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Abstract

This chapter is devoted to the topics of not yet marketed HSV vaccine, which is still in the focus of interest, especially from the point of immunotherapeutic use. To understand the principles of vaccination strategies (prophylactic and/or immunotherapeutic), the pathogenesis of herpes simplex virus 1 (HSV-1) and/or HSV-2 infections in animal models is briefly outlined. Even when both herpesviruses may spread via bloodstream, which is especially true in the immunocompromised host, the main route of their transmission is along peripheral nerves. Both viruses establish latency in ganglion cells, and after reactivation, they spread along axons back to the site of primary infection. Since neither the establishment of latency nor its reactivation can be fully controlled by virus-neutralizing antibodies, the outcome of immune response greatly depends on the activity of cytotoxic CD8+ T lymphocytes. The majority of important antigenic epitopes is located in envelope glycoproteins (such as gB, gD, gE, gC and gG) that are related to virus adsorption and penetration into susceptible cells. The HSV-1 and/or HSV-2 experimental vaccines designed so far were either purified virion products derived from infected cells (subunit vaccines), purified recombinant immunogenic herpes simplex virus HSV-coded proteins (especially gD), and/or attenuated live viruses lacking some of virulence tools (such as gH and/or gE). We bring a comprehensive overview of the efficacy of experimental HSV-1/HSV-2 vaccines and discuss our own data. In conclusion, we believe in the continued demand of HSV-1 and HSV-2 vaccines, at least for their immunotherapeutic use, suggesting unified evaluation criteria for clinical trials to reach consent at their interpretation.

Keywords: herpes simplex virus 1 and herpes simplex virus 2, candidate vaccine, clinical trials, efficacy in animals, humoral and cell-mediated immune response, antigenic epitopes
1. Introduction

This review will be devoted to the topics of not yet marketed HSV vaccine(s), which design is still in the focus of interest, especially from the point of view of immunotherapeutic use. The human herpesvirus 1 (common name herpes simplex virus 1, HSV-1) is one of the first human viruses discovered [1] and belongs among the most intensively investigated viruses. In the span of the last 45 years, the PubMed portal has registered nearly 11,000 papers devoted to various aspects of HSV-1/HSV-2, starting from virus structure, continued by the molecular mechanisms of lytic replication versus latency maintenance, through the virus spread in the body and the eliciting of different forms of immune response, not omitting many clinical and epidemiological studies. In the second half of the last century, two subtypes of herpes simplex virus (HSV) have been described [2] and were designated HSV type 1 and HSV type 2 [3]. While the former (HSV type 1) has been predominantly isolated from the orofacial area and upper respiratory airways, the latter was believed to infect the urogenital tract and occasionally the newborn. According to recent classification, the HSV-2 represents a distinct species of the Simplexvirus genus, which along with the Varicellovirus genus belongs to subfamily Herpesvirinae of the Herpesviridae family. Nevertheless, both species are closely related, since they differ only in a few antigenic domains (and/or epitopes) located in the envelope glycoproteins, namely, in an entirely distinct gG and/or in the partially unrelated gC [4].

2. The molecular biology of HSV: virion structure, lytic replication and latency

The HSV virion consists of four elements: (a) a core containing the viral DNA (vDNA), (b) an icosahedral capsid surrounding the core, (c) an unstructured proteinaceous layer called the tegument that surrounds the capsid, and (d) an outer lipid bilayer envelope exhibiting spikes on its surface. The core contains the double-stranded (ds) DNA (dsDNA) genome wrapped as a toroid or spool in a liquid crystalline state. A small fraction of the virion DNA may be circular, but the bulk of packaged HSV DNA is linear and double-stranded. The enveloped virion is a spherical particle with an average diameter of 186 nm, which might extend to 225 nm with the spikes included, while the internal capsid has a constant diameter of 110 nm (reviewed at [5]). The HSV virion contains more than 30 proteins that were designated as virion polypeptides (VPs). Out of these approximately known and additional 10 suspected virion proteins (VPs), at least 11 are located within the envelope at the surface of virion (predominantly accessible to antibody), from which at least 10 are glycosylated (reviewed at [6]).

The HSV genes were classified into at least three general kinetic classes: alpha or immediate-early (IE), beta or early (E), and gamma or late (L) genes. The IE mRNAs are transcribed by the help of a transcription-inducing cofactor present in the virion tegument, also called alpha-TIF/VP16. In brief, the tegument protein VP16 is becoming a part of a tripartite complex comprised of octamer-binding protein 1 (Oct-1) and host cell factor 1 (HCF-1). At least two of the IE proteins (ICP0 and ICP4; Figure 1) are transactivators needed for initiation of transcrip-
tion of the E/beta mRNAs (the first checkpoint) and later also for transcription of gamma/L mRNAs. The latter are mainly structural virion proteins in contrast to E transcripts specifying nonstructural polypeptides. The structural proteins are transcribed in two waves, called gamma1/L and gamma2/L. The former occurs more closely related with the E transcription phase (second checkpoint) and requires predominantly ICP4. The expression of beta polypeptides as well as that of group gamma1/L genes requires ICP4 and at low multiplicities of infection also ICP0. The expression of gamma2/L genes, controlled at the third checkpoint, requires ongoing DNA synthesis as well as the presence of ICP4 and ICP0 [7].

The HSV genome consists of two covalently linked sequence components, designated as L (long) and S (short). Each component consists of unique (U) sequences bracketed by inverted repeats (IR) [6].

The entry of HSV into cells involves the interaction of at least five virion surface glycoproteins (gB, gC, gD, gH and gL) with several receptors on the cell surface following fusion of the envelope with a cellular membrane. The HSV glycoproteins along with other proteins important for pathogenicity are listed in Table 1. The first step in the process of entry is the binding of virions to glycosaminoglycans (GAGs) on the cell surface. The interaction of virions with GAGs is mediated by two glycoproteins: gC and gB. The next step in virus entry consists of the interaction of gD with its receptors and execution of fusion between the envelope and the cellular membrane by the heterodimer gH/gL and also by gB. The interaction of gD with its receptors has been extensively studied in several laboratories. The consensus is that, for entry, gD interacts with one of three natural receptors (reviewed at [8]). The receptors are nectins, a protein-designated herpesvirus entry mediator (HVEM) and a selected form of 3-O-sulfated heparan sulfate (3-OS HS). Nectins are intracellular adhesion molecules expressed on epithelial and neural cells and are members of the extended immunoglobulin (Ig) family. Earlier studies showed that gD of HSV-1 interacts with the amino-terminal V1 domain of nectin 1. The wild-type HSV-2 can enter susceptible cells via an alternative receptor, namely, nectin 2. HVEM is a member of the extended tumor necrosis factor receptor (TNFR) family, expressed mainly on T lymphocytes but occasionally also on natural killer (NK) cells. Its natural ligand is LIGHT (homologous to lymphotoxins, exhibits inducible expression), which is constitutively expressed on T and natural killer (NK) cells and appears to be a regulator of mucosal immune system. The LIGHT receptor competes with HVEM for the gD (reviewed at [9]). The role of gD with respect to gB and gH/gL is to recruit and position them properly to enable interaction with GAGs and with the lipid bilayers of the cellular membranes [10].
<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL1</td>
<td>γ34.5</td>
<td>L protein, cofactor for ribosomal translation</td>
</tr>
<tr>
<td>RL2</td>
<td>ICP0</td>
<td>IE protein, spliced, three exons, a RING finger motif, acts as a nonspecific transactivator of any beta and gamma gene</td>
</tr>
<tr>
<td>UL1</td>
<td>gL</td>
<td>Late glycoprotein, forms the gH/gL complex, syn mutation</td>
</tr>
<tr>
<td>UL5</td>
<td></td>
<td>E class protein, part of the helicase/primase complex</td>
</tr>
<tr>
<td>UL9</td>
<td></td>
<td>E/L class, ori-binding protein, cooperates with ICP8 at initiation of vDNA replication</td>
</tr>
<tr>
<td>UL10</td>
<td>gM</td>
<td>L class, glycosylated polypeptide, interacts with gN, participates in exocytosis and in cell-to-cell spread</td>
</tr>
<tr>
<td>UL18</td>
<td>VP24</td>
<td>L class, capsid component, participates in triplex formation</td>
</tr>
<tr>
<td>UL19</td>
<td>VP5</td>
<td>L class protein, the major capsid component (149/150K)</td>
</tr>
<tr>
<td>UL21</td>
<td></td>
<td>L class tegument protein weakly capsid-associated, binds to microtubules at axonal transport</td>
</tr>
<tr>
<td>UL22</td>
<td>gH</td>
<td>L class, glycosylated protein, essential for penetration to cells, needs complexing with gL</td>
</tr>
<tr>
<td>UL23</td>
<td>TK</td>
<td>E class, tegument protein, thymidine kinase, virulence factor</td>
</tr>
<tr>
<td>UL26</td>
<td></td>
<td>L class, “scaffolding” capsid protein, the N-terminus required for virion assembly</td>
</tr>
<tr>
<td>UL27</td>
<td>gB</td>
<td>L class glycoprotein, essential for adsorption and membrane fusion, syn3 locus</td>
</tr>
<tr>
<td>UL29</td>
<td>ICP8</td>
<td>E class, ssDNA binding, required for vDNA synthesis, keeping the DNA fork apart</td>
</tr>
<tr>
<td>UL30</td>
<td>DNA pol</td>
<td>E/L class protein, DNA polymerase (elongation enzyme), virulence factor</td>
</tr>
<tr>
<td>UL35</td>
<td>VP26</td>
<td>L class virion protein, hexon component, also termed NC7</td>
</tr>
<tr>
<td>UL36</td>
<td>VP1/VP2</td>
<td>L class, large tegument protein, important for egress through cytoplasm and re-envelopment</td>
</tr>
<tr>
<td>UL38</td>
<td>VP19C</td>
<td>L class capsid protein, triplet component with VP23 (1:2), connects the hexons and pentons</td>
</tr>
<tr>
<td>UL39</td>
<td>RR</td>
<td>E class protein, large subunit of RR, membrane-anchored protein kinase</td>
</tr>
<tr>
<td>UL40</td>
<td>RR</td>
<td>E class protein, the small subunit of RR</td>
</tr>
<tr>
<td>UL41</td>
<td>vhs</td>
<td>L class, tegument protein, interferes with the host cell proteosynthesis, virulence factor</td>
</tr>
<tr>
<td>UL44</td>
<td>gC</td>
<td>L class, glycoprotein, reacts with GAG on cell surface, binds C3 and/or C5, virulence factor</td>
</tr>
<tr>
<td>UL48</td>
<td>Alpha-TIF/VP16</td>
<td>L class tegument protein, alpha-transinducing factor</td>
</tr>
<tr>
<td>UL49.5</td>
<td>gN</td>
<td>L class membrane-associated small glycoprotein, complexes with gM</td>
</tr>
<tr>
<td>UL53</td>
<td>gK</td>
<td>L class glycoprotein (40K), localizes to Golgi and ER, involved in egress, syn1 locus</td>
</tr>
<tr>
<td>UL54</td>
<td>ICP27/IE63</td>
<td>IE class protein, blocks cellular mRNA transport to cytoplasm</td>
</tr>
<tr>
<td>RS1</td>
<td>ICP4</td>
<td>IE class protein, transactivator for E and L promoters</td>
</tr>
<tr>
<td>US1</td>
<td>ICP22</td>
<td>IE class regulatory protein, essential in animal experiments, cellular cyclin A and B degradation</td>
</tr>
</tbody>
</table>
### Table 1. HSV-1 genes and corresponding proteins regarded for importance in virulence.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>US4</td>
<td>gG</td>
<td>L class, glycosylated protein and envelope component, HSV-2 specific</td>
</tr>
<tr>
<td>US6</td>
<td>gD</td>
<td>E/L class, glycosylated protein, 56K, adsorption to nectin 1 and HVEM protein receptors, essential for entry to cells, neural uptake</td>
</tr>
<tr>
<td>US7</td>
<td>gI</td>
<td>L class glycoprotein, forms heterodimer with gE, the complex facilitates cell-to-cell spread, neural uptake</td>
</tr>
<tr>
<td>US8</td>
<td>gE</td>
<td>L class glycoprotein, complexes with gI, essential for neural uptake</td>
</tr>
<tr>
<td>US12</td>
<td>ICP47</td>
<td>IE class protein, blocks the transport of viral antigenic epitopes</td>
</tr>
<tr>
<td>LAT</td>
<td></td>
<td>Three categories of RNAs (8.5 kb, 2.0, and 1.5 kb), the small LATs terminate antisense to ICP0 ORF. Latency regulator</td>
</tr>
</tbody>
</table>

Abbreviations: vDNA = viral DNA; ICP = infected cell protein; VP = virion protein (structural); IE = immediate-early kinetics (class); E = early kinetics (class); L = late kinetics (class); K = 1,000 kDa (Mr); RR = ribonucleotide reductase; vhs = virion host shutoff; LAT = latency-associated transcript(s); RNA molecules without capping, do not interact with ribosomes; UL = unique long (DNA segment); US = unique short; RL = repeat long; RS = repeat short; HVEM = herpes virus entry mediator.

*Modified according to Roizman et al. [6].

The gB belongs to class III fusion protein that after GAG interaction facilitates fusion of the virion envelope bilayer with the cellular membrane. A recent study showed that gB can also bind to the paired immunoglobulin-like type 2 receptor-α (PILRα) to trigger viral fusion in certain cell types; however, the precise role of this interaction in viral entry remains to be determined [11]. The gH and gL appear to form a tight complex, since neither protein is stable without the other. In contrast to gB, the structural studies of gH/gL showed no homology with fusion domains of other viral glycoproteins. The exact role of gH/gL has not been determined; however, one hypothesis is that the interaction of gD with its receptors changes the conformation of gH/gL, which in turn induces gB to adopt its fusion conformation [12]. Even that gE had been regarded for not essential in cell culture, it has been shown very important at entering nerve endings (neural uptake) and subsequent neural spread [13].

The latency of HSV can be characterized by circularized state of the HSV genome in the absence of IE and/or E transcription, especially lacking the expression of the two most important beta/gamma transcription activators, namely, ICP0 and ICP4 (reviewed at [14]). We pointed at the possibility that the IE block in question might be “leaky” since small amounts of ICP4 mRNA could be found in the non-cultured ganglion explants which would later on yield infectious virus [15]. Overwhelming literature deals with the hypotheses that the small latency-associated transcripts RNA (LATs), RNA molecules expressed during latency contribute to the silencing of ICP0 mRNA transcription as well as to the maintenance of latency [16]. It has been shown that LAT can reduce the expression of viral genes and suppress HSV replication in cultured cells. In addition, LAT probably protects the HSV DNA carrying neuron from apoptosis. The anti-apoptosis activity of LAT has been independently confirmed in tissue...
culture and in the mouse ocular model [17]. Further data suggest the participation of LAT in reactivation of the latent genome [18, 19].

3. Pathogenesis of HSV-1 and/or HSV-2 infections

The crucial event in HSV pathogenesis is, when the virus, which has reached the pseudounipolar neuron of the regional sensory ganglion via the quick axonal transmission, may but need not undergo productive replication. Thus, latency can be established from the very beginning, that is, in the absence of any transcription provided by the virus-coded alpha-transinducing factor (alpha-TIF). As described by Efstathiou et al. [20], the latent genome resides in the nuclei of neurons in the form of a circularized nonintegrated plasmid-like structure. This structure survives within the carrier neuron, the carrier neuron, so that neural cells harbor the viral DNA for their lifetime. The tegument-bound alpha-transinducing factor (alpha-TIF) might be “lost” during intra-axonal transport [21]. Some axons might be as long as 500 mm (as a rule about 100 mm), while the minimum speed for HSV capsid transmission is about 1–2 mm/h. The quick axonal transport along neurotubules is provided by the dynein molecule, which binds the capsid component UL21. The environment within sensory neurons favors the establishment of latency rather than productive virus replication also because the neuronal transcription cofactors such as Oct-2 and/or Brn-3 repress the IE promoters [22, 23].

During reactivation, the blocked IE checkpoint of virus replication must be overcome especially by overwhelming expression of ICP0 protein in order to achieve productive virus replication and infectious HSV production [24]. The reactivation process can be hampered by two different mechanisms. One depends on viral genome products interacting with cellular cofactors of transcription within neurons. It may not be excluded that due to the existence of at least two activation checkpoints, controlling the expression gamma1 or E/L proteins (glycoproteins) and the gamma2/L proteins (glycoproteins), also incomplete virus particles may be formed. The E and/or E/L proteins may be synthesized in the absence of complete virion formation, or assembly of virions with defective vDNA occurs. However, the presence of virus-coded antigens (even in the absence of infectious virus particles) could induce a potent immune response (including the accumulation of cytotoxic T cells in the vicinity of HSV carrier neurons). It should be also mentioned that the IE protein ICP47 acts by immune evasion. Alternative effects inhibiting the antiviral defense can be exerted by gC [25], which binds complement, and by the gE molecule, which binds the Fc fragment of immunoglobulins.

If reactivated virus reaches the peripheral tissues by retrograde axonal transport (skin squamous epithelium, non-cornified squamous epithelium of mouth mucous membrane, corneal epithelium), then it starts to replicate and causes inapparent virus shedding or blister formation. A nice example for creating favorable conditions of virus replication at peripheral skin was described in the ear model [26, 27]. These investigators found that prostaglandins produced after skin trauma or UV light irradiation would enhance the replication of recurrent HSV-1. As shown by Walz et al. [28], neurectomy of the trigeminal nerve root reactivated the latent HSV harbored within ganglion cells. The round trip of reactivated virus
usually ends with reinfection of additional neurons within the regional sensory ganglion. Less frequently, the retrograde axonal transport may continue in centripetal direction, that is, to brain stem. Furthermore, the central nervous system (CNS) may become infected via bloodstream (in newborn) or along the olfactory route (reviewed at [29]).

The different manifestations of clinical disease reflect the above-described mechanisms of HSV spread in the body. Essentially, HSV-1 can induce the acute primoinfection (gingivostomatitis) and recurrent disease (classical labial herpes). Alternatively, the ocular herpes, often manifested as herpes keratitis, occurs rather as recurrent disease than primoinfection. In newborn as well as in the immunocompromised host and/or in the case of local inflammation in the skin (e.g., due to allergic manifestations such as atopic eczema), the HSV might cause generalized skin disease or even viral sepsis (i.e., the hepatoadrenal necrosis in newborn or severe meningoencephalitis). In newborn, perinatal infection causes an acute dissemination of HSV-2 via bloodstream. Meningitis and meningoencephalitis of neonates, similarly as various forms of genital infection of man and women, are mainly HSV-2-related. In women, the outer but also the inner genital tract (vagina or cervical mucosa covered with non-cornified squamous epithelium) might become infected. In the skin as well as at the mucous membranes covered by squamous epithelium, both HSV-1 and HSV-2 species replicate within the lower and/or medium squamous cell layers causing blister formation [30].

4. Mechanisms of the immune response to HSV-1 or HSV-2

The immune response to HSV-1 and/or HSV-2 is induced in an early and a late phase. During early phase, the nonspecific antiviral mechanisms are activated, while in the late phase, the HSV-specific reaction is mobilized. The specific immune response culminates by recruiting the CD8+ cytotoxic T lymphocytes important for extracellular latency control, in contrast to the intracellular latency control which has been discussed above. The specific cell-mediated immune response may begin in the regional lymph nodes draining the virus inoculation site (both T-lymphocyte lines are engaged) as well as in the trigeminal ganglia which harbor the latent virus (mainly cytotoxic CD8+ T cells take part).

4.1. The innate immune response

The innate antiviral immunity to HSV-1 and/or HSV-2 is provided by alpha/beta type I interferons (IFNs) as well as by activation of natural killer (NK) cells. The role of type I IFNs in anti-HSV defense was demonstrated in knockout mice deficient in type I IFN production [31, 32]. The type I IFN release is induced by virus constituents called pathogen-associated molecular patterns (PAMPs) interacting with the corresponding pattern recognition receptors (PRRs) at the surface of responding cells. The best known examples are the Toll-like receptors (TLR). For example, the envelope glycoprotein D molecule of the HSV-1 interacts with the TLR-2 receptor on the target cell membrane [33], while the TLR-9 receptor binds to non-methylated CpG motifs of the vDNA present in the nuclei of infected cells [34]. It has been also shown that children with TLR-3 defects are more susceptible to herpes encephalitis [35,
Both types I of IFNs act on the receptors in neighboring cells eliciting the synthesis of antiviral substances such as the 2′,5′-oligoadenylate synthase, RNase L, and/or RNA-dependent protein kinase. The type I IFN also inhibits cell proliferation and enhances the activation of NK cells as well as the expression of MHC class I molecules, but downregulates MHC class II expression. The type III IFNs, also designated as IFN-λ1, IFN-λ2 and IFN-λ3 (IL-29, IL-28A, and IL-28B), have been recently characterized; alike to classical type I IFNs, also IFN-λ induces production of antiviral substances in the infected cells [37]. The participation of type III IFNs in inhibition of HSV replication has been confirmed in vitro [38, 39].

In addition to type I IFN formation, the activation of NK cells represents another important arm of nonspecific immune response to HSV-1 and HSV-2 infection [40]. The nonspecific killers belong to the group of class I innate lymphoid cells. Morphologically, they belong to large granular lymphocytes (LGLs), which cytoplasm contains azurophilic granules with perforins and granzymes A and B. They exert cytolytic activities helpful at the elimination of infected cells. The perforins create a transmembrane channel piercing the cell membrane, while granzymes activate the apoptosis of target cells. The NK cells also produce IFN-gamma, which promotes their activation along with the activation of macrophages. In association with this, enhanced death rates were noted in NK-deficient mice infected with HSV [41].

The elimination of HSV-1 and/or HSV-2 infected cells at the portal of entry is mediated by additional immune cells such as neutrophils, dendritic cells (DCs), and macrophages. The neutrophils secrete tumor necrosis factor (TNF) which induces apoptosis of infected cells via caspase-8 activation [42]. Blood monocytes when entering the connective and/or other tissues are altered into macrophages able to engulf the extracellular virus particles but also residues of apoptotic cells. The active macrophages belong to the group of antigen-presenting cells (APCs) that present the external immunogenic peptides to T lymphocytes. This process called antigen presentation launches the specific immune response. Activated macrophages also release several pro-inflammatory cytokines like TNF and IL-6 (participates in B-cell activation), type I IFNs, and several chemokines such as RANTES regulated upon activation normal T-cell expressed and secreted. Finally, nitric oxide (NO) and other substances are produced by macrophages. It has been demonstrated in experiments in vitro that the replication of HSV-1 in infected cells may be considerably inhibited in the presence of NO released from macrophages [43]. It should be mentioned here that IFN-gamma (belongs to type II IFNs) is in fact a cytokine released from activated NK cells but also produced by the helper CD4+ T lymphocytes as well as by cytotoxic CD8+ T lymphocytes [44]. The IFN-gamma considerably promotes function of activating macrophages and NK cells, in which it induces a production of cytolytic substances. Taken together, both NK cells and macrophages represent an important first-line defense at the early stage of HSV infection, that is, before the onset of specific immune response.

As already mentioned above, important immune cells participating in the initiation of antigen processing are the dendritic cells (DCs). They represent a relatively heterogenous cell population, from which the most active in antiviral defense are the myeloid (conventional) and the plasmacytoid DCs (pDCs), both derived from the myeloid progenitor cells. The myeloid DCs act as antigen-presenting cells (APCs), which carry the processed antigenic
peptides into lymph nodes, where the peptides in question are presented to the T lymphocytes. Based on this presentation event, the specific immune response is induced (activation of T and B cells). In DC-depleted mice infected with HSV-1, encephalitis developed with a significantly higher frequency as compared to conventional mice [45]. The plasmacytoid DCs under physiological conditions participate in the development of peripheral immune tolerance. Upon HSV infection, plasmacytoid DCs start to produce high amounts of IFN-alpha, which not only inhibits the virus replication in the otherwise surrounding susceptible cells but also activates lymphocytes and additional DCs. The HSV dsDNA binds to TLR-9 of the plasmacytoid DC which initiates IFN-alpha production [46].

4.2. The acquired immune response

The acquired immune response encounters the activation of both T-lymphocyte lines, namely, those differentiated either in direction of CD4+ or CD8+ T cells. The acquired immune response then is triggered by the activation of T-cell receptors (TCRs), which recognize the HLA I/CD8 and/or the HLA II/CD4 bound antigenic peptides. While the former T cells are involved in the destruction and elimination of HSV-infected cells, the latter acts as helper T cells at inducing the specific antibody production by B cells. At primary infection, the HSV-1 or HSV-2 particles are engulfed by DCs, which move the viral peptides to the regional lymph nodes, where the naïve (unprimed) lymphocytes get first stimulated. The presentation of exogenous viral peptides is achieved by means of the HLA class II molecules, which are recognized by the TCR of the CD4+ T lymphocytes. In contrast, the immunogenic peptides of the newly (de novo) synthesized HSV proteins are presented by HLA class I molecules at the surface of infected cells where they interact with the TCR of cytotoxic CD8+ T lymphocytes [47]. In certain extent, the so-called cross presentation occurs, at which the exogenous viral antigens are binding into the HLA class I molecules that are recognized by the CD8+ T lymphocytes [48]. The activated helper CD4+ T cells release cytokines such as IL-2 (inducing T-lymphocyte proliferation) and IL-4, IL-5 and IL-6 (promoting the differentiation of B cells into plasma cells).

The plasma cells synthesize the specific virus-neutralizing antibodies reacting mainly with the envelope glycoproteins such as gB and gD. The antibodies may also activate complement; on the other hand, the gC molecule of the HSV-1 envelope binds C3b and in less extent also C5b reducing the availability of complement components for virus neutralization. The low-pathogenic HSV-1 gC minus strains may become pathogenic as was confirmed in a C3 knockout murine model [49]. The HSV-1 antibodies belong to IgM as well as IgG class; the HSV-2 antibodies may be also of the secretory IgA class; the latter participates in virus clearance at the genital tract mucosa [50]. Even though the serum antibodies are of importance at the acute phase of infection, they may not fully eliminate the HSV since it invades the nerve endings and spreads to regional sensory ganglia before the development of antibody response. Some studies have shown that passive immunization with immune serum did not prevent latency after genital infection with HSV-2 [51, 52, 53]. Since the latent HSV (episomal vDNA) is frequently harbored in neural tissue for lifelong, the presence of HSV antibodies in the serum of healthy subjects may be interpreted as infectious immunity.
The cytotoxic CD8+ T lymphocytes represent the mainstream of the specific immune response providing clearance of HSV-1- or HSV-2-infected cells from the body. The activation of cytotoxic CD8+ T cells takes place in the regional lymph nodes by means of viral antigenic peptides presented by HLA class I molecules expressed at the surface of DCs. The IL-2 released from helper T cells acts on the precursor cytotoxic T lymphocytes which differentiate into mature cytotoxic CD8+ T cells. These mature CD8+ T cells accumulate at the peripheral virus inoculation site, where they eliminate the HSV-infected cells. The activated cytotoxic T lymphocytes release substances such as perforins, granzyme and granulysin, which destroy the infected target cell and/or induce apoptosis acting on their FAS receptors. They also release cytokines such as IFN-gamma and the tumor necrosis factor (TNF). IFN-gamma has a multiple effect, since it enhances the expression of HLA class I as well as HLA class II molecules [54] and also induces the expression of antiviral substances such as protein kinase R (PKR). This substance causes inhibition of translation of many viral but also cellular proteins. The increased TNF production also enhances the number and activity of HLA class I molecules.

The clearance of HSV-2 from peripheral tissues such as genital mucosa may be also provided by CD4+ T cells. Transgenic mice, which revealed defects in their CD8+ T-lymphocyte activity, have been still well protected against a lethal dose of HSV-2. In contrast, mice with depleted CD4+ T lymphocytes showed slower clearance and less protection against HSV-2 [55–57]. It can be concluded that the clearance of HSV-2 from genital mucosa requires cooperation of both the helper and cytotoxic T lymphocytes. Both T-cell populations can be found in the skin, in the genital and oral mucosa, in the ocular tissue, and also in the trigeminal ganglia surrounding the infected neurons [58–60].

After healing of the acute phase of HSV infection, about 0.1–1% of memory T cells, which may remain within the circulation, are still able to recognize the HSV antigenic epitopes. These epitopes being recognized by CD4+ and/or CD8+ lymphocytes were not all exactly mapped yet, but until now, 22 of them were defined. The HSV-specific epitopes can be found mainly among VP’s (structural envelope glycoproteins, tegument proteins and capsid polypeptides), but are also present on the ICPs, that is, on nonstructural enzymes and regulatory proteins. The CD4+ T lymphocytes generated against the HSV-2-specified structural proteins recognize the epitopes of the RR1/UL39 polypeptide, the epitopes of UL46 tegument protein, and several ones on glycoproteins gD and gB [61].

The CD8+ T lymphocytes, surrounding the neurons of the regional sensory ganglia of mice, rabbits, and humans in which viral latency had been established [62–64], are believed to control reactivation [65]. It was demonstrated, for example, that the ICP4/145K IE protein can be digested by granzyme B, which would prevent virus reactivation [66]. The accumulated CD8+ T cells produce IFN-gamma as well, inhibiting the expression of ICP0/110K IE protein [67].

The most intriguing still unexplained question is why do the CD8+ T lymphocytes accumulated in the neighborhood of latent HSV-harboring neurons, since the expression of IE and E polypeptides is widely hampered in them. Interestingly enough, the great majority of CD8+ T lymphocytes in the trigeminal ganglion of mice in which latency had been established possess a TCR which reacts with gB epitope at aa 498–505, while a smaller proportion of them may be stimulated with RR1 epitope aa 822–829. The reactivity of the other T cells was not identified.
In human trigeminal ganglia, both the CD4+ and CD8+ T cells were described that react with many antigenic epitopes present in the IE polypeptides ICP0 and ICP4, in the E polypeptides such as thymidine kinase (TK) and RR1, as well as with antigenic domains from structural proteins such as the tegument proteins VP11/12 and VP13/14 and envelope glycoproteins gB, gK, and gL, even though an exact identification of all antigenic epitopes in the HSV proteins was not done yet [70, 71].

The phenotype of CD8+ T lymphocytes which can accumulate within the ganglia was not characterized. The permanently present cytotoxic T lymphocytes might belong to the population of so-called tissue-resident memory T lymphocytes (TRM) or to the population referred as T-effector memory cells (TEM cells). Additional markers such as CD44, CD69, CD62L, and CCR7 were described at their surface, but their full definition is still the matter of investigation [72].

5. Survey of experimental HSV-1 and HSV-2 vaccines and their design

Though the first vaccines against HSV-1 and/or HSV-2 were prepared in the sixties of last century [73], no accepted and fully efficient HSV vaccine is available till now. The main problem is the incomplete understanding of the role of cytotoxic CD8+ T cells in the maintenance and/or of elimination neurons carrying the silenced HSV DNA. Another serious problem is the production of immune evasion polypeptides (reviewed at [74]). The optimal HSV vaccine should overcome the ICP47-mediated interference with TAP, which transports the viral peptides inside ER to bind with HLA molecules. Finally, the last but not negligible problem is that at least some of over 20 immunogenic epitopes, which are distributed within about 80 HSV proteins (both structural and nonstructural), should participate in the vaccine but in an optimal composition. An effective HSV vaccine might differ depending of its purpose, which could be either preventive or immunotherapeutic. An ideal vaccine should not allow the acute disease to develop, and it should be able to prevent the establishment of latency. These goals, especially the latter, are difficult to achieve. The minimum effect provided by an immunotherapeutic vaccine would be to minimize the extent of recurrent lesion originating from the reactivation of the latent viral genome. As described above, the cytotoxic T lymphocytes accumulating in vicinity of HSV DNA carrier cells in the regional sensory ganglion seem of great importance when keeping a state of equilibrium probably inhibiting the transcription of IE genes by an unknown mechanism. One could estimate that in the case of the inefficiency and/or complete failure of the function of cytotoxic T-cell virus, reactivation and subsequent retrograde axonal transport of virions occur. In this section, we describe the various HSV vaccines which have been used under experimental conditions, while the next paragraph will describe their efficacy in human volunteers.

5.1. Inactivated virion and subunit vaccines

The inactivated HSV-1 and/or HSV-2 vaccines consist of infected cell extracts, which have been introduced in the 1980s of last century, partially purified and inactivated either by UV light,
by thermal treatment, or with formalin [75–77]. The efficacy of these first vaccines was tested mainly in mice, but some of them were used also in human trials (see Section 6). Especially in animal models, a good protective effect against acute virus challenge has been found not to prevent the establishment of latency. However, when testing the effect of a virion-free HSV vaccine prepared by extraction of infected LEP cells with Nonidet P-40 [78] in the rabbit corneal model, Rajčáni et al. [79] concluded that even though vaccination prior to infection did not fully prevent latency, it considerably reduced the number of regional sensory (trigeminal) ganglion cells (neurons), which became the HSV genome carriers. Recently, a formalin-inactivated HSV-2 (FI-HSV2) vaccine mixed with Al(OH)$_3$ adjuvant was tested in combination with monophosphoryl lipid A (MPL) [80]. The latter authors showed that the vaccine protected mice against local vaginal challenge with HSV-2 as well as reduced the extent of latency established in the sensory ganglia of lumbosacral nerve roots.

The subunit vaccines were mainly glycoprotein mixes purified on lectins (e.g., lentil lectin) showing high affinity to the HSV virion envelope antigens. The dominant protective antigens among the 11 HSV glycoproteins are gB and gD, which possess important immunogenic epitopes. The abovementioned glycoproteins elicit virus-neutralizing antibodies, antibodies participating in the antibody-dependent cellular cytotoxic (ADCC) response, and they also activate the T lymphocytes. Several reports described the efficacy of the subunit vaccines of various purity based on either HSV-1 and/or HSV-2 envelope glycoproteins [81–84]. For example, the subunit HSV-1 vaccine (strain HSZP Immuno) had been prepared from chick embryo cells infected with the low-virulent HSZP strain [85]. Tested in cooperation with the Research and Development Department of the former Immuno AG Company in Vienna, the infected cell extract in question was purified on lentil lectin to obtain a glycoprotein mix containing at least four envelope glycoproteins (gB, gC, gD and gG). This subunit vaccine was immunogenic and protective in mice as well as rabbits and showed at least partial cross protection in the HSV-2-challenged guinea pigs infected by the vaginal route.

5.2. Recombinant HSV-1 and HSV-2 vaccines

The next step of HSV vaccine development proceeded from purified (nearly cell DNA-free) subunit vaccines to those composed of recombinant HSV-1/HSV-2 polypeptides [86–90]. Great majority of the recombinant vaccines contained the gB and/or gD polypeptides, and their efficacy was done in mice. The first purified glycoprotein vaccines (gB1 and gD1) were prepared on immunoaffinity columns (with specific-bound MoAbs); such vaccines were found to protect mice against intracerebral challenge with HSV-1 [91–93]. Manservigi et al. [94] expressed the gB ectodomain in mammalian cells. After immunization with the purified gB fragment, the mice developed virus-neutralizing antibodies not only against HSV-1 but also cross-reacting antibodies to HSV-2. In addition, the authors also showed that the animals were resistant to the challenge with a lethal HSV dose. Later on, the recombinant proteins were prepared by transfection of plasmids (carrying the gD and/or gB ORFs) into competent *Escherichia coli* cells, which expressed the corresponding not glycosylated polypeptides [90, 95–100]. Recombinant glycoproteins (including gC) were also prepared in insect and/or mammalian cells, in which the recombinant products could be glycosylated [101].
Immunization with the recombinant gD1 or gD2 alone, or in combination with the gB protected mice against infectious virus challenge given by intraperitoneal or subcutaneous routes and in the case of gD1 vaccination cross protection to HSV-2 was observed as well. Immunization with gD alone was more effective than that with gB alone [87], while immunity provided by gC alone was negligible [95]. In contrast, very high effect was achieved following immunization with the mix of gB1, gD1, and gE1 glycoproteins in combination with the Al(OH)₃ adjuvant including the cross protection of guinea pigs challenged with HSV-2 at genital route [99]. A trivalent vaccine containing gD2, gC2 and gE2 prepared in the baculovirus expression system was more immunogenic for mice than the subunit vaccine containing gC2 and gD2 [102]. Furthermore, Awasthi et al. [103] immunized the guinea pigs with recombinant proteins gC2 and gD2 prepared in a baculovirus system (which activated antibody production), with the adenovirus vector carrying the VP5 capsid protein and with the tegument proteins VP13 and VP14 (which activated the T-cell-mediated response). However, no significant difference was detected, which confirmed that the glycoprotein mix gD2/gB2 was sufficient enough.

The alum adjuvant in combination with the recombinant gD polypeptide activates the Th2-type humoral response and therefore may be less suitable for immunotherapeutic use, for which the Th1-type response is essential. To stimulate the Th1 response predominantly, cytokines as adjuvants were tested, such as IL-2 with good results after corneal challenge with the virulent HSV-1 CHR3 strain [104]. The so-called immune-stimulating lipid complexes (ISCOM) combined with HSV-2 immunogenic polypeptides induced both Th1- and Th2-type responses when showing a highly neutralizing antibody response along with the production of IL-2, IFN-gamma, and TNF [105]. Similar strong potentiating effect was observed at immunization with gD2 polypeptide adjuvanted with modified lipid A (AS04) [98]. Furthermore, when using the gD2 fusion polypeptide along with MF59 (squalene) as adjuvant, the local mucosa immune response could neither be stimulated properly, nor the latency reactivation prevented [106]. There is worth mentioning that the so-called autoimmune/inflammatory syndrome induced by adjuvants (ASIA) is an inflammatory syndrome associated with certain adjuvants (such as MF59/squalene); the latter potentiates pathological autoimmune reactions, which may be evident in the case of prophylactic mass immunization campaigns [107].

In contrast to T-lymphocyte stimulation, the local IgA secretion can be enhanced by mistletoe lectins such as ML-1, ML-2, and/or ML-3, the application of which in combination with the gD2 polypeptide elicited a good secretory IgA response at intranasal challenge in mice [108]. The authors also used non-ionized liposome like particles carrying the gB ectodomain and additional polylysine-rich polypeptides. Intranasal application of the product in question provided protection against lethal challenge with HSV-2 and induced a strong Th1-type immune response [109]. Skoberne et al. [110] prepared an experimental vaccine named GEN-003/MM-2, which contained gD2 and the IE protein ICP4/175K expressed in a baculovirus system. In combination with MM2 adjuvants (also called matrix M2 composed of cholesterol, phospholipids, and saponin), the vaccine showed immunotherapeutic effect in guinea pigs when it reduced the frequency of recurrent genital lesions upon HSV-2 challenge.
5.3. Viral vectored and DNA vaccines

The recent idea of DNA vaccines represents a progressive approach for immunization (reviewed at [111]). The advantage of DNA vaccines is that the immune response resembles that following the administration of live-attenuated virus vaccines, but without the risk of reversion to viral phenotype. The viral DNA vaccines fall into two categories: viral DNA carriers and the recombinant plasmid vaccines (classical DNA vaccines). The viral DNA carriers are in fact nonpathogenic attenuated viruses that genome contains the inserted ORF fragments encoding the desired antigen. The best examples are either the recombinant adenovirus or vaccinia virus, which carry the HSV gD or gB genes. The latter glycoproteins become expressed in the immunized animal, that is, in the mice, which are then protected against HSV challenge [112–115]. The recombinant vesicular stomatitis virus (VSV) was prepared from a plasmid containing the cDNA of the VSV RNA genome and the gD2 ORF US6. This recombinant virus induced a good cell-mediated immune response as well as anti-gD antibody formation. Immunized mice were protected against acute HSV-2 challenge, and the establishment of latency was also reduced [116].

Chiuppesi et al. [117] described the effect of immunization with feline lentiviral vector-based, herpes simplex virus 1 (HSV-1) glycoprotein B vaccine. This lentivirus construct induced HSV-1 antibody formation and also provided cross protection against lethal HSV-2 infection.

Many classical plasmid DNA vaccines were prepared with the inserted HSV DNA fragment. The majority of them encoded HSV-1 and/or HSV-2 glycoproteins such as gB, gD, gC, and gE; their efficacy was tested in mice, guinea pigs, and rabbits [90, 118–124]. The results were obtained after immunization with plasmids encoding the glycoproteins gD and/or gB of either HSV-1 or HSV-2 alone or in combination. Especially the immunization of mice and/or guinea pigs with the gD-expressing plasmid protected against challenge with a lethal dose of HSV-2 [124–128], but without clear-cut reduction of the latency rate. Better results were obtained with plasmids encoding both gB and gD [119, 129, 130]. The efficiency of DNA vaccines could be higher when adding cytokines such as IL-12 which induced the Th type 1 immune response [131, 132] similarly as the presence of IL-18 and/or RANTES [133, 134]. In contrast, the Th2-type response could be better achieved if the gD and IL-4-coding plasmid was used for immunization [133, 135]. The Th type 2 immune response is important for virus clearance from peripheral tissues including the infection of the eye, while the Th1-type response was involved in preventing the latency establishment [135]. The DNA vaccine-encoding gB when combined with DNA plasmid coding for cytokines IL-12 and IL-18 as adjuvant was efficient in prevention of the vaginal infection in mice: gB1/IL-12 and/or gB1/IL-18 elicited a local resistance of genital mucosa and protected mice against lethal HSV-2 challenge. The best results were observed with IL-12, while additional adjuvants did not enhance protection [136].

Another adjuvant tested with the DNA vaccines was the lipid adjuvant Vaxfectin®. In guinea pigs immunized with plasmid-expressing gD2 and VP11/VP12 as well as VP13/14 polypeptides along with a lipid adjuvant, a great prophylactic effect and the reduced HSV-2 replication in the genital tract of experimental animals was observed. The vaccine also showed immunotherapeutic properties when reducing the extent of latency (vDNA contents) in the dorsal root ganglia [137, 138]. Immunization with the recombinant plasmid encoding the
fusion protein consisting of the gB and the CCL19 chemokine ORF induced both the Th type 1 and the Th type 2 responses including an increased local secretory IgA antibody production protecting mice against HSV-2 challenge [139]. The enhanced efficiency of DNA vaccines could be achieved by combining genes encoding the envelope glycoproteins with certain nonstructural HSV genes. The recombinant plasmid consisting of gD2, gB2, and ICP27 ORFs elicited a higher cellular as well as humoral response than the plasmid construct encoding the glycoproteins only. The construct in question also provided a higher protection against vaginal challenge with HSV-2 [140].

5.4. Live-attenuated HSV-1 and HSV-2 vaccines

Attenuated HSV vaccines are live HSV-1 and/or HSV-2 viruses derived from the wild-type strains by modifications of their virulence but keep the immunogenic properties. The best known deletion for HSV-1 was the removal of the UL22 ORF-encoding gH glycoprotein [141, 142] and/or the removal of the IR1 sequence encoding the protein γ34.5 gamma, a neurovirulence factor [143]. Furthermore, the deletion of the gE encoding by US8 gene, which is not needed for virus replication in cell culture but is inevitable for neural uptake [144, 145] along with the deletion of the UL41 gene, encoding the virion host shutoff (vhs) protein [146] might be an excellent solution. In this respect, the non-virulent HSZP strain with natural mutations altering the vhs protein, if deleted in the gE gene, would be of special advantage [147]. Another attempts to prepare a live-attenuated HSV strain were made by deleting nonstructural genes, such as the ORF UL54 (encodes the ICP27 IE polypeptide), the ORF RS1 (encodes the transactivation protein ICP4) [146, 148], and/or the ORF RL2 (encoding the ICP0 transactivation protein) as described by Halford et al. [149]. All the above-mentioned attenuated viruses was protective against HSV challenge in various animal models. The HSV-2 deleted in the gH gene designated as disabled infectious single cycle (DISC) and tested as therapeutic vaccine, but no convincing protection was found [150].

Several other attenuated HSV strains were prepared by deleting the nonstructural genes important for virulence. Deleted ORFs for such purpose were the following: (1) the UL23 ORF encoding the thymidine kinase (TK) as described by Morrison and Knipe [151], (2) the UL39 ORF encoding the large RR1 subunit [152], (3) the UL29 ORF encoding the ssDNA-binding ICP8 polypeptide [153], (4) the UL5 ORF encoding a protein of the primase/helicase complex [154, 155], and finally (5) the UL9 ORF encoding the ori-binding protein [156]. The TK minus recombinant R7017 was prepared from the w.t. strain F, which genome was, in addition, deleted in the IR1 ORF sequence encoding the γ34.5 and furthermore manipulated by inserting the gD2, gI2, and gG2 sequences along with a portion of the gE ORF (US4–US8). The TK-reversed recombinant virus was further modified by reinserting the TK gene (R7020 TK plus). Both recombinant viruses protected mice as well as guinea pigs against HSV-2 challenge [148]. The next UL39-/RR1-deleted virus was tested not only in animals but also in man [152, 157]. The phase I and phase II clinical trials showed partial protection against recurrences (in 37.5% of immunized individuals as compared with the placebo group) [157]. The attenuated strain deleted in the genes UL29 and UL5 (called dl29-5) protected mice against ocular infection with HSV-1 and guinea pigs against genital challenge with HSV-2 [158, 159]. Stanfield et al. [160]
prepared an attenuated strain of HSV-2 called VC2, which had mutations in the gK gene and in the UL20 ORF (which is membrane protein inhibiting the neural uptake). This vaccine prevented genital infection upon vaginal challenge with HSV-2 and also inhibited latent infection in the lumbosacral dorsal root ganglia. Finally, the defective HSV-2 designated CJ9-gD2 was used to immunize mice. This virus had mutation in the UL9 ORF which encodes a vDNA replication protein and was deleted in the gD2 ORF (US9). The attenuated vaccine induced a higher antibody response in comparison with the gD2-alum/MPL subunit vaccine (used in a human trial) and protected mice against lethal challenge with HSV-2 [161].

6. Human vaccination trials: prophylactic and therapeutic HSV vaccines

Some of the vaccines mentioned above were tested not only in animal models but also in man at clinical trials. They were designed either as prophylactic vaccines, or they were destined for immunotherapeutic use, having been suggested for prevention or modulation of genital infection predominantly caused by HSV-2. The aim of prophylactic vaccine against HSV-2 infection is not only to prevent a clinical disease at primoinfection but also to interfere with the subsequent establishment of latency. The latter is a hard task, since the majority of vaccines just reduce the number of neurons which are getting HSV DNA carriers but does not fully prevent latent infection. Therefore, it is more reasonable to design a vaccine for immunotherapeutic use aimed to reduce the extent and the frequency of recurrent lesions.

The prophylactic vaccine should induce a satisfactory humoral as well as cell-mediated immune response. For such purpose, subunit, recombinant and DNA vaccines were tested. The first subunit vaccine tested was an HSV-2 glycoprotein mix (gB, gC, gG, gD, and gE) purified from infected chick embryo cells (this product is essentially similar to the subunit HSZP vaccine immuno mentioned above). As shown in 22 seronegative volunteers, this particular vaccine induced both the humoral and the cell-mediated specific response [162]. Alternatively, a similar vaccine (containing gB2 and gG2) was used to immunize 161 seronegative individuals with a less encouraging effect at phase II clinical trial [163]. The company Chiron Corporation sponsored the testing of a recombinant gB2/gG2 vaccine mixed with the MF59 adjuvant used for immunization of 137 persons. The efficiency of this vaccine was reported 9% only [53, 164]. Another study by GlaxoSmithKline uses a recombinant gD2 in combination with alum adjuvant and/or ASO₄; in the latter trial, 7460 volunteers were selected as seronegative before the onset of the phase II trial. Immunization with the vaccine in question induced higher antibody titers than natural infection and also conferred partial protection against natural infection that had a milder course as compared to non-immunized controls [165]. At phase III trial, 847 seronegative individuals (no antibodies to HSV-1 as well as to HSV-2) and 1867 women showing antibodies to HSV-1 but not to HSV-2 were immunized with the same vaccine [166]. The results showed that 73–74% immunized women which were seronegative against both antigens have been protected against HSV-2 primoinfection (as detected by natural seroconversion), but the vaccine was not effective in man and in HSV-1 seropositive women. The GlaxoSmithKline company provided another trial, during which together 8323 seronegative women (for HSV-1 as well as HSV-2) were immunized. This follow-
up was less encouraging when showing only 20% efficiency when measured by the seroconversion rate against natural genital HSV-2 infection and about 58% protection against HSV-1 genital infection [167]. In a multicenter study from 2013, the possible role of the abovementioned gD2-ASO4 vaccine in the etiology of stillbirth was evaluated. Together 19,727 pregnant women were immunized, from which 13.3% really had stillbirth in comparison with 11.00% of pregnant women in the control group that shows a clearly not significant effect [168].

The HSV DNA vaccine was also tested for prophylactic use. In the phase I trial, 62 seronegative women were immunized with the gD2 recombinant plasmid GENEVAX. T-cell-mediated response was found in one out of four volunteers [169]. Another prophylactic candidate vaccine tested was the HSV-2/HSV529-attenuated virus (deleted in genes UL5 and and UL29, also called dl5-29). This vaccine when tested in guinea pigs elicited a satisfactory humoral as well as T-cell response, conferred protection to HSV-2 challenge, and reduced the extent of latency in the regional lumbosacral ganglia [170]. The vaccine efficiency in human will be evaluated by the end of 2016.

As already mentioned, the therapeutic vaccines should decrease the frequency of recurrence episodes as well as their severity. To achieve this, the therapeutic vaccine must induce a potent and specific T-cell-mediated response. Several subunit vaccines were tested with an immunotherapeutic purpose, namely, the semipurified inactivated infected cell extracts, recombinant vaccines, and attenuated viruses. Probably, the first of the purified inactivated HSV-1-infected cell extract tested was the vaccine by Kutinová et al. [171]. A similar vaccine introduced by Skinner et al. [172] was used for immunization of 316 subjects with herpes disease who had recurrent blisters at least six times per year. Though this vaccine induced a detectable increase in the antibody levels and a satisfactory T-cell-mediated response, no difference in the frequency of recurrences was noted during the 1-year observation period. However, the vaccination accelerated the healing of the lesions, which had a less severe course in the immunized persons. Among the recombinant vaccines tested in a therapeutic context, the gD2 vaccine containing alum adjuvant should be mentioned first. This was used for immunization of 98 HSV-2 seropositive persons who had at least four but up to 14 recurrence episodes per year [173]. In this trial, the average of six recurrences per year in the placebo group was significantly higher than the average of four recurrences per year in the immunized group (p = 0.039). Furthermore, the gB2/gD2 vaccine mixed with the MF59 adjuvant was applied in another trial, in which 202 volunteers were immunized. At the phase II clinical trial, no difference was found in the titers of neutralizing antibodies during the observation period of 8 months, but there was a significant difference in the increased interval between the occurrences of the recurrent lesions following the immunization procedure [174]. Further trials were made using peptide vaccines. An overview of defined T-cell-based epitopes from HSV proteins was reported by Laing et al. ([175], Table 2).

Not all known T-cell based epitopes can induce protective immunity. Mapping of T cells in seropositive population found out that CD4+ T cells were mainly stimulated by tegument proteins UL21, UL46, UL47, UL49 and envelope glycoproteins gB and gD. It was observed that gB stimulates both CD4+ and CD8+ T cells, whereas gD induces stronger CD4+ T-cell-dependent immunity [58]. CD8+ T cells were stimulated by gD53–61, gD70–78, and gD278–
286 peptides mainly in HLA-A*02:01-positive HSV-1 and HSV-2 seropositive healthy individuals [190]. Moreover, CD8+ T cells were also induced by tegument protein VP11/VP12 (gene UL46) that would account for another candidate protein to prepare effective HSV vaccine [61].

Table 2. Overview of T- and B-cell HSV-1/HSV-2–specific epitopes as tested in human, mice, and rabbits⁹.

Vaccine design also requires involvement of epitopes suitable for HLA binding by most people. In most ethnic groups, HLA-A*02:01 and HLA-B*07:02 belong to the most abundant HLA

<table>
<thead>
<tr>
<th>Protein (gene)</th>
<th>HSV-1 T-cell epitopes</th>
<th>HSV-2 T-cell epitopes</th>
<th>B-cell epitopes</th>
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<tr>
<td></td>
<td>CD4</td>
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*Modified according to Laing et al. [175].
alleles [194]. HLA-A*02:01-restricted epitopes have been found in glycoprotein B (UL27 442–451), tegument protein VP13/VP14 (UL47 551–559), and tegument protein coded for UL25 (UL25 372–380) of HSV-2, and they stimulated CD8+ T-cell response [176, 195]. HLA-B*07:02-restricted epitopes have been reported for tegument protein VP22 (gene UL49) (HSV-2 UL49 49–57, HSV-2 UL49 82–90, HSV-2 UL49 99–108, HSV-2 UL49 131–140, and HSV-1 UL49 291–290) and stimulated CD8+ T-cell response [176]. Samandary et al. [196] found association with high prevalence of herpes infection and disease with the frequency of HLA-A*24, HLA-B*27, and HLA-B*58 alleles. In contrast, low prevalence of herpes infection and disease appeared associated with the high frequency of HLA-B*44 allele.

The effectiveness of immune response is also depending on adjuvant type. Cooper et al. [189] found out that vaccine design and adjuvant type can have a significant effect on T-cell epitope utilization. Four epitopes within the gD2 molecule gD2 49–63, gD2 105–119, gD2 245–259 and gD2 333–347 were administered to mice with alum or IL-12. CD4+ T-cell response was induced in mice immunized with gD HSV-2 epitope gD2 245–259 and adjuvant alum. Mice immunized with IL-12 stimulate CD4+ T-cell response to HSV-2 epitope gD2 245–259 as well as to gD2 333–347 [189].

“Epitope” (peptide) vaccines that selectively stimulate T and B cells belong to other herpes simplex vaccine candidates. Wang et al. [180] prepared multi-epitope peptide vaccine that contained six B-cell epitopes from different glycoproteins of HSV-2 (gB2 466–473, gC2 216–223, gD2 6–18, gE2 483–491, gG2 572–579 and gI2 286–295), four CD4+ T-cell-based epitopes (gD2 21–28, gD2 205–224, gD2 245–259 and gB2 162–177) and two CD8+ T-cell-based epitopes (gD2 10–20 and gD2 268–276). All above-described epitopes were inserted into the extracellular fragment (1–290) of HSV-2 glycoprotein D to construct multi-epitope assembly peptides (MEAPs) by replacing some non-epitope amino acid sequences. The genes of the selected peptides were inserted into recombinant plasmid and expressed in E. coli strain BL21.

The multi-epitope vaccine elicited in mice production of virus-neutralizing antibodies induced Th1 and Th2 immune response and protected mice against intravaginally induced lethal challenge of HSV-2 [180].

Many studies have focused on mapping of protective epitopes that stimulate immunity in asymptomatic individuals, that is, individuals without clinical findings of herpes infection. Analysis of IFN-γ-producing CD4+ T cells in HSV-1 seropositive individuals revealed that gB peptide epitopes (aa 166–180 and aa 666–680) were strongly recognized by CD4+ T cells from asymptomatic individuals, but not from symptomatic individuals. Inversely, CD4+ T cells from symptomatic individuals preferentially recognized gB (aa 661–675) [179]. Another study identified asymptomatic CD8+ T-cell epitopes from glycoprotein D (gD53–61, gD70–78 and gD278–286) [191]. It can be hypothesized that repertoire of T-cell-based epitopes determines either the development of HSV-1 (-2) clinical symptoms or asymptomatic viral shedding.

Finally, some investigators preferred the genetically attenuated virus vaccines, because they induce an immune response essentially similar to that following natural infection. For example, Casanova et al. [157] immunized the volunteers (32 persons having at least five recurrent lesions per year) with an attenuated HSV-2 virus deleted in the UL39 gene (the RR1 protein ORF). The phase I and phase II clinical trials showed the reduction of recurrent clinical
manifestations by 37.5% as compared with the placebo-immunized group. Another clinical trial, which was performed in 2006, took the advantage of HSV-2-attenuated virus deleted in the gH gene (the so-called disabled infectious single cycle [DISC] virus). Unfortunately, no significant difference was found in the number of recurrences between the immunized and control mock-immunized subjects during the 1 year follow-up period [150]. The last phase I and II clinical trial which results will be announced nowadays concerns volunteers who had from two to nine recurrent lesions per year; they were immunized with a DNA vaccine-expressing gD2 in combination with the lipid adjuvant Vaxfectin [197].

7. Future perspectives of HSV-1/HSV-2 vaccination with the emphasis on the T-cell response

As described above, an effective vaccine against HSV-1 and HSV-2 infection that would prevent virus reactivation (therapeutic vaccine) should stimulate both humoral and cellular immunity mediated by CD4+ and CD8+ T cells. This immune response can be induced by live-attenuated virus, but such viruses are not safe because of their possible reversion back to the wild-type virus. Therefore, alternative approaches to develop effective herpes simplex vaccine have been attempted during the last decades. Nowadays, T-cell-inducing herpes simplex vaccines bearing “asymptomatic” immunodominant epitopes derived from HSV proteins were designed and tested [180]. Such vaccines possess many advantages over traditional vaccine like (1) induction of specific T-cell-based immunity, (2) inhibition of pathogenic immune response, and (3) safety.

Only few asymptomatic T-cell epitope-based vaccines were prepared and tested until now. The vaccine used by Wald et al. [198] contained 32 synthetic immunogenic HSV-2 peptides, linked to the heat shock protein Hsp 70 in combination with the QS-21 (contains a saponin) adjuvant. In 32 immunized volunteers, the vaccine elicited no complication, but induced a satisfactory CD4+/CD8+ T-lymphocyte response against a wide range of immunogenic HSV-2 peptides used in the stimulation tests in vitro. Chentoufi et al. [191] prepared CD8+ T-cell epitope-based vaccine containing three separate pairs of CD4–CD8 lipopeptides. Each of the lipopeptide contained one of the three asymptomatic immunodominant human CD8+ T-cell peptide epitopes from HSV-1 glycoprotein D (gD53–61, gD70–78, and gD278–286) that were joined with a human CD4+ T-cell peptide epitope (gD49–82). Humanized HLA-A*02:01 transgenic rabbits were immunized with a mixture of the three CD4–CD8 HSV-1 gD lipopeptides. Immunization induced an increased production of CD4+ and CD8+ T cells and protected rabbits against ocular HSV disease [191]. The same ASYMP vaccine was used for therapeutic vaccination of HLA transgenic rabbits infected by HSV-1. The vaccine induced production of HSV-specific CD8+ T cells that prevent HSV-1 reactivation ex vivo from latently infected explanted trigeminal ganglia. Moreover, the vaccine significantly reduced HSV-1 shedding and boosted the function of HSV-1 gD epitope-specific CD8+ T cells in draining lymph nodes, conjunctiva, and trigeminal ganglion [199].
The lipopeptide vaccines belong to another herpes simplex vaccine candidate. The lipopeptide vaccine can be easily produced and possesses some advantages over traditional vaccine such as safety and tolerance, bearing protective T-cell epitopes derived from HSV antigens, missing of non-immunogenic harmful epitopes, and missing of viral pathogenic proteins such as ICP47, and lipids have functioned as adjuvant. Previous studies observed that lipopeptide vaccine injected intranasally into mice induced mucosal and systemic B and Th1 immune response [191]. Other authors prefer attenuated genetically modified herpes simplex viruses as successfully vaccine candidates (Section 5.4).

8. Conclusions

In conclusion, the development of successfully therapeutical vaccine against HSV infection should respect the following recommendations: (1) the assessment of putative differences in the recognition of T- and/or B-cell epitopes from envelope, tegument, and regulatory HSV proteins in patients with recurrent herpes disease versus asymptomatic individuals; (2) the production of new safer adjuvants avoiding those claimed to cause the ASIA syndrome; (3) the induction of local mucosal immunity mediated by lipopeptides; (4) the use of humanized susceptible HLA transgenic mice as well as rabbits before human trials; and (5) at last but not least, the efficacy of human trials for an immunotherapeutic vaccine that should be made according to internationally accepted unified criteria in at least three groups of subjects (seronegative individuals, seropositive individuals without recurrent herpes disease, and volunteers with such disease). The tests should include the demonstration of elevated antibody titers after vaccination and in vitro testing of the T-lymphocyte response in a blastic transformation test as well as production of selected cytokines; 6. Finally, the application of the therapeutic vaccine in future human trials should be intracutaneous rather than by using other administration routes.

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