Claims

1. An influenza virus hemagglutinin (HA) molecule having a HA1 domain, wherein said HA1 domain is totally or partially free of N-linked glycosylation.

2. The influenza virus hemagglutinin (HA) molecule as defined in claim 1, wherein one or more of N-linked glycosylation sites from HA1 domain is abolished, whereby the glycosylation site that is abolished is originally present on an immunogenic globular head portion of the protein.

3. The influenza virus hemagglutinin (HA) molecule as defined in claim 2, wherein said abolished glycosylation site is within or near a receptor binding subdomain of said immunogenic globular head or within or near an F'2 subdomain of the HA1 domain.

4. The HA molecule as defined in claim 3, wherein said glycosylation site is consisting of a glycosylation recognition triad N-X-S/T wherein N is asparagine, X is any amino acid except proline, S is serine and T is threonine.

5. The HA molecule as defined in claim 4, wherein one or more asparagine residue comprised in a glycosylation recognition triad N-X-S/T is substituted for a non-asparagine amino acid.

6. The HA molecule according to claim 5, wherein said asparagine residue is substituted for an amino acid selected from the group consisting of: Leu, Ile, Val, Thr, Ser and Ala.

7. The HA molecule according to claim 6, wherein said asparagine residue is substituted for an alanine.

8. The HA molecule as defined in claim 4 or 5 wherein one or more serine or threonine residue comprised in a glycosylation recognition triad N-X-S/T is substituted for a non-serine and non-threonine amino acid.

9. The HA molecule as defined in claim 8, wherein one or more serine or threonine residue comprised in a glycosylation recognition triad N-X-S/T is substituted for an amino acid selected from the group consisting of: Ala, Val, Ile and Leu.

10. The HA molecule according to claim 9, wherein said serine or threonine residue is substituted for an alanine.

11. The HA molecule according to any one of claims 1 to 10, wherein said glycosylation site is at a position corresponding to any triad located between amino acids 39 to 331 wherein numbering is in accordance with strain A/Vietnam/1194/04, whereby said abolished N-glycosylation site makes the HA molecule more antigenic with respect to antibodies specific for a native HA molecule.

12. The HA molecule according to any one of claims 11, wherein said glycosylation site is at a position selected from the group consisting of: 154, 165 and 286.

13. The HA molecule according to claim 12, having one glycosylation site abolished.

14. The HA molecule according to claim 12, having two glycosylation sites abolished.

15. The HA molecule according to claim 12, having three glycosylation sites abolished.

16. The influenza hemagglutinin (HA) according to any one of claims 1 to 15, wherein said influenza is of type A and type B.

17. The influenza hemagglutinin (HA) according to claim 16, wherein said influenza is of type B.

18. The influenza hemagglutinin (HA) according to claim 16, wherein said HA is from one or more than one A subtype selected from the group consisting of: H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16.

19. The influenza hemagglutinin (HA) according to claim 18, wherein said HA subtype is one or more than one of subtype selected from the group consisting of: H1, H2, H3, H5, H7, H9, and H10.

20. The influenza hemagglutinin (HA) according to claim 19, wherein said HA subtype is one or more than one of subtype selected from the group consisting of: H1, H3, H5 and H7.

21. The influenza hemagglutinin (HA) of claim 16, wherein the influenza hemagglutinin is of type B or of type A subtype H3.

22. A nucleic acid comprising a nucleotide sequence encoding an influenza HA molecule according to anyone of claims 1 to 21.

23. A nucleic acid as defined according to SEQ ID NO. 17, wherein one or more residue encoding amino acids selected from the group consisting of: 154, 165 and 286 is encoding a non-asparagine.

24. The nucleic acid as defined in claim 23, wherein said one or more residue encoding amino acids selected from the group consisting of: 154, 165, and 286 is encoding alanine.

25. A nucleic acid as defined according to SEQ ID NO. 17, wherein one or more residue encoding amino acids selected from the group consisting of: 154+2, 165+2 and 286+2 is encoding a non-serine and non-threonine.

26. The nucleic acid as defined in claim 25, wherein said one or more residue encoding amino acids selected from the group consisting of: 154+2, 165+2, and 286+2 is encoding alanine.

27. A nucleic acid having 90% identity to the nucleic acid as defined in claim 23, 24, 25 or 26.

28. A nucleic acid as defined according to SEQ ID NO. 29.

29. A nucleic acid having 90% identity to SEQ ID NO. 29.

30. An expression cassette comprising a nucleic acid according to any one of claims 22 to 29, operatively linked to a regulatory region active in a non-sialylating host organism.

31. The expression cassette according to claim 30, wherein said regulatory region is selected from the group consisting of : plastocyanin regulatory region; napin promoter, the cruciferin promoter, or a regulatory region obtained from Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO), chlorophyll a/b binding protein, ST-LS, polyhedron promoter, and the gp64 promoter.

32. The expression cassette according to claim 31, wherein said plastocyanin regulatory region is from alfalfa (*Medicago sativa*).

33. A method of producing influenza virus like particles (VLPs) in a non-sialylating host organism, said method comprising:

a) introducing a nucleic acid according to any one of claims 22 to 29, operatively linked to a regulatory region active in a non-sialylating host organism or a portion thereof, and

b) incubating the host or a portion therefore under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

34. The method according to claim 33, wherein, in the step of introducing (step a), the nucleic acid may be either transiently expressed in the host, or stably expressed in the host.

35. The method according to claim 33 or 34, wherein said non-sialylating host organism is selected from the group consisting of: plant, insect and yeast.

36. The method according to claim 35, wherein said plant is selected from the group consisting of: a plant cell, a whole plant and a portion thereof.

37. The nucleic acid according to claim 36, wherein said plant cell is from *N. benthiamina.*

38. The method of claim 33 further comprising a step of : c) harvesting the host and purifying the VLPs.

39. A virus like particle (VLP) comprising an influenza hemagglutinin molecule having a HA1 domain, wherein one or more of the N-linked glycosylation site from said HA1 subdomain has been abolished.

40. The VLP according to claim 39, wherein said VLP is immunogenic.

41. A virus like particle (VLP) encoded by the nucleic acid according to any one of claims 22 to 29.

42. A virus like particle (VLP) comprising an influenza virus HA protein according to any one of claims 1 to 21, and one or more than one host lipid.

43. The virus-like particle according to claim 41 or 42, wherein said host lipid is a plant lipid.

44. A virus like particle (VLP) according to claims 41, 42 or 43, comprising an influenza virus HA bearing plant-specific N-glycans, or modified N-glycans.

45. The virus like particle (VLP) of claim 44 wherein the influenza HA protein is H5 Indonesia.

46. Use of the VLP according to any one of claims 39 to 45 for the manufacture of a vaccine for the prevention or treatment of a viral infection in a subject.

47. Use according to claim 46, wherein said subject is selected from the group consisting of: human or birds.

48. Use of the VLP according to any one of claims 39 to 45 for the prevention or treatment of an influenza viral infection in a subject.

49. Use according to claim 48, wherein said subject is selected from the group consisting of: human or birds.

50. A composition comprising an effective dose of a VLP according to any one of claims 39 to 45, in admixture with a pharmaceutically acceptable carrier.

51. The composition according to claim 50, wherein said pharmaceutically acceptable carrier is suitable for oral, intradermal, intranasal, intramuscular, intraperitoneal, intravenous, or subcutaneous administration.

52. A composition for immunizing an individual at risk of contracting an influenza viral disease, said composition comprising: a VLP according to any one of claims 39 to 45 in admixture with a pharmaceutically acceptable carrier.

53. The composition according to claim 52 wherein said composition is in liquid, suspension, solid or powder form.

54. The composition according to claim 53 wherein said composition is suitable for oral, intradermal, intranasal, intramuscularly, intraperitoneal, intravenous, or subcutaneous administration.

55. A vaccine composition comprising an immumologically effective does of a VLP according to any one of claims 39 to 45, in admixture with a pharmaceutically acceptable carrier with or without the presence of an adjuvant.

56. The vaccine according to claim 55, wherein said pharmaceutically acceptable carrier is suitable to be administered orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

57. The vaccine according to claim 56, devoid of said adjuvant.

58. A viral vaccine comprising: a VLP according to any one of claims 39 to 45 in admixture in a physiologically acceptable excipient or adjuvant.

59. The vaccine according to claim 58, being a human or avian vaccine.

60. The vaccine according to claim 59, being suitable for prophylaxis prior to appearance of symptoms; or for treatment of infection after appearance of symptoms

61. A method of inducing immunity to an influenza virus infection in a subject, the method comprising administering the virus like particle comprising an influenza virus HA protein according to any one of claim 1 to 21, or encoded by the nucleic acid according to any one of claims 22 to 29, one or more than one host lipid, and a pharmaceutically acceptable carrier.

62. The method according to claim 61, wherein said virus-like particle is administered to a subject orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

63. A method for inducing immunity to influenza virus infection in a subject comprising administering an effective dose of a composition according to claim 50, or a vaccine according to claim 55.

64. A method of inducing immunity to an influenza virus infection in a subject, comprising administering the virus-like particle according to any one of claims 39 to 45.

65. The method of claim 64, wherein the virus like particle is administered to a subject orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

66. The method according to any one of claims 61 to 65, wherein said subject selected from the group comprising humans, primates, horses, pigs, birds (avian) water fowl, migratory birds, quail, duck, geese, poultry, chicken, camel, canine, dogs, feline, cats, tiger, leopard, civet, mink, stone marten, ferrets, house pets, livestock, mice, rats, seal, whales and the like.

67. The method according to claim 66, wherein the subject is selected from the group consisting of: human and birds.

68. The method according to claim 67, wherein the subject is human.

69. A container comprising the vaccine according to claim 55.

70. The container according to claim 69, said container containing a single unit dose.

71. The container according to claim 69, said container being a in multiple dosage

form.

72. The container according to claim 69, 70 or 71, said container being a syringe.

73. A syringe pre-filled with the vaccine according to claim 55.

74. A kit comprising a container according to any one of claims 69 to 73, and instructions on how to use/administer said vaccine.

75. A plant cell transformed with a nucleic acid according to any one of claims 22 to 29.

76. The plant cell according to claim 75, being transiently or stably transformed.

77. A portion of a plant transformed with a nucleic acid according to any one of claims 22 to 29.

78. The portion of a plant according to claim 77, being transiently or stably transformed.

79. A plant transformed with a nucleic acid according to any one of claims 22 to 29.

80. The plant according to claim 79 being transiently or stably transformed.

81. An *Agrobacterium* cell transfected with an expression cassette according to claim 30, 31 or 32.

Claims

1. A nucleic acid comprising a nucleotide sequence having about 90% to 100% identity to SEQ ID NO. 17, the nucleotide sequence encoding an influenza virus hemagglutinin (HA) comprising a HA1 domain, wherein said HA1 domain is modified to be free of N-linked glycosylation sites at position 154 and/or 156 and free of N-linked glycosylation sites at position 165 and/or 167 and free of N-linked glycosylation sites at position 286 and/or 288, by modifying position 154, 165 or 286 to encode a non-asparagine, or by modifying position 156, 167 or 288 to encode a non-serine or non-threonine, wherein numbering is in accordance with strain A/Vietnam/1194/04, when compared to an original native HA sequence and wherein the nucleotide sequence is operatively linked to a regulatory region active in a plant.

2. The nucleic acid of claim 1, wherein the nucleotide sequence has from about 90% to 100% identity to SEQ ID NO. 29, whereby residues encoding amino acids 154, 165 and 286 are identical to those of SEQ ID NO. 29.

3. The nucleic acid of claim 1, wherein amino acids at positions 154, 165 or 286 are selected from the group consisting of: Leu, Ile, Val, Thr, Ser and Ala.

4. The nucleic acid of claim 1, wherein amino acids at positions 156, 167 or 288 are selected from the group consisting of Ala, Val, Ile and Leu.

5. The nucleic acid according to claim 1, wherein said regulatory region is selected from the group consisting of: plastocyanin regulatory region; napin promoter, the cruciferin promoter, or a regulatory region obtained from Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO), chlorophyll a/b binding protein, ST-LS, polyhedron promoter, and the gp64 promoter.

6. The nucleic acid according to claim 5, wherein said plastocyanin regulatory region is from alfalfa (Medicago sativa).

7. The nucleic acid according to any one of claims 1-4, wherein each of residues encoding amino acids at positions 154, 165 and 286 is modified to encode a non-asparagine.

8. The nucleic acid as defined in claim 1, wherein each residues encoding amino acids at positions 154, 165 and 286 is modified to encode alanine.

9. The nucleic acid as defined in claim 1, wherein each residues encoding amino acids at positions 156, 167 and 288 is modified to encode alanine.

10. The nucleic acid as defined in claim 1, wherein the nucleic acid is as defined according to SEQ ID NO. 29.

11. A method of producing influenza virus like particles (VLPs) in a plant, a portion thereof, or a plant cell, said method comprising:

a. introducing said nucleic acid according to any one of claims 1 to 10, into said plant, portion thereof, or said plant cell and

b. incubating said plant, portion thereof, or said plant cell under conditions that permit expression of the nucleic acid, thereby producing said VLPs.

12. The method according to claim 11, wherein, in the step of introducing (step a), said nucleic acid is either transiently expressed in said plant, portion thereof, or said plant cell, or stably expressed in said plant, portion thereof, or said plant cell.

13. The method of claim 12, wherein said plant, portion thereof, or the plant cell is from N. benthiamina.

14. The method of claim 12, further comprising a step of: c) harvesting said plant, portion thereof, or said plant cell and purifying the VLPs.

15. A virus like particle (VLP) produced by the method according to any one of claims 11-14.

16. A virus like particle (VLP) comprising an influenza virus hemagglutinin (HA) molecule encoded by the nucleotide sequence as defined in any one of claims 1-10.

17. The virus-like particle (VLP) according to claims 15 or 16, comprising an influenza virus HA bearing plant-specific N-glycans, or modified N-glycans.

18. The VLP according to claim 15, wherein said influenza virus hemagglutinin is of type A or type B.

19. The VLP according to claim 16, wherein said influenza virus hemagglutinin is of type A or type B.

20. The VLP according to claim 18 or 19, wherein said influenza virus hemagglutinin is of type B.

21. The VLP according to claim 18 or 19, wherein said HA is from one or more than one A subtype selected from the group consisting of: H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16.

22. The VLP according to claim 21, wherein said HA subtype is one or more than one of subtype selected from the group consisting of: H1, H2, H3, H5, H7, H9, and H10.

23. The VLP according to claim 22, wherein said HA subtype is one or more than one of subtype selected from the group consisting of: H1, H3, H5 and H7.

24. The VLP of claim 18 or 19, wherein the influenza hemagglutinin is of type B or of type A subtype H3.

25. The virus like particle (VLP) of claim 23, wherein the influenza HA protein is H5 Indonesia.

26. The VLP according to any one of claims 15-25, wherein said VLP is immunogenic.

27. The virus like particle (VLP) according to claim 15, comprising influenza virus hemaglutinin (HA) and one or more than one plant lipid.

28. The virus like particle (VLP) according to claim 16, comprising influenza virus hemaglutinin (HA) and one or more than one plant lipid.

29. The VLP according to claim 15 for use as a vaccine for the prevention or treatment of an influenza infection in a subject.

30. The VLP according to claim 16 for use as a vaccine for the prevention or treatment of an influenza infection in a subject.

31. The VLP according to claim 29 or 30, wherein said subject is selected from the group consisting of: human or birds.

32. A composition comprising an effective dose of a VLP according to claim 15 or 16, in admixture with a pharmaceutically acceptable carrier.

33. The composition according to claim 32, wherein said pharmaceutically acceptable carrier is suitable for oral, intradermal, intranasal, intramuscular, intraperitoneal, intravenous, or subcutaneous administration.

34. The composition according to claim 33, wherein said composition is in liquid, suspension, solid or powder form.

35. The composition according to claim 34, wherein said composition is suitable for oral, intradermal, intranasal, intramuscularly, intraperitoneal, intravenous, or subcutaneous administration.

36. A vaccine composition comprising an immumologically effective dose of a VLP according to claim 15 or 16, in admixture with a pharmaceutically acceptable carrier with or without the presence of an adjuvant.

37. The vaccine according to claim 36, wherein said pharmaceutically acceptable carrier is suitable to be administered orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

38. The vaccine according to claim 37, devoid of adjuvant.

39. The vaccine according to claim 35, being a human or avian vaccine.

40. The vaccine according to claim 39, being suitable for prophylaxis prior to appearance of symptoms; or for treatment of infection after appearance of symptoms.

41. A use of the VLP according to claim 15 or 16 and a pharmaceutically acceptable carrier for the manufacture of a composition for inducing immunity to an influenza virus infection in a subject.

42. The use according to claim 41, wherein said virus-like particle is suitable for oral, intradermal, intranasal, intramuscular, intraperitoneal, intravenous, or subcutaneous administration.

43. The use according to claim 41, wherein said subject selected from the group comprising primates, horses, pigs, birds (avian), camel, canidae, felidae, civet, mink, stone marten, ferrets, mice, rats, seal, whales and the like.

44. The use according to claim 41, wherein the subject is human.

45. A container comprising the vaccine according to claim 36.

46. The container according to claim 45, said container containing a single unit dose.

47. The container according to claim 45, said container being in a multiple dosage form.

48. The container according to any one of claims 45-46, said container being a syringe.

49. A syringe pre-filled with the vaccine according to any one of claim 38-40.

50. A kit comprising a container according to any one of claims 45-48, and instructions on how to use/administer said vaccine.

51. An Agrobacterium cell transfected with a nucleic acid according to any one of claims 1 to 10.