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(54) **VACCINE AGAINST BETA-HERPESVIRUS INFECTION AND USE THEREOF**

(76) Inventors: **Christian Thirion**, Munich (DE); **Ulrich Koszinowski**, Feldafing (DE); **Christian A. Mohr**, Munchen (DE); **Zsolt Ruzsics**, Diessen am Ammersee (DE)

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(57) **ABSTRACT**

The present invention is related to a beta-herpesvirus, wherein the beta-herpesvirus is spread-deficient.

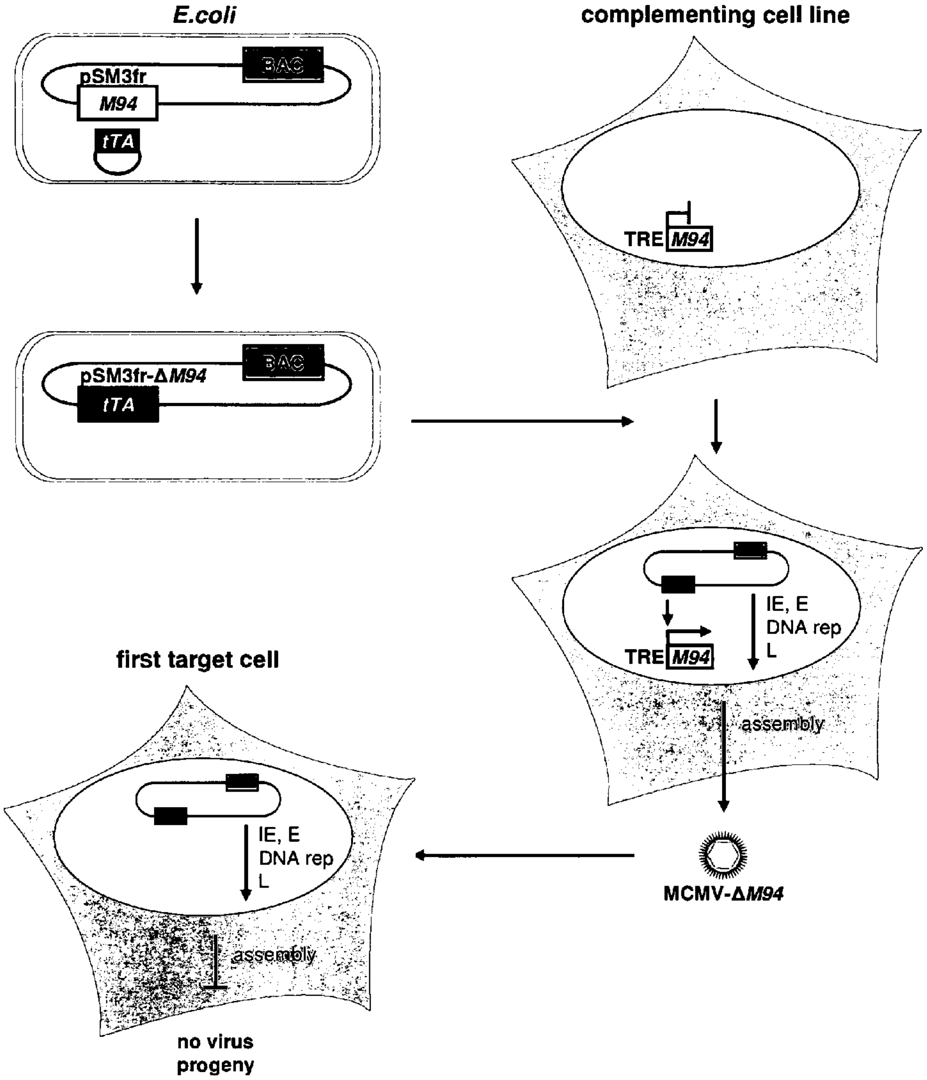


Figure 1

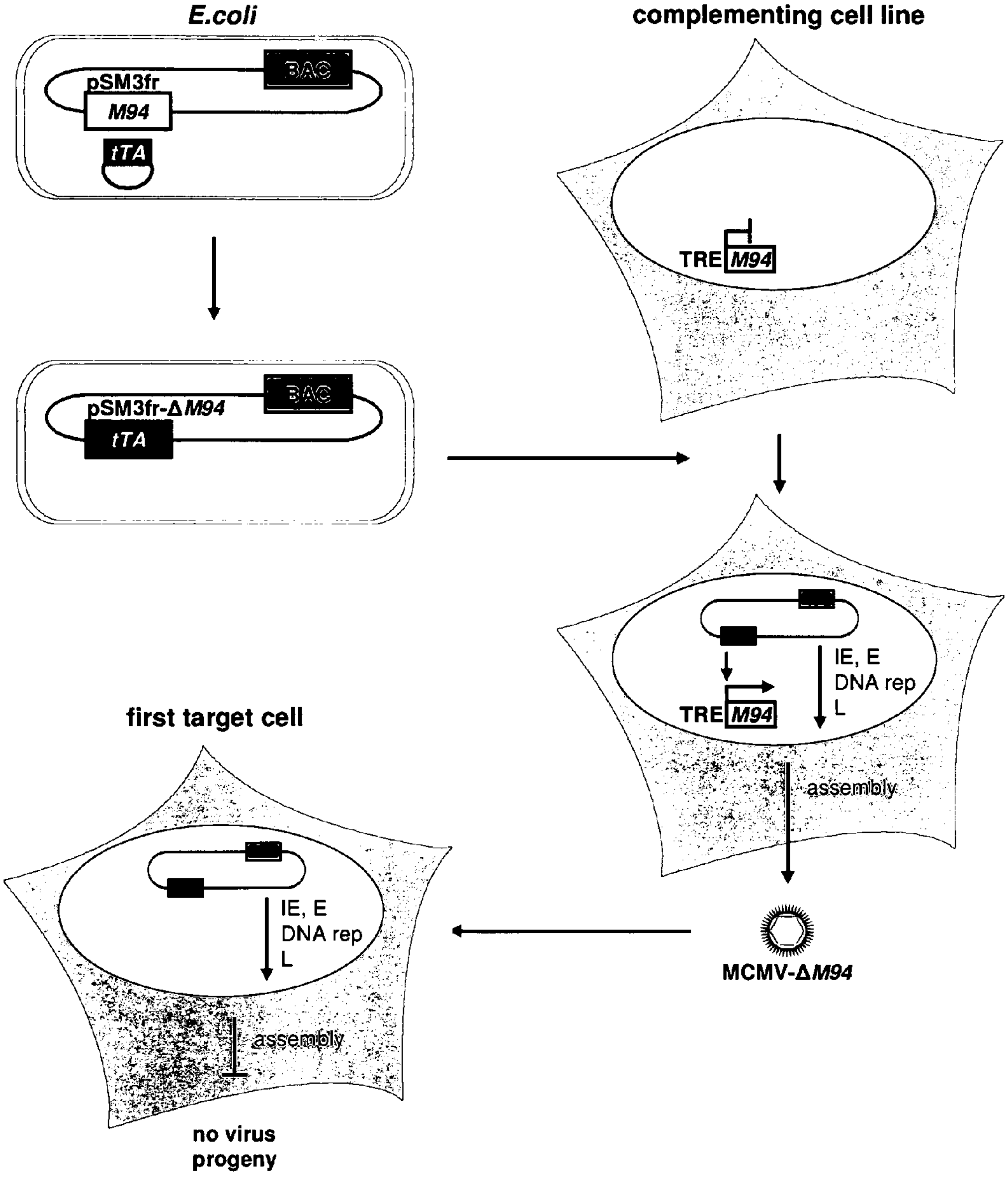

E.coli
pSM3fr
BAC
M94
tTA
pSM3fr-ΔM94
BAC
tTA
complementing cell line
TRE
M94
IE, E
DNA rep
L
TRE
M94
assembly
MCMV-ΔM94
first target cell
IE, E
DNA rep
L
assembly
no virus
progeny

Figure 2

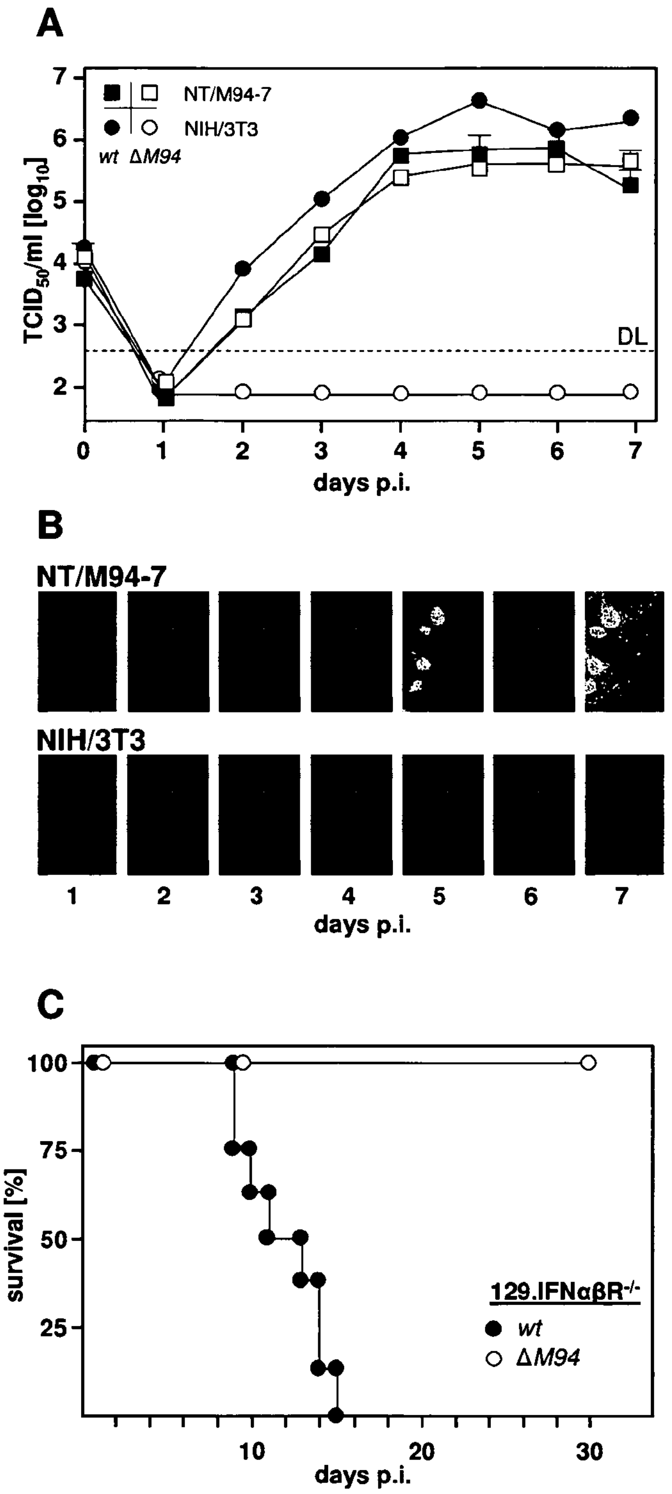

A
NT/M94-7
NIH/3T3
wt ΔM94
TCID50/ml [log10]
7
6
5
4
3
2
DL
0
1
2
3
4
5
6
7
days p.i.
B
NT/M94-7
NIH/3T3
1
2
3
4
5
6
7
days p.i.
C
survival [%]
100
75
50
25
129.IFNαβR-/-
wt
ΔM94
10
20
30
days p.i.

Figure 3

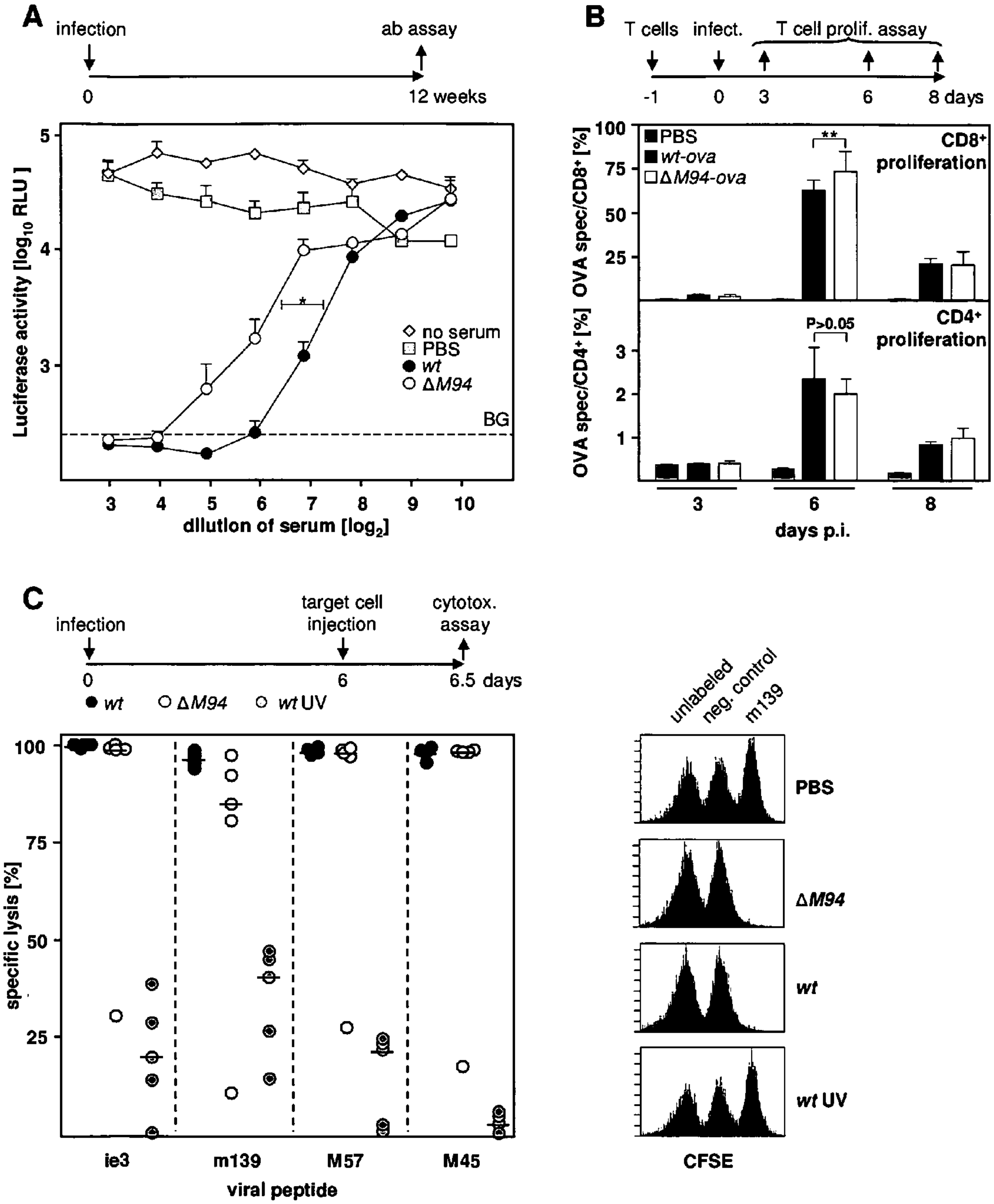

A
infection
ab assay
0
12 weeks
Luciferase activity [$\log_{10}$ RLU]
5
4
3
*
◇ no serum
□ PBS
● wt
○ ΔM94
BG
3
4
5
6
7
8
9
10
dilution of serum [$\log_2$]
B
T cells
infect.
T cell prolif. assay
-1
0
3
6
8 days
OVA spec/CD8+ [%]
100
75
50
25
■PBS
■wt-ova
□ΔM94-ova
**
CD8+ proliferation
OVA spec/CD4+ [%]
3
2
1
P>0.05
CD4+ proliferation
3
6
8
days p.i.
C
infection
target cell injection
cytotox. assay
0
6
6.5 days
● wt
○ ΔM94
○ wt UV
100
75
50
25
specific lysis [%]
ie3
m139
M57
M45
viral peptide
unlabeled
neg. control
m139
PBS
ΔM94
wt
wt UV
CFSE

Figure 4

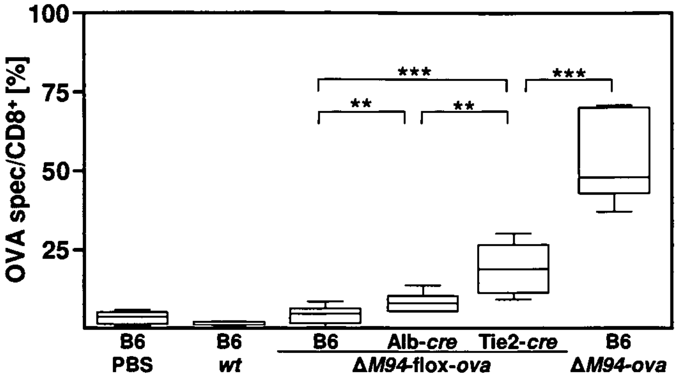

100
75
50
25
OVA spec/CD8+ [%]
***
**
**
***
B6
PBS
B6
wt
B6
Alb-cre
Tie2-cre
ΔM94-flox-ova
B6
ΔM94-ova

Figure 5

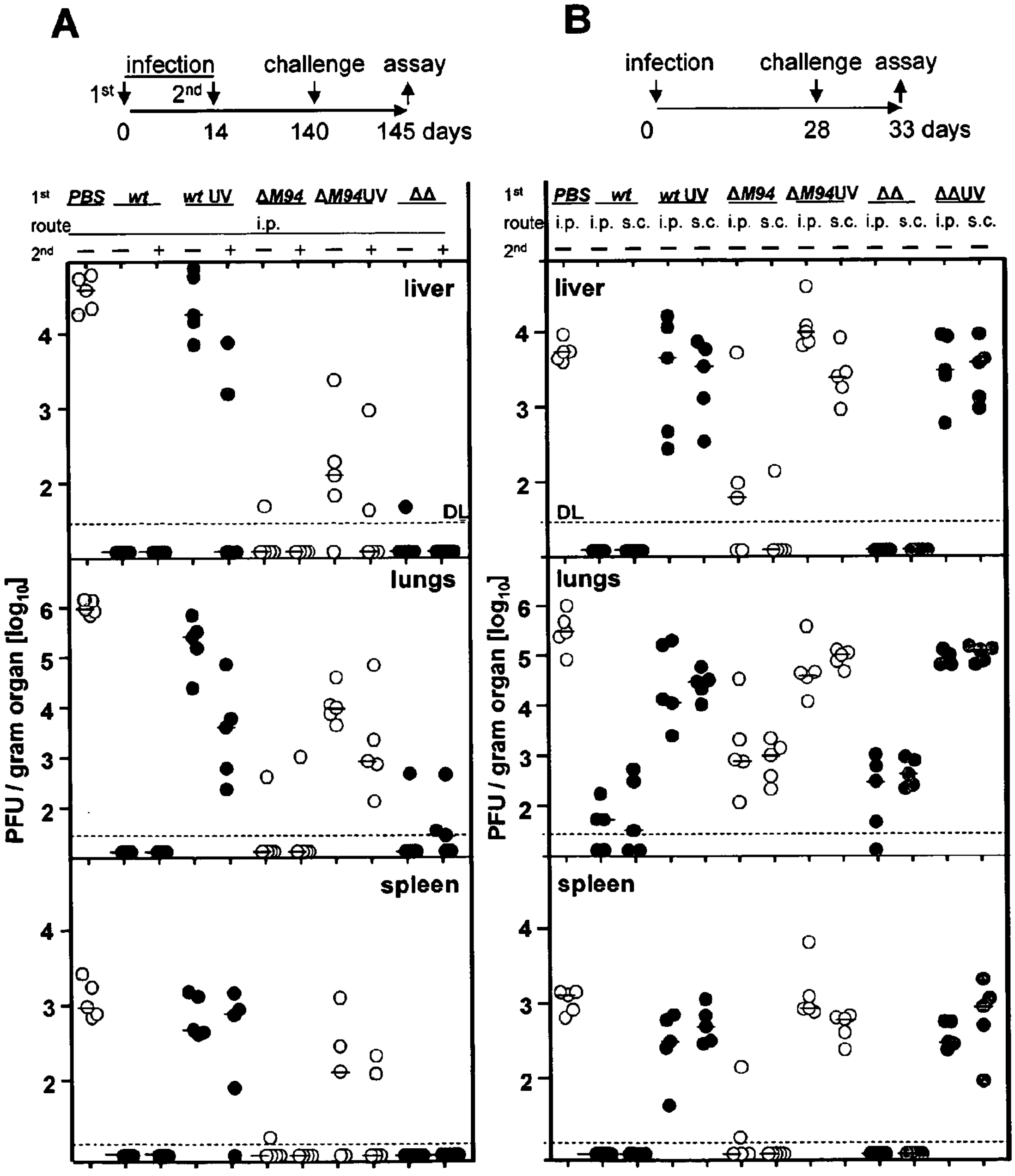

A
infection
challenge
assay
1st
2nd
0
14
140
145 days
1st PBS wt wt UV ΔM94 ΔM94UV ΔΔ
route i.p.
2nd — — + — + — + — + — +
liver
lungs
spleen
DL
PFU / gram organ [log10]
B
infection
challenge
assay
0
28
33 days
1st PBS wt wt UV ΔM94 ΔM94UV ΔΔ ΔΔUV
route i.p. i.p. s.c. i.p. s.c. i.p. s.c. i.p. s.c. i.p. s.c. i.p. s.c.
2nd — — — — — — — — — — — — —

Figure 6

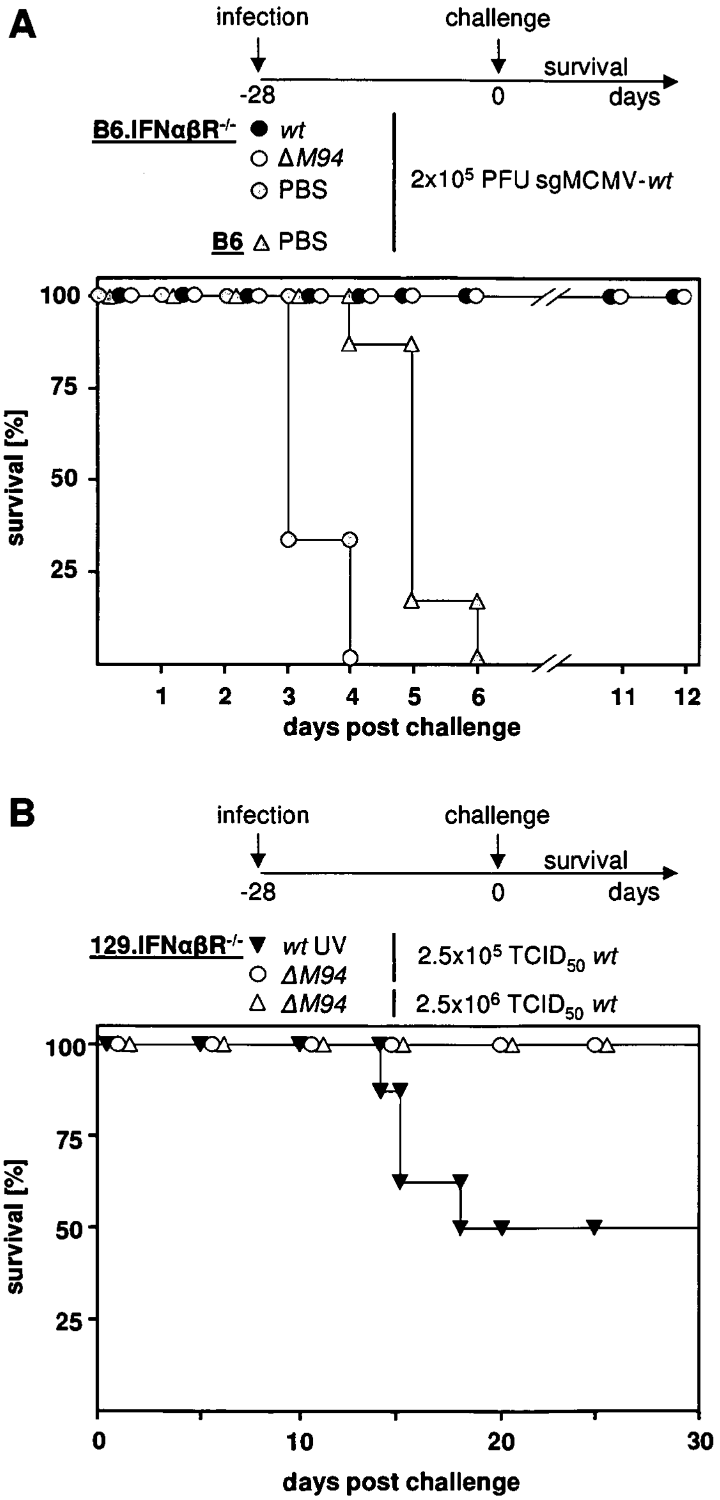

A
infection
challenge
survival
-28
0
days
B6.IFNαβR$^{-/-}$ ● wt
○ ΔM94
⊙ PBS
B6 △ PBS
2x10^5 PFU sgMCMV-wt
100
75
50
25
survival [%]
1 2 3 4 5 6 11 12
days post challenge
B
infection
challenge
survival
-28
0
days
129.IFNαβR$^{-/-}$ ▼ wt UV
○ ΔM94
△ ΔM94
2.5x10^5 TCID$_{50}$ wt
2.5x10^6 TCID$_{50}$ wt
100
75
50
25
survival [%]
0 10 20 30
days post challenge

Figure 7

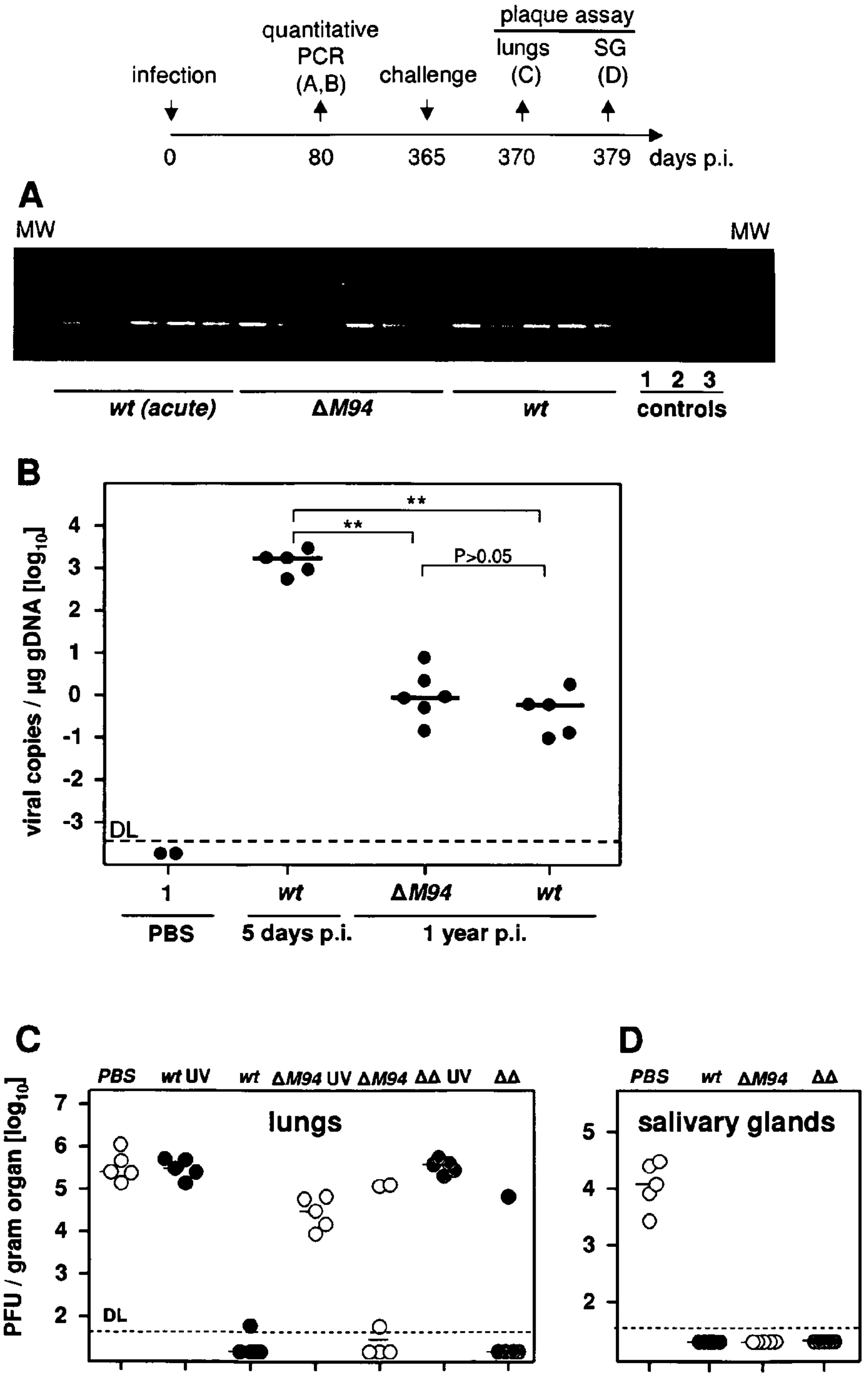
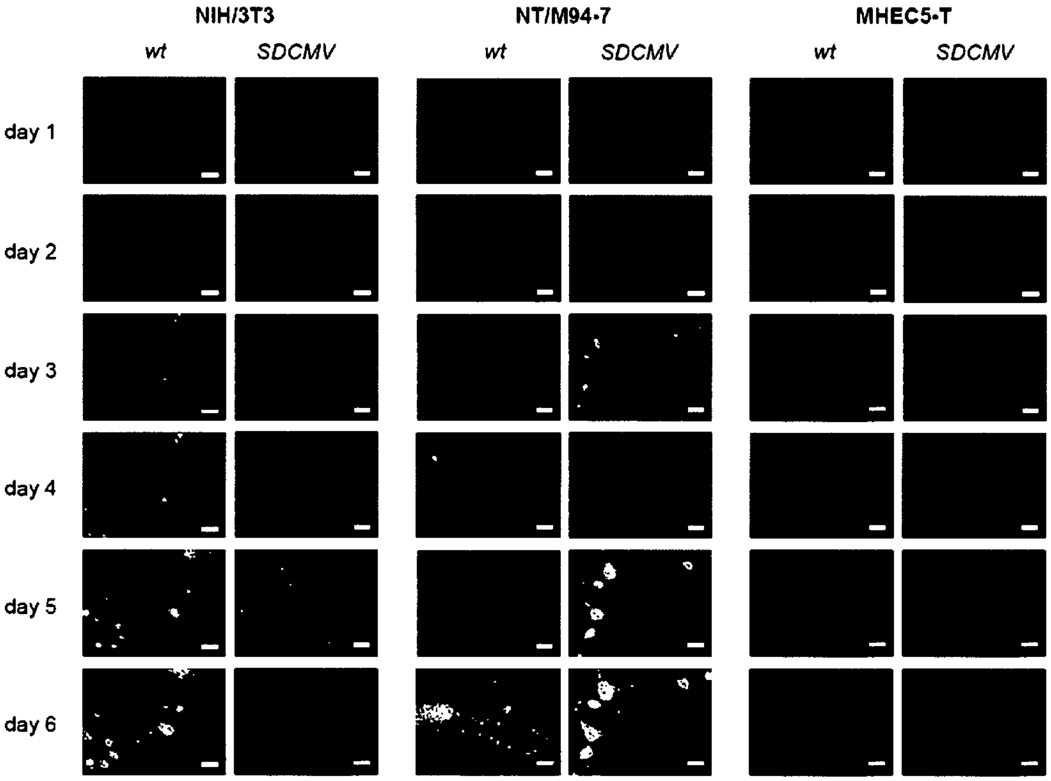

plaque assay
quantitative PCR (A,B)
infection
challenge
lungs (C)
SG (D)
0
80
365
370
379
days p.i.
A
MW
MW
1 2 3
wt (acute)
ΔM94
wt
controls
B
**
**
P>0.05
viral copies / μg gDNA [log10]
4
3
2
1
0
-1
-2
-3
DL
1
wt
ΔM94
wt
PBS
5 days p.i.
1 year p.i.
C
PBS
wt UV
wt
ΔM94 UV
ΔM94
ΔΔ UV
ΔΔ
lungs
PFU / gram organ [log10]
7
6
5
4
3
2
DL
D
PBS
wt
ΔM94
ΔΔ
salivary glands
5
4
3
2

Figure 8



day 5: MCMV-Δ*M94* higher magnification

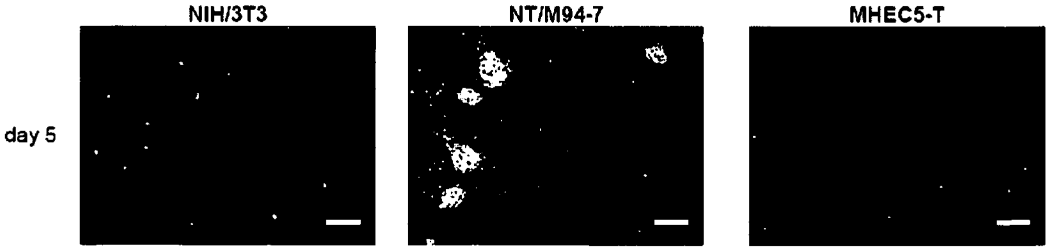
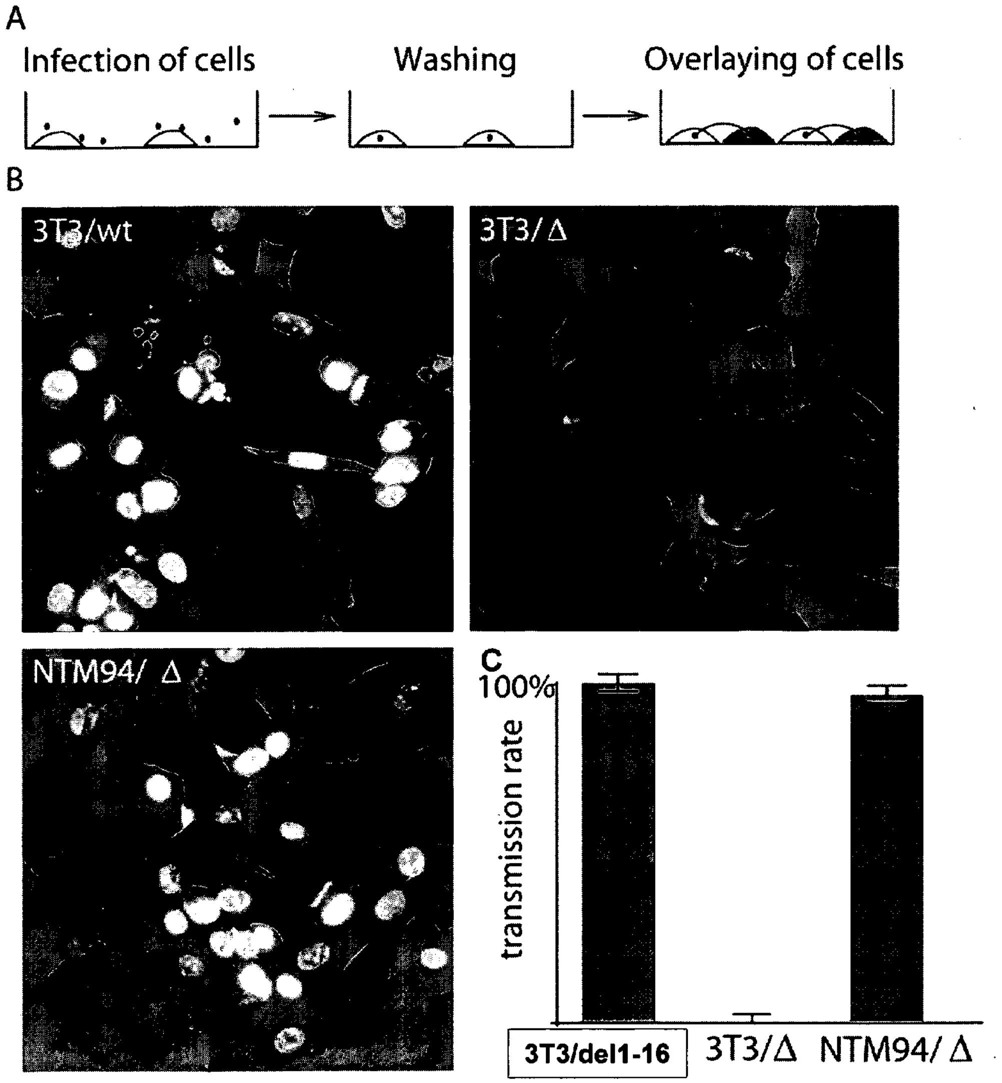


Figure 9


A
Infection of cells
Washing
Overlaying of cells
B
3T3/wt
3T3/Δ
NTM94/ Δ
C
100%
transmission rate
3T3/del1-16
3T3/Δ
NTM94/ Δ

VACCINE AGAINST BETA-HERPESVIRUS INFECTION AND USE THEREOF

[0001] The present invention is related to a beta-herpesvirus, preferably a recombinant beta-herpesvirus, the use of the beta-herpesvirus for the manufacture of a medicament, the use of the beta-herpesvirus for the manufacture of a vaccine, a nucleic acid coding for the beta-herpesvirus, a vector comprising the nucleic acid coding for the beta-herpesvirus, and a host cell comprising the nucleic acid coding for the beta-herpesvirus or the vector. In a preferred embodiment, the beta-herpesvirus is a human cytomegalovirus.

[0002] Human cytomegalovirus (CMV), a member of the beta-herpesvirus subfamily is the medically most significant herpesvirus infecting humans (Arvin et al. 2004 Clin. Infect. Dis. 39:233-239.; Stratton et al. 1999 Vaccines for the 21st Century: A Tool for Decisionmaking National Academy Press). Most of the human CMV infection is acquired without symptomatic disease via breast feeding or saliva/urine contact in early childhood. This results in nearly 100% prevalence of HCMV in developing countries. In industrialized countries about 30% of the population gets infected in the childhood and the prevalence of human CMV infection increases up to ~50% by early adulthood.

[0003] Human CMV can also be transmitted from the mother to the fetus during pregnancy leading to mental retardation and developmental disabilities in the infected child. Human CMV is the most important causative agent of congenital infections in industrialized countries with one out of 1000 newborn affected. To date 30,000-40,000 infants are annually born with congenital cytomegalovirus infection in the United States, making cytomegalovirus by far the most common and important of all congenital infections. The likelihood of congenital infection and the extent of disease in the newborn depend on the maternal immune status. If primary maternal infection occurs during pregnancy, the average rate of transmission to the fetus is 40%; about 65% of these newborns will have congenital inclusion disease (CID). With recurrent maternal infection going along with reactivation from latency, the risk of transmission to the fetus becomes lower ranging only from 0.5 to 1.5% and the majority of these infants will also be symptomless. Although natural infections before pregnancy cause a risk of reactivation associated feto-maternal transmission the induced immunity is a major protective factor against CID.

[0004] The infection at birth bears the risk of serious complications; the primary infection with HCMV is generally symptomless in immunologically competent individuals. The major risk groups comprise organ transplant recipients and acquired immunodeficiency syndrome (AIDS) patients in which human CMV induces life-threatening inflammatory diseases with high probability. Moreover, after primary infection at any age, CMV establishes lifelong latency, leaving the infected individuals at danger of later reactivation upon immune suppression.

[0005] Although enormous progress has recently been made in molecular biology and immunology of cytomegaloviruses (Murphy et al. 2008 Curr. Top. Microbiol. Immunol. 325:1-19), to date there is no commercially available vaccine and the single hit chemotherapy is the only way of controlling acute HCMV infection (Mocarski et al. 2007, p. 2701-2772 in D. M. Knipe and P. M. Howley (eds.), Fields Virology, Lippincott Williams and Wilkins, a Wolters Kluwer Business, Philadelphia, Pa.). This chemotherapy causes severe side effects and application is often restricted to the most severe cases.

[0006] The development of vaccines against CMV infection is reviewed in Schleiss et al. (Schleiss et al. 2005 Herpes. 12:66-75; Schleiss et al. 2008 Curr. Top. Microbiol. Immunol. 325:361-382.).

[0007] One strategy for the development of a human CMV vaccine is the use of live attenuated HCMV. Live attenuated CMV are generated by multiple cell culture passages. In accordance therewith, in live attenuated vaccines the administered viruses are infectious. However due to the adaptation to the cell culture a loss of functional genes occurs whereby the lost genes are not required for virus propagation in vitro, but are important for virus infection in vivo. Such live attenuated CMV are therefore less pathogenic to the host.

[0008] The first human CMV vaccine candidate which was tested in clinical trials was a live attenuated vaccine. This was the AD169 strain of HCMV which was attenuated by extensive tissue culture passages in human primary fibroblasts. This attenuation is a result of a selective adaptation of the virus to the conditions of the cell and cell culture. It is likely that the loss of virulence is the result of affecting genes not relevant for the in vitro situation but important for the virus in its natural host. Therefore, it is not surprising that AD169, extensively passaged on fibroblasts, lost its ability to infect endothelial cells and monocytes. The majority of seronegative adults inoculated with AD169 vaccine developed HCMV specific immune response. This vaccine was found to be safe and generally well tolerated. However, injection site reactions were common, and several patients developed mild systemic symptoms consisting of fever, headache, fatigue and myalgia.

[0009] Since the AD169 strain was too aggressive, a more attenuated preparation of laboratory adapted HCMV, the Towne strain, was developed in a manner similar to AD169 as a potential live attenuated vaccine. This strain was more extensively passaged in cell culture and in vitro appeared to be also phenotypically similar to AD169.

[0010] The initial human trial showed that, as expected, the Towne strain was much better tolerated than the AD169. After this positive initial test the efficacy of the Towne vaccine was extensively studied. These studies showed that the Towne vaccine is safe and well tolerated in humans and induces both humoral and cellular immunity specific to human CMV. Although the Towne vaccine appears to provide some protection against human CMV disease in certain settings, unfortunately, vaccination is less protective than natural immunity. Therefore, the Towne strain is most likely over-attenuated rendering it of suboptimal efficacy as a vaccine.

[0011] Consequently, new human CMV strains with intermediate attenuation have been produced. Chimeric viruses have been constructed by genetic recombination between Towne strain and Toledo strain, which is a wild type like clinical isolate of human CMV not attenuated by tissue culture passages.

[0012] Interestingly, an essential feature of the Towne strain and the vaccine based thereon is its incapability of efficiently infecting endothelial cells. Furthermore, vaccination with the Towne strain does not induce antibodies that are capable of neutralizing endotheliotropic CMV infection, more specifically Towne does not induce antibodies against endotheliotropic human CMV strains (Cui et al. 2008 Vaccine 26:5760-5766.).

[0013] To differentiate between neutralization of endotheliotropic and non-endotheliotropic viruses, Gerna et al. (Gerna et al. 2008 J Gen Virol 89:853-865.) proposed the testing of human sera and quantification of the neutralizing potency against human CMV clinical isolates via propagation and testing in endothelial (or epithelial) cells and against the same virus infecting human fibroblasts (Gerna et al. supra).

[0014] It is important to note that in addition to the inability of the Towne strain to infect endothelial cells and the inability of the Towne strain to induce antibodies that are capable of neutralizing endotheliotropic human CMV infection, the Towne strain is lacking genes compared with clinical wild type human CMV isolates. More specifically, the Towne strain is lacking the genes UL133, UL134, UL135, UL136, UL137, UL138, UL139, UL140, UL141, UL142, UL143, UL144, and UL145 as also described by Cha et al. (Cha et al. 1996 J. Virol Vol. 70, No. 1 p. 78-83).

[0015] A further strategy for developing a HCMV vaccine is based on the deletion of an essential gene from a viral genome and was described for many viruses such as adenoviruses, alpha-herpesviruses, and retroviruses. Immunization trials using replication defective or single-cycle viruses as vaccines against herpesviruses were, to date, only described for alpha-herpesviruses (Dudek et al. 2006 Virology 344:230-239). The propagation of these viruses is facilitated by complementing cells that express the lacking genomes and support the growth of the defective viruses. Propagation of such viruses with the deletion of a gene on complementing cells results in vaccine-virus particles that possess a wild type virion surface and a tropism like wild type virus for the first target cells. These viruses are infectious upon vaccination for the first line target cells. In said first line target cells, the deleted or inactivated gene leads to either the abrogation of virus replication or the formation of virus particles with diminished infectivity or tropism.

[0016] The design of an alpha-herpesvirus vaccine by deletion of one gene essential for DNA replication or the abrogation of production of infectious particles by deletion of the targeting complex, namely glycoprotein gB is reviewed in Dudek et al. (Dudek et al. supra). Two types of these viruses were described: the so called “replication-defective” and the so called “single-cycle” viruses.

[0017] Replication-defective alpha-herpesviruses were generated by the deletion of genes essential for the DNA replication cycle. The deletion of genes essential for the viral DNA replication e.g. the major DNA binding protein ICP8, was used to generate respective deletion viruses. Said viruses can be propagated in vitro by using stably transformed cells that complement the product of the lacking gene (Forrester et al. 1992 J Virol 66:341-348.). Several publications from Knipe and colleagues prove that such viruses can induce protective immune responses (see Morrison et al. 1998 Virology 243:178-187; Morrison et al. 1994 J Virol 68:689-696.; Morrison et al. 1996 Virology 220:402-413; Morrison et al. 1997 Virology 239:315-326.).

[0018] Single-cycle viruses lack glycoproteins of targeting complexes e.g. glycoprotein gB or fusion complexes e.g. gH/gL (Dudek et al. supra). Such virus mutants are described in U.S. Pat. No. 7,374,768 by Inglis et al. Said complexes are described to be important for the attachment to the cell and/or fusion of virus and cell, as initiation steps for infection of this cell. The deletion of said glycoproteins will generate single-cycle vaccine virus particles that infect first line target cells. It is important to note that said cells in the host form virus particles which do not possess a wild type-like virion surface since they lack the glycoprotein mentioned above. These particles lacking the glycoprotein, are not infectious or at least possess limited tropism or infectivity for the next cells to be infected. Further, the deletion of said glycoprotein leads to a lacking expression of said glycoprotein preferably being effective as an antigen in the infected cell.

[0019] Due to society costs caused by human CMV infection in both morbidity groups and the emerging epidemiological situation the development of an effective HCMV vaccine has been emphasize as a highest level priority by the National Vaccine Committee of the Institute of Medicine (US) (Stratton et al. supra).

[0020] Thus the problem underlying the present invention was to provide an effective HCMV vaccine and a beta-herpesvirus contained in such vaccine, respectively.

[0021] This problem is solved by the attached independent claims. Preferred embodiments may be taken from the attached dependent claims.

[0022] The claims are recited in the following as embodiments. It will be acknowledged that further embodiments may result from the disclosure of the instant specification which is insofar not limited to the embodiments being a recitation of the claims.

EMBODIMENT 1

[0023] A beta-herpesvirus, preferably a recombinant beta-herpesvirus, wherein the beta-herpesvirus is spread-deficient.

EMBODIMENT 2

[0024] The beta-herpesvirus according to embodiment 1, wherein the beta-herpesvirus is endotheliotropic and/or has a wild type-like virion surface.

EMBODIMENT 3

[0025] The beta-herpesvirus according to any one of embodiments 1 to 2, wherein the beta-herpesvirus is endotheliotropic and has a wild type-like virion surface.

EMBODIMENT 4

[0026] The beta-herpesvirus according to any one of embodiments 1 to 3, wherein the beta-herpesvirus is suitable to or capable of inducing an immune response, wherein preferably the immune response comprises neutralizing antibodies against beta-herpesvirus and $CD4^+$ and $CD8^+$ T-cells directed against epitopes of beta-herpesvirus.

EMBODIMENT 5

[0027] The beta-herpesvirus according to embodiment 4, wherein the immune response comprises neutralizing antibodies, wherein beta-herpesvirus is prevented from infecting endothelial cells and/or epithelial cells by the neutralizing antibodies.

EMBODIMENT 6

[0028] The beta-herpesvirus according to embodiment 5, wherein beta-herpesvirus which is prevented from infecting endothelial cells and/or epithelial cells by the neutralizing antibodies, is a pathogen, preferably a human pathogen.

EMBODIMENT 7

[0029] The beta-herpesvirus according to any one of embodiments 1 to 6, wherein the beta-herpesvirus is a human beta-herpesvirus.

EMBODIMENT 8

[0030] The beta-herpesvirus according to any one of embodiments 1 to 7, wherein the beta-herpesvirus is a cytomegalovirus.

EMBODIMENT 9

[0031] The beta-herpesvirus according to any one of embodiments 7 and 8, wherein the beta-herpesvirus is a human cytomegalovirus.

EMBODIMENT 10

[0032] The beta-herpesvirus according to any one of embodiment 1 to 9, preferable embodiment 9, wherein the beta-herpesvirus is deficient in at least one gene product involved in primary and/or secondary envelopment.

EMBODIMENT 11

[0033] The beta-herpesvirus according to embodiment 10, wherein the at least one gene product is involved in primary envelopment

EMBODIMENT 12

[0034] The beta-herpesvirus according to embodiment 11, wherein the at least one gene product is encoded by a gene selected from the group comprising UL50 and UL 53 and homologs of each thereof.

EMBODIMENT 13

[0035] The beta-herpesvirus according to embodiment 10, wherein the at least one gene product is involved in secondary envelopment.

EMBODIMENT 14

[0036] The beta-herpesvirus according to embodiment 13, wherein the at least one gene product is encoded by a gene selected from the group comprising UL94 and UL99 and homologs each thereof.

EMBODIMENT 15

[0037] The beta-herpesvirus according to any one of embodiments 1 to 14, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20 and a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 16

[0038] The beta-herpesvirus according to any one of embodiments 1 to 14, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and a fourth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.NO:34.

EMBODIMENT 17

[0039] The beta-herpesvirus according to embodiment 16, wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 34, wherein nucleotide 252 of the nucleotide sequence according to SEQ.ID.No: 34 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 18

[0040] The beta-herpesvirus according to any one of embodiments 1 to 14, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 130670 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 131243 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20 and a fourth nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20, wherein the nucleotide 130670 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 131243 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein the nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 19

[0041] The beta-herpesvirus according to any one of embodiments 1 to 14, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 130670 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 131243 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20, a fourth nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20, a fifth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 34 and a sixth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 35.

EMBODIMENT 20

[0042] The beta-herpesvirus according to embodiment 19, wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 34, wherein nucleotide 252 of the nucleotide sequence according to SEQ.ID.No: 34 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20, wherein nucleotide 130670 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 35, wherein nucleotide 67 of the nucleotide sequence according to SEQ.ID.NO:35 is covalently linked to nucleotide 131243 of the nucleotide sequence according to SEQ.ID.No: 20, and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 21

[0043] The beta-herpesvirus according to any one of embodiments 1 to 14, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20 and a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 22

[0044] The beta-herpesvirus according to any one of embodiments 1 to 14, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and a fourth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 32.

EMBODIMENT 23

[0045] The beta-herpesvirus according to embodiment 22, wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 32, wherein nucleotide 179 of the nucleotide sequence according to SEQ.ID.No: 32 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 24

[0046] The beta-herpesvirus according to any one of embodiments 1 to 14, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 63261 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20 and a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein the nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 25

[0047] The beta-herpesvirus according to any one of embodiments 1 to 14, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 63261 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and a fourth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 33.

EMBODIMENT 26

[0048] The beta-herpesvirus according to embodiment 25, wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 33, wherein nucleotide 38 of the nucleotide sequence according to SEQ.ID.No: 33 is covalently linked to nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 27

[0049] The beta-herpesvirus according to any one of embodiments 1 to 14, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 632161 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20 and a fourth nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20, wherein the nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein the nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 28

[0050] The beta-herpesvirus according to any one of embodiments 1 to 14, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 63261 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20, a fourth nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20, a fifth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 32 and a sixth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 33.

EMBODIMENT 29

[0051] The beta-herpesvirus according to embodiment 28, wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 32, wherein nucleotide 179 of the nucleotide sequence according to SEQ.ID.No: 32 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20, wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 33, wherein nucleotide 38 of the nucleotide sequence according to SEQ.ID.NO:33 is covalently linked to nucleotide 63261 of the nucleotide sequence according to SEQ.ID.No: 20, and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 30

[0052] The beta-herpesvirus according to any one of embodiment 1 to 29, wherein the beta-herpesvirus comprises one or more genes selected from the group comprising UL133, UL134, UL135, UL136, UL137, UL138, UL139, UL140, UL141, UL142, UL143, UL144 and UL145

EMBODIMENT 31

[0053] The beta-herpesvirus according to any one of embodiment 1 to 30, wherein the beta herpesvirus comprises the nucleotide sequence according to SEQ.ID.NO:23.

EMBODIMENT 32

[0054] The beta-herpesvirus according to any one of embodiments 1 to 31, wherein the beta-herpesvirus is deficient in at least one gene product encoded by an immune evasive gene.

EMBODIMENT 33

[0055] The beta-herpesvirus according to embodiment 32, wherein the at least one gene product encoded by an immune evasive gene is selected from the group comprising gene products regulating MHC class I presentation and gene products regulating NK cell response.

EMBODIMENT 34

[0056] The beta-herpesvirus according to embodiment 33, wherein the at least one gene product encoded by an immune evasive gene is a gene product regulating MHC class I presentation.

EMBODIMENT 35

[0057] The beta-herpesvirus according to embodiment 34, wherein the gene product regulating MHC class I presentation is selected from the group comprising US6, US3, US2, UL18, US11, UL83 and UL40.

EMBODIMENT 36

[0058] The beta-herpesvirus according to embodiment 33, wherein the at least one gene product encoded by an immune evasive gene is a gene product regulating NK cell response.

EMBODIMENT 37

[0059] The beta-herpesvirus according to embodiment 36, wherein the gene product regulating NK cell response is selected from the group comprising gene products encoded by the genes UL40, UL16 and UL18.

EMBODIMENT 38

[0060] The beta-herpesvirus according to any one of embodiments 1 to 37, wherein the beta-herpesvirus encodes a heterologous nucleic acid.

EMBODIMENT 39

[0061] The beta-herpesvirus according to embodiment 41, wherein the heterologous nucleic acid is a functional nucleic acid, preferably selected from the group comprising antisense molecules, ribozymes and RNA interference mediating nucleic acids.

EMBODIMENT 40

[0062] The beta-herpesvirus according to embodiment 38, wherein the nucleic acid is a nucleic acid coding for a peptide, oligopeptide, polypeptide or protein.

EMBODIMENT 41

[0063] The beta-herpesvirus according to embodiment 40, wherein the peptide, oligopeptide, polypeptide or protein comprises at least one antigen.

EMBODIMENT 42

[0064] The beta-herpesvirus according to embodiment 41, wherein the antigen is an antigen selected from the group comprising viral antigens, bacterial antigens and parasite antigens.

EMBODIMENT 43

[0065] The beta-herpesvirus according to any one of embodiments 1 to 42 for or suitable for use in a method for the treatment of a subject and/or for use in a method for the vaccination of a subject.

EMBODIMENT 44

[0066] The beta-herpesvirus according to embodiment 43, wherein the subject is a mammal, preferably a human.

EMBODIMENT 45

[0067] The beta-herpesvirus according to embodiment 43 or 44, wherein the beta-herpesvirus is human cytomegalovirus.

EMBODIMENT 46

[0068] The beta-herpesvirus according to any one of embodiments 43 to 45, wherein the subject is suffering from a disease or is at risk of suffering from a disease.

EMBODIMENT 47

[0069] The beta-herpesvirus according to any one of embodiments 43 to 46, wherein the vaccination is a vaccination against a disease.

EMBODIMENT 48

[0070] The beta-herpesvirus according to any one of embodiments 46 and 47, wherein the disease is a disease or condition which is associated with beta-herpesvirus infection, preferably human cytomegalovirus infection.

EMBODIMENT 49

[0071] The beta-herpesvirus according to embodiment 48, wherein the disease or condition is selected from the group comprising congenital inclusion disease.

EMBODIMENT 50

[0072] The beta-herpesvirus according to any one of embodiment embodiments 43 to 49, wherein the subject is a pregnant female or female of reproductive age, preferably a pregnant woman or a woman of reproductive age.

EMBODIMENT 51

[0073] The beta-herpesvirus according to embodiment 50, wherein the treatment is or is suitable for or capable of preventing the transfer of a beta-herpesvirus, preferably human cytomegalovirus, from the female to a fetus and/or to an embryo carried or to be carried in the future by the female.

EMBODIMENT 52

[0074] The beta-herpesvirus according to embodiment 50, wherein the treatment is for or is suitable for the generation of or capable of generating an immune response in the female body or the immune response in the female body, whereby preferably such immune response confers protection to a fetus and/or to an embryo carried or to be carried in the future by the female against beta-herpesvirus, preferably human cytomegalovirus, and/or a disease or condition associated with beta-herpesvirus infection, preferably human cytomegalovirus infection.

EMBODIMENT 53

[0075] Use of a beta-herpesvirus according to any of embodiments 1 to 47 for the manufacture of a medicament.

EMBODIMENT 54

[0076] Use according to embodiment 53, wherein the medicament is for the treatment and/or prevention of beta-herpesvirus infection.

EMBODIMENT 55

[0077] Use according to embodiment 53, wherein the medicament is for the treatment and/or prevention of a disease or condition associated with beta-herpesvirus infection, preferably human cytomegalovirus infection.

EMBODIMENT 56

[0078] Use of a beta-herpesvirus according to any of embodiments 1 to 47 for the manufacture of a vaccine.

EMBODIMENT 57

[0079] Use according to embodiment 56, wherein the vaccine is for the treatment and/or prevention of beta-herpesvirus infection.

EMBODIMENT 58

[0080] Use according to embodiment 57, wherein the vaccine is for the treatment and/or prevention of a disease or condition associated with beta-herpesvirus infection, preferably human cytomegalovirus infection.

EMBODIMENT 59

[0081] Use according to any one of embodiments 56 to 58, wherein the vaccine is or is suitable for the administration to a subject, whereby the subject is selected form the group comprising a pregnant female, a female of reproductive age, a donor of a transplant, a recipient of a transplant and a subject being infected with HIV or being at risk of being infected with HIV.

EMBODIMENT 60

[0082] Use according to embodiment 59, wherein the donor is a potential donor and/or the recipient is a potential recipient.

EMBODIMENT 61

[0083] A nucleic acid coding for a beta-herpesvirus according to any of the preceding embodiments.

EMBODIMENT 62

[0084] A vector comprising the nucleic acid according to embodiment 61.

EMBODIMENT 63

[0085] A vector comprising the nucleic acid according to embodiment 62, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 123688 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 64

[0086] A vector comprising the nucleic acid according to embodiment 62, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and a third nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 34.

EMBODIMENT 65

[0087] The vector according to embodiment 64, wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 34 and wherein nucleotide 252 of the nucleotide sequence according to SEQ.ID.No: 34 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO: 20.

EMBODIMENT 66

[0088] A vector comprising the nucleic acid according to embodiment 62, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 130670 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 131243 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein the nucleotide 130670 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 131243 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 67

[0089] A vector comprising the nucleic acid according to embodiment 62, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 130670 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 131243 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 34 and a fourth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 35

EMBODIMENT 68

[0090] The vector according to embodiment 67, wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 34, wherein nucleotide 252 of the nucleotide sequence according to SEQ.ID.No: 34 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20, wherein nucleotide 130670 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 35 and wherein nucleotide 67 of the nucleotide sequence according to SEQ.ID.NO:35 is covalently linked to nucleotide 131243 of the nucleotide sequence according to SEQ.ID.No:20

EMBODIMENT 69

[0091] A vector comprising the nucleic acid according to embodiment 62, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 70

[0092] A vector comprising the nucleic acid according to embodiment 62, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and a third nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 32.

EMBODIMENT 71

[0093] The vector according to embodiment 70, wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 32 and wherein nucleotide 179 of the nucleotide sequence according to SEQ.ID.No: 32 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO: 20.

EMBODIMENT 72

[0094] A vector comprising the nucleic acid according to embodiment 62, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 63261 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 73

[0095] A vector comprising the nucleic acid according to embodiment 62, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 63261 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and a third nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 33

EMBODIMENT 74

[0096] The vector according to embodiment 73, wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 33 and wherein nucleotide 38 of the nucleotide sequence according to SEQ.ID.No: 33 is covalently linked to nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO: 20.

EMBODIMENT 75

[0097] A vector comprising the nucleic acid according to embodiment 62, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 63261 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein the nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO:20.

EMBODIMENT 76

[0098] A vector comprising the nucleic acid according to embodiment 62, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 63261 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20, a fourth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 32 and a fifth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 33.

EMBODIMENT 77

[0099] The vector according to embodiment 76, wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 32, wherein nucleotide 179 of the nucleotide sequence according to SEQ.ID.No: 32 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20, wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 33 and wherein nucleotide 38 of the nucleotide sequence according to SEQ.ID.NO:33 is covalently linked to nucleotide 632161 of the nucleotide sequence according to SEQ.ID.No: 20.

EMBODIMENT 78

[0100] A host cell comprising a nucleic acid according to embodiment 61 or a vector according to any one of embodiments 62 to 77.

EMBODIMENT 79

[0101] A pharmaceutical composition comprising a beta-herpesvirus according to any one of the preceding embodiments, a nucleic acid according to embodiment 61 and/or a vector according to any one of the preceding embodiments, and a pharmaceutically acceptable carrier.

[0102] The present inventors have surprisingly found that the infection of endothelial cells of a host organism such as man by beta-herpesvirus and more specifically CMV of the invention will result in eliciting an immune response against CMV. More specifically, the immune response is an anti-CMV response which comprises neutralizing antibodies against beta-herpesvirus and CD4$^+$ and CD8$^+$ T-cells directed against epitopes of beta-herpesvirus. Furthermore, the present inventors have surprisingly found that such immune response can be elicited by the beta-herpesvirus and more specifically the human cytomegalovirus of the invention being spread-deficient. It has to be acknowledged that any characteristic feature, embodiment of and any statement made in relation to beta-herpesviruses such as murine CMV equally applies to human CMV. Furthermore, it will be acknowledged that the beta-herpesvirus according to the present invention will, in a preferred embodiment, exhibit the following characteristics as observed for human and murine, respectively, CMV: multiple infections occur with mouse and human CMV, in mouse and human, respectively, (Boppana, S. B. et al., 2001. Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. N. Engl. J. Med 344:1366-1371; Cicin-Sain, L. et al., 2005. Frequent coinfection of cells explains functional in vivo complementation between cytomegalovirus variants in the multiply infected host. J Virol 79:9492-9502.); an unusually high response of neutralizing antibodies against CMV is caused by infection with mouse and human CMV, in mouse and human, respectively (Farrell, H. E. and G. R. Shellam, 1990. Characterization of neutralizing monoclonal antibodies to murine cytomegalovirus. J. Gen. Virol. 71 (Pt 3):655-664; Farrell, H. E. and G. R. Shellam, 1991. Protection against murine cytomegalovirus infection by passive transfer of neutralizing and non-neutralizing monoclonal antibodies. J. Gen. Virol. 72 (Pt 1):149-156; Gerna, G., A. et al., 2008. Human cytomegalovirus serum neutralizing antibodies block virus infection of endothelial/epithelial cells, but not fibroblasts, early during primary infection. J. Gen. Virol. 89:853-865); memory inflation, which represents a very characteristic CD8+ T cell response, is caused by infection with mouse and human CMV, in mouse and human, respectively, and has almost identical kinetics (Karrer, U. et al., 2003. Memory inflation: continuous accumulation of antiviral CD8+ T cells over time. J. Immunol. 170:2022-2029; Karrer, U. et al. 2004. Expansion of protective CD8+ T-cell responses driven by recombinant cytomegaloviruses. J. Virol. 78:2255-2264; Klenerman, P. and P. R. Dunbar, 2008. CMV and the art of memory maintenance. Immunity. 29:520-522; Komatsu, H. et al., 2003. Population analysis of antiviral T cell responses using MHC class I-peptide tetramers. Clin. Exp. Immunol. 134:9-12;). In connection with the present invention a person skilled in the art will also acknowledge that a murine CMV gene can replace a homolog of said murine CMV gene in a human CMV. (Schnee, M. et al., 2006. Common and specific properties of herpesvirus UL34/UL31 protein family members revealed by protein complementation assay. J Virol 80:11658-11666)

[0103] In a preferred embodiment the beta-herpesvirus according to the present invention is different from the Towne strain as described by Liu et al. in U.S. Pat. No. 7,407,744, i.e. a Towne strain where the genes UL133, UL134, UL135, UL136, UL137, UL138, UL139, UL140, UL141, UL142, UL143, UL144, and UL145 are deleted, preferably compared to wild type. A person skilled in the art will further acknowledge that the Towne strain is not endotheliotropic and has also a defective gH/gL complex.

[0104] In a further preferred embodiment the beta-herpesvirus according to the present invention comprises a nucleotide sequence according to SEQ.ID.No:23.

[0105] In still further preferred embodiment the beta-herpesvirus according to the present invention is different form the Toledo strain.

[0106] Spread-deficient as used herein, preferably means that the virus which is spread-deficient infects a cell and no viral particle is released from the infected cell, whereby the viral DNA is replicated, the viral proteins except those which are deleted in accordance with the present invention are expressed in the infected cell, preferably all viral glycoproteins are expressed, more preferably all viral glycoproteins are expressed, that mediate entry of the virus into a cell, whereby, preferably, the cell is an endothelial and/or an epithelial cell. The assay which is preferably used in accordance with the present invention so as to determine whether or not a virus is spread-deficient, is described herein as Example 1.

[0107] A wild type CMV strain as preferably used herein means that the virus is a beta-herpesvirus strain which has been isolated from its native host and which has maintained its ability to infect endothelial cells in tissue culture. More specifically the wild type human CMV strain as preferably used herein contains, among others, the genes UL133, UL134, UL135, UL136, UL137, UL138, UL139, UL140, UL141, UL142, UL143, UL144, and UL145 (Cha et al. supra) and more specifically the wild type CMV strain as preferably used herein is TB40/E and FIX-BAC (Sinzger et al. 1999 Journal of General Virology, 80, 2867-2877; Hahn et al. 2002 J. Virol. 76(18): 9551-9555) and/or TB40E-BAC4-FRT (SEQ.ID.NO:20) (Scrivano, L. et al., 2011. HCMV spread and cell tropism are determined by distinct virus populations. PLoS. Pathog. 7:e1001256) for human CMV or Smith strain for MCMV (Rawlinson et al. 1996 J Virol 70:8833-8849). In a preferred embodiment of the present invention the wild type CMV strain as preferably used herein comprises a nucleotide sequence according to SEQ.ID.No: 23. The sequence of the pTB40E-BAC4-FRT, which is the molecular infectious BAC plasmid according to TB40E-BAC4-FRT has the nucleotide sequence according to SEQ.ID.NO:20.

[0108] Said pTB40E-BAC4-FRT is consisting of viral sequences encoded by nt 1-181652 and by nt 189192-233681, as well as BAC sequences represented by nt 181653-189191. A person skilled in the art will acknowledge that a BAC plasmid such as pTB40E-BAC4-FRT comprising a virus genome such as the virus genome of TB40E-BAC4-FRT is circular in *E. coli* therefore the nucleotide 233681 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.NO:20. A person skilled in the art will know methods for reconstitute a virus from a BAC plasmid comprising the viral genome of said virus, for example for reconstitute TB40E-BAC4-FRT from pTB40E-BAC4-FRT comprising the viral genome of TB40E-BAC4-FRT. Such methods comprise among others transfection of cells, comprising complementing cells.

[0109] As used herein, the term "deficient in at least one gene product" preferably means that the at least one gene product which is a biochemical material such as a nucleic acid, DNA, RNA or a peptide, polypeptide or protein, resulting from expression of the gene does not show at least one of the functions displayed by said gene product in the wild type strain. Preferably, said at least one of the functions not shown is the function which is responsible for spread of the beta-herpesvirus. Also preferably, all of the functions of said gene product in the wild type strain are not shown. This may be the result of a complete or partial deletion or mutation of the gene coding for said gene product, of a complete or partial deletion of a mutation, of the nucleic acid controlling the expression of the gene coding of said gene product, of a truncation of said gene product, or of the inhibition of the otherwise compete gene product.

[0110] As used herein, the term "DNA is replicated" preferably means that the replication occurs like replication of a wild type virus.

[0111] As used herein, a wild type-like virion surface is preferably a surface displayed by a beta-herpesvirus of the wild type as defined herein, more specifically by a cytomegalovirus wild type strain as defined herein. The molecules which are used to define the surface displayed by a beta-herpesvirus of the wild type are glycoproteins expressed by said wild type virus mediating the entry of said wild type virus into a cell, preferably an endothelial cell. In other words, a virus according to the present invention having a wild type-like virion surface has a virion surface which, after infection of primary fibroblasts, displays or expresses the same glycoproteins identical to, essentially identical to or at least not significantly different from the wild type virus based on which the deletions were or may be made to generate the virus of the present invention. The determination of the expression of glycoproteins is known to the ones skilled in the art and may be performed by a quantitative RT-PCR or mass spectrometry (Britt et al. 1990. J Virol 64:1079-1085) although other methods suitable for such purpose are knoen to the person skilled in the art.

[0112] So as to determine whether the beta-herpesvirus of the invention and particularly the human cytomegalovirus of the invention is endotheliotropic, preferably, the assay as described in Example 2 is used.

[0113] So as to determine whether the immune response elicited by the beta-herpesvirus of the invention and particularly the human cytomegalovirus of the invention comprises at least neutralizing antibody, and whereby the at least neutralizing antibody is preventing said viruses from infecting endothelial cells and/or epithelial cells, the assay described by Cui et al. (Cui et al. supra) may preferably be used.

[0114] It will be acknowledged that viral DNA replication is abrogated in replication-defective virus mutants and therefore gene expression does not exploit the total set of viral epitopes. Especially glycoproteins and structural virion components are not expressed.

[0115] In order to further illustrate the present invention the biology of human cytomegalovirus will be outlined in the following.

[0116] Human cytomegalovirus is one of eight human herpesviruses, which are clustered in three subfamilies (alpha (α), beta (β), gamma (γ)) based on biological properties and molecular phylogenetic relationships to other herpesviruses. Cytomegalovirus belongs to the beta-herpesvirus subfamily and possesses the largest genome in the herpesvirus families: its genome of 240 kbp is capable of encoding more than 200 potential gene products (Murphy et al. supra).

[0117] The viral particle of cytomegaloviruses consist of three major constituents, namely the internal icosahedral capsid, which packages the double stranded linear DNA genome; the tegument which is a less organized protein meshwork surrounding the capsid; and the outermost envelop which is a lipid bilayer embedded with viral glycoprotein complexes.

[0118] The infection of a host cell by the virus particles is mediated by the contact of the viral glycoproteins with the molecular structures of the host cell surface. CMVs can infect many different cell types and the mechanism of virus entry is known to be dependent on the specific cell type and can occur via two major routes: (a) the free, i.e. non-cell associated virus particles can encounter the host cell directly, or (b) the virus is transferred from the infected cell to a non-infected one by a preformed, i.e. non-virus-induced cell-cell contact, or virus induced cell-cell contact, the so called cell to cell spread.

[0119] After attachment with high affinity to a set of cellular receptors the viral glycoproteins induce fusion between the viral envelope and a host cell membrane. After entry of an CMV particle into the host cell the HCMV genome is targeted to the nucleus where it either establishes latency which is characterized by a symptomless maintenance of the more or less silent genome, or induces a lytic infection leading to propagation of new infectious CMV particles.

[0120] The lytic replication cycle of CMV is divided into three phases of regulated gene expression: immediate early, early, and late. The hallmarks of the replication stages are the specific gene clusters which are expressed with characteristic kinetics. Immediate early gene transcription occurs at first and leads to synthesis of viral master regulators that reprogram the host cell according to the needs of virus production. Following the synthesis of immediate early gene products, the early genes are transcribed. Early gene products include DNA replication proteins and regulators and enzymes which are important in nucleotide metabolism. Finally, the late genes are transcribed after the onset of DNA replication, and the gene products of said late genes are mainly structural proteins that are involved in the assembly of and egress of new infectious virus particles.

[0121] The late gene products comprise many viral antigens including the viral glycoproteins such as the gB and the gH/gL complex, which are the major targets of neutralizing antibodies against CMV (Schleiss et al. 2008 supra) and the major tegument protein the phosphoprotein 65 (pp65) and the immediate early 1 protein which are the major targets of the cellular immune response to CMV.

[0122] A further step in the lytic replication cycle of CMV is the maturation of novel infectious virus particles which comprises steps of envelopment of the pre-mature virus particle with membrane structures. The steps of envelopment comprise a primary envelopment, de-envelopment and secondary envelopment.

[0123] The primary envelopment at the membranes of the nucleus is crucial for the egress of virus capsids out of the nucleus. Proteins as part of the protein complex which is also referred to as nuclear egress complex (NEC) playing an essential role in this primary envelopment, were recently identified as M50 and M53 of mouse CMV (Lotzerich et al. 2006 J Virol 80:73-84.) or as UL50 and UL53 being their homologs in human CMV.

[0124] A homologues gene as used herein is preferably the gene of one herpesvirus referred to be a homolog of the gene of another herpesvirus according to Fossum et al. (Fossum et al. PLoS Pathog. 2009 September; 5(9): e1000570) or Davison et al. (Davison et al. (2010) Vet Microbiol. 2010 Feb. 11. Herpesvirus systematics; and Davison et al. 2004 Compendium of Human Herpesvirus gene names; Reno).

[0125] Further, homologs of UL50 are listed in the EMBL-EBI InterPro database (http://www.ebi.ac.uk/interpro/) under accession number IPR007626 and UL53 homologs are found similarly under entry IPR021152.

[0126] The secondary envelopment occurs at the membranes of the Golgi-apparatus and/or the endoplasmatic reticulum. In connection with said secondary envelopment a protein complex which is also referred to as secondary envelopment complex (SEC), was identified comprising at least the gene product of M94 of mouse CMV or its homolog in human CMV, i.e. UL94. The gene UL94 of HCMV is conserved in all herpesvirus sub-families (Chee et al. 1991 Transplant Proc 23:174-80; Chee et al. 1990 Curr Top Microbiol Immunol 154:125-169; Higgins et al. 1989 Comput. Appl. Biosci. 5:151-153) and was found only at a late stage of infection (Scott et al. 2002 Virus Genes 24:39-48; Wing et al. 1996 J Virol 70:3339-3345). It was recently shown that UL94 is part of the virion (Kalejta et al. 2008 Microbiol Mol Biol Rev 72:249-65; Kattenhorn et al. 2004 J Virol 78:11187-11197; Wing et al. op.cit). UL94 is essential in the infection of the Towne strain of HCMV shown by transposon-mediated mutagenesis (Dunn et al. 2003 Proc Natl Acad Sci USA 100:14223-14228. That M94 is essential in mouse CMV infection is disclosed herein in the example part.

[0127] Homologs of UL94 are listed in the EMBL-EBI InterPro database (http://www.ebi.ac.uk/interpro/) under accession number IPR004286.

[0128] The high viral load of CMV in salivary glands indicates the transmission of CMV by direct contact via secretions. After initial replication in the first target cells at the entry site, CMV is disseminated through the body by blood and lymph. Most likely the virus is taken up by white blood cells which carry the virus from the primary infection site to almost every internal organ.

[0129] The interplay between the CMV and its host, i.e. humans or mice, is very complex. On the one hand, the immune response of the host is controlling the virus replication very efficiently. Therefore, most of the CMV infections are symptomless which means that virus replication is controlled before the tissue damage reaches an observable pathological level of local or systemic inflammation. On the other hand, the virus itself is controlling the immune response resulting in efficient clearance of the virus from the host. In almost all cases of immune competence natural CMV infection ends up with a situation where the virus is controlled by the immune system without being totally cleared from the host (Reddehase et al. 2002 J Clin Virol 25 Suppl 2:S23-S36).

[0130] In recent years an impressive body of knowledge was generated by studying the molecular mechanisms of immune suppressive functions of CMV. It is acknowledged that more than half of the CMV genes encode gene products interfering with different immune mechanisms at all stages of the immune system, the so-called immune evasive genes. There is evidence that neither the humoral nor the cellular immune response alone is sufficient to control CMV infection; a concerted action of both is needed to keep the balance with the viral immune evasion (Adler et al. 1995 J Infect Dis 171:26-32; Reddehase et al. 1987 J Virol 61:3102-3108).

[0131] Diseases and conditions of a subject which is infected by beta-herpesvirus and human CMV, respectively, are, among others, mononucleosis-like symptoms, splenomegaly, pneumonitis, blindness, hearing loss, congenital inclusion disease, and organ damage and organ failure, respectively, of the organ infected by HCMV. It is to be acknowledged that said diseases and conditions are diseases and conditions which can be treated and/or prevented by the beta-herpesvirus of the present invention.

[0132] Typically, human CMV infection becomes clinically apparent only if the host immune system is vulnerable or suppressed. There are several major risk groups of public health importance.

[0133] One situation where the host immune system is vulnerable, is where non-pregnant women of reproductive age or women being pregnant get infected by human CMV. If the human CMV infection is transmitted from the mother to the fetus and embryo, respectively, during pregnancy, due to the immature immune system of the fetus and embryo, respectively, direct cytotoxic pathology of the human CMV infection can develop which is called congenital inclusion disease (CID). The symptoms of CID are dominated by the cause that the human CMV infects the central nervous system comprising microcephaly, cerebral atrophy, chorioretinitis, and sensorineural hearing loss, which are typically combined with consequences of infection of other visceral organs including intrauterine growth retardation, hepatosplenomegaly, hematological abnormalities such as thrombocytopenia, and various cutaneous manifestations appearing as rushes, i.e. petechiae and purpura. CID is the most frequent infectious congenital disorder in developed countries. Furthermore, human CMV infection is the major cause of hearing loss acquired after viral infection.

[0134] A second scenario of clinically significant human CMV infection is formed by immunocompromised or immunosuppressed patients. This kind of patient is, e.g., a HIV-positive patient or a transplant recipient. In these patients the disease manifestations vary depending on the quality and the degree of immune dysfunction. Infection mostly occurs because of reactivation of latent viral infection, however, may be as well newly acquired via virus reactivation from organ or bone marrow transplant derived from an already infected donor in case of a transplant recipient.

[0135] In the absence of sufficient immune control CMV infection leads to inflammatory diseases of various organs. In connection therewith the most frequent clinical manifestations consist of pneumonitis, gastrointestinal diseases, hepatitis, and retinitis. In bone marrow transplant recipients HCMV pneumonitis occurs with mortality rates of 90%. It is to be acknowledged that said diseases and conditions are diseases and conditions which can be treated and/or prevented by the beta-herpesvirus of the present invention.

[0136] In AIDS patients opportunistic human CMV infection is common and occurs at a frequency of almost 100%, if anti-retroviral therapy fails or not applicable/available. This is still the case in non-industrialized countries were an effective therapy is not yet available. Before the availability of highly active anti-retroviral therapy for human immunodeficiency virus (HIV) infection, HCMV retinitis was the most common cause of blindness in adult patients with acquired immunodeficiency syndrome (AIDS), with an overall lifetime prevalence of more than 90%.

[0137] In an embodiment of the beta-herpesvirus of the invention the beta-herpesvirus is used as a vaccine and/or vector. In a further embodiment thereof such beta-herpesvirus encodes for a heterologous nucleic acid. Preferably such heterologous nucleic acid codes for an antigen, more preferably an antigen of a pathogen. Because of this such vaccine and vector, respectively, is suitable for the treatment and/or prevention of a disease caused by or associated with said pathogen. Such pathogens preferably comprise viruses and bacteria. In an embodiment the antigen is NP-NT60 of Influenza, whereby the vector then is useful in the treatment of influenza. In a further embodiment the antigen is ORF Rv3407 from *Mycobacterium tuberculosis* strain H37Rv, whereby the vector then is useful in the treatment of tuberculosis.

[0138] In an embodiment the beta-herpesvirus of the present invention is a recombinant beta-herpesvirus.

[0139] In a further embodiment the beta-herpesevirus of the present invention is a human beta-herpesvirus, preferably a recombinant human beta-herpesvirus.

[0140] In a preferred embodiment the individual nucleotides of the beta-herpesvirus of the invention are linked, preferably covalently linked, through phosphodiester bonds. Such phosphodiester bonds are those phosphodiester bonds which are contained in nucleic acid molecules contained or produced in biological material such as cells.

[0141] It will be acknowledged that the beta-herpesvirus of the present invention is part of a pharmaceutical composition. Preferably, such pharmaceutical composition contains, a part from the beta-herpesvirus of the present invention and/or a nucleic acid coding for the same, a pharmaceutically acceptable carrier. The ingredients of such pharmaceutical composition and their respective contents are known to a person skilled in the art. It will be further acknowledged that such pharmaceutical composition is for or is for use in the treatment of the diseases and conditions as disclosed herein in connection with the beta-herpesvirus of the present invention.

[0142] It will be acknowledged by a person skilled in the art that the experimental evidence provided in the example part of the instant application is based on murine CMV, but that such evidence can be directly and immediately transferred to HCMV, so that the present invention is plausible to a person skilled in the art. The reason for this being that the genomes of different herpesvirus strains including CMV are linearly correlated and the mode of action of human CMV in a human host and of mouse CMV in a murine host are essentially identical.

[0143] The various SEQ.ID. Nos., the chemical nature of the nucleic acid molecules, proteins and peptides according to the present invention, the actual sequence thereof and the internal reference number is summarized in the following table. To the extent that the particular sequences are not displayed in this table they are contained in the attached sequence listing which is part of the instant specification.

SEQ. ID. No.	Sequence	internal reference number
1	GTGGGATCCACCATGTACCCCTACGACGT GCCCGACTACGCCACGTCCAGACTATCC	HAM94for
2	ACTCTAGAGTCGACTTCACATGTGCTCGA GAACA	M94rev
3	AATTCATGATAACTTCGTATAGCATACAT TATACGAAGTTATCCGGAGATATCCACCG GTCTGGCGGCCGC	ATGlox1
4	TCGAGCGGCCGCCAGACCGGTGGATATCT CCGGATAACTTCGTATAATGTATGCTATA CGAAGTTATCATG	ATGlox2
5	CGT GGT CAA GCC GGT CGT GTT GTA CCA GAA CTC GAC TTC GGT CGC GTT GCT TAC AAT TTA CGC GCG GG	5'-Δm157-pCR3-FRT-Kan^r-FRT
6	CCC CGA TAT TTG AGA AAG TGT ACC CCG ATA TTC AGT ACC TCT TGA CTA AGA AGC CAT AGA GCC CAC CGC	3'-Δm157-flox-egfp
7	TGC TTC CCG GCG GCT TCT GCG CGA CCT TCC AGC TGC AGG TAG ACC ACG GCG ACG TCC AGA CTA TCC GTG AAA AGT TTG AGA AGC ATC AGT AGC CGA TTT CGG CCT ATT GGT T	5'ΔM94-pO6-tTA
8	CAT GGA TGG GTT GGT TGA TTT GTA TGT CTG TTG GCT ACT CAC ATG TGC TCG AGA AGC CAG TGT GAT GGA TGA TCC TC	3'-ΔM94-pO6-tTA
9	SIINFEKL	OVA-MHC-I Peptide
10	TVYGFCLL	m139 MHC-I Peptide
11	RALEYKNL	ie3 MHC-I Peptide
12	SCLEFWQRV	M57 MHC-I Peptide
13	HGIRNASFI	M45 MHC-I Peptide
14	FAM-AACGTACATCGCTCTCTGCTGGCCG- TAMRA	Taqman-Probe M54
15	Ttactgggtgctgccgggcggctttgccgtctcttcgcgcgtcactct tcacggcctggcccagcgagccctgcgggaccggttccaaaacttc gaggccgtgctggcccggggcatgcacgtggaggccggccggca ggagcccgagacccccccgggtgagcggccggcggctgcccttcg acgacctgtgatccggaggacgacggctcgtgtatcttgtgccaatt gctgttgctctaccgcgacggcgaatggatcctctgtctttgctgcaa cggccgttatcaaggccactatggcggggtctgacagttcacgggg agaagaaacaagaaacaacaaaaaaaaaagaggagatctgcggc cgctagggataacagggtaatcgatgttgacaattaatcatcggcata gtatatcggcatagtataatacgacaaggtgaggaactaaaccatgg caaaactgaccagcgcagttccggttctgaccgcacgtgatgttgcc ggtgccgttgaattttggaccgatcgtctgggttttagccgtgattttgt ggaagatgattttgccggtgttgttcgtgatgatgttaccctgtttattag cgcagttcaggatcaggttgttccggataataccctggcatgggtttg ggttcgtggtctggatgaactgtatgcagaatggtcagaagttgtgag caccaattttcgtgatgcaagcggtccggcaatgaccgaaattggtg aacagccgtggggtcgtgaatttgcactgcgtgatccggcaggtaat tgtgttcattttgttgcagaagaacaggattaacctcgattaattaattgt	LIFdelUL94

-continued

SEQ. ID. No.	Sequence	internal reference number
	aacattaccctgttatccctaccggtgtcctaggcggggtctgacagt tcacggggagaagaaacaagaaacaacaaaaaaaaaagagg	
16	cgtgttagaccgttggagtcgcgacctgtcccgcaagacgaaccta ccgatctgggtcgccaacagcgccaacgagtacgtcgtcagctccg tgccccgccccgtcagtccgtagaagtaactcataaactttcaggtct cgcgtacgattcgcgagtcgggaatgtagggataacagggtaatcg atgttgacaattaatcatcggcatagtatatcggcatagtataatacga caaggtgaggaactaaaccatggcaaaactgaccagcgcagttcc ggttctgaccgcacgtgatgttgccggtgccgttgaattttggaccga tcgtctgggttttagccgtgattttgtggaagatgattttgccggtgttgt tcgtgatgatgttaccctgtttattagcgcagttcaggatcaggttgttc cggataataccctggcatgggtttgggttcgtggtctggatgaactgt atgcagaatggtcagaagttgtgagcaccaattttcgtgatgcaagc ggtccggcaatgaccgaaattggtgaacagccgtggggtcgtgaat ttgcactgcgtgatccggcaggtaattgtgttcattttgttgcagaaga acaggattaacctcgattaattaattgtaacattaccctgttatccctaa agtaactcataaactttcaggtctcgcgtacgattcgcgagtcgggaa tg	LIF-delUL99
17	as contained in the sequence listing	pCB-Ubic-UL94-IRES-mChe
18	as contained in the sequence listing	pCB-Ubic-UL99-IRES-gfp
19	as contained in the sequence listing	pLV-Ubiqc-BLAs-IRES-Puro
20	as contained in the sequence listing	pTB40E-BAC4-FRT
21	as contained in the sequence listing	pBSK-OVA
22	as contained in the sequence listing	pTRE-HAM94
23	as contained in the sequence listing	Unique\in\TB40\(UL133-UL145)
24	MGSGIGAASMEFCFDVFKELKVHHANENIFYCPIAIMSAL AMVYLGAKDSTRTQINKVVRFDKLPGFGDSIEAQCGTSVN VHSSLRDILNQITKPNDVYSFSLASRLYAEERYPILPEYL QCVKELYRGGLEPINFQTAADQARELINSWVESQTNGIIR NVLQPSSVDSQTAMVLVNAIVFKGLWEKTFKDEDTQAMPF RVTEQESKPVQMMYQIGLFRVASMASEKMKILELPFASGT MSMLVLLPDEVSGLEQLESIINFEKLTEWTSSNVMEERKI KVYLPRMKMEEKYNLTSVLMAMGITDVFSSSANLSGISSA ESLKISQAVHAAHAEINEAGREVVGSAEAGVDAASVSEEF RADHPFLFCIKHIATNAVLFFGRCVSP	OVA
25	MSGQGTKRSYEQMETDGERQNATEIRASVGKMIGGIGRFY IQMCTELKLSDYEGRLIQNSLTIERMVLSAFDERRNKYLE EHPSAGKDPKKTGGPIYRRVNGKWMRELILYDKEEIRRIW RQTNNGDDATAGLTHMMIWHSNLNDATYQRTRALVRTGMD PRMCSLMQGSTLPRRSGAAGAAVKGVGTMVMELVRMIKRG INDRNFWRGENGRKTRIAYERMCNILKGKFQTAAQKAMMD QVRESRNPGNAEFEDLTFLARSALILRGSVAHKSCLPACV YGPAVASGYDFEREGYSLVGIDPFRLLQNSQVYSLIRPNE NPAHKSQLVWMACHSAAFEDLRVLSFIKGTKVLPRGKLST RGVQIASNENMDAMESSTLELRSRYWAIRTRSGGNTNQQR ASAGQISIQPTFSVQRNLPFDRTTIMAAFNGNTEGRTSDM RTEIIRMMESARPEDVSFQGRGVFELSDEKAASPIVPSFD MSNEGSYFFGDNAEEYDN	NP-NT60 of Influenza
26	MRATVGLVEAIGIRELRQHASRYLARVEAGEELGVTNKGR LVARLIPVQAAERSREALIESGVLIPARRPQNLLDVTAEP ARGRKRTLSDVLNEMRDEQ	ORF Rv3407 from *Mycobacterium tuberculosis* strain H37Rv
27	MAWRSGLCETDSRTLKQFLQEECMWKLVGKSRKHREYRAV ACRSTIFSPEDDGSCILCQLLLLYRDGEWILCLCCNGRYQ GHYGVGHVHRRRRRICHLPTLYQLSFGGPLGPASIDFLPS FSQVTSSMTCDGITPDVIYEVCMLVPQDEAKRILVKGHGA MDLTCQKAVTLGGAGAWLLPRPEGYTLFFYILCYDLFTSC GNRCDIPSMTRLMAAATACGQAGCSFCTDHEGHVDPTGNY VGCTPDMGRCLCYVPCGPMTQSLIHNDEPATFFCESDDAK YLCAVGSKTAAQVTLGDGLDYHIGVKDSEGRWLPVKTDVW DLVKVEEPVSRMIVCSCPVLKNLVH	UL94

-continued

SEQ. ID. No.	Sequence	internal reference number
28	VTLGGAGAWLLP	SSc cross-reactive UL94 peptide
29	MGGELCKRICCEFGTTSGEPLKDALGRQVSLRSYDNIPPT SSSDEGEDDDDGEDDDNEERQQKLRLCGSGCGGNDSSSGS HREATHDGPKKNAVRSTFREDKAPKPSKQSKKKKKPSKHH HHQQSSIMQETDDLDEEDTSIYLSPPPVPPVQVVAKRLPR PDTPRTPRQKKISQRPPTPGTKKPAAPLSF	UL99
30	MATSRLSVKSLRSISRFVQWECCWMLVNKSARYREFRAVT SQSPGLGKVSSTDDGRCLAASMMLFRRDGNFVLCLVVNKE PVGQFGCSGMRREKMVIDGLQEPVYVMRLLAPLIPVKLGF SPYMLPPKSIGGSGGLDPSVIYQNASVVTPEEAATVTMQG SGIVTVGLSGVGSWVQIKDGGNMKLFVFALCFDVFTACCD RLAFPSLAKIYSETVSCEADKCGFCRDSGRHVDPTGRFVG CVPDSGVCLCYSPCRGTDAAVSVRSWLPYLELEDGANTHS LFVRRYDGRKGLPATISDYLGARNSEGDEIPLRTEPWQLL KIEPTLSAMIIMACPLLKKIVLEHM	M94
31	MYPYDVPDYATSRLSVKSLRSISRFVQWECCWMLVNKSAR YREFRAVTSQSPGLGKVSSTDDGRCLAASMMLFRRDGNFV LCLVVNKEPVGQFGCSGMRREKMVIDGLQEPVYVMRLLAP LIPVKLGFSPYMLPPKSIGGSGGLDPSVIYQNASVVTPEE AATVTMQGSGIVTVGLSGVGSWVQIKDGGNMKLFVFALCF DVFTACCDRLAFPSLAKIYSETVSCEADKCGFCRDSGRHV DPTGRFVGCVPDSGVCLCYSPCRGTDAAVSVRSWLPYLEL EDGANTHSLFVRRYDGRKGLPATISDYLGARNSEGDEIPL RTEPWQLLKIEPTLSAMIIMACPLLKKIVLEHM	HA-M94
32	gaccgcgccacagcagagccagcaccagcagaagagccagcac cagcgggcccagagtcgcaaagcgcgcgggcagccacggccca gactgcggtcgcgatggcccggagcgcgctcgccaccacgatgac ggtgcccaacgataaccagtccgctcccgcaccgacgccaccgcc gat	delUL50S
33	atgtctagcgttttctcaacagcattcgtgcgccttga	delUL53S
34	cacggcctggcccagcgagccctgcgggaccggttccaaaacttc gaggccgtgctggcccggggcatgcacgtggaggccggccggca ggagcccgagacccccccgggtgagcggccggcggctgcccttcg acgacctgtgatccggaggacgacggctcgtgtatcttgtgccaatt gctgttgctctaccgcgacggcgaatggatcctctgtctttgctgcaa cggccgttatcaaggccactatgg	delUL94S
35	ctgggtcgccaacagcgccaacgagtacgtcgtcagctccgtgccc cgccccgtcagtccgtagaag	delUL99S

[0144] It will be acknowledged by a person skilled in the art and is in so far also within the scope of the present invention that each and any of the above nucleic acid sequences can be replaced by nucleic acid sequences which, due to the degeneracy of the genetic code, code for the same or functionally homolog peptides and proteins, respectively, as the above indicated nucleic acid sequences.

[0145] The present invention is now further illustrated by the following figures and examples from which further features, embodiments and advantages may be taken.

[0146] More specifically,

[0147] FIG. **1** is a schematic illustration of the concept of inducible trans-complementation;

[0148] FIG. **2**A is a diagram indicating $TCID_{50}$ as a function of time;

[0149] FIG. **2**B is a series of microphotographs;

[0150] FIG. **2**C is a survivorship curve indicating survival of mice as a function of time;

[0151] FIG. **3**A is a diagram indicating virus neutralizing antibody response as luciferase activity as a function of dilution of serum;

[0152] FIG. **3**B is a diagram indicating the percentage of adaptively transferred T cells at various time points;

[0153] FIG. **3**C is a diagram indicating the percentage of specific lysis of transferred cells loaded with various viral peptides by $CD8^+$ T-cells specific for the viral peptides;

[0154] FIG. **4** is a Whisker blot indicating the percentage of adaptively transferred T cells in different mouse strains being infected with different virus mutants;

[0155] FIGS. **5**A and **5**B are diagrams indicating the challenge virus load in different organs of vaccinated mice;

[0156] FIGS. **6**A and **6**B are survivorship curves indicating survival of vaccinated mice as a function of time;

[0157] FIG. **7**A is an agarose gel showing the result of a PCR detecting viral gene M54 in lungs of infected mice with either wild type or MCMV-ΔM94;

[0158] FIG. **7**B is a diagram indicating the result of a quantitative PCR detecting viral gene M54 in lungs of infected mice with either wild type or MCMV-ΔM94;

[0159] FIGS. **7**C and D are diagrams indicating the challenge virus load in different organs of vaccinated mice;

[0160] FIG. **8** is a series of microphotographs of cells of different cell-lines infected with and MCMV-Δm157-rec-egfp-ΔM94.

[0161] FIG. **9**A is a schematic overview of a spread-assay

[0162] FIG. **9**B is a series of microphotographs

[0163] FIG. **9**C is a diagram showing the results of a spread-assay

EXAMPLE 1

Spread Assay

[0164] The spread assay described herein may be used in connection with the characterization of a beta-herpesvirus and a human cytomegalovirus so as to determine whether such virus is spread-deficient.

[0165] Primary fibroblast cell lines MRCS for human CMV and NIH/3T3 for mouse CMV and complementing cell lines TCL94/99-BP and NTM94-7, respectively, are plated and infected at an MOI of about 0.25 for 1 h and then washed twice with D-PBS. Cells are incubated for 6 h and afterwards washed four times with D-PBS. Equal numbers of non-infected cells were stained with 5 μM CFSE for 8 min and blocked by 2% FCS/D-PBS, then washed twice with 2% FCS/D-PBS and subsequently seeded on top of the unstained but infected cells.

[0166] 48 (mouse CMV) and 72 hours (human CMV) after infection the co-cultures were fixed with 4% Paraformaldehyde (PFA) in D-PBS for 10 min at 37° C. and washed and permeablized with 0.1% Triton X100 for 10 min. After triple washing, cells were blocked with 3% BSA/D-PBS for 1 h. Immediate early staining was performed by incubating fixed cells with a primary antibody against the immediate early gene product of the CMV, more specifically monoclonal antibody Croma 101 ((IgG1 isotype) specific for the immediate-early protein 1 of mouse CMV designated as antibody 6/20/1 in Keil et al. (Keil et al. 1987 J. Virol. 61(2): 526-533.) and monoclonal antibody designated as CH160 in Plachter et al. specific for the human CMV immediate early 1 ((Plachter et al. 1993 Virology 193, 642-652), commercially available from Virusys Co.) in 3% BSA/D-PBS. After three D-PBS washes, cells were incubated with an Alexa Fluor 555-coupled secondary antibody directed against the primary antibody Croma 101 in case of MCMV and CH160 in case of human CMV in 3% BSA/D-PBS. Finally, cells were washed three times and imaged by confocal microscopy using an LSM 510 Meta (Zeiss). To determine whether a CMV strain or mutant is spread-deficient cells infected with wild type CMVs are used as positive control. Spread-deficient Virus transmission is determined by counting immediate early- and CFSE-positive cells using the ImageJ Cell Counter plugin (Rasband, W S. ImageJ 2009. Bethesda, Md., USA, U.S. National Institutes of Health. This program is a freely accessible standard at NCBI (http://rsbweb.nih.gov/ij/) and accepted as a reference in scientific publications). CFSE stained cells, immediate early-positive cells and cells showing both signals were counted. Virus transmission was determined by calculating the ration between immediate early-positive/CFSE stained cells to immediate-early-negative/CFSE stained cells.

EXAMPLE 2

Assay for Determining Whether a Virus is Endotheliotropic

[0167] The assay described herein is used for determining whether a virus is endotheliotropic.

[0168] As to determine whether a human CMV is endotheliotropic a primary human fibroblast cell line, a complementing cell line which complements the product of the gene in relation to which the HCMV of the invention is deficient, and a human endothelial cell line are plated and infected at an MOI of about 0.1 with HCMV wild type or the virus of the present invention. 24 hours after infection immediate early staining is performed by incubating fixed cells with a monoclonal antibody against immediate early gene product of the beta-herpesvirus of the invention, more specifically CMV IE 1/2 monoclonal Antibody CH160 (Plachter et al. supra), commercially available from Virusys Co. in 3% BSA/D-PBS. After three D-PBS washes, cells are incubated with an Alexa Fluor 555-coupled secondary antibody directed against the monoclonal antibody against human immediate early 1 of HCMV in 3% BSA/D-PBS. Finally cells are washed three times and imaged by UV microscopy. Cells infected with wild type HCMV are used as positive control and counted immediate early 1- and CFSE-positive cells using the ImageJ Cell Counter plugin (Rasband supra).

[0169] As to determine whether a mouse CMV is endotheliotropic a primary mouse fibroblast cell line, a complementing cell line which complements the product of the gene in relation to which the MCMV of the invention is deficient, and a mouse endothelial cell line are plated and infected at an MOI of about 0.1 with MCMV wild type or the virus of the present invention. 24 hours after infection immediate early staining is performed by incubating fixed cells with a monoclonal antibody against immediate early gene product of the beta-herpesvirus of the invention, more specifically Croma 101 designated as antibody 6/20/1 in Keil et al. (Keil et al., supra) in 3% BSA/D-PBS. After three D-PBS washes, cells are incubated with an Alexa Fluor 555-coupled secondary antibody directed against the mouse monoclonal antibody against immediate early 1 of mouse CMV in 3% BSA/D-PBS. Finally cells are washed three times and imaged by UV microscopy. Cells infected with wild type mouse CMV are used as positive control and counted immediate early 1 positive cells using the ImageJ Cell Counter plugin (Rasband supra).

EXAMPLE 3

Materials and Methods

[0170] Cells and Mice

[0171] The fibroblast cell line NIH/3T3 and BALB/c derived murine embryonic fibroblasts (MEF) were cultured as described in Cicin-Sain et al., (Cicin-Sain et al. 2005 J Virol 79:9492-9502.). C57BL/6 (B6) mice, B6.SJL-Ptpr^c (Ptpr^c) mice and 129.IFNαβR$^{-/-}$ mice were purchased from Elevage Janvier (Le Genest Saint Isle, France), Jackson Laboratories (Bar Harbor, Me., USA) and B&K Universal Limited (Grimston, England), respectively. 129.IFNαβR$^{-/-}$ mice (Muller et al. 1994 Science 264:1918-1921.) were back-crossed on the B6 background (B6.IFNαβR$^{-/-}$). T cell receptor transgenic mice OT-I (Hogquist et al. 1994 Cell 76:17-27.) and OT-II (Barnden et al. 1998 Immunol Cell Biol 76:34-40.) were backcrossed to Ptpr^c (CD45.1) or Thy1.1 (CD90.1) congenic mice, respectively. Alb-cre (Postic et al. 1999 J Biol Chem 274:305-315.) and Tie2-cre (Constien et al. 2001 Genesis 30:36-44.) were maintained on the B6 background. Mice were kept under specified pathogen free conditions. Animal experiments were approved by the responsible office of the state of Bavaria (approval no. 55.2-1-54-2531-111-07) or by the Ethics Committee at the University of Rijeka.

[0172] Generation of the Trans-Complementing Cell Line NT/M94-7

[0173] The conditional trans-complementing cell line NT/M94-7 was generated according to (Lotzerich et al. supra). Briefly, the M94 ORF was amplified from pSM3fr (Sacher et al. 2008 Cell Host Microbe 3:263-272.) using primers HΔM94for (SEQ.ID.No.1) and M94rev (SEQ.ID. No.2) thereby introducing an HA tag at the N-terminus. The PCR product was digested with BamHI and XbaI and inserted into the BamHI- and NheI-cleaved pTRE2Hyg vector (BD Biosciences Clontech, Heidelberg, Germany), resulting in pTRE-HΔM94 (SEQ.ID.NO:22) putting HΔM94 expression, the HΔM94 protein is depicted in SEQ.ID.NO:31, under the control of the tetracycline (tet) inducible promoter. Stable NIH/3T3 transfectants harboring pTRE-HΔM94 were selected with 50 µg/ml Hygromycin B. The deletion virus MCMV-ΔM94 was reconstituted by transfecting different NT/M94 cell clones with the respective BAC. The most productively infected trans-complementing cell line NT/M94-7 was subcloned using limiting dilution. The trans-complementing cell line was deposited under the Budapest Treaty with the DSZM, Germany on May 5, 2010.

[0174] Generation of Recombinant Viruses

[0175] Recombinant mouse CMV (MCMV) mutants were derived from the MCMV bacterial artificial chromosome (BAC) clone pSM3fr, originated from Smith strain (Messerle et al. 1997 Proc Natl Acad Sci USA 94:14759-14763.). Nucleotide positions are given according to Rawlinson et al. (Rawlinson et al. supra). The 1.4 kilo base pair (bp) SmaI fragment of pCP15 carrying the FRT flanked kanamycin resistance gene (Kan^r) was introduced into the BssHII site of pCR3 (Invitrogen, Basel, Switzerland) resulting in pCR3-FRT-Kan^r-FRT. A fragment containing an ATG start codon and a loxP site was generated by annealing the oligonucleotides ATGlox1 (SEQ.ID.No.3) and ATGlox2 (SEQ.ID. No.4). This fragment was inserted into the EcoRI and XhoI site positioned between the major immediate early promoter of HCMV (IEP) and the polyA signal of the bovine growth hormone of pCR3-FRT-Kan^r-FRT to obtain pCR3-FRT-Kan^r-FRT-ATG-loxP. The ovalbumin gene (ova) was synthesized as contained in pBSK-OVA (SEQ.ID.NO: 21) introducing GGAA after nt position 9 resulting in a BspEI restriction site for further cloning. Ova was inserted in frame using BspEI and NotI of pCR3-FRT-Kan^r-FRT-ATG-loxP resulting in a full length ova with inserted loxP site after the initial ATG under control of IEP named pCR3-FRT-Kan^r-FRT-ATG-loxP-ova. To obtain a construct with Cre inducible ovalbumin (OVA) expression (SEQ.ID.NO: 24) a floxstop cassette (Sacher et al. supra) was inserted into the EcoRI and BspEI sites of pCR3-ATG-loxP-ova resulting in pCR3-ATG-flox-stop-ova. Using these constructs as templates and oligonucleotides 5'-Δm157-pCR3-FRT-Kan^r-FRT (SEQ.ID.No.5) (nt position 216243 to 216290) and 3'-Δm157-flox-egfp (SEQ.ID.No.6) (nt position 216885 to 216930) as primers a linear DNA fragment containing the IEP-ova cassette, the FRT flanked Kan^r, and the viral homology sequences to the MCMV genome target site m157 was generated. In a similar procedure the firefly luciferase gene (luc) was cloned under control of the IEP into pCP 15 carrying the FRT flanked Kan^r. These fragments were introduced into m157 of pSM3fr as described (Sacher et al. supra) resulting in pSM3fr-Δm157-ova, pSM3fr-Δm157-flox-ova and pSM3fr-Δm157-luc. For excision of the FRT flanked Kan^r FLP recombinase was transiently expressed from plasmid pCP20.

[0176] Generation of Spread-Deficient Virus Mutants

[0177] As shown in FIG. **1** in *E. coli* the BAC pSM3fr-ΔM94 was generated by insertion of the tTA transactivator cassette into pSM3fr thereby deleting M94. The trans-complementing cell line NT/M94-7 expresses pM94 under control of the Tet inducible promoter. Upon transfection with pSM3fr-ΔM94 expression of tTA by the viral genome induces expression of pM94 by the cell leading to the production of trans-complemented MCMV-ΔM94. This virus is able to infect non complementing first target cells. Due to the lack of the essential gene M94 the release of infectious virus particles is impossible although immediate early (IE), early (E) and late (L) viral gene expression as well as DNA replication (DNA rep) occur.

[0178] For generation of the recombinant MCMV lacking the M94 sequence the parental MCMV BACs pSM3fr (MCMV-wt), pSM3fr-Δm157-ova (MCMV-ova) and pSM3fr-Δm157-rec-egfp (MCMV-Δm157-rec-egfp) (Sacher et al. supra) were applied to a second mutagenesis step. Therefore, the plasmid pO6-tTA-mFRT-Kan^r-mFRT was obtained by insertion of the Kan^r, on both sides flanked by mutant 34 bp FRT sites from pO6ie-F5 into pO6-tTA (Lotzerich et al. supra) to express the tTA transactivation gene under control of the IEP necessary for trans-complementation of pM94 (SEQ.ID:NO: 30). A linear DNA fragment containing the tTA cassette, the Kan^r and viral homology sequences to the MCMV genome target site (MCMV upstream-homology: nt position 136189 to 136234 and MCMV downstream-homology: nt position 137256 to 137309) was generated using primer 5' ΔM94-pO6-tTA (SEQ.ID.No.7), primer 31-ΔM94-pO6-tTA (SEQ.ID.No.8) and plasmid pO6-ITA-mFRT-Kan^r-mFRT as template. This PCR fragment was inserted into the different parental pSM3fr clones, hereby deleting the M94 gene. Since ORFs of M94 and M93 are overlapping 47 bp of homology had to be left at the 5'-end of M94 to keep the M93 ORF intact and 17 bp homology are still present at the former 3'-end of M94. Again FLP recombinase was expressed for excision of the Kan^r. Construction of pSM3fr-ΔM94, pSM3fr-ova-ΔM94, pSM3fr-flox-ova-ΔM94 and pSM3fr-Δm157-rec-egfp-ΔM94 was confirmed by restriction digest analysis and sequencing.

[0179] Viruses were reconstituted from BAC DNA, propagated on NT/M94-7 complementing cells and purified on a sucrose cushion as previously described (Sacher et al. supra). For analysis of virus replication supernatants from infected cells were taken every 24 h. Quantification of infectious virus was done using $TCID_{50}$ (median tissue culture infectious dose) method on NIH/3T3 or complementing NT/M94-7 cells. For the determination of virus replication in vivo virus load was determined by standard plaque assay as plaque forming units (PFU) per gram organ as described (Sacher et al. supra). Spread-deficiency of each virus stock of M94 deficient mutants (MCMV-ΔM94, MCMV-ova-ΔM94, MCMV-flox-ova-ΔM94 and MCMV-Δm157-rec-egfp-ΔM94) was confirmed by the absence of plaque formation after infection of non-complementing MEF, although CPE of individually infected cells was detectable. The *E. coli* containing the pSM3fr-ΔM94 BAC of the spread-deficient MCMV-ΔM94 was deposited under the Budapest Treaty with the DSZM on Apr. 28, 2010 as DSM 23561.

[0180] UV Inactivation of Virus

[0181] For in vivo application, a fraction of the MCMV-wt virus preparation used for immunization was inactivated by exposure to 1.5 kJ/cm^{2} UV light at a distance of 5 cm in a UV-crosslinker (Stratagene, Amsterdam, Netherlands) at 4° C. Viral infectivity was decreased by factor 2.4×10^{7}. The same treatment was sufficient to abolish viral gene expression when MCMV-Δm157-rec-egfp was subjected to different doses (0.5, 1.0 and 1.5 kJ/cm^{2}) of UV light and subsequently titrated on MEF. After 4 days post infection (p.i.) EGFP expression was monitored in single infected cells if virus was irradiated with low dose (0.5 kJ/cm^{2}) of UV and no EGFP expression was seen after strong irradiation (1.5 kJ/cm^{2}). Untreated MCMV-Δm157-rec-egfp formed EGFP^{+} plaques.

[0182] Immunization and Challenge of Mice

[0183] 8 to 10 weeks old female B6 mice were immunized by intraperitoneal (i.p.) or subcutaneous (s.c.) injection of either MCMV-wt or mutant MCMV. Each mouse received 100 µl of virus suspension s.c. or 300 µl i.p. C57BL/6 mice were immunized with 1×10^{5} TCID$_{50}$ MCMV-wt or MCMV-deltaM94, 129.IFNαβR$^{-/-}$ with 2.5×10^{5} TCID$_{50}$ of MCMV-deltaM94 or UV irradiated MCMV-wt, and B6.IFNαβR$^{-/-}$ with 3×10^{5} TCID$_{50}$ of MCMV-ΔM94 or MCMV-wt. Mock treated mice received same volumes of PBS. To boost mice, this procedure was repeated 14 days p.i. Sera collected from mice 12 weeks p.i. were used to determine amounts of virus specific antibodies by virus neutralization assay, as described below.

[0184] 28 days or 20 weeks post priming, mice were challenged by intravenous (i.v.) injection of 10^{6} PFU of tissue culture derived MCMV-wt. Five days post challenge lungs, liver and spleen were collected under sterile conditions and stored at −80° C. Organ homogenates were analyzed for infectious virus load by standard plaque assay on MEF cells. Salivary glands derived MCMV (sgMCMV-wt) was generated as a homogenate of salivary glands from mice infected with tissue culture derived MCMV-wt as described in Trgovcich et al. (Trgovcich et al. 2000 Arch Virol 145:2601-2618). The isolated sgMCMV-wt is more virulent compared to tissue culture derived MCMV-wt (Pilgrim et al. 2007 Exp Mol. Pathol. 82:269-279). Vaccinated B6.IFNαβR$^{-/-}$ mice were challenged with 2×10^{5} PFU sgMCMV-wt and 129.IFNαβR$^{-/-}$ mice were challenged with 2.5×10^{5} TCID$_{50}$ tissue culture derived MCMV-wt.

[0185] Virus Neutralization Assay

[0186] Heat inactivated serum (56° C., 30 min) from 5 immunized mice 12 weeks p.i. were pooled and serially diluted 1:2 in DMEM containing a final concentration of 10% guinea-pig complement. Each dilution was mixed with 50 PFU of MCMV-luc and incubated for 90 min at 37° C. and subsequently added to NIH/3T3 cells in a 96 well format. After 1 h at 37° C. the virus inoculum was removed and NIH/3T3 medium added. The cultures were incubated for 24 h and luciferase activity was determined in cell extracts using the luciferase assay (Promega, Mannheim, Germany) in a luminometer (Berthold, Bad Wildbad, Germany) according to the supplier's and manufacturer's instructions, respectively.

[0187] In Vivo Cytotoxicity Assay

[0188] To evaluate CD8^{+} T cell effector function in vivo, splenocytes of congenic CD45.1^{+} Ptpr^{c} mice were incubated with 2 µM of the indicated peptide and stained with 2 µM, 0.7 µM, or 0.1 µM carboxyfluorescein succinimidyl ester (CFSE) and PKH26 Red Fluorescent Cell Linker Mini Kit according to the manufacturer's instructions (Sigma-Aldrich). At day 6 p.i., labeled CD45.1^{+} cells were transferred into B6 (CD45.2^{+}) recipients. After 16 h spleens of recipient mice were removed and flow cytometrical analysis of the target cells was performed. Specific cytotoxicity of target cells was calculated using the equation: % spec lysis=(1−ratio unprimed/ratio primed)*100; ratio=(% CFSE low/% CFSE high) (Lauterbach et al. 2005 J Gen Virol 86:2401-2410.). The OVA derived class I peptide (SEQ.ID.NO.9) and MCMV specific peptides derived from m139 (SEQ.ID.No.10), ie3 (SEQ.ID.No.11), M57 (SEQ.ID.No.12) and M45 (SEQ.ID.No.13) (Snyder et al. 2008 supra) were purchased from Metabion, Germany and were dissolved and stored according to manufacturer's device.

[0189] Adoptive Transfer and Flow Cytometrical Analysis

[0190] OVA specific CD8^{+} T cells were isolated from spleen and cervical, axillary, brachial and inguinal lymph nodes of OT-I TCR transgenic mice backcrossed to congenic CD45.1^{+} mice. OT-I cells were purified by negative selection via the CD8α^{+} T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). 3×10^{5} transgenic T cells were injected i.v. into recipient B6 mice one day prior to i.p. infection with 10^{5} TCID$_{50}$ MCMV. To follow expansion of the transferred OT-I T cells 100 µl blood was taken 3, 6 and 8 days p.i., erythrocytes were lysed (PharmLyse, BD Biosciences, Heidelberg, Germany) and remaining cells were incubated with PE-TexasRed coupled α-CD8α (5H10; Caltag, Sacramento, Calif., USA) and PE coupled α-CD45.1 antibodies (A20; BD Biosciences Pharmingen). Flow cytometrical acquisition was performed using an Epics XL-MCL (Beckman-Coulter) and data were analyzed using FlowJo software (Tristar, Ashland, Oreg., USA).

[0191] OVA specific CD4^{+} T cells were isolated from spleen and cervical, axillary, brachial and inguinal lymph nodes of OT-II TCR transgenic mice backcrossed to congenic CD90.1^{+} mice. After lysis of erythrocytes 3×10^{5} transgenic T cells were injected i.v. into recipient mice one day prior to infection with 10^{5} TCID$_{50}$ MCMV. Spleens were removed and splenocytes were incubated with Fc block (2.4G2; BD Biosciences) and subsequently stained with PE conjugated α-CD90.1 (HIS51; eBioscience) and PE-Cy5.5 coupled α-CD4 (RM 4-5; eBioscience). Flow cytometrical acquisition was performed using a FACS Calibur (BD Biosciences) and data were analyzed using FlowJo software.

[0192] Quantification of Viral Genomes in Organ Homogenates

[0193] Lungs were removed from mice twelve month after infection. Organs were homogenized and DNA was extracted using the DNeasy Blood & Tissue Kit from Qiagen (Hilden, Germany). Elution was done with 100 µl of the supplied elution buffer and genomic DNA concentration of each sample was quantified in duplicates using a NanoDrop ND-1000 UV-Vis Spectrophotometer. To quantify the viral DNA a quantitative realtime PCR specific for the MCMV M54 gene (Cicin-Sain et al. 2005 supra) was performed using a specific Taqman-Probe (SEQ.ID.No.14) and the Taqman 1000 RXN PCR Core Reagents kit on an ABI PRISM 7700 Sequence Detector (Applied Biosystems, Carlsbad, Calif., USA). To calculate the viral genome copy number, a standard curve of the BAC plasmid pSM3fr containing the M54 gene was included.

[0194] Statistical Analysis

[0195] Statistical analyses were done using GraphPad Prism 4 (GraphPad Software La Jolla, Calif., USA). For in vitro growth comparison of viruses, neutralizing antibody assay, realtime PCR and T cell proliferation the mean was calculated with standard deviation (SD). In all figures depicting virus load in organs and in vivo cytotoxicity, the median is given. Comparison of the neutralizing antibody response in mice vaccinated with MCMV-wt or MCMV-ΔM94 was performed with the two-way ANOVA test. Comparison of percentage of T cell proliferation and quantification of virus in organs or viral genomes was done with the two-tailed Wilcoxon rank sum test using website http://elegans.swmed.edu/~leon/stats/utest.cgi. Asterisks denote statistical differences (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

EXAMPLE 4

MCMV-ΔM94 is Spread-Deficient

[0196] The HCMV virion protein pUL94 is essential for virus replication (Dunn et al. supra) and is expressed with late kinetics (Wing et al. supra). It has been found that pM94, the MCMV homolog, is also essential and plays a crucial role in a post nuclear step of virus maturation. In order to trans-complement the essential M94 gene product and reconstitute an M94 deletion mutant the NIH/3T3 derived complementing cell line NT/M94-7 harbouring the M94 gene under control of the TRE promoter was generated. The TRE promoter is only active in the presence of the Tet trans-activator (tTA). To provide the tTA for trans-complementation of pM94 the tTA expression cassette was introduced into pSM3fr (Messerle et al. supra) disrupting M94 generating pSM3fr-ΔM94. MCMV-ΔM94 virus was reconstituted by transfecting NT/M94-7 cells (FIG. **1**). Next, multistep growth analysis infecting NT/M94-7 cells as well as parental NIH/3T3 fibroblasts with MCMV-ΔM94 or MCMV-wt were performed.

[0197] The results of this Example are shown in FIG. **2**. In FIG. **2**A Parental NIH/3T3 (circles) and NT/M94-7 fibroblasts (boxes) were infected at 0.1 TCID$_{50}$/cell with MCMV-wt (wt; closed symbol) or MCMV-ΔM94 (ΔM94; open symbol). At indicated days, infectious virus in the supernatant was quantified on NT/M94-7 cells by TCID$_{50}$ endpoint titration. Shown is the mean+/−SD of titrated duplicates. At day 5 p.i. supernatants were additionally titrated on MEF. No PFU was found within 1 ml supernatant of MCMV-ΔM94 infected NT/M94-7. p.i.=post infection; DL=detection limit.

[0198] As shown in FIG. **2**B Parental NIH/3T3 (lower panel) and NT/M94-7 (upper panel) fibroblasts were infected with MCMV-Δm157-rec-egfp-ΔM94. At indicated time points EGFP expressing cells were monitored. hpi=hours post infection.

[0199] As shown in FIG. **2**C 129.IFNαβR$^{-/-}$ mice (n=15 for MCMV-ΔM94, open symbols; n=8 for MCMV-wt, closed symbols) were infected with 2.5×10^{5} TCID$_{50}$ i.p. and survival was followed for 30 days p.i.

[0200] While MCMV-ΔM94 replicated to MCMV-wt-like titers on NT/M94-7 cells, no infectious virus was detectable in the supernatant of NIH/3T3 cells (FIG. **2**A). As the defect of MCMV-ΔM94 to release infectious virus particles into the supernatant does not exclude cell-associated virus spread, a ΔM94 mutant expressing the enhanced green fluorescent protein EGFP (MCMV-Δm157-rec-egfp-ΔM94) was constructed. While MCMV-Δm157-rec-egfp-ΔM94 spread with kinetics comparable to MCMV-wt on NT/M94-7 cells, MCMV-Δm157-rec-egfp-ΔM94 remained strictly confined to the first infected NIH/3T3 cells (FIG. **2**B). This result was confirmed also in endothelial cells (FIG. **8**). In summary, M94 is essential and deletion abrogates virus release and cell-to-cell spread. In addition, MCMV-ΔM94 can be efficiently produced by trans-complementation.

[0201] Complementing NT/M94-7, parental NIH/3T3 fibroblasts and myocardium-derived endothelial cells MHEC5-T were infected with 0.1 TCID50/cell MCMV-ΔM94-Δm157-rec-egfp (MCMV-ΔM94) or MCMV-Δm157-rec-egfp (wt). At indicated time points EGFP expressing cells were monitored. Scale bar represents 100 μm.

EXAMPLE 5

MCMV-ΔM94 does not Revert to Replication Competent Virus

[0202] A major safety concern is reversion of vaccine strains to replication competent viruses during preparation (Roizman et al. 1982 Dev Biol Stand. 52:287-304) or in the vaccinated patient (Iyer et al. 2009 Ann. Emerg. Med. 53:792-795). To exclude acquisition of the M94 gene through recombination via homologous sequences between MCMV-ΔM94 and the complementing cell line homologies were carefully avoided during virus construction. Replication competent virus indicative of recombination between the deletion virus and the M94 gene expressed by NT/M94-7 was never observed. In order to investigate the safety of MCMV-ΔM94 for vaccination studies in a highly susceptible mouse strain, 129.IFNαβR$^{-/-}$ mice were infected with MCMV-wt or MCMV-ΔM94. While all IFNαβR$^{-/-}$ mice died within 14 days upon infection with MCMV-wt, after infection with MCMV-ΔM94 all mice survived with no or only minimal weight loss (FIG. **2**C). In conclusion, MCMV-ΔM94 could be safely produced and even immune deficient mice tolerated MCMV-ΔM94 infection.

EXAMPLE 6

MCMV-ΔM94 Induces Neutralizing Antibody and T Cell Responses

[0203] Poor induction of neutralizing antibodies that prevent viral entry is a problem in HCMV infection (Landini et al. 1991 Comp Immunol Microbiol Infect Dis 14:97-105). Therefore, the neutralizing antibody response to MCMV-wt and MCMV-ΔM94 was compared 12 weeks post immunization. Serial dilutions of sera were mixed with a luciferase expressing MCMV (MCMV-luc) prior to infection of NIH/3T3. The reduction of the luciferase signal reflected the neutralizing capacity of the antisera. Immunization with MCMV-ΔM94 induced a slightly lower amount of neutralizing antibodies than with MCMV-wt (FIG. **3**A, $p<0.05$) whereas immunization with UV irradiated MCMV-wt abolished the induction of neutralizing antibodies confirming published observations (Gill et al. supra).

[0204] The results of this example are shown in FIG. **3**. In FIG. **3**A B6 mice were immunized i.p. with 10^{5} TCID$_{50}$ MCMV-wt (wt; closed circles), MCMV-ΔM94 (ΔM94; open circles) or mock infected (PBS; gray squares). Blood was collected 12 weeks p.i. and virus neutralizing capacity of the serum was determined using MCMV-luc. Neutralizing antibody levels of MCMV-ΔM94 immunized mice were significantly lower than antibody levels of MCMV-wt immunized mice using two-way ANOVA testing (P=0.04). Values represent the mean±SD of measured serum pools. RLU=Relative Luciferase Units, BG=background.

[0205] In FIG. **3**B after adoptive transfer of 3×10^{50} T-I CD8^{+} T cells (upper panel), B6 mice (n=5) were infected i.p.

with 10^5 $TCID_{50}$ MCMV-ova (wt-ova; closed bars), MCMV-ova-ΔM94 (ΔM94-ova; open bars) or PBS (gray bars). At day 3, 6 and 8 p.i. flow cytometrical analysis was performed on blood for the congenic marker CD45.1 and CD8. After adoptive transfer of 3×10^{50} T-II $CD4^+$ T cells (lower panel), B6 mice (n=5) were infected i.p. as above. At day 3, 6 and 8 p.i. flow cytometrical analysis was done on splenocytes for CD90.1 and CD4. Each bar represents the mean±SD of the indicated group; (**, P<0.01).

[0206] In FIG. **3**C B6 mice (n=5) were infected i.p. with 10^5 $TCID_{50}$ MCMV-wt (wt; closed symbols), MCMV-ΔM94 (ΔM94; open symbols) or UV irradiated MCMV-wt (wt UV; gray symbols). At day 6 p.i. in vivo cytotoxicity assay was performed using splenocytes labeled with carboxyfluorescein succinimidyl ester (CFSE) and the indicated viral peptides. Symbols represent the specific lysis activity against the indicated peptide in individual animals. The cross bar indicates the median of the analyzed group. The right panel shows an exemplary set of flow cytometric data.

[0207] Both $CD4^+$ and $CD8^+$ T cells play important roles in host defense against CMV. Antiviral $CD8^+$ T cells are effective in controlling MCMV during acute infection and mediate protection after immunization (Reddehase et al. supra). In addition, $CD4^+$ T helper cells are required for virus clearance in salivary glands (Jonjic et al. 1989 J Exp Med 169:1199-1212). In order to compare the level of $CD4^+$ and $CD8^+$ T cell responses induced by MCMV-wt and MCMV-ΔM94, OVA as a model antigen was chosen to be expressed by the vaccine. B6 mice were infected with MCMV-ova and MCMV-ova-ΔM94 one day after adoptive transfer of OVA specific $CD4^+$ or $CD8^+$ T cells. For MCMV-ova the expansion of OVA specific $CD4^+$ and $CD8^+$ T cells peaked at day 6 p.i., concordant with published data (Karrer et al, 2004 J Virol 78:2255-2264). Remarkably, MCMV-ova-ΔM94 also stimulated the proliferative response of OVA specific $CD8^+$ and $CD4^+$ (FIG. **3**B) T cells to a degree comparable to the spread competent MCMV-ova. The amount of $CD8^+$ T cells was even slightly higher than with MCMV-wt (P<0.01).

[0208] This observation was to be confirmed for native MCMV antigens. B6 mice were infected with MCMV-ΔM94 or MCMV-wt. At six days p.i., target cells loaded with viral peptides derived from either m139, ie3, M57, or M45 (Snyder et al. 2008 supra) were injected and their cytolysis in vivo was analyzed (FIG. **3**C). The cytolytic $CD8^+$ T cell response induced by MCMV-ΔM94 turned out to be comparable to MCMV-wt. In contrast, B6 mice injected with UV irradiated MCMV generated no or only poor lysis of targets. UV inactivation of MCMV-ΔM94 or MCMV-wt also abolished OVA specific T cell expansion and the virus neutralizing capacity of sera. Thus, viral gene expression appeared to be crucial for the induction of the adaptive immune response. Altogether, spread-deficient MCMV induced an immune response comparable to MCMV-wt.

EXAMPLE 7

Role of Viral Target Cell Types in $CD8^+$ T Cell Activation

[0209] The strong adaptive immune response against MCMV-ΔM94 was surprising, since MCMV-ΔM94 gene expression is limited to the first target cells. Induction of a specific T cell response is dependent on antigen presentation by infected cells and by professional antigen presenting cells (Villadangos et al. 2008 Immunity. 29:352-361). In order to assess the contribution of infection of different cell types in the generation of an efficient $CD8^+$ T cell response the replication deficient MCMV was combined with conditional activation of a marker gene (Sacher et al. supra). MCMV-flox-ova-ΔM94 was constructed which expresses OVA only after Cre-mediated recombination.

[0210] One day prior to i.p. injection of 10^5 $TCID_{50}$ of MCMV-flox-ova-ΔM94 (ΔM94-flox-ova), MCMV-ova-ΔM94 (ΔM94-ova), MCMV-wt (wt) or PBS 3×10^5 congenic OT-I $CD8^+$ T-cells were transferred i.v. into B6, Alb-cre and Tie2-cre mice. At day 6 p.i. a flow cytometrical analysis was performed on PBL for the congenic marker CD45.1 and CD8. Boxes represent the ratio of OT-I cells per $CD8^+$ cells as a pool of 3 independent experiments and extend from the 25 to the 75 percentile. The lines indicate the median. Whiskers extend to show the extreme values. The P-values were obtained applying a two-tailed Wilcoxon rank sum test, (**, P<0.01; ***, P<0.001). The results are shown in FIG. **4**

[0211] Endothelial cells (EC) and hepatocytes (Hc) are among the first target cells infected by MCMV in vivo (Sacher et al. supra). Whether these cell types contribute to $CD8^+$ T cell activation was addressed by infecting mice that express Cre recombinase selectively in vascular EC (Tie2-cre) or Hc (Alb-cre). One day after adoptive transfer of OVA specific $CD8^+$ T cells mice were infected with 10^5 $TCID_{50}$ of spread-deficient MCMV-flox-ova-ΔM94. Hc are the main producers of infectious virus during the first few days of infection and are highly effective in activating a conditional marker gene by Cre recombinase (Sacher et al. supra). Yet, selective induction of OVA expression in MCMV infected Hc resulted in only weak proliferation of OVA specific $CD8^+$ T cells (FIG. **4**). In contrast, a significantly (P<0.001) higher proliferative response of OVA specific $CD8^+$ T cells was observed upon OVA expression in EC. Therefore, infection of EC make a stronger contribution to the induction of an antiviral $CD8^+$ T cell response than infection of Hc. As infection of C57BL/6 mice with MCMV-ΔM94-ova that leads to expression of OVA in all infected cells induces a higher proportion of OVA specific $CD8^+$ T cells than expression selectively in EC (Tie2-cre mice infected with MCMV-ΔM94-flox-ova; P<0.01) additional cell types seem to be involved in antigen expression and T cell stimulation. In addition, the significant different T cell responses after cell type specific recombination in vivo prove that MCMV-ΔM94 is unable to spread from cell to cell.

[0212] The experimental details in connection with this example were, in addition to the ones outlined in Example 3, as follow and the results of this example are depicted in FIG. **5**.

[0213] B6 mice (n=5) were immunized (1^{st}) s.c. or. i.p. with 10^5 $TCID_{50}$ MCMV-wt (wt; closed symbols), MCMV-ΔM94 (ΔM94; open symbols), Δm01-17+m144-158-MCMV (AA; gray symbols) or PBS (light gray symbols). Virus preparations were UV irradiated before immunization (UV) as indicated. Optionally, mice were boosted (2^{nd}) two weeks later with the same dose, route and virus. Challenge infection was applied i.v. 20 (A) or four weeks (B) post prime with 10^6 PFU MCMV-wt. Five day post challenge plaque assay was performed. Horizontal bars show the median of each group. Each symbol represents one individual mouse. DL=detection limit.

EXAMPLE 8

MCMV-ΔM94 Protects Against Challenge with MCMV-wt

[0214] In order to test protection of MCMV-ΔM94 against lethal challenge, B6 mice were infected with either spread-deficient MCMV-ΔM94, the attenuated strain Δm01-17+m144-158-MCMV (Cicin-Sain et al. 2007 J Virol 81:13825-13834) or MCMV-wt. A boost infection was applied 4 weeks later with the same dose. 20 weeks after priming mice were challenged i.v. with 10^6 $TCID_{50}$ tissue culture derived MCMV-wt. Most remarkably, already a singular immunization dose of MCMV-ΔM94 was already sufficient to strongly suppress MCMV-wt replication by 10,000 fold in lungs, 1,000 fold in liver and at least 100 fold in spleen, whereas non-immunized controls had high virus loads in all organs tested (all $P<0.01$; FIG. **5**A). Overall, the protection mediated by MCMV-ΔM94 vaccination was comparable to MCMV-wt or Δm01-17+m144-158-MCMV vaccination (all $P>0.05$). Due to the strong protection achieved already after one administration, a boosting effect could not be detected. However, there was weak protective effect after a singular dose when UV inactivated MCMV-wt or UV inactivated MCMV-ΔM94 virus was administered. Only after a boost with UV inactivated viruses the effect was slightly improved but still remained lower than that of a singular dose of MCMV-ΔM94 ($P<0.05$).

[0215] It was asked, whether the strong protection after singular administration of MCMV-ΔM94 could also be realized in a short-term vaccination protocol. In addition, the influence of two different application routes was tested. B6 mice were injected either i.p. or s.c. followed by challenge infection with MCMV-wt only 4 weeks later. Here, vaccination with MCMV-ΔM/94 resulted in about 100 fold reduction of challenge virus load in liver ($P<0.05$), lungs ($P<0.01$) and spleen ($P<0.01$; FIG. **5**B) comparable to immunization with Δm01-17+m144-158-MCMV. MCMV-wt vaccination resulted in reduction of challenge virus load by 1,000 fold ($P<0.01$). Generally, there was no significant difference between the i.p. or s.c. vaccination route although s.c. injection appeared to induce slightly better protection in spleen ($P>0.05$) FIG. **5**B) and hearts.

[0216] Summarized, vaccination with the spread-deficient MCMV-ΔM94 was able to efficiently protect immunocompetent mice against challenge with MCMV-wt after vaccination with a singular dose. Remarkably, vaccination with MCMV-ΔM94 was as efficient as vaccination with MCMV-wt concerning long-term vaccination, whereas the use of UV inactivated virus could not compete even after a second application.

EXAMPLE 9

Protection of Severely Immune Compromised Recipients

[0217] Type I interferons are key cytokines in the immune response against CMV and deletion of their receptor results in a mouse (IFNαβ$R^{-/-}$) that is severely immunocompromised and at least 1.000-fold more susceptible to MCMV infection than the parental mouse strain (Presti et al. 1998 J Exp Med 188:577-588). Since spread-deficient MCMV-ΔM94 was proven to be well tolerated by IFNαβ$R^{-/-}$ mice (FIG. **2**C), it was tested whether MCMV-ΔM94 could even protect IFNαβ$R^{-/-}$ mice against lethal MCMV-wt challenge (see FIG. **6**A). B6.IFNαβ$R^{-/-}$ mice were immunized with MCMV-ΔM94 or a sublethal dose of MCMV-wt. Both groups survived and mice immunized with MCMV-ΔM94 showed no significant weight loss, whereas MCMV-wt infected mice lost approximately 15% of their body weight. Four weeks later, mice were challenged by infection with a lethal dose of more virulent salivary glands derived MCMV (as described in Example 3). Most strikingly, the vaccination with both, MCMV-ΔM94 as well as MCMV-wt was protective and all animals survived (FIG. **6**A).

[0218] The results of this Example are shown in FIG. **6**.

[0219] In FIG. **6**A B6.IFNαβ$R^{-/-}$ (n=6) mice were immunized i.p. with 3×10^5 $TCID_{50}$ MCMV-wt (wt; black circles) or MCMV-ΔM94 (ΔM94; open circles). Control groups of B6.IFNαβ$R^{-/-}$ (gray circles) or B6 (gray triangles) were treated with PBS. Four weeks later challenge infection was performed by i.p. injection of 2×10^5 PFU salivary glands derived MCMV (sgMCMV-wt) mice and survival was monitored.

[0220] In FIG. **6**B 129.IFNαβ$R^{-/-}$ mice 4 weeks previously immunized with 2.5×10^5 $TCID_{50}$ of MCMV-ΔM94 (ΔM94; open circles, n=8), or UV irradiated MCMV-wt (wt UV; closed triangles down, n=8) were challenged with a lethal dose of MCMV-wt (see FIG. **2**C) and survival was monitored. A 10 fold higher dose of MCMV-wt was applied to mice immunized with MCMV-ΔM94 (n=7) (open triangles).

[0221] B6 mice profit from an Ly49H-dependant activation of natural killer cells resulting in a strong innate immune response stimulated by the MCMV protein encoded by m157 (Sun et al. 2008. J. Exp. Med. 205:1819-1828.). 129.IFNαβ$R^{-/-}$ mice do not express Ly49H and are even more susceptible to MCMV infection than B6.IFNαβ$R^{-/-}$ mice. 129.IFNαβ$R^{-/-}$ mice were vaccinated with MCMV-ΔM94 and challenged 4 weeks later with a dose of 2.5×10^5 $TCID^{50}$ tissue culture derived MCMV-wt (FIG. **6**B). In line with the earlier data (Cicin-Sain et al. 2007 supra), vaccination with UV inactivated virus mediated only partial protection and could delay death for a short period. MCMV-ΔM94 vaccinated mice survived the lethal challenge even with a dose of 2.5×10^6 $TCID^{50}$. In summary, vaccination with MCMV-ΔM94 is able to protect even highly susceptible immune compromised mice against lethal MCMV challenge.

EXAMPLE 10

Maintenance of the MCMV-ΔM94 Genome In Vivo

[0222] One argument against the application of attenuated life vaccines is their ability to establish a latent infection that bears the risk of reactivation (Iyer et al. supra). On the other hand non-productive reactivation episodes might result in endogenous boosts of the antiviral immune response (Snyder et al. 2008 Immunity 29:650-659). Thus, it was intriguing to test whether MCMV-ΔM94 genome is maintained in vaccinated hosts. Quantitative PCR analysis on total DNA extracted from lungs, a key manifestation site of CMV disease (Balthesen et al. 1993 J Virol 67:5360-5366), was performed. Twelve months p.i. genomes of MCMV-ΔM94 could be detected in all mice tested (FIGS. **7**A and B) proving that the genome of MCMV-ΔM94 is maintained. Interestingly, the genome numbers detected in lungs one year after infection with MCMV-ΔM94 and MCMV-wt were not significantly different ($P>0.05$). This finding proved that at least some of the first target cells are not lost after infection either due to virus-induced cell death or elimination by the immune response. In summary, these data also provide first evidence that virus spread is not necessary for long-term genome maintenance and that first target cells of MCMV-ΔM94 may be able to contribute to a more sustained immune response.

[0223] The results of this example are shown in FIG. **7**.

[0224] B6 mice were infected i.p. with 10^5 $TCID_{50}$ MCMV-wt (wt) (n=5) or MCMV-ΔM94 (ΔM94) (n=6).

Twelve months p.i. total DNA was extracted from lungs. (FIG. 7A) PCR analysis was performed obtaining a specific 246 bp fragment of the polymerase gene M54. As controls DNA from lungs five days after infection with 10^5 $TCID_{50}$ MCMV-wt (wt acute) (n=5), PBS (1), no template (2) or the BAC plasmid pSM3fr (3) were used. (FIG. 7B) Quantitative realtime PCR analysis was performed and viral M54 gene copies were calculated per μg genomic DNA. Each symbol represents one individual mouse. Horizontal bars show the median of each group. Genome copy numbers of MCMV-wt (wt) and MCMV-ΔM94 (ΔM94) are not significantly different ($P>0.05$). Both groups are significantly different compared to acutely infected lungs (wt acute) (**, $P<0.01$). MW=molecular weight marker; DL=detection limit. (FIG. 7C and FIG. 7D) B6 mice (n=5) were immunized i.p. with 10^5 $TCID_{50}$ MCMV-wt (wt; closed symbols), MCMV-ΔM94 (ΔM94; open symbols), Δm01-17+m144-158-MCMV (AA; gray symbols) or PBS (light gray symbols). Virus preparations were UV-irradiated before immunization (UV) as indicated. Challenge infection was applied i.v. one year post prime with 10^6 PFU MCMV-wt. Plaque assay was performed (FIG. 7C) five days post challenge with lungs and (FIG. 7D) 14 days post challenge with salivary glands (SG). Horizontal bars show the median of each group. Each symbol represents one individual mouse. DL=detection limit.

EXAMPLE 11

Vaccination with MCMV-ΔM94 Prevents Replication of Virus in the Respiratory Tract

[0225] From epidemiological studies it was suggested that saliva is an important route of transmission of HCMV (Pass et al. 1986 N. Engl. J. Med 314:1414-1418.). To test whether the vaccine MCMV-ΔM94 is able to block virus replication in salivary glands and lungs C57BL/6 mice were immunized with MCMV-ΔM94 or control viruses and received twelve months later a challenge infection with 10^6 PFU MCMV-wt i.v. (FIGS. 7C and D). A single application of MCMV-ΔM94 was sufficient to suppress challenge virus replication by more than factor 1,000 in lungs in 4 out of 6 animals (FIG. 7C). Further, no challenge virus could be isolated from salivary glands 14 days after challenge (FIG. 7D). This implies that shedding of virus from the respiratory tract via saliva and therefore horizontal transmission via this route is abrogated by vaccination with spread-deficient MCMV.

EXAMPLE 12

Discussion

[0226] It is reported herein on the vaccination against a beta-herpesvirus using a spread-deficient vaccine. The vaccine induced a strong adaptive immune response comparable to MCMV-wt conferring protection even in highly immune compromised mice. This means that infection of the first target cells is sufficient for successful vaccination.

[0227] An intact immune system usually protects against HCMV disease. Hence, the antigenic capacity of the wild type virus is sufficient for the induction of a protective immune response. The inability of UV inactivated virus to protect efficiently against challenge infection demonstrated the need for viral antigen expression including nonstructural antigens (Cicin-Sain et al. 2007 supra; Gill et al. 2000 J Med Virol 62:127-139). As a consequence an ideal vaccine should exploit the full immunogenic but avoid the pathogenic potential of the wild type virus.

[0228] The alpha-herpesvirus field has pioneered the use of replication defective viruses as vaccines (Dudek et al. supra). These vaccines were generated by the deletion of genes essential for virus replication and are thus apathogenic (Dudek et al. supra). Now, to construct a spread-deficient beta-herpesvirus vaccine deletion of M94 was chosen for the following reasons. First, M94 is essential for spread of MCMV and inferred from studies of HCMV it should be expressed with late kinetics during virus replication (Scott et al. supra; Wing et al. supra). Second, pM94 does not belong to the group of glycoproteins which comprise major targets for the neutralizing antibody response of HCMV. Third, M94 of MCMV is the homolog of UL94 in human CMV (Wing et al. supra) that in principle allows translation to the human pathogen. Finally, the deletion of UL94 of HCMV might even be of advantage because pUL94 induces autoreactive antibodies that are associated with systemic sclerosis (Lunardi et al. 2000 Nat Med 6:1183-1186). The SSc cross-reactive UL94 peptide is depicted in SEQ.ID:NO: 28. Interestingly, genomes of the spread-deficient MCMV-ΔM94 were detected in lungs after i.p. infection, showing that virus can disseminate either as free particles (Hsu et al. 2009 J Gen Virol 90:33-43) or associated to cells. Monocytes and macrophages were shown to be attracted to the peritoneal cavity after infection and transport the virus in blood (Stoddart et al. 1994 J Virol 68:6243-6253; van der Strate et al. 2003 J Virol 77:11274-11278). These cells could also release virus at distant sites to infect EC or other cell types, a process called trans infection (Halary et al. 2002 Immunity 17:653-664).

[0229] The spread-deficient betea-herpesvirus vaccine presented here, has a strong protective capacity similar to wild type CMV infection. The immune response of the vaccinee controls virus replication in all analysed organs preventing overt CMV-disease. The absence of detectable amounts of infectious virus in salivary glands of long-term vaccinated mice two weeks after challenge implies that also horizontal transmission to other individuals via saliva is abrogated. Because of this it is plausible that such an equivalent vaccine will protect against HCMV-disease, similar to the protective effect of a pre-existing infection. This is supported by the observation that women who were exposed to HCMV were at lower risk to give birth to children with symptomatic disease compared to non-infected women (Fowler et al. 2003 JAMA 289:1008-1011.). The seropositivity of the mother could not prevent infection but pathogenesis of the children. In addition, frequent exposure to different CMV strains could further increase immunity against reinfection (Adler et al. supra). It is therefore again plausible that a spread-deficient human CMV vaccine induces an immune response equal to natural infection which will protect against symptomatic human CMV infection without the risk for reactivation and pathogenesis.

[0230] The immune response to MCMV-ΔM94 reached a level comparable to MCMV-wt. Protection was similar to the recently generated vaccine Δm01-17+m144-158-MCMV (Cicin-Sain et al. 2007 supra) which lacks 32 viral genes but which is not spread-deficient in vitro. In Δm01-17+m144-158-MCMV immune evasive genes were deleted to increase the antiviral immune response and thereby to attenuate the virus (Scalzo et al. 2007 Immunol Cell Biol 85:46-54.).

[0231] It is within embodiments of the present invention that (a) at least one essential gene and (b) at least one immune evasive gene is deleted, whereby it is preferred that the deleted at least one immune evasive gene is selected from the group comprising genes encoding gene products affecting antigen presentation, interaction with cytokines, the complement system and humoral immunity. More preferably, the deleted at least one immune evasive gene is selected from the group comprising genes encoding gene products that down-regulate MHC I to avoid CTL response, gene products that evade the NK cell response, gene products that interfere with MHC II presentation, down-regulate adhesion molecules, gene products that interact with IL-1, gene products that activate TGF-β.

[0232] Infection of susceptible IFNαβR$^{-/-}$ mice with spread-deficient MCMV proved the safety of the vaccination concept. Furthermore, IFNαβR$^{-/-}$ mice were protected against otherwise lethal challenge, similar to other infection models (Calvo-Pinilla et al. 2009 PLoS One. 4:e5171; Paran et al. 2009 J Infect Dis 199:39-48). Although recent work revealed the capacity of MCMV to efficiently induce type I interferon (Hokeness-Antonelli et al. 2007 J Immunol 179: 6176-6183), the efficacy of the spread-deficient MCMV vaccine in IFNαβR$^{-/-}$ mice implies that type I interferon-dependent immunity is not essential in the protection conferred by short term vaccination.

[0233] Interestingly, the spread-deficient MCMV induced an adaptive immune response with similar efficiency as MCMV-wt. The CD4$^+$ and CD8$^+$ T cell response was on the same level as MCMV-wt and the neutralizing antibody response was only marginally reduced. This slightly lower neutralizing capacity might be caused by the smaller number of infected cells and by the therefore reduced amount of antigen that is released after infection with MCMV-ΔM94. A lower number of antigen-antibody complexes might lead to less efficient affinity maturation creating antibodies of lower neutralizing capacity. Nevertheless, the neutralization of virus appears sufficient to control virus replication.

[0234] Why did the adaptive immune response to the vaccine reach a level near to MCMV-wt infection despite the inability to spread? MCMV-ΔM94 was able to establish viral genome maintenance as efficient as MCMV-wt. The classical definition of herpesviral latency includes the potential for reactivated gene expression with subsequent release of infectious virus (Roizman et al. 1987 Annu Rev Microbiol 41:543-571.). Although the term “latency” is formally not applicable to the situation with MCMV-ΔM94 in the absence of productive infection, there is no evidence that pM94 affects reactivation of gene expression. Because the protective effect of MCMV-ΔM94 rather increased than faded over time, the inventors believe that periodic restimulation of the immune response due to reactivation of gene expression contributed to the sustained protection induced by MCMV-ΔM94. Interestingly, virus infected cells are not eliminated by the activated immune response. This means that the first target cells that are infected by the spread-deficient vaccine are resistant to elimination. Similarly, cells infected with a spread-deficient mutant of the gamma herpesvirus MHV-68 were not attacked by the adaptive immune response (Tibbetts et al. 2006 Virology 353:210-219.). For MCMV-wt it was shown that T cells are activated against a highly antigenic virus epitope of M45 presented by professional APC but the activated T cells did not eliminate infected target cells in organs of C57BL/6 mice (Holtappels et al. 2004 J Exp Med 199:131-136). This protection was caused by m152, that is known to downmodulate MHC class I. The target cells that are protected from CD8$^+$ T cell elimination were not identified and it could be shown that at least some of these protected cells are first target cells of MCMV.

[0235] Endothelial cells (EC), hepatocytes (Hc) and macrophages are first target cells for HCMV and MCMV in vivo (Hsu et al. supra; Sacher et al. supra). In addition, EC have recently been identified as sites of virus latency (Seckert et al. 2009 J Virol 83:8869-8884), and at least liver EC are able to directly stimulate a cytotoxic T cell response (Kern et al. 2010 Gastroenterology 138(1):336-46). Using MCMV-ΔM94 constructs for conditional gene expression, substantial differences were noticed in the ability of EC and Hc to activate a CD8$^+$ T cell response. In contrast to EC, Hc one of the most important first targets for MCMV during acute infection (Sacher et al. supra), induced only a poor CD8$^+$ T cell response. This lack of stimulatory capacity is apparently not compensated by cross presentation through professional antigen presenting cells. Cross presentation was shown to be important for the induction of a T cell response against fibroblasts infected with a single-cycle MCMV (Snyder et al. 2010 PLoS One. 5:e9681). On the other hand, bone marrow derived APC, that are thought to be important cross presenting cells, seem not to be necessary for the activation of a CD8$^+$ T cell response via cross presentation against MCMV infection (Kern et al. supra). In addition to EC also other cell types seem to contribute to CD8$^+$ T cell stimulation as antigen expression in most infected cells led to a stronger T cell response than expression in infected EC only. Infected dendritic cells and macrophages were described to activate a T cell response against MCMV in vitro (Mathys et al. 2003 J Infect Dis 187:988-999) and are infected in vivo (Andrews et al. 2001 Nat Immunol 2:1077-1084). Therefore, it suggests itself that infected professional APC contribute to immune stimulation against MCMV in addition to EC. It appears noteworthy that the attenuated human CMV strains such as Towne and AD169 which are characterized by a 20-fold reduction of immunogenicity and the inability to confer immune protection (Adler et al. supra) accumulated mutations resulting in their inability to infect EC, epithelial cells, smooth muscle cells and macrophages (Hahn, G. et al. 2004 J Virol 78:10023-10033). Thus, it appears likely that the restricted cell tropism may in fact represent the cause for their failure as human CMV vaccines.

EXAMPLE 13

Spread-Assay of MCMV-ΔM94

[0236] The phenotype of MCMV-ΔM94 was analyzed in cell-to-cell spread. This was investigated by an in vitro spread assay as essentially described herein in Example 1 with the following mo modifications

[0237] The results of this Example are shown in FIG. **9**.

[0238] NIH/3T3 and NT/M94-7 cells were plated and infected with MCMVΔ1-16-FRT (dell-16) and MCMVΔM94tTA (Δ) at an MOI of 0.25 for 1 h and then washed twice with D-PBS. Cells were incubated for 6 h and afterwards washed four times with D-PBS. Equal numbers of non-infected cells were stained with 5 μM Carboxyfluorescein succinimidyl ester (CFSE) for 8 min and blocked by 2% FCS/D-PBS, then washed twice with 2% FCS/D-PBS, and subsequently seeded on top of the unstained but infected cells. Cells were fixed 48 hours post infection with 4% PFA in D-PBS for 10 min at 37° C. and washed and permeablized with 0.1% Triton X-100 for 10 min. After triple washing cells were blocked with 3% BSA/D-PBS for 1 h. Staining of immediate early gene products was performed by incubating fixed cells with a monoclonal antibody to MCMV immediate-early 1 in 3% BSA/D-PBS. After three D-PBS washes, cells were incubated with an Alexa Fluor 555-coupled anti-mouse secondary antibody (Invitrogen) in 3% BSA/D-PBS. Finally, cells were washed three times and imaged by confocal microscopy using a LSM 510 Meta (Zeiss). Virus transmission was determined by counting immediate-early 1- and CFSE-positive cells using the ImageJ Cell Counter plugin.

[0239] FIG. **9** shows that infection of NIH/3T3 and NT/M94-7 (NTM94) cells with MCMVΔ1-16-FRT (Mohr C A et al., Engineering of cytomegalovirus genomes for recombinant live herpesvirus vaccines; Int J Med. Microbiol. 2008 January; 298(1-2):115-25. Epub 2007 Aug. 16. Review) and MCMV-ΔM94, followed by removal of excess virus by extensive washes after infection. Next, CFSE stained NIH/3T3 were added and virus replication was permitted. After additional 48 h the culture was fixed and stained for immediate-early 1. This resulted in cells which were either immediate-early 1-positive, CFSE-positive or positive for both stains (FIG. **9** A). Stained cells were counted and cell-to-cell spread was determined by calculating the ratio between immediate-early 1-positive/CFSE stained cells to immediate-early 1-positive/CFSE-negative cells (FIG. **9** C). The spread rate of the MCMVΔ1-16-FRT was set as 100%. MCMVΔ1-16-FRT infection spreads rapidly throughout the cell culture as indicated by the large number double stained nuclei (FIG. **9** B). In contrast, the M94 deletion mutant did not infect the newly added cells. Only one double stained nucleus was seen after counting 416 immediate-early 1+/CFSE negative cells. Its ability to infect fresh cells was, however, restored to a transmission rate of 97% when the mutant was grown on complementing NT/M94-7 cells. It is thus evident that the effect of the M94 deletion on secondary envelopment of mouse CMV also resulted in a deficiency of cell-to-cell spread.

EXAMPLE 14

Propagation of Spread-Deficient Human CMV

[0240] Generation of the Trans-Complementing Cell Line TCL94/99-BP

[0241] Recombinant lentiviruses expressing a) UL99 coupled with EGFP (encoded by pCB-Ubic-UL99-IRES-gfp; SEQ.ID.No:18), b) UL99 coupled with UL94 mCherry (encoded by pCB-Ubic-UL94-IRES-mChe; SEQ.ID.No:17) and c) beta-lactamase coupled with puromycine resistance gene (encoded by pLV-Ubiqc-BLAs-IRES-Puro; SEQ.ID.No:19) were constructed and propagated by Sirion GmbH using ViraPower lentiviral packaging mix (Invitrogen) in 293FT cells (Invitrogen). 2×10^6 MRCS fibroblasts (ATCC CCL-171) were transduced by 5 TDU/cell (transduction units/cell) of each lentivirus by spin infection according to the manufacturer's protocol. The transduced cells were plated out on a 10 cm dish and were selected for 5 days with 20 μg/ml puromycin in OPTI-MEM 5% FCS. The tranduced cells were passaged (1:2) one time in the presence of 20 μg/ml puromycin and the double positive (mCherry+EGFP) cells were purified by fluorescence associated cell sorting and re-plated at density of 2.5×10^4 cell/cm^2. 48 h after confluency the cells were passaged (1:5) two more times in the presence of 20 μg/ml puromycin and re-sorted as above. After one more passage in OPTI-MEM 5% FCS+20 μg/ml puromycin the cells were aliquoted to 0.7×10^7 cell/vial and were deep frozen in OPTI-MEM supplemented with 10% FCS and 10% DMSO.

[0242] Construction of Spread-Deficient Human CMV

[0243] To generate a non-functional UL94 locus pTB40E-BAC4-FRT; SEQ.ID.No:20 (Scrivano L, et al., 2011. HCMV spread and cell tropism are determined by distinct virus populations. PLoS. Pathog. 7:e1001256; Sinzger, C. et al., 2008. Cloning and sequencing of a highly productive, endotheliotropic virus strain derived from human cytomegalovirus TB40/E. J. Gen. Virol. 89:359-368.) was introduced in GS1783 *E. coli* strain (Tischer, B. K. et al., 2010. En passant mutagenesis: a two step markerless red recombination system. Methods Mol. Biol. 634:421-430.). (a) Red-recombination was induced by electro-transformation of the synthetic DNA fragment LIFdel94; SEQ.ID.No:15 according to the standard protocol (Tischer, B. K. et al., supra) resulting in pTB40E-BAC4-delUL94-SZeo. Recombinants were selected by picking single clones after plating the transformants on LB agar plates in the presence of 25 μg/ml chloramphenicol and 30 μg/ml zeocin. The correct replacement of the BAC sequences from nt122630 to 123668 referring to SEQ.ID.No:20 with LIFdelUL94, SEQ.ID.No:15 was confirmed by restrictions pattern analysis and sequencing. (b) To remove the zeocin cassette from the UL94 locus, a second round of Red recombination was induced in liquid culture of pTB40E-BAC4-delUL94-Szeo according to the standard protocol (Tischer, B. K. et al., supra) in presence of 25 μg/ml chloramphenichol and 2% of L-arabinose. Recombinants, which were coined pTB40E-BAC4-del94, were selected by picking single clones after plating of the recombinants on LB agar plates in the presence of 25 μg/ml chloramphenicol 1% of L-arabinose. The correct removal of the operational sequences were confirmed by restrictions pattern analysis and sequencing. (c) A next red-recombination was induced by electro-transformation of the synthetic mutagenesis fragment LIFdel99, SEQ.ID.No:16, as described above (see a) herein) resulting in pTB40E-BAC4-delUL94-del99-SZeo. Recombinants were selected by picking single clones after plating the transformants on LB agar plates in the presence of 25 μg/ml chloramphenicol and 30 μg/ml zeocin. The correct replacement of the sequences from nt 130670 to 131243 (according to the numbering of the BAC referred to herein as SEQ.ID.No:20) was confirmed by restrictions pattern analysis and sequencing. (d) To remove the zeocin cassette from the UL99 locus, a final round of red-recombination was induced in liquid culture of pTB40E-BAC4-delUL94-delUL99-Szeo as above (see b) herein). Recombinants, which were coined pTB40E-BAC4-del94-del99, were selected by picking single clones after plating of the recombinants on LB agar plates in the presence of 25 μg/ml chloramphenicol 1% of L-arabinose. The correct removal of the operational sequences from the UL99 locus were confirmed by restrictions pattern analysis and sequencing. 1. The description of the BAC modifications in the new way are the following:

[0244] M1) To generate a non-functional UL94 (or inactivate the UL94 gene) the nt sequence of pTB40E-BAC4-FRT (SEQ.ID.No:20) between nt 122630 and nt 123668 is replaced by the synthetic fragment delUL94S (SEQ.ID.No:34).

[0245] M2) To generate a non-functional UL99 (or inactivate the UL99 gene) the nt sequence of pTB40E-BAC4-FRT (SEQ.ID.No:20) between nt 130670 and nt 131243 is replaced by the synthetic fragment delUL99S (SEQ.ID.No:

35). For the double mutant of UL94-UL99 this has to be done in addition to modification M1.

[0246] M3) To generate a non-functional UL50 (or inactivate the UL50 gene) the nt sequence of pTB40E-BAC4-FRT (SEQ.ID.No:20) between nt 58442 and nt 59622 is replaced by the synthetic fragment delUL50S (SEQ.ID.No:32).

[0247] M4) To generate a non-functional UL53 (or inactivate the UL53 gene) the nt sequence of pTB40E-BAC4-FRT (SEQ.ID.No:20) between nt 62129 and nt 63261 is replaced by the synthetic fragment delUL53S (SEQ.ID.No:33). For the double mutant of UL50-UL53 this has to be done in addition to modification M3.

[0248] Reconstitution of Spread-Deficient Human CMV.

[0249] 0.7×10^7 frozen TCL94/99-BP cells were plated on a 10 cm dish in OPTI-MEM 5% FCS containing 0.2 µg/ml puromycin and two days later the adherent cell were split and plated on 6 cm dishes at densities of 2×10^6 cells per dish. On the next day two 6 cm cultures were transfected with 2 µg of purified pTB40E-BAC4-FRT-del94-del99-DNA each by Lipofectamin 2000 (Invitrogen) according to the manufacturer's protocol. 24 h later the two culture were combined and plated on a 10 cm dish in OPTI-MEM 5% FCS. After 10 days the reconstitution of the recombinant TB40E-BAC4-FRT-del94-del99 virus was evident by plaque formation. After 14-16 days the most of the cells in the transfected cultures showed CPE the entire culture was harvested. The amounts of the viable viruses was determined by limiting dilution on sub-confluent TCL94/99-BP cell in 96 well plates using TCID50 (median tissue culture infectious dose) method as described in Mohr et al (Mohr, C. A. et al., 2010. A spread-deficient cytomegalovirus for assessment of first-target cells in vaccination. Virol. 2010 August; 84(15):7730-42. Epub 2010 May 12.). The spread-deficient human CMV reconstituted from TB40E-BAC4-FRTdel94-99, can be propagated using TCL94/99-BP cells after infection with 0.1 MOI per cell using standard protocols for propagation of human CMV as described by Scrivano et al. (Scrivano et al., supra).

[0250] HCMV lacking secondary envelopment complex, i.e. UL99 and UL94, is spread-deficient.

[0251] The phenotype of the UL94-UL99 double deletion CMV reconstituted from TB40E-BAC4-FRTdel94-99 was tested in cell-to-cell spread. This was investigated by infection of MRCS and TCL94/99-BP cells as essentially described in Example 1 herein, with CMVs reconstituted from TB40E-BAC4-FRT-del94-del99 and TB40E-BAC4-FRT, respectively, followed by removal of excess virus by extensive washing after infection. Next, CFSE stained MRCS cells were added and virus replication was permitted. After additional 72 h the culture was fixed and stained for immediate-early 1 expression as described in Example 1 herein. This resulted in cells which were either "immediate-early 1"-positive, CFSE-positive or positive for both stains. These cells were counted in each preparation. The missing increase of double positive cells in MRCS after infection with TB40E-BAC4-FRT-del94-del99 is conclusive to a deficiency in cell-to-cell spread.

EXAMPLE 15

Immunization with Spread-Deficient Human CMV

[0252] After primary immunization with an additional boost with spread-deficient human CMV the human sera exhibit at least 64-fold higher neutralizing potency against endotheliotropic a human CMV strains such as TB40E or VR1814 assayed on endothelial- or epithelial cells (such as HUVEC [ATCC CRL 1730]- or ARPE-19 [ATCC CRL2302], respectively, than against the same virus assayed on human fibroblasts cell line (such as MRCS, ATCC CLL-171). In addition, specific antibody response is detectable against the gene products of UL130, UL128, or UL131A by Western blot (whereby it is sufficient that at least one specificity is seen).

[0253] The following deletions of the indicated genes result in recombinant human beta-herpesviruses which are spread-deficient:

Effector complex	UL50 gene	UL53 gene	UL94 gene	UL99 gene
NEC	+			
NEC		+		
NEC	+	+		
SEC			+	
SEC			+	+

[0254] The features of the present invention disclosed in the specification, the claims, the sequence listing and/or the drawings may both separately and in any combination thereof be material for realizing the invention in various forms thereof. It has to be acknowledged that the sequence listing is part of the instant specification.

SEQUENCE LISTING

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1 5

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1               5


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cgtggaggcc ggccggcagg agcccgagac cccccgggtg agcggccggc ggctgcccctt    180

cgacgacctg tgatccggag gacgacggct cgtgtatctt gtgccaattg ctgttgctct     240

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cagaatggtc agaagttgtg agcaccaatt ttcgtgatgc aagcggtccg gcaatgaccg 540

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atgttcccat agtaacgcca atagggactt tccattgacg tcaatgggtg gagtatttac 180

ggtaaactgc ccacttggca gtacatcaag tgtatcatat gccaagtacg ccccctattg 240

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gtaacaactc cgccccattg acgcaaatgg gcggtaggcg tgtacggtgg gaggtctata 540

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cgtgccaatg gtaagctaga tagcagagaa tggccacgat cagcacgagc atcacgccga   6540

tgatgggtaa cccgacgttc agcggcagat cgtccatggt gaccgtcctc tgtccggatc   6600

tacgtcccag tctctctctt ttgtacagca ctcgcgcggg aacggccccc tcaaccctct   6660

tacgtagcgg gagatacggc gttctcccgc gggccactta cttgcacggt cgcttgaacg   6720

gcggcttgga ccgccacatg taccgcatcc atccattctg gcagcagcgc gttcgacgac   6780

gtcgtacgag tcgcggatga tgttaccccg ccagcacctc cgccggcaac cgcgtcgtcg   6840

ttgctatcgt cgccggtttc gggcgatgac agcgccggcg gcgcgggtct cgtctcgtcc   6900

accatttcca ccgtgtcgaa gcgacagccg ctgccgtagt acatggcccc gttcaacggc   6960

cggcgggccg ggtcgccgag ttccgggtcg ggcacatcca tggctcgccg tctgcttctc   7020

tgccgctcgt ggtgccgacg gcacttctca ggataatgac agccgcaaaa tagatcgtgg   7080

agcatgtctc gccaactgtc ctggtggtaa tatcttaagt acgcgatgag cgcgccgatg   7140

gccataatca taagcgtaag caaaacggca cagataacgt gaaacaccgc ggtcatccaa   7200

gtcgggcggc gtcggggacg cggtgggtcg gtttctctta cgccggcgtc actcagccac   7260

cacacccgta gtcgacattc ccagaaccgg tgaatgcgac tcagggcctt tcgacgccgc   7320

catttatttc caacgtccaa gtcccacgtc atttctggca tctccacgcc cttgactgac   7380

atactctctt tctctctctt agctgcggtg aaaaagaggg aaggcgtgtg ctgctataca   7440

actgtacaac ggacgcgctc gctgtttcgg tctcaggtca tctgcattga ctcggcgtcc   7500

ttcatgacgc tctgcaccgc cttttccaag agttcctcga tgtccgacca tcgaggaggc   7560

ggggctaact cggaaaccga cacgataggc agcgtggtcg gctccgtcgg cgtgcggggt   7620

cggggacagg gacacgagag tcccaccttc gagagattct ccagcccgac ggtgcgcggc   7680

agtctcggat tccgcggtgg cttttgtggc gtcggcgttt tcgggaaggg cctgggcgtc   7740

accggcggtg tccagccgac cggcttgggt ttcgtgggcg gcggtgtttt cttggtgggc   7800

ggcgtgctca ggttcttacg cggcgcgggt atcggcgtcg gggcctgtg cgacgacagc   7860

cgcgtggtgg gggcccggac cggcggcgta ggcggccgct tcttgcgccc gggcggcgga   7920

ggtggcttcc aggatggcgg cggctgatgc agtaccgtgt cgacgctggc cgaggacgac   7980

aaagagctcg acgaggagca atgcgacgga gatcggccga tgctggtcgg cgttcccggc   8040

gtggatacgt cggggatctc gaatcgcgcc ggaggaaact cgggtttatc tatcggcaga   8100

ccatcctctc ctatgtagag cgacgtacac cgcggcacct gcggcgtcgg cgggtgggtg   8160

gccacccgca tgagccccag ttccagatcc agcggctcga cgacgtcttc tttcggaatt   8220

cgatagcagc acgcgcaggc accacgctta tcagaagcag cacccgggag ccggcctcgc   8280

gacgaagtct cgtcggatcg cttgcggcct cggcgctggg taaataagga aatggccagg   8340

accagggaag ccagtccggt accgccgagg agcccgacgc cgagccacag ccacaccatg   8400

atcttctctc ctgcttggaa tctcaaactc cgtgtcggga agggccggtg tacggacatt   8460

tatgccttgg atttctggaa acgtcatttt ttggcaagga atgtgtttat tgtccaaaca   8520

ctgaggaagg agatgtgagc caagtcggaa aattccttat cacaccgggg gcgggttacg   8580

ttccggtctg atgctgctgc tgttgttgta gagccgcggc catggccgcc tgcacggcag   8640

cttgtaccgc ctcggccacg ccgggtggca tctgcggcat ggcgggggga gacgcgtcgg   8700
```

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gcggaccgcc gggcatcgcc gtcggctgcg acggtggttg tgaactcacc gtcggctcgc   8760

acggaggttt gtccttcggt ttgctcttcg gtttatcttt cgccctacct ttcttcggtt   8820

tgggttccga tgtcggtgtt ggcggctgcg gtgggatgac gggctggtgg gactcctccg   8880

acggcggggg gacgaatact gtcggcgccg aaaccggggg actctcgact atctcgcaga   8940

tcaccctgtc gggatcgtcg ccgtgtccgg gacgccgtcg atgaccatat tgaaccatgt   9000

cgtaaatcat cgtctccttg taacacgctg aacagcagcg gctacaagga cccgaaatgc   9060

atttgcagct gcacttacag ctgcagctgc agtagcgcac ccatcggcag gtgaagacgt   9120

cgattacgga gtccttgaag aattcccggt aacggatgag atacgcgcag aggaaaatca   9180

tgaaaacaga acagccgact acggctgcga tgccgggtcc cgaaaacgta ttcggtgatc   9240

ctaccaaaca ccaaattccc agggccgcgc atgttatcca ggccacaata atcgtgggaa   9300

cgccccattg gcattgccac gaaggatcgt gcacgtcgca acccatcgct actgcgttct   9360

cccacaaacg ccatcgcact atttatccct acagcggctg                         9400
```

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<210> SEQ ID NO 24
<211> LENGTH: 387
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 24

Met Gly Ser Gly Ile Gly Ala Ala Ser Met Glu Phe Cys Phe Asp Val
1               5                   10                  15

Phe Lys Glu Leu Lys Val His His Ala Asn Glu Asn Ile Phe Tyr Cys
            20                  25                  30

Pro Ile Ala Ile Met Ser Ala Leu Ala Met Val Tyr Leu Gly Ala Lys
        35                  40                  45

Asp Ser Thr Arg Thr Gln Ile Asn Lys Val Val Arg Phe Asp Lys Leu
    50                  55                  60

Pro Gly Phe Gly Asp Ser Ile Glu Ala Gln Cys Gly Thr Ser Val Asn
65                  70                  75                  80

Val His Ser Ser Leu Arg Asp Ile Leu Asn Gln Ile Thr Lys Pro Asn
                85                  90                  95

Asp Val Tyr Ser Phe Ser Leu Ala Ser Arg Leu Tyr Ala Glu Glu Arg
            100                 105                 110

Tyr Pro Ile Leu Pro Glu Tyr Leu Gln Cys Val Lys Glu Leu Tyr Arg
        115                 120                 125

Gly Gly Leu Glu Pro Ile Asn Phe Gln Thr Ala Ala Asp Gln Ala Arg
    130                 135                 140

Glu Leu Ile Asn Ser Trp Val Glu Ser Gln Thr Asn Gly Ile Ile Arg
145                 150                 155                 160

Asn Val Leu Gln Pro Ser Ser Val Asp Ser Gln Thr Ala Met Val Leu
                165                 170                 175

Val Asn Ala Ile Val Phe Lys Gly Leu Trp Glu Lys Thr Phe Lys Asp
            180                 185                 190

Glu Asp Thr Gln Ala Met Pro Phe Arg Val Thr Glu Gln Glu Ser Lys
        195                 200                 205

Pro Val Gln Met Met Tyr Gln Ile Gly Leu Phe Arg Val Ala Ser Met
    210                 215                 220
```

```
Ala Ser Glu Lys Met Lys Ile Leu Glu Leu Pro Phe Ala Ser Gly Thr
225                 230                 235                 240

Met Ser Met Leu Val Leu Leu Pro Asp Glu Val Ser Gly Leu Glu Gln
                245                 250                 255

Leu Glu Ser Ile Ile Asn Phe Glu Lys Leu Thr Glu Trp Thr Ser Ser
            260                 265                 270

Asn Val Met Glu Glu Arg Lys Ile Lys Val Tyr Leu Pro Arg Met Lys
        275                 280                 285

Met Glu Glu Lys Tyr Asn Leu Thr Ser Val Leu Met Ala Met Gly Ile
    290                 295                 300

Thr Asp Val Phe Ser Ser Ser Ala Asn Leu Ser Gly Ile Ser Ser Ala
305                 310                 315                 320

Glu Ser Leu Lys Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile
                325                 330                 335

Asn Glu Ala Gly Arg Glu Val Val Gly Ser Ala Glu Ala Gly Val Asp
            340                 345                 350

Ala Ala Ser Val Ser Glu Glu Phe Arg Ala Asp His Pro Phe Leu Phe
        355                 360                 365

Cys Ile Lys His Ile Ala Thr Asn Ala Val Leu Phe Phe Gly Arg Cys
    370                 375                 380

Val Ser Pro
385


<210> SEQ ID NO 25
<211> LENGTH: 498
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 25

Met Ser Gly Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp
1               5                   10                  15

Gly Glu Arg Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Lys Met
            20                  25                  30

Ile Gly Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
        35                  40                  45

Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu
    50                  55                  60

Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
65                  70                  75                  80

Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
                85                  90                  95

Tyr Arg Arg Val Asn Gly Lys Trp Met Arg Glu Leu Ile Leu Tyr Asp
            100                 105                 110

Lys Glu Glu Ile Arg Arg Ile Trp Arg Gln Thr Asn Asn Gly Asp Asp
        115                 120                 125

Ala Thr Ala Gly Leu Thr His Met Met Ile Trp His Ser Asn Leu Asn
    130                 135                 140

Asp Ala Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
145                 150                 155                 160

Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
                165                 170                 175

Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Met Val Met Glu
```

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            180                 185                 190

Leu Val Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
        195                 200                 205

Gly Glu Asn Gly Arg Lys Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn
    210                 215                 220

Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Lys Ala Met Met Asp
225                 230                 235                 240

Gln Val Arg Glu Ser Arg Asn Pro Gly Asn Ala Glu Phe Glu Asp Leu
                245                 250                 255

Thr Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His
            260                 265                 270

Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Pro Ala Val Ala Ser Gly
        275                 280                 285

Tyr Asp Phe Glu Arg Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe
    290                 295                 300

Arg Leu Leu Gln Asn Ser Gln Val Tyr Ser Leu Ile Arg Pro Asn Glu
305                 310                 315                 320

Asn Pro Ala His Lys Ser Gln Leu Val Trp Met Ala Cys His Ser Ala
                325                 330                 335

Ala Phe Glu Asp Leu Arg Val Leu Ser Phe Ile Lys Gly Thr Lys Val
            340                 345                 350

Leu Pro Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn
        355                 360                 365

Glu Asn Met Asp Ala Met Glu Ser Ser Thr Leu Glu Leu Arg Ser Arg
    370                 375                 380

Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Arg
385                 390                 395                 400

Ala Ser Ala Gly Gln Ile Ser Ile Gln Pro Thr Phe Ser Val Gln Arg
                405                 410                 415

Asn Leu Pro Phe Asp Arg Thr Thr Ile Met Ala Ala Phe Asn Gly Asn
            420                 425                 430

Thr Glu Gly Arg Thr Ser Asp Met Arg Thr Glu Ile Ile Arg Met Met
        435                 440                 445

Glu Ser Ala Arg Pro Glu Asp Val Ser Phe Gln Gly Arg Gly Val Phe
    450                 455                 460

Glu Leu Ser Asp Glu Lys Ala Ala Ser Pro Ile Val Pro Ser Phe Asp
465                 470                 475                 480

Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr
                485                 490                 495

Asp Asn


<210> SEQ ID NO 26
<211> LENGTH: 99
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 26

Met Arg Ala Thr Val Gly Leu Val Glu Ala Ile Gly Ile Arg Glu Leu
1               5                   10                  15

Arg Gln His Ala Ser Arg Tyr Leu Ala Arg Val Glu Ala Gly Glu Glu
            20                  25                  30
```

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```
Leu Gly Val Thr Asn Lys Gly Arg Leu Val Ala Arg Leu Ile Pro Val
        35                  40                  45

Gln Ala Ala Glu Arg Ser Arg Glu Ala Leu Ile Glu Ser Gly Val Leu
    50                  55                  60

Ile Pro Ala Arg Arg Pro Gln Asn Leu Leu Asp Val Thr Ala Glu Pro
65                  70                  75                  80

Ala Arg Gly Arg Lys Arg Thr Leu Ser Asp Val Leu Asn Glu Met Arg
                85                  90                  95

Asp Glu Gln


<210> SEQ ID NO 27
<211> LENGTH: 345
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 27

Met Ala Trp Arg Ser Gly Leu Cys Glu Thr Asp Ser Arg Thr Leu Lys
1               5                   10                  15

Gln Phe Leu Gln Glu Glu Cys Met Trp Lys Leu Val Gly Lys Ser Arg
            20                  25                  30

Lys His Arg Glu Tyr Arg Ala Val Ala Cys Arg Ser Thr Ile Phe Ser
        35                  40                  45

Pro Glu Asp Asp Gly Ser Cys Ile Leu Cys Gln Leu Leu Leu Leu Tyr
    50                  55                  60

Arg Asp Gly Glu Trp Ile Leu Cys Leu Cys Cys Asn Gly Arg Tyr Gln
65                  70                  75                  80

Gly His Tyr Gly Val Gly His Val His Arg Arg Arg Arg Arg Ile Cys
                85                  90                  95

His Leu Pro Thr Leu Tyr Gln Leu Ser Phe Gly Gly Pro Leu Gly Pro
            100                 105                 110

Ala Ser Ile Asp Phe Leu Pro Ser Phe Ser Gln Val Thr Ser Ser Met
        115                 120                 125

Thr Cys Asp Gly Ile Thr Pro Asp Val Ile Tyr Glu Val Cys Met Leu
    130                 135                 140

Val Pro Gln Asp Glu Ala Lys Arg Ile Leu Val Lys Gly His Gly Ala
145                 150                 155                 160

Met Asp Leu Thr Cys Gln Lys Ala Val Thr Leu Gly Gly Ala Gly Ala
                165                 170                 175

Trp Leu Leu Pro Arg Pro Glu Gly Tyr Thr Leu Phe Phe Tyr Ile Leu
            180                 185                 190

Cys Tyr Asp Leu Phe Thr Ser Cys Gly Asn Arg Cys Asp Ile Pro Ser
        195                 200                 205

Met Thr Arg Leu Met Ala Ala Ala Thr Ala Cys Gly Gln Ala Gly Cys
    210                 215                 220

Ser Phe Cys Thr Asp His Glu Gly His Val Asp Pro Thr Gly Asn Tyr
225                 230                 235                 240

Val Gly Cys Thr Pro Asp Met Gly Arg Cys Leu Cys Tyr Val Pro Cys
                245                 250                 255

Gly Pro Met Thr Gln Ser Leu Ile His Asn Asp Glu Pro Ala Thr Phe
            260                 265                 270

Phe Cys Glu Ser Asp Asp Ala Lys Tyr Leu Cys Ala Val Gly Ser Lys
        275                 280                 285
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```
Thr Ala Ala Gln Val Thr Leu Gly Asp Gly Leu Asp Tyr His Ile Gly
    290                 295                 300

Val Lys Asp Ser Glu Gly Arg Trp Leu Pro Val Lys Thr Asp Val Trp
305                 310                 315                 320

Asp Leu Val Lys Val Glu Glu Pro Val Ser Arg Met Ile Val Cys Ser
                325                 330                 335

Cys Pro Val Leu Lys Asn Leu Val His
            340                 345


<210> SEQ ID NO 28
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 28

Val Thr Leu Gly Gly Ala Gly Ala Trp Leu Leu Pro
1               5                   10


<210> SEQ ID NO 29
<211> LENGTH: 190
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 29

Met Gly Gly Glu Leu Cys Lys Arg Ile Cys Cys Glu Phe Gly Thr Thr
1               5                   10                  15

Ser Gly Glu Pro Leu Lys Asp Ala Leu Gly Arg Gln Val Ser Leu Arg
            20                  25                  30

Ser Tyr Asp Asn Ile Pro Pro Thr Ser Ser Ser Asp Glu Gly Glu Asp
        35                  40                  45

Asp Asp Asp Gly Glu Asp Asp Asp Asn Glu Glu Arg Gln Gln Lys Leu
    50                  55                  60

Arg Leu Cys Gly Ser Gly Cys Gly Gly Asn Asp Ser Ser Ser Gly Ser
65                  70                  75                  80

His Arg Glu Ala Thr His Asp Gly Pro Lys Lys Asn Ala Val Arg Ser
                85                  90                  95

Thr Phe Arg Glu Asp Lys Ala Pro Lys Pro Ser Lys Gln Ser Lys Lys
            100                 105                 110

Lys Lys Lys Pro Ser Lys His His His His Gln Gln Ser Ser Ile Met
        115                 120                 125

Gln Glu Thr Asp Asp Leu Asp Glu Glu Asp Thr Ser Ile Tyr Leu Ser
    130                 135                 140

Pro Pro Pro Val Pro Pro Val Gln Val Val Ala Lys Arg Leu Pro Arg
145                 150                 155                 160

Pro Asp Thr Pro Arg Thr Pro Arg Gln Lys Lys Ile Ser Gln Arg Pro
                165                 170                 175

Pro Thr Pro Gly Thr Lys Lys Pro Ala Ala Pro Leu Ser Phe
            180                 185                 190


<210> SEQ ID NO 30
<211> LENGTH: 345
<212> TYPE: PRT
<213> ORGANISM: artificial
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<220> FEATURE:
<223> OTHER INFORMATION: syntehtic

<400> SEQUENCE: 30

Met Ala Thr Ser Arg Leu Ser Val Lys Ser Leu Arg Ser Ile Ser Arg
1               5                   10                  15

Phe Val Gln Trp Glu Cys Cys Trp Met Leu Val Asn Lys Ser Ala Arg
            20                  25                  30

Tyr Arg Glu Phe Arg Ala Val Thr Ser Gln Ser Pro Gly Leu Gly Lys
        35                  40                  45

Val Ser Ser Thr Asp Asp Gly Arg Cys Leu Ala Ala Ser Met Met Leu
    50                  55                  60

Phe Arg Arg Asp Gly Asn Phe Val Leu Cys Leu Val Val Asn Lys Glu
65                  70                  75                  80

Pro Val Gly Gln Phe Gly Cys Ser Gly Met Arg Arg Glu Lys Met Val
                85                  90                  95

Ile Asp Gly Leu Gln Glu Pro Val Tyr Val Met Arg Leu Leu Ala Pro
            100                 105                 110

Leu Ile Pro Val Lys Leu Gly Phe Ser Pro Tyr Met Leu Pro Pro Lys
        115                 120                 125

Ser Ile Gly Gly Ser Gly Gly Leu Asp Pro Ser Val Ile Tyr Gln Asn
    130                 135                 140

Ala Ser Val Val Thr Pro Glu Glu Ala Ala Thr Val Thr Met Gln Gly
145                 150                 155                 160

Ser Gly Ile Val Thr Val Gly Leu Ser Gly Val Gly Ser Trp Val Gln
                165                 170                 175

Ile Lys Asp Gly Gly Asn Met Lys Leu Phe Val Phe Ala Leu Cys Phe
            180                 185                 190

Asp Val Phe Thr Ala Cys Cys Asp Arg Leu Ala Phe Pro Ser Leu Ala
        195                 200                 205

Lys Ile Tyr Ser Glu Thr Val Ser Cys Glu Ala Asp Lys Cys Gly Phe
    210                 215                 220

Cys Arg Asp Ser Gly Arg His Val Asp Pro Thr Gly Arg Phe Val Gly
225                 230                 235                 240

Cys Val Pro Asp Ser Gly Val Cys Leu Cys Tyr Ser Pro Cys Arg Gly
                245                 250                 255

Thr Asp Ala Ala Val Ser Val Arg Ser Trp Leu Pro Tyr Leu Glu Leu
            260                 265                 270

Glu Asp Gly Ala Asn Thr His Ser Leu Phe Val Arg Arg Tyr Asp Gly
        275                 280                 285

Arg Lys Gly Leu Pro Ala Thr Ile Ser Asp Tyr Leu Gly Ala Arg Asn
    290                 295                 300

Ser Glu Gly Asp Glu Ile Pro Leu Arg Thr Glu Pro Trp Gln Leu Leu
305                 310                 315                 320

Lys Ile Glu Pro Thr Leu Ser Ala Met Ile Ile Met Ala Cys Pro Leu
                325                 330                 335

Leu Lys Lys Ile Val Leu Glu His Met
            340                 345


<210> SEQ ID NO 31
<211> LENGTH: 353
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
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-continued

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<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 31

Met Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Thr Ser Arg Leu Ser Val
1               5                   10                  15

Lys Ser Leu Arg Ser Ile Ser Arg Phe Val Gln Trp Glu Cys Cys Trp
            20                  25                  30

Met Leu Val Asn Lys Ser Ala Arg Tyr Arg Glu Phe Arg Ala Val Thr
        35                  40                  45

Ser Gln Ser Pro Gly Leu Gly Lys Val Ser Ser Thr Asp Asp Gly Arg
    50                  55                  60

Cys Leu Ala Ala Ser Met Met Leu Phe Arg Arg Asp Gly Asn Phe Val
65                  70                  75                  80

Leu Cys Leu Val Val Asn Lys Glu Pro Val Gly Gln Phe Gly Cys Ser
                85                  90                  95

Gly Met Arg Arg Glu Lys Met Val Ile Asp Gly Leu Gln Glu Pro Val
            100                 105                 110

Tyr Val Met Arg Leu Leu Ala Pro Leu Ile Pro Val Lys Leu Gly Phe
        115                 120                 125

Ser Pro Tyr Met Leu Pro Pro Lys Ser Ile Gly Gly Ser Gly Gly Leu
    130                 135                 140

Asp Pro Ser Val Ile Tyr Gln Asn Ala Ser Val Val Thr Pro Glu Glu
145                 150                 155                 160

Ala Ala Thr Val Thr Met Gln Gly Ser Gly Ile Val Thr Val Gly Leu
                165                 170                 175

Ser Gly Val Gly Ser Trp Val Gln Ile Lys Asp Gly Gly Asn Met Lys
            180                 185                 190

Leu Phe Val Phe Ala Leu Cys Phe Asp Val Phe Thr Ala Cys Cys Asp
        195                 200                 205

Arg Leu Ala Phe Pro Ser Leu Ala Lys Ile Tyr Ser Glu Thr Val Ser
    210                 215                 220

Cys Glu Ala Asp Lys Cys Gly Phe Cys Arg Asp Ser Gly Arg His Val
225                 230                 235                 240

Asp Pro Thr Gly Arg Phe Val Gly Cys Val Pro Asp Ser Gly Val Cys
                245                 250                 255

Leu Cys Tyr Ser Pro Cys Arg Gly Thr Asp Ala Ala Val Ser Val Arg
            260                 265                 270

Ser Trp Leu Pro Tyr Leu Glu Leu Glu Asp Gly Ala Asn Thr His Ser
        275                 280                 285

Leu Phe Val Arg Arg Tyr Asp Gly Arg Lys Gly Leu Pro Ala Thr Ile
    290                 295                 300

Ser Asp Tyr Leu Gly Ala Arg Asn Ser Glu Gly Asp Glu Ile Pro Leu
305                 310                 315                 320

Arg Thr Glu Pro Trp Gln Leu Leu Lys Ile Glu Pro Thr Leu Ser Ala
                325                 330                 335

Met Ile Ile Met Ala Cys Pro Leu Leu Lys Lys Ile Val Leu Glu His
            340                 345                 350

Met


<210> SEQ ID NO 32
<211> LENGTH: 179
<212> TYPE: DNA
<213> ORGANISM: artificial
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<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 32

gaccgcgcca cagcagagcc agcaccagca gaagagccag caccagcggg cccagagtcg     60

caaagcgcgc gggcagccac ggcccagact gcggtcgcga tggcccggag cgcgctcgcc    120

accacgatga cggtgcccaa cgataaccag tccgctcccg caccgacgcc accgccgat     179


<210> SEQ ID NO 33
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 33

atgtctagcg ttttctcaac agcattcgtg cgccttga                             38


<210> SEQ ID NO 34
<211> LENGTH: 252
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 34

cacggcctgg cccagcgagc cctgcgggac cggttccaaa acttcgaggc cgtgctggcc     60

cggggcatgc acgtggaggc cggccggcag gagcccgaga ccccccgggt gagcggccgg    120

cggctgccct tcgacgacct gtgatccgga ggacgacggc tcgtgtatct tgtgccaatt    180

gctgttgctc taccgcgacg gcgaatggat cctctgtctt tgctgcaacg gccgttatca    240

aggccactat gg                                                        252


<210> SEQ ID NO 35
<211> LENGTH: 67
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 35

ctgggtcgcc aacagcgcca acgagtacgt cgtcagctcc gtgccccgcc ccgtcagtcc     60

gtagaag                                                               67
```

1. A beta-herpesvirus, preferably a recombinant beta-herpesvirus, wherein the beta-herpesvirus is spread-deficient.

2. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus is endotheliotropic and/or has a wild type-like virion surface.

3. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus is endotheliotropic and has a wild type-like virion surface.

4. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus is suitable to or capable of inducing an immune response, wherein preferably the immune response comprises neutralizing antibodies against beta-herpesvirus and $CD4^+$ and $CD8^+$ T-cells directed against epitopes of beta-herpesvirus.

5. The beta-herpesvirus according to claim **4**, wherein the immune response comprises neutralizing antibodies, wherein beta-herpesvirus is prevented from infecting endothelial cells and/or epithelial cells by the neutralizing antibodies.

6. The beta-herpesvirus according to claim **5**, wherein beta-herpesvirus which is prevented from infecting endothelial cells and/or epithelial cells by the neutralizing antibodies, is a pathogen, preferably a human pathogen.

7. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus is a human beta-herpesvirus.

8. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus is a cytomegalovirus.

9. The beta-herpesvirus according to claim **7**, wherein the beta-herpesvirus is a human cytomegalovirus.

10. The beta-herpesvirus according to claim **1**, preferable claim **9**, wherein the beta-herpesvirus is deficient in at least one gene product involved in primary and/or secondary envelopment.

11. The beta-herpesvirus according to claim **10**, wherein the at least one gene product is involved in primary envelopment

12. The beta-herpesvirus according to claim **11**, wherein the at least one gene product is encoded by a gene selected from the group comprising UL50 and UL 53 and homologs of each thereof.

13. The beta-herpesvirus according to claim **10**, wherein the at least one gene product is involved in secondary envelopment.

14. The beta-herpesvirus according to claim **13**, wherein the at least one gene product is encoded by a gene selected from the group comprising UL94 and UL99 and homologs each thereof.

15. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20 and a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

16. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and a fourth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.NO:34.

17. The beta-herpesvirus according to claim **16**, wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 34, wherein nucleotide 252 of the nucleotide sequence according to SEQ.ID.No: 34 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

18. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 130670 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 131243 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20 and a fourth nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20, wherein the nucleotide 130670 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 131243 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein the nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

19. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 130670 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 131243 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20, a fourth nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20, a fifth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 34 and a sixth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 35.

20. The beta-herpesvirus according to claim **19**, wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 34, wherein nucleotide 252 of the nucleotide sequence according to SEQ.ID.No: 34 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20, wherein nucleotide 130670 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 35, wherein nucleotide 67 of the nucleotide sequence according to SEQ.ID.NO:35 is covalently linked to nucleotide 131243 of the nucleotide sequence according to SEQ.ID.No: 20, and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

21. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20 and a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

22. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and a fourth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 32.

23. The beta-herpesvirus according to claim **22**, wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 32, wherein nucleotide 179 of the nucleotide sequence according to SEQ.ID.No: 32 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

24. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 63261 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20 and a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein the nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

25. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 63261 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and a fourth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 33.

26. The beta-herpesvirus according to claim **25**, wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 33, wherein nucleotide 38 of the nucleotide sequence according to SEQ.ID.No: 33 is covalently linked to nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

27. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 632161 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20 and a fourth nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20, wherein the nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein the nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to the nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

28. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence represented by nucleotides 63261 to 181652 of the nucleotide sequence according to SEQ.ID.NO:20, a fourth nucleotide sequence represented by nucleotides 189192 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20, a fifth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 32 and a sixth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 33.

29. The beta-herpesvirus according to claim **28**, wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 32, wherein nucleotide 179 of the nucleotide sequence according to SEQ.ID.No: 32 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20, wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 33, wherein nucleotide 38 of the nucleotide sequence according to SEQ.ID.NO:33 is covalently linked to nucleotide 63261 of the nucleotide sequence according to SEQ.ID.No: 20, and wherein nucleotide 181652 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 189192 of the nucleotide sequence according to SEQ.ID.NO:20.

30. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus comprises one or more genes selected from the group comprising UL133, UL134, UL135, UL136, UL137, UL138, UL139, UL140, UL141, UL142, UL143, UL144 and UL145

31. The beta-herpesvirus according to claim **1**, wherein the beta herpesvirus comprises the nucleotide sequence according to SEQ.ID.NO:23.

32. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus is deficient in at least one gene product encoded by an immune evasive gene.

33. The beta-herpesvirus according to claim **32**, wherein the at least one gene product encoded by an immune evasive gene is selected from the group comprising gene products regulating MHC class I presentation and gene products regulating NK cell response.

34. The beta-herpesvirus according to claim **33**, wherein the at least one gene product encoded by an immune evasive gene is a gene product regulating MHC class I presentation.

35. The beta-herpesvirus according to claim **34**, wherein the gene product regulating MHC class I presentation is selected from the group comprising US6, US3, US2, UL18, US11, UL83 and UL40.

36. The beta-herpesvirus according to claim **33**, wherein the at least one gene product encoded by an immune evasive gene is a gene product regulating NK cell response.

37. The beta-herpesvirus according to claim **36**, wherein the gene product regulating NK cell response is selected from the group comprising gene products encoded by the genes UL40, UL16 and UL18.

38. The beta-herpesvirus according to claim **1**, wherein the beta-herpesvirus encodes a heterologous nucleic acid.

39. The beta-herpesvirus according to claim **41**, wherein the heterologous nucleic acid is a functional nucleic acid, preferably selected from the group comprising antisense molecules, ribozymes and RNA interference mediating nucleic acids.

40. The beta-herpesvirus according to claim **38**, wherein the nucleic acid is a nucleic acid coding for a peptide, oligopeptide, polypeptide or protein.

41. The beta-herpesvirus according to claim **40**, wherein the peptide, oligopeptide, polypeptide or protein comprises at least one antigen.

42. The beta-herpesvirus according to claim **41**, wherein the antigen is an antigen selected from the group comprising viral antigens, bacterial antigens and parasite antigens.

43. The beta-herpesvirus according to claim **1** for or suitable for use in a method for the treatment of a subject and/or for use in a method for the vaccination of a subject.

44. The beta-herpesvirus according to claim **43**, wherein the subject is a mammal, preferably a human.

45. The beta-herpesvirus according to claim **43**, wherein the beta-herpesvirus is human cytomegalovirus.

46. The beta-herpesvirus according to claim **43**, wherein the subject is suffering from a disease or is at risk of suffering from a disease.

47. The beta-herpesvirus according to claim **43**, wherein the vaccination is a vaccination against a disease.

48. The beta-herpesvirus according to claim **46**, wherein the disease is a disease or condition which is associated with beta-herpesvirus infection, preferably human cytomegalovirus infection.

49. The beta-herpesvirus according to claim **48**, wherein the disease or condition is selected from the group comprising congenital inclusion disease.

50. The beta-herpesvirus according to claim **43**, wherein the subject is a pregnant female or female of reproductive age, preferably a pregnant woman or a woman of reproductive age.

51. The beta-herpesvirus according to claim **50**, wherein the treatment is or is suitable for or capable of preventing the transfer of a beta-herpesvirus, preferably human cytomegalovirus, from the female to a fetus and/or to an embryo carried or to be carried in the future by the female.

52. The beta-herpesvirus according to claim **50**, wherein the treatment is for or is suitable for the generation of or capable of generating an immune response in the female body or the immune response in the female body, whereby preferably such immune response confers protection to a fetus and/or to an embryo carried or to be carried in the future by the female against beta-herpesvirus, preferably human cytomegalovirus, and/or a disease or condition associated with beta-herpesvirus infection, preferably human cytomegalovirus infection.

53. Use of a beta-herpesvirus according to claim **1**, for the manufacture of a medicament.

54. Use according to claim **53**, wherein the medicament is for the treatment and/or prevention of beta-herpesvirus infection.

55. Use according to claim **53**, wherein the medicament is for the treatment and/or prevention of a disease or condition associated with beta-herpesvirus infection, preferably human cytomegalovirus infection.

56. Use of a beta-herpesvirus according to claim **1**, for the manufacture of a vaccine.

57. Use according to claim **56**, wherein the vaccine is for the treatment and/or prevention of beta-herpesvirus infection.

58. Use according to claim **57**, wherein the vaccine is for the treatment and/or prevention of a disease or condition associated with beta-herpesvirus infection, preferably human cytomegalovirus infection.

59. Use according to claim **56**, wherein the vaccine is or is suitable for the administration to a subject, whereby the subject is selected form the group comprising a pregnant female, a female of reproductive age, a donor of a transplant, a recipient of a transplant and a subject being infected with HIV or being at risk of being infected with HIV.

60. Use according to claim **59**, wherein the donor is a potential donor and/or the recipient is a potential recipient.

61. A nucleic acid coding for a beta-herpesvirus according to claim **1**.

62. A vector comprising the nucleic acid according to claim **61**.

63. A vector comprising the nucleic acid according to claim **62**, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 123688 of the nucleotide sequence according to SEQ. ID.NO:20.

64. A vector comprising the nucleic acid according to claim **62**, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and a third nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 34.

65. The vector according to claim **64**, wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO: 20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 34 and wherein nucleotide 252 of the nucleotide sequence according to SEQ.ID. No: 34 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO: 20.

66. A vector comprising the nucleic acid according to claim **62**, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 130670 of the nucleotide sequence according to SEQ.ID.NO: 20, a third nucleotide sequence represented by nucleotides 131243 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein the nucleotide 130670 of the nucleotide sequence according to SEQ.ID.NO: 20 is covalently linked to the nucleotide 131243 of the nucleotide sequence according to SEQ.ID.NO:20.

67. A vector comprising the nucleic acid according to claim **62**, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 122630 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 123668 to 130670 of the nucleotide sequence according to SEQ.ID.NO: 20, a third nucleotide sequence represented by nucleotides 131243 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20, a third nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 34 and a fourth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 35

68. The vector according to claim **67**, wherein nucleotide 122630 of the nucleotide sequence according to SEQ.ID.NO: 20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 34, wherein nucleotide 252 of the nucleotide sequence according to SEQ.ID.No: 34 is covalently linked to nucleotide 123668 of the nucleotide sequence according to SEQ.ID.NO:20, wherein nucleotide 130670 of the nucleotide sequence according to SEQ.ID.NO: 20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 35 and wherein nucleotide 67 of the nucleotide sequence according to SEQ.ID.NO: 35 is covalently linked to nucleotide 131243 of the nucleotide sequence according to SEQ.ID.No:20

69. A vector comprising the nucleic acid according to claim **62**, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20.

70. A vector comprising the nucleic acid according to claim **62**, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and a third nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 32.

71. The vector according to claim **70**, wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO: 20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 32 and wherein nucleotide 179 of the nucleotide sequence according to SEQ.ID.No: 32 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO: 20.

72. A vector comprising the nucleic acid according to claim **62**, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 63261 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO:20.

73. A vector comprising the nucleic acid according to claim **62**, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 62129 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 63261 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and a third nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 33

74. The vector according to claim **73**, wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO: 20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 33 and wherein nucleotide 38 of the nucleotide sequence according to SEQ.ID.No: 33 is covalently linked to nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO: 20.

75. A vector comprising the nucleic acid according to claim **62**, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 62129 of the nucleotide sequence according to SEQ.ID.NO: 20, a third nucleotide sequence represented by nucleotides 63261 to 233681 of the nucleotide sequence according to SEQ.ID.NO: 20 and wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO:20 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20 and wherein the nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO: 20 is covalently linked to the nucleotide 63261 of the nucleotide sequence according to SEQ.ID.NO:20.

76. A vector comprising the nucleic acid according to claim **62**, wherein the vector comprises a nucleotide sequence, wherein the nucleotide sequence comprises a first nucleic acid sequence represented by nucleotides 1 to 58442 of the nucleotide sequence according to SEQ.ID.NO:20, a second nucleotide sequence represented by nucleotides 59623 to 62129 of the nucleotide sequence according to SEQ.ID.NO: 20, a third nucleotide sequence represented by nucleotides 63261 to 233681 of the nucleotide sequence according to SEQ.ID.NO:20, a fourth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 32 and a fifth nucleotide sequence comprising a nucleotide sequence according to SEQ.ID.No: 33.

77. The vector according to claim **76**, wherein nucleotide 58442 of the nucleotide sequence according to SEQ.ID.NO: 20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 32, wherein nucleotide 179 of the nucleotide sequence according to SEQ.ID.No: 32 is covalently linked to nucleotide 59623 of the nucleotide sequence according to SEQ.ID.NO:20, wherein nucleotide 62129 of the nucleotide sequence according to SEQ.ID.NO: 20 is covalently linked to nucleotide 1 of the nucleotide sequence according to SEQ.ID.No: 33 and wherein nucleotide 38 of the nucleotide sequence according to SEQ.ID.NO: 33 is covalently linked to nucleotide 632161 of the nucleotide sequence according to SEQ.ID.No: 20.

78. A host cell comprising a nucleic acid according to claim **61**.

79. A pharmaceutical composition comprising a beta-herpesvirus, preferably a recombinant beta-herpesvirus, wherein the beta-herpesvirus is spread-deficient, and a pharmaceutically acceptable carrier.

80. The pharmaceutical composition of claim **79**, further comprising a nucleic acid encoding from a beta-herpesvirus and/or a vector encoding the nucleic acid encoding for beta-herpesvirus.

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