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(54) **Title**: IMPROVED VACCINES COMPRISING MULTIMERIC HSP60 PEPTIDE CARRIERS

(57) **Abstract**: The present invention provides improved vaccine compositions having enhanced immunogenicity and methods of using same. The compositions and methods include immunogenic conjugates containing peptide carriers derived from heat shock protein 60 (HSP60). The known synthetic peptide carrier, p458, provides significantly improved immunogenicity when provided as a multimeric conjugate comprising a plurality of peptide carrier units conjugated to each antigen. Cell vaccine compositions comprising antigen presenting cells loaded with multimeric p458 conjugates are also provided.

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1. A conjugate comprising an antigen covalently attached to HSP60-derived peptide sequences selected from the group consisting of :

(a) NEDQKIGIEIIKRTLKI (p458h; SEQ ID NO: 1),
(b) NEDQKIGIEIIKRALKI (p458; SEQ ID NO:2),
(c) EGDEATGANIVKVALEA (p458mt; SEQ ID NO:3),
(d) NEDQNVGIKVALRAMEA (p458e; SEQ ID NO:4),
(e) an analog or derivative of p458h (SEQ ID NO: 1) that has at least 70 % of the electric and hydrophilicity/hydrophobicity characteristic of human HSP60 from position 458 to position 474, said peptide, analog or derivative being capable of increasing substantially the immunogenicity of the antigen when the conjugate is administered in vivo;

wherein the conjugate comprises a weight ratio of HSP60-derived peptide to antigen in excess of 1:1 (w/w);

with the proviso that said antigen is other than the capsular polysaccharide (CPS) Vi of Salmonella typhi.

2. The conjugate of claim 1, comprising an antigen covalently attached to a multimeric carrier, in which the carrier comprises a plurality of peptide units comprising HSP60-derived sequences selected from the group of peptides consisting of:

(a) NEDQKIGIEIIKRTLKI (p458h; SEQ ID NO: 1),
(b) NEDQKIGIEIIKRALKI (p458; SEQ ID NO:2),
(c) EGDEATGANIVKVALEA (p458mt; SEQ ID NO:3),
(d) NEDQNVGIKVALRAMEA (p458e; SEQ ID NO:4),
(e) an analog or derivative of p458h (SEQ ID NO: 1) that has at least 70 % of the electric and hydrophilicity/hydrophobicity characteristic of human HSP60 from position 458 to position 474, said peptide, analog or derivative being capable of increasing substantially the immunogenicity of the antigen when the conjugate is administered in vivo;

wherein the antigen is a bacterial capsular polysaccharide (CPS) other than the CPS Vi of Salmonella typhi; and wherein said conjugate has a peptide/polysaccharide ratio (w/w) of at least 1:1.

3. The conjugate of claim 1, wherein the antigen is a polysaccharide or oligosaccharide.

4. The conjugate of claim 3, wherein said antigen is a homopolymer of sialic acid selected from an α(2-8) sialic acid homopolymer and an α(2-9) sialic acid homopolymer.

5. A conjugate comprising an antigen covalently attached to a multimeric carrier, in which the carrier comprises a plurality of peptide units comprising heat shock protein 60 (HSP60)-derived sequences selected from the group of peptides consisting of:

(a) NEDQKIGIEIIKRTLKI (p458h; SEQ ID NO: 1),
(b) NEDQKIGIEIIKRALKI (p458; SEQ ID NO:2),
(c) EGDEATGANIVKVALEA (p458mt; SEQ ID NO:3),
(d) NEDQNVGIKVALRAMEA (p458e; SEQ ID NO:4),
(e) an analog or derivative of p458h (SEQ ID NO: 1) that has at least 70 % of the electric and hydrophilicity/hydrophobicity characteristic of human HSP60 from position 458 to position 474, said peptide, analog or derivative being capable of increasing substantially the immunogenicity of the antigen when the conjugate is administered in vivo;

wherein the conjugate comprises at least about 30 peptide units per antigen molecule;

with the proviso that said antigen is other than the capsular polysaccharide (CPS) Vi of Salmonella typhi.

6. The conjugate of claim 5, wherein said conjugate comprises at least about 40 peptide units per antigen molecule.

7. The conjugate of claim 5, wherein said conjugate comprises at least about 80 peptide units per antigen molecule.

8. The conjugate of claim 5, wherein said conjugate comprises between about 50 and about 200 peptide units per antigen molecule.

9. The conjugate of claim 5, wherein said conjugate comprises at least one analog of p458h (SEQ ID NO: 1): ^{458}NEDQKIGIEIIKRTLKI^{474} in which the residue E^{459} is either E or D; the residue D^{460} is either D or E; the residue K^{462} is either K or R or ornithine (Orn); the residue I^{463} is either I or L, V, M, F,

norleucine (Nle) or norvaline (Nva); the residue I^{465} residue is either I or L, V, M, F, Nle or Nva; the residue E^{466} is either E or D; the residue I^{467} is either I or L, V, M, F, Nle or Nva; the residue I^{468} is either I or L, V, M, F, Nle or Nva; the residue K^{469} is either K or R or Orn; the residue R^{470} is either R, K or Orn; the residue L^{472} in either L or I, V, M, F, Nle or Nva; the residue K^{473} is either K or R or Orn; and the residue I^{474} is either I or L, V, M, F, Nle or Nva.

10. The conjugate of claim 5, wherein the antigen is selected from the group consisting of polypeptides, peptides, peptide derivatives, saccharides, lipoproteins, glycolipids and antibodies.

11. The conjugate of claim 5, wherein the antigen is selected from the group consisting of antigens derived from *Streptococcus pneumoniae, Neisseria meningitidis, Staphylococcus aureus, Shigella flexneri, Klebsiella pneumoniae* and enterotoxigenic *Escherichia coli.*

12. The conjugate of claim 10, wherein said antigen is a bacterial CPS or a fragment thereof.

13. The conjugate of claim 12, wherein the antigen is a *Streptococcus pneumoniae* (*S. pneumoniae*) CPS.

14. The conjugate of claim 13, wherein the CPS is selected from the group consisting of CPS derived from *S. pneumoniae* serotypes as detailed in Table 1.

15. The conjugate of claim 14, wherein the *S. pneumoniae* CPS is a *S. pneumoniae* CPS type 4 (PS4).

16. The conjugate of claim 11, wherein the antigen is a *Neisseria meningitidis* (*N. meningitidis)* CPS.

17. The conjugate of claim 16, wherein the CPS is selected from the group consisting of CPS derived from *N. meningitidis* Group A, B, C, W-135 and Y.

18. The conjugate of claim 17, wherein said *N. meningitidis* CPS is a *N. meningitidis* group B CPS (MnB).

19. The conjugate of claim 17, wherein said *N. meningitidis* CPS is a *N. meningitidis* group C CPS (MnC).

20. The conjugate of any one of claims 12-19, wherein said conjugate has a peptide/polysaccharide ratio (w/w) of at least 1:1.

21. The conjugate of claim 20, wherein said conjugate has a peptide/polysaccharide ratio (w/w) of at least 2:1.

22. The conjugate of claim 20, wherein said conjugate has a peptide/polysaccharide ratio (w/w) of between about 1.5:1 and 6:1.

23. A vaccine composition comprising at least one conjugate according to any one of claims 1-22 and a pharmaceutically acceptable carrier, excipient or diluent.

24. A vaccine composition comprising at least one multimeric carrier in admixture with at least one antigen, and a pharmaceutically acceptable carrier, excipient or diluent, wherein:

the multimeric carrier comprises a plurality of covalently attached peptide units comprising HSP60-derived sequences, in which each peptide unit is selected from the group of peptides consisting of:

(a) NEDQKIGIEIIKRTLKI (p458h; SEQ ID NO: 1),
(b) NEDQKIGIEIIKRALKI (p458; SEQ ID NO:2),
(c) EGDEATGANIVKVALEA (p458mt; SEQ ID NO:3),
(d) NEDQNVGIKVALRAMEA (p458e; SEQ ID NO:4),
(e) an analog or derivative of p458h (SEQ ID NO: 1) that has at least 70 % of the electric and hydrophilicity/hydrophobicity characteristic of human HSP60 from position 458 to position 474, said peptide, analog or derivative being capable of increasing substantially the immunogenicity of the antigen when the conjugate is administered in vivo;

wherein the multimeric carrier comprises at least about 30 covalently attached peptide units.

25. The vaccine composition of claim 24, wherein the multimeric carrier comprises at least about 50 covalently attached peptide units.

26. The vaccine composition of claim 24, wherein the multimeric carrier comprises at least about 100 covalently attached peptide units.

27. The vaccine composition of claim 24, wherein the antigen is selected from the group consisting of polypeptides, peptides, peptide derivatives, saccharides, lipoproteins, glycolipids and antibodies.

28. The vaccine composition of claim 24, wherein the antigen is selected from the group consisting of antigens derived from *Streptococcus pneumoniae, Neisseria meningitidis, Staphylococcus aureus, Shigella flexneri, Klebsiella pneumoniae* and enterotoxigenic *Escherichia coli*.

29. The vaccine composition of claim 24, wherein said antigen is a bacterial CPS or a fragment thereof.

30. The vaccine composition of claim 29, wherein the antigen is a *Streptococcus pneumoniae* (*S. pneumoniae*) CPS.

31. The vaccine composition of claim 30, wherein the CPS is selected from the group consisting of CPS derived from *S. pneumoniae* serotypes as detailed in Table 1.

32. The vaccine composition of claim 31, wherein the *S. pneumoniae* CPS is a *S. pneumoniae* CPS type 4 (PS4).

33. The vaccine composition of claim 29, wherein the antigen is a *Neisseria meningitidis* (*N. meningitidis*) CPS.

34. The vaccine composition of claim 33, wherein the CPS is selected from the group consisting of CPS derived from *N. meningitidis* Group A, B, C, W-135 and Y.

35. The vaccine composition of claim 34, wherein said *N. meningitidis* CPS is a *N. meningitidis* group B CPS (MnB).

36. The vaccine composition of claim 34, wherein said *N. meningitidis* CPS is a *N. meningitidis* group C CPS (MnC).

37. A vaccine composition comprising a conjugate according to claim 18, the composition comprising between about 0.1 and about 1000 μg of said conjugate.

38. The composition of claim 37, comprising between about 0.1 and about 100 μg of said conjugate or between about 0.2 and about 25 μg of said conjugate.

39. The composition of claim 37, wherein said conjugate comprises about 30 to about 200 peptide units per antigen molecule or about 40 to about 100 peptide units per antigen molecule.

40. The composition of claim 37, further comprising a second conjugate according to claim 19.

41. The composition of claim 40, wherein said second conjugate comprises about 30 to about 200 peptide units per antigen molecule or about 40 to about 100 peptide units per antigen molecule.

42. The composition of claim 40, comprising between about 10 and about 1000 μg of the second conjugate, between about 20 and about 500 μg of the second conjugate or between about 40 and about 250 μg of the second conjugate.

43. The composition of claim 37, wherein said composition does not comprise an additional adjuvant.

44. The composition of claim 23, further comprising at least one peptide derived from E. coli HSP60 (GroEL), the peptide selected from the group consisting of: KARVEDALHATRAAVEEGV (SEQ ID NO:5; Ec27), KKDRVTDALNATRAAVEEGI (SEQ ID NO:6; Ec27h), and analogs, fragments, derivatives and salts thereof.

45. The composition of claim 24, further comprising at least one peptide derived from E. coli HSP60 (GroEL), the peptide selected from the group consisting of: KARVEDALHATRAAVEEGV (SEQ ID NO:5; Ec27), KKDRVTDALNATRAAVEEGI (SEQ ID NO:6; Ec27h), and analogs, fragments, derivatives and salts thereof.

46. A vaccine composition comprising at least one bacterial CPS and at least one peptide derived from E. coli HSP60 (GroEL), the peptide selected from the group consisting of: KARVEDALHATRAAVEEGV (SEQ ID NO:5; Ec27), KKDRVTDALNATRAAVEEGI (SEQ ID NO:6; Ec27h), and analogs, fragments, derivatives and salts thereof;
wherein the CPS is selected from *N. meningitidis* CPS and *S. pneumoniae* CPS.

47. The composition of claim 46, selected from the group consisting of:

a) a composition wherein the capsular polysaccharide is conjugated to said peptide; and

b) a composition comprising an admixture of the capsular polysaccharide and said peptide.

48. The composition of claim 46, wherein the composition comprises a plurality of covalently attached peptides selected from the group consisting of: KARVEDALHATRAAVEEGV (SEQ ID NO:5; Ec27), KKDRVTDALNATRAAVEEGI (SEQ ID NO:6; Ec27h), and analogs, fragments, derivatives and salts thereof.

49. The composition of claim 46, wherein the CPS is selected from the group consisting of MnB, MnC and mixtures thereof.

50. The composition of claim 49, wherein said CPS is MnB.

51. The composition of claim 46, wherein the CPS is PS4.

52. The composition of claim 46, wherein the CPS is further conjugated to a carrier comprising HSP60-derived sequences selected from the group of peptides consisting of:

(a) NEDQKIGIEIIKRTLKI (p458h) (SEQ ID NO: 1),
(b) NEDQKIGIEIIKRALKI (p458) (SEQ ID NO:2),
(c) EGDEATGANIVKVALEA (p458mt) (SEQ ID NO:3),
(d) NEDQNVGIKVALRAMEA (p458e) (SEQ ID NO:4),
(e) an analog of p458h (SEQ ID NO: 1) that has at least 70 % of the electric and hydrophilicity/hydrophobicity characteristic of human HSP60 from position 458 to position 474, said peptide or analog being capable of increasing substantially the immunogenicity of the capsular polysaccharide when the conjugate is administered in vivo.

53. The composition of claim 52, wherein said carrier is a multimeric carrier comprising at least about 30 copies of said HSP60-derived sequence.

54. A cell vaccine composition comprising a population of antigen presenting cells (APC) exposed in culture, under conditions promoting uptake and processing of an antigen, to an immunogenic conjugate, to provide antigen-expressing APC,

wherein said conjugate comprises an antigen covalently attached to a multimeric carrier, in which the carrier comprises a plurality of peptide units comprising HSP60-derived sequences selected from the group of peptides consisting of:

(a) NEDQKIGIEIIKRTLKI (p458h; SEQ ID NO: 1),
(b) NEDQKIGIEIIKRALKI (p458; SEQ ID NO:2),
(c) EGDEATGANIVKVALEA (p458mt; SEQ ID NO:3),
(d) NEDQNVGIKVALRAMEA (p458e; SEQ ID NO:4),
(e) an analog or derivative of p458h (SEQ ID NO: 1) that has at least 70 % of the electric and hydrophilicity/hydrophobicity characteristic of human HSP60 from position 458 to position 474, said peptide, analog or derivative being capable of increasing substantially the immunogenicity of the antigen when the conjugate is administered in vivo;

wherein the conjugate comprises at least about 30 peptide units per antigen molecule.

55. The composition of claim 54, wherein the APC are selected from macrophages, B-cells and dendritic cells.

56. A method for enhancing the immunogenicity of an antigen comprising conjugating the antigen to a plurality of peptide units comprising HSP60-derived sequences, in which each peptide unit is selected from the group of peptides consisting of:

(a) NEDQKIGIEIIKRTLKI (p458h; SEQ ID NO: 1),
(b) NEDQKIGIEIIKRALKI (p458; SEQ ID NO:2),
(c) EGDEATGANIVKVALEA (p458mt; SEQ ID NO:3),
(d) NEDQNVGIKVALRAMEA (p458e; SEQ ID NO:4),
(e) an analog or derivative of p458h (SEQ ID NO: 1) that has at least 70 % of the electric and hydrophilicity/hydrophobicity characteristic of human HSP60 from position 458 to position 474, said peptide, analog or derivative being capable of increasing substantially the immunogenicity of the antigen when the conjugate is administered in vivo;

wherein the antigen is conjugated to at least about 30 peptide units;

with the proviso that said antigen is other than the capsular polysaccharide (CPS) Vi of Salmonella typhi.

57. A method for immunizing a subject in need thereof, comprising administering to the subject an effective amount of a vaccine composition according to claim 23.

58. A method for immunizing a subject in need thereof, comprising administering to the subject an effective amount of a vaccine composition according to claim 24.

59. A method for immunizing a subject in need thereof, comprising administering to the subject an effective amount of a vaccine composition according to claim 44.

60. A method for immunizing a subject in need thereof, comprising administering to the subject an effective amount of a vaccine composition according to claim 45.

61. A method for immunizing a subject in need thereof, comprising administering to the subject an effective amount of a vaccine composition according to claim 46.

62. A method for immunizing a subject in need thereof against *Neisseria meningitidis* infection, comprising the step of administering to the subject an effective amount of a vaccine composition according to claim 37.

63. A method of treating cancer in a subject in need thereof, comprising the step of administering to the subject a therapeutically effective amount of a vaccine composition according to claim 35, 37 or 50.

64. The method of claim 63, wherein the cancer is selected from the group consisting of malignancies of neuroectodermal origin (e.g. small cell lung cancer), neuroblastoma, thyroid carcinoma, primary C-cell hyperplasia, rhabdomyosarcoma, natural killer cell derived-lymphoma, and Wilm's tumor.

65. A method of inhibiting tumor metastasis in a subject in need thereof, comprising the step of administering to the subject a therapeutically effective amount of a vaccine composition according to claim 35, 37 or 50.

66. A method of stimulating an immune response in a subject in need thereof, comprising the steps of:

(i) obtaining antigen presenting cells (APC) from the subject, or from a donor histocompatible with said subject;

(ii) exposing the APC in culture, under conditions promoting uptake and processing of an antigen, to an immunogenic conjugate, to provide antigen-expressing APC; and

(iii) administering an effective amount of the antigen-expressing APC to said subject;

wherein said conjugate comprises an antigen covalently attached to a multimeric carrier, in which the carrier comprises a plurality of peptide units comprising HSP60-derived sequences selected from the group of peptides consisting of:

(a) NEDQKIGIEIIKRTLKI (p458h; SEQ ID NO: 1),
(b) NEDQKIGIEIIKRALKI (p458; SEQ ID NO:2),
(c) EGDEATGANIVKVALEA (p458mt; SEQ ID NO:3),
(d) NEDQNVGIKVALRAMEA (p458e; SEQ ID NO:4),
(e) an analog or derivative of p458h (SEQ ID NO: 1) that has at least 70 % of the electric and hydrophilicity/hydrophobicity characteristic of human HSP60 from position 458 to position 474, said peptide, analog or derivative being capable of increasing substantially the immunogenicity of the antigen when the conjugate is administered in vivo;

wherein the conjugate comprises at least about 30 peptide units per antigen molecule.

67. The method of claim 66, wherein the APC are selected from macrophages, B-cells and dendritic cells.

Statement under Article 19(1)

Claims of International Application No. PCT/IL2007/000468 were amended to better define certain embodiments of the claimed invention as described in the specification as filed. The claims were merged to reduce the number of claims and no new matter has been added.