Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are the causative agents of fever blisters and genital herpes. In immunocompromised persons, HSV can cause more severe infections, including encephalitis. HSV-1 and HSV-2 belong to the family Herpesviridae, a group of double stranded DNA viruses. Virions of HSV-1 and HSV-2 are surrounded by a membrane, which consists of a lipid bilayer. In this membrane, at least 11 viral glycoproteins are present which have a role in a wide variety of viral processes. They are essential for the attachment of virions to cells, for the entry of virions into cells, and for the cell-to-cell spread of virions. In addition, the viral glycoproteins have functions in viral egress and immune evasion.

Two of these glycoproteins, glycoproteins H (gH) and L (gL) are the subject of this thesis. gH and gL are present as gH:gL heterodimers in the envelopes of virions and in the membranes of infected cells. The gH:gL complex functions in the fusion of the virion envelope with the membrane of the cell and in the fusion of the membrane of an infected cell with the membrane of an uninfected cell. The gH:gL is required for efficient entry of virions into cells, most likely because its essential role in membrane fusion events. Homologs of the HSV-1 gH:gL complex have been found in all herpesviruses investigated till now. This indicates the importance of this complex in the functionality of a herpesvirus.

This thesis describes structural and immunological aspects of the gH:gL complex. These aspects of the gH:gL complex were examined with the use of the baculovirus expression system and an inducible mammalian expression system.

Structural aspects of the gH:gL complex

Expression of the gH:gL complex

The glycoproteins H and L were expressed in insect cells (chapters 2, 3, 4, and 5) and in chinese hamster ovary cells (chapter 6). Correctly folded and transported gH:gL complex is obtained only when both gH and gL are expressed in a single cell. When gH is expressed without gL, it is retained in the cell, probably in the endoplasmic reticulum (ER) or cis-Golgi, and contains not fully processed oligosaccharides. In addition, some gH-specific monoclonal antibodies do not recognize gH when expressed without gL. Upon coexpression with gL, gH is transported to the cell surfaces, it contains fully processed oligosaccharides and is recognized by all gH-specific monoclonal antibodies.

Chapter 2 describes the formation of gH:gL hetero-oligomers and cell surface expression of the gH:gL complex in insect cells. By using recombinant baculoviruses, gL, gH, and gL plus gH were expressed in insect cells. This study showed that recombinant gH:gL hetero-oligomers were produced in insect cells. In addition, gH showed gL-dependent transport and folding when expressed in insect cells. Recombinant gH was not detected on the surfaces of insect cells in the absence of gL. When co-expressed with gL, recombinant gH was displayed on the surfaces of insect cells. These results indicate that the process of folding and intracellular transport of glycoproteins gH and gL is similar in insect cells and mammalian cells. Therefore, the structural aspects of the gH:gL complex can be studied by using expression in insect cells.

In addition to the expression in insect cells, gH and gL were expressed in the mammalian chinese

hamster ovary cells (chapter 6). A stable cell line which expresses gH of HSV-1 was established (CHOgH-A16). The gH expression by CHOgH-A16 is regulated by the tetracycline concentration in the culture medium. Glycoprotein H produced by this cell line showed gL-dependent folding and gL-dependent cell surface expression. This indicates that gH is folded and transported similar to viral gH. The established cell line provides a powerful tool for further studies on the biological function and the molecular mechanism of the gH:gL complex formation.

Expression of a secreted form of the gH:gL complex

Truncated forms of gH which lacked the transmembrane region and the cytoplamic tail were co-expressed in insect cells. The truncated gH was either untagged (gH_t, chapter 2) or tagged at the C-terminus with a six-histidine sequence ($gH_{t(His)}$, chapter 4). Recombinant gH_t:gL and recombinant $gH_{t(His)}$:gL complexes were secreted into the culture medium upon coexpression (chapters 2 and 4). In addition, the secreted recombinant gH_t:gL and $gH_{t(His)}$:gL complexes were recognized by all available gH-specific monoclonal antibodies. This indicated that gHt and $gH_{t(His)}$ are transported to the surfaces of insect cells in a gL-dependent manner similar to full-length gH and that the gH_t:gL complex and the gH_t:gL complex are folded similar to authentic gH:gL.

Domains of gH involved in the interaction with gL

The amino acids involved in the interaction between gL and gH are not yet known. Until now, two studies are reported in which the gL-binding site on HSV-1 gH is investigated. In chapter 3, the complex formation between glycoproteins H (gH) and L (gL) of herpes simplex virus type 1 (HSV-1) was studied by using five recombinant baculoviruses expressing open reading frames that contain deletions in the coding region of the extracellular domain of gH. The data from this study did allow at least two interpretations. There is either one binding site for gL binding on gH between gH residues 300 and 473 or gL contacts multiple regions of gH. Another study used truncated forms of gH to determine the minimal region of gH required for gH:gL complex formation and secretion [166]. The data from this study demonstrated that the N-terminal 323 amino acids of gH were still able to form a stable complex with gL and that this complex was secreted into the culture medium. This latter finding is not in contrast with the findings in chapter 3 because the gH region 1 to 323 contains an overlap of 23 amino acids with the region identified in chapter 3. The three-dimensional structure of the gH:gL complex will eventually reveal the residues involved in the gH:gL interaction. Highly purified recombinant $gH_{t(His)}$:gL complex (chapter 5) could enable studies for the determination of the molecular structure of gH:gL by crystallographic methods.

Immunological aspects of gH:gL

Purification of secreted recombinant gH:gL complex

The humoral and T-cell responses were studied using purified recombinant $gH_{t(His)}$:gL complex (chapter 5). This complex was isolated from insect cell culture medium to a high degree of purity by immobilized metal affinity chromatography (IMAC). IMAC is widely used for the purification of recombinant proteins that contain a polyhistidine tag. Recombinant $gH_{t(His)}$:gL complex contains a six histidine tag at the C-terminus of truncated gH and was purified to near homogeneity by

using IMAC. A colum
chelating groups, whic
recombinant $gH_{t(His)}$:gL
contaminant proteins
EDTA (0.5 mM) in on

Humoral and T-cell re

As described in chap
responses to the $gH_{t(His)}$
seropositive and three
which was purified fro
most likely orchestrate
T cells stimulated with
detectable interleukin

In 1998, it was shown
complex of HSV-1 cou
with the finding that n
the glycoprotein $gH_{t(His)}$
important candidate a
control of HSV infecti

using IMAC. A column was used that consisted of nickel ions immobilized to iminodiacetic acid chelating groups, which were coupled via a linker to highly cross-linked agarose beads. Not only recombinant $gH_{t(His)}$:gL complex bound to the column but also contaminant proteins. These contaminant proteins could be separated from $gH_{t(His)}$:gL complex using low concentrations of EDTA (0.5 mM) in one of the washing buffers.

Humoral and T-cell responses to the gH:gL complex

As described in chapter 5, naturally acquired HSV-1 infection induces humoral and T cell responses to the $gH_{t(His)}$:gL complex of HSV-1. These responses were studied in seven HSV-1 seropositive and three HSV-1 seronegative healthy donors using recombinant $gH_{t(His)}$:gL complex which was purified from the culture medium of insect cells. The measured T cell responses were most likely orchestrated by lymphocytes with a Th1 phenotype because culture supernatants of PB T cells stimulated with recombinant $gH_{t(His)}$:gL contained high levels of gamma interferon and no detectable interleukin 4.

In 1998, it was shown by others that immunization with soluble recombinant glycoprotein gH_t:gL complex of HSV-1 could protect mice from a HSV type 1 challenge [149]. This, in combination with the finding that naturally acquired HSV-1 infection induces humoral and T cell responses to the glycoprotein $gH_{t(His)}$:gL complex indicates that the gH:gL complex of HSV-1 may be an important candidate as a component of an effective subunit vaccine for the prevention and/or control of HSV infections.