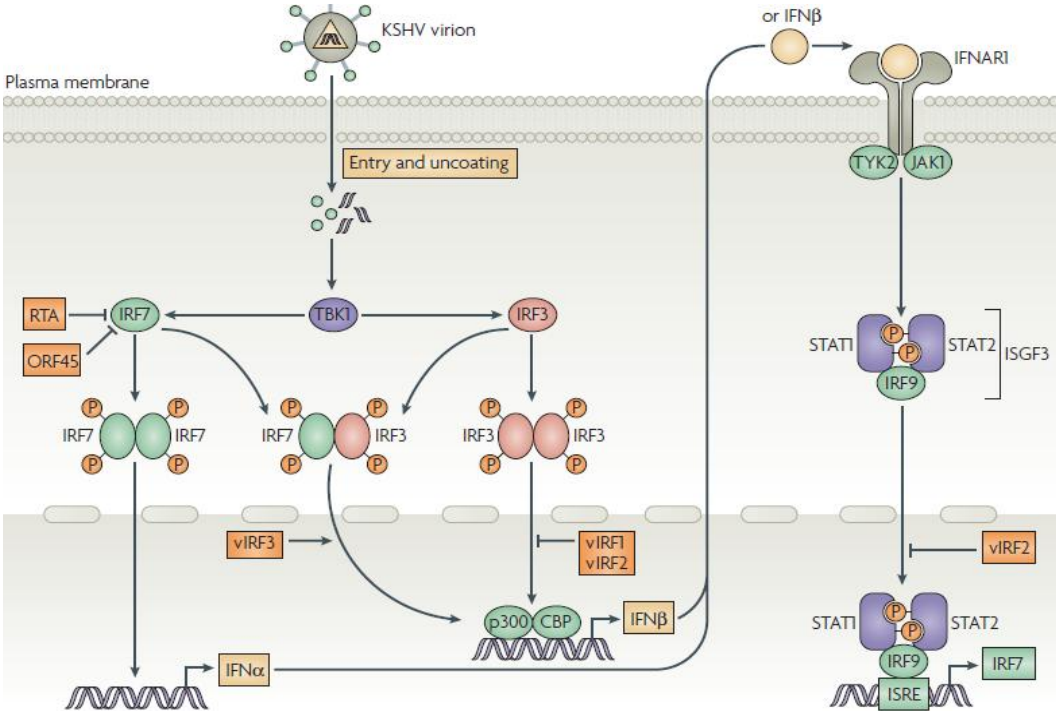

Figure 9. A simplified overview of the type I IFN signaling pathway and the interfering proteins encoded by KSHV [85].

Sensing of viral products by the host cell results in activation of TANK binding kinase 1 (TBK1). Subsequently, TBK1 phosphorylates IRF3 leading to IFN-β production. The secreted IFN-β adheres to the type I IFN receptor (IFNAR1) which activates the JAK/ STAT signaling pathway. The phosphorylated STAT-1/-2 complex recruits IRF9 which will adhere to the complex, forming the IFN-stimulated gene factor 3 (ISGF3) complex. This complex will stimulate gene production of genes containing IFN-stimulated response elements (ISRE) in their promoter region. One of these genes is IRF7. After production, IRF7 is phosphorylated by TBK1, dimerizes and contributes to the production of IFN-β and IFN-α [57, 85].

The KSHV encoded IRFs function as transcription regulators while RTA and ORF45 specifically target IRF7 activation [85].

Another protein aiding in prolonged cell survival is vFLIP (viral Fas-associated death domain like IL-1 β-convertase enzyme (FLICE) inhibitory protein) also known as K13. This protein interferes in the interaction of FADD (Fas-associated death domain) and caspase 8 in death receptor signaling. Thereby the apoptosis signal is inhibited, promoting cell survival [7, 108]. Similar to LANA, vFLIP interferes with multiple cellular pathways. For instance, vFLIP is also able to associate with the IKK (I kappa B kinase) complex and HSP (heat shock protein) 90 to induce NF-κB signaling. This signal is important for viral latency and oncogenesis [7, 109, 110].

These are just two of the several oncogenic proteins KSHV encodes, other proteins involved in prolonged cell survival are K1, vGPCR and vIRF1 (Table 7) [7].

In this thesis, just a few examples of immune evasion by KSHV are discussed. Table 7 provides an overview of more proteins involved in immune evasion/ modulation.

Table 7. An overview of the immune evasive proteins of KSHV [7, 85]

ORF	Gene product	Function
Intracellular communication		
K2	vIL-6	IL-6 homolog
K4	vCCL2	CCR3 and 8 agonist CCR1, 2, 5, 10, CXCR4, CX_3CR1 and XCR1 antagonist angiogenesis, monocyte and Th_2 chemotractant
K4.1	vCCL3	CCR4 agonist Angiogenesis and VEGF-A induction, Th_2 chemotractant
K6	vCCL1	CCR8 agonist, CCR5 binding, Th_2 chemotractant
K13	vFLIP	Apoptosis prevention in some cells
Intracellular defense (inhibit type I IFN)		
ORF45	ORF45	Prevents IRF7 activation
ORF50	RTA	Promotes IRF7 ubiquitination and degradation
K9	vIRF1	Prevents IRF3 mediated transcription
K10.5-K10.6	vIRF3	Enhances IRF3 and 7 mediated transcription
K11.1-K11	vIRF2	Prevents IRF1 and 3 mediated transcription, binds and inhibits PKR
Apoptosis		
K1	VIP	Transformation, B cell activation, apoptosis inhibition, downregulation BCR and activation PI3 K, AKT and mTOR kinases
K2	vIL-6	Inhibits IFN mediated apoptosis
K7	vIAP	Links BCL2 to effector caspase and inhibition of vGPCR expression and function
ORF16	vBCL2	BCL2 homolog
ORF45	ORF45	Prevents IRF7 activation
ORF50	RTA	Inhibits p53 transcriptional activity
K9	vIRF1	Inhibits p53, p300, TGF-β and transformation, binds ATM kinase and GRIM19
K10.5-K10.6	vIRF3	Binds and inhibits p53 and NF-κB, inhibition of CD95L surface expression
K11.1-K11	vIRF2	Inhibition of CD95L surface expression
K13	vFLIP	Blocks recruitment and/or activation of caspases, transactivator NF-κB and transformation
ORF73	LANA	Binds p53
Intracellular interactions		
K1	K1	Downregulates BCR surface expression
K3	MIR1	Downregulates MHC-A, -B, -C, -E and CD1d
K5	MIR2	Downregulates MHC-A, -B, CD86, ICAM1, PECAM1 and CD1d
K14	vCD200	CD200 homolog, inhibits myeloid functions and regulation of inflammatory cytokines
K15	LAMP	Induce IL-6, -8 and COX-2 and inhibits B cell signaling
Others		
ORF4	KCP	Inhibitor of complement activation
ORF37	SOX	Induce host shutoff (promotes mRNA degradation)
K8	K-bZIP	RTA repression
K12	Kaposin	Transformation (KaposinA), stabilization AU rich and cytokine mRNA (KaposinB)

Vaccine

There are two vaccination strategies one can think of; either vaccination against primary infection and the establishment of latency or vaccination against the complications associated with primary infection and an immunocompromised status. Unfortunately, there is not much known about the immune response KSHV elicits in humans, making the development of a vaccine challenging.

KSHV is the cause of KS in immunocompromised individuals like AIDS patients. A large fraction of the HIV infected individuals in the homosexual community is also infected with KSHV (60%). Consequently, the HIV infected homosexual community forms a good target group for vaccination against both primary infection and vaccination against the development of KS [111].
However, vaccination against KSHV in HIV infected individuals might encounter some problems. First, KSHV-associated diseases only evolve in immunocompromised individuals, at this stage a vaccine might not work. However, there is evidence that asymptomatic HIV infected individuals respond similar to influenza vaccination as healthy individuals [112], suggesting that HIV infected individuals might be susceptible for vaccination [111]. Second, cells isolated from KS lesions are mainly latently infected with KSHV while vaccination is usually to prevent reactivation or primary infection [111, 113]. Lastly, because a good animal model is lacking, it is difficult to evaluate a newly designed KSHV vaccine [111].

Although a good animal model for KSHV is not available, there is a mouse γ herpesvirus, murine gammaherpesvirus 68 (MHV68) with 80% sequence homology with KSHV. Additionally, many proteins required for lytic and latent infection with KSHV are conserved in MHV68. Consequently, mice can function as an animal model for testing different vaccine strategies, although one should take into account that there are many differences between men and mice regarding their immune system [114].
A recent study has indicated that a recombinant mutant MHV68 strain can provide protection against infection with the wildtype virus [114]. The mutant MHV68 virus lacked ORF72, ORF73 and M11, proteins required during latency and reactivation. Furthermore the mutant had the RTA gene under control of a highly active promoter. Thus, a virus only able to induce a lytic infection was produced, named AC-RTA. The study indicated that this virus indeed did not induce latency, caused milder symptoms during primary infection and most importantly prevented infection with the wildtype virus three months after vaccination [114]. Whether this approach would work for KSHV remains unclear. However, even if vaccination only reduced the latent viral load in humans, it still contributes to a decreased disease burden [114].

The problems encountered by a lacking animal model, might belong to the past. Last year, Chang and colleagues discovered that marmosets (New World primates) could be infected with human KSHV. Subsequenlty, KSHV established a persistent infection which could occasionally lead to KS-like skin lesions. Thus, these monkeys might provide a good model for immunotherapy and vaccine development.

Conclusion

In conclusion, the 8 known human herpesviruses, HSV-1, HSV-2, VZV, HCMV, HHV-6, HHV-7, EBV and KSHV, are very common viruses [8]. In our lifetime we will encounter at least one, if not several of these viruses. During primary infection a strong immune response is elicited. However, this immune response is unable to clear the infection. It merely drives the virus into a latent state [3]. Viral persistence is facilitated by an extensive repertoire of immune evasive and modulatory proteins encoded by these viruses [4, 34, 49]. Despite the fact that all human herpesviruses give rise to a persistent infection, infections are generally fairly harmless. However, in immunocompromised individuals these viruses are associated with the development of severe diseases. These diseases include, among others, postherpetic neuralgia, pneumonitis, enterocolitis, Kaposi sarcoma and post-transplantation lymphoproliferative disorder [7, 11, 25, 29, 30]. The association of these diseases with a deficient immune system implicates the presence of a balance between viral pathogenesis and the immune response.

The phenomenon of a strong immune response which is unable to clear viral infection lies at the basis of the problems encountered during vaccine development against primary infections. This problem is best illustrated for HCMV; during primary HCMV infection a massive immune response is elicited, even when the virus is no longer lytically active, over 10% of the circulating memory T cell population is HCMV specific [68]. However, despite the presence of this huge memory cell population, newly encountered HCMV viral particles reinfect the HCMV seropositive host just as easily as a naïve host [68]. Therefore it is very unlikely that vaccination against primary infection with HCMV is possible.
On the other hand, VZV elicits an immune response which is able to protect the host from infection by new VZV viral particles. Hence, for this virus a vaccine has successfully been developed [22].
In conclusion, the ability of the immune system to develop protection against superinfection is crucial for the development of a successful vaccine against primary infection. Another factor to take into account when developing a vaccine, is the age one usually contracts the wildtype virus.

Alternatively, one could also vaccinate against the symptoms associated with primary infection. This strategy is used for targeting infectious mononucleosis caused by a primary EBV infection during adolescence or adulthood [31, 83, 93]. So far, a phase I clinical trail with a gp350 recombinant vaccine gave promising results though, additional research is necessary to determine the vaccines effect on other factors of EBV infection [93].

Besides vaccinating against primary infections or the complications associated with a primary infection, one can vaccinate against reactivation and the complications associated with this. This strategy has already been proven to be useful in VZV infections. VZV recrudescence's occur in immunocompromised and elderly individuals, whose VZV specific T cell responses have decreased [22]. The use of a life attenuated VZV strain to boost the immune system of elderly individuals significantly reduces the number of herpes zoster cases compared to the control group [23]. Although successful in elderly individuals, it seems unlikely that the compromised immune system in AIDS or transplant patients can be boosted by a vaccine. For these patients immunotherapy or addressing the cause of immune suppression appears to be a more appropriate.

An example supporting this suggestion, is the study performed by Rooney and colleagues. They indicated that administration of EBV specific CTLs to bone marrow transplant patients reduced the number of PTLD cases. Additionally, patients who developed EBV associated lymphoma after bone marrow transplantation, fully recovered after treatment with these CTLs [95].

In summary, the immune evasive properties of the human herpesviruses make them a difficult target for vaccine development. For vaccination against primary infection, it is important to determine whether the immune response induced upon infection, provides protection against superinfections. When superinfections are possible, as seen for HCMV, the change of developing a successful vaccine is very small.
Alternatively, one could vaccinate against the complications or symptoms caused by a primary infection, as is studied for EBV.
Vaccinating against complications associated with a deficient immune system is even more challenging. Boosting a deficient immune system seems unfeasible, therefore immunotherapy or addressing the cause of the immune deficiency appear to be more appropriate in these cases.

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