CLAIMS

1. A mutant of a wild-type cytomegalovirus (CMV) glycoprotein B (gB) protein, which mutant comprises at least two amino acid mutations relative to the amino acid sequence of the wild-type CMV gB protein, and wherein the amino acid mutation is selected from the group consisting of:

(1) an engineered disulfide bond mutation;

(2) an additional mutation; and

(3) a combination of at least one engineered disulfide mutation and at least one additional mutation.

2. The mutant according to claim 1, wherein the amino acid mutations comprise a combination of at least two engineered disulfide mutations and at least one additional mutation.

3. The mutant according to claim 1, which is in the form of a trimer.

4. The mutant according to claim 1, which has increased stability in prefusion form as compared with the corresponding wild-type CMV gB protein, wherein the stability is measured by binding of a prefusion-specific antibody, thermal shift assay or EM imaging.

5. The mutant according to any one of claims 1-4, wherein the wild-type CMV gB is Towne strain.

6. The mutant according to any one of claims 1-5, wherein the engineered disulfide mutation is selected from the group consisting of: D217C and Y589C; M371C and W506C; and N524C and M684C.

7. The mutant according to any one of claims 1-5, wherein the additional mutation is selected from the group consisting of:

(1) substitution of YIH at positions 155-157 with GHR;

(2) substitution of W at position 240 with A;

(3) substitution of C at position 246 with S;

(4) substitution of P at position 655 with S;

(5) substitution of F at position 678 with S; and

(6) substitution of L at position 680 with T;

(7) substitution of R at position 685 with A;

(8) substitution of MIALDI at positions 648-653 with GSGKDG;

(9) substitution of R at position 693 with V;

(10) substitution of I at position 675 with S;

(11) substitution of I at positions 767 and 768 with C;

(12) substitution of D at position 703 and P at position 704 with C; and

(13) substitution of Y at position 696 and V at position 697 with C.

8. The mutant according to any one of claims 1-5, wherein the additional mutation is selected from the group consisting of:

(1) substitution of YIH at positions 155-157 with GHR;

(2) substitution of W at position 240 with A;

(3) substitution of C at position 246 with S; and

(4) substitution of I at position 675 with S.

9. The mutant according to any one of claims 1-5, wherein the amino acid mutation is a combination of at least two engineered disulfide mutations and at least one additional mutation, and wherein:

(i) the engineered disulfide mutations are selected from the group consisting of: D217C and Y589C; M371C and W506C; and N524C and M684C; and

(ii) the additional mutation is selected from the group consisting of:

(1) substitution of YIH at positions 155-157 with GHR;

(2) substitution of W at position 240 with A;

(3) substitution of C at position 246 with S;

(4) substitution of P at position 655 with S;

(5) substitution of F at position 678 with S; and

(6) substitution of L at position 680 with T;

(7) substitution of R at position 685 with A;

(8) substitution of MIALDI at positions 648-653 with GSGKDG;

(9) substitution of R at position 693 with V;

(10) substitution of I at position 675 with S;

(11) substitution of I at positions 767 and 768 with C;

(12) substitution of D at position 703 and P at position 704 with C; and

(13) substitution of Y at position 696 and V at position 697 with C.

10. The mutant according to claim 9, wherein the amino acid mutations are a combination of mutations selected from the group consisting of:

(1) combination of D217C and Y589C, M371C and W506C, and I675S;

(2) combination of D217C and Y589C, N524C and M684C, and I675S;

(3) combination of D217C and Y589C, M371C and W506C, Y155G I156H H157R, W240A, C246S and I675S; and

(4) combination of D217C and Y589C, N524C and M684C, Y155G I156H H157R, W240A, C246S and I675S.

11. The mutant according to claim 1, wherein the mutant comprises a cysteine (C) at position 217 (217C) and at position 589 (589C), a cysteine (C) at position 371 (371C) and at position 506 (506C), and a serine (S) at position 675 (675S), and wherein the mutant is selected from the group consisting of:

(1) a mutant comprising the amino acid sequence set forth in SEQ ID NO:257; and

(2) a mutant comprising an amino acid sequence that is at least 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:257.

12. The mutant according to claim 1, wherein the mutant comprises a cysteine (C) at position 217 (217C) and at position 589 (589C), a cysteine (C) at position 524 (524C) and at position 684 (684C), and a serine (S) at position 675 (675S), and wherein the mutant is selected from the group consisting of:

(1) a mutant comprising the amino acid sequence of SEQ ID NO: 259; and

(2) a mutant comprising an amino acid sequence that is at least 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 259.

13. The mutant according to claim 1, wherein the mutant comprises a cysteine (C) at position 217 (217C) and at position 589 (589C), a cysteine (C) at position 371 (371C) and at position 506 (506C), a serine (S) at position 675 (675S), a glycine (G) at position 155 (155G), a histidine at position 156 (156H), an arginine at position 157 (157R), an alanine at position 240 (240A), and a serine at position 246 (246S), and wherein the mutant is selected from the group consisting of:

(1) a mutant comprising the amino acid sequence set forth in SEQ ID NO:261; and

(2) a mutant comprising an amino acid sequence that is at least 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 261; (1) a mutant comprising the amino acid sequence set forth in SEQ ID NO: 261;

(3) a mutant comprising the amino acid sequence set forth in SEQ ID NO: 265; and

(4) a mutant comprising an amino acid sequence that is at least 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:265.

14. The mutant according to claim 1, wherein the mutant comprises a cysteine (C) at position 217 (217C) and at position 589 (589C), a cysteine (C) at position 524 (524C) and at position 684 (684C), a serine (S) at position 675 (675S), a glycine (G) at position 155 (155G), a histidine at position 156 (156H), an arginine at position 157 (157R), an alanine at position 240 (240A), and a serine at position 246 (246S), and wherein the mutant is selected from the group consisting of:

(1) a mutant comprising the amino acid sequence of SEQ ID NO: 263; and

(2) a mutant comprising an amino acid sequence that is at least 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:263.

15. The mutant according to any one of claims 1-11, wherein the amino acid sequence of the wildtype CMV gB polypeptide is set forth in SEQ ID NO: 1.

16. The mutant of any one of claims 1-15, wherein the amino acid sequence of the mutant does not comprise a signal sequence.

17. The mutant of claim 16, wherein the mutant comprises residues 23-907 of SEQ ID NO: 1.

18. The mutant of any one of claims 1-17, wherein the amino acid sequence of the mutant does not comprise an MPR, TM or CT domain.

19. The mutant of claim 18, wherein the mutant comprises residues 23-707 of SEQ ID NO: 1.

20. The mutant of any one of claims 1-19, wherein the amino acid sequence of the mutant comprises a truncated Domain V region.

21. The mutant of claim 20, wherein the mutant comprises residues 23-702 or 23-703 of SEQ ID NO: 1.

22. The mutant of any one of claims 1-19, wherein the amino acid sequence of the mutant does not comprise a Domain V region.

23. The mutant of claim 22, wherein the mutant comprises residues 23-646 of SEQ ID NO: 1.

24. The mutant of any one of claims 20-23, wherein the mutant further comprises a trimerization motif linked to the C terminus of the mutant.

25. The mutant of claim 24, wherein the trimerization motif is selected from the group consisting of:

(i) an inter-protomer disulfide ring;

(ii) GCN4;

(iii) T4 fibritin foldon; and

(iv) C-terminus fusion sequence.

26. The mutant of claim 25, wherein

(i) the GCN4 comprises an amino acid sequence as set forth in SEQ ID NOs: 269, 272 or 273;

(ii) the T4 fibritin foldon comprises an amino acid sequence as set forth in SEQ ID NO: 271; or

(iii) the C-terminus fusion sequence comprises an amino acid sequence as set forth in SEQ ID NO: 274.

27. The mutant of claim 25, wherein the inter-protomer disulfide ring comprises at least two engineered cysteine mutations selected from:

(i) 696C and 697C;

(ii) 703C and 704C; or

(iii) 767C and 768C.

28. The mutant of any one of claims 18-27, wherein the mutant is secreted.

29. The mutant of any one of claims 1-17, wherein the mutant is soluble.

30. The mutant of any one of claims 1-14 and 16-19, wherein the wildtype CMV gB polypeptide sequence is selected from SEQ ID NOs: 107-140 or 224.

31. The mutant of any one of claims 1-14 and 16-19, wherein the wildtype CMV gB polypeptide sequence is encoded by the polynucleotide sequences set forth in SEQ ID NOs: 225-254.

32. A nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of a CMV gB protein mutant according to any one of claims 1-31.

33. The nucleic acid molecule of claim 32, wherein the nucleic acid comprises nucleotides having a sequence set forth in SEQ ID NOs: 225-254.

34. A pharmaceutical composition comprising (i) a CMV gB protein mutant according to any one of claims 1-31 and (ii) a pharmaceutically acceptable carrier.

35. The pharmaceutical composition according to claim 34, which is a vaccine.

36. Use of an effective amount of the vaccine according to claim 35 in the manufacturing of a medicament for reducing CMV infection in a subject, wherein the medicament is to be administered to the subject.

37. Use of an effective amount of the vaccine according to claim 35 in the manufacture of a medicament for eliciting an immune response to CMV infection in a subject, wherein the medicament is to be administered to the subject .

38. Use of an effective amount of the vaccine according to claim 35 in the manufacture of a medicament for preventing CMV infection in a subject, wherein the medicament is to be administered to the subject.

39. The use of any one of claims 36-38, wherein the subject is a human.