as VP1, VP2, VP3 and/or VP4, or immunologically or biologically active fragments thereof which elicit neutralizing antibodies against enteroviruses.

[0037] The present invention relates to vaccines in which the picornavirus antigens are present in the form of one or more picornavirus polypeptides, especially human *Enterovirus* peptides VP2, VP3 or VP0, immunogenic fragments thereof, and/or antigenic determinants thereof. The picornavirus polypeptides may be obtained by chemical synthesis or by means of recombinant DNA techniques using known human *Enterovirus* amino acid or nucleic acid sequences.

SUMMARY OF THE INVENTION

[0038] In one embodiment there is provided, a vaccine comprising a virus-like particle, the virus-like particle comprising an Enterovirus polypeptide, the Enterovirus polypeptide comprising any combination of a P1 polypeptide, a VP0 polypeptide, a VP1 polypeptide, a VP2 polypeptide, a VP3 polypeptide, a VP4 polypeptide, or immunologically active fragments thereof, wherein the vaccine elicits a protective and/or neutralizing immune response directed against a human Enterovirus.

[0038a] In one embodiment there is provided, an expression cassette comprising a promoter operably linked to a nucleic acid encoding a virus-like particle, the virus-like particle comprising an Enterovirus P1 polypeptide, an Internal Ribosome Entry Site (IRES), and an Enterovirus 3CD protease.

[0038b] In one embodiment there is provided, a method of making a vaccine comprising the step of culturing a host cell comprising an expression cassette as described herein for a period of time sufficient to produce the Enterovirus P1 polypeptide and Enterovirus 3CD protease and form virus like particles, capsomers, complexes and/or aggregates.

[0038c]] In one embodiment there is provided, a vaccine comprising a virus-like particle produced according to a method as described herein.

[0038d] In one embodiment there is provided, use of a vaccine as described herein in the manufacture of a medicament for vaccinating a subject against an Enterovirus infection.

[0038e] In one embodiment there is provided, use of a vaccine as described herein for vaccinating a subject against an Enterovirus infection

[0038f] A vaccine comprising one or more immunologically active antigens comprising one or more human *Enterovirus* polypeptides selected from VP0, VP1, VP2, VP3, VP4, and immunologically active fragments thereof, such a

[0039] vaccine, which elicits a protective and/or neutralizing immune response directed against a human *Enterovirus*, such a

[0040] vaccine, wherein the human *Enterovirus* is selected from *Human enterovirus A, Human enterovirus B*, *Human enterovirus C* and *Human enterovirus D*, such a

[0041] vaccine, wherein the *Human enterovirus A* is selected from *Human enterovirus* 71 (HEV71) and *Coxsackievirus* A16, wherein the *Human enterovirus B* is selected from *Coxsackievirus* B and echovirus, wherein the *Human enterovirus C* is selected from *Human poliovirus* 1, *Human poliovirus* 2 and *Human poliovirus* 3, and wherein the *Human enterovirus D* is *EV* 68, such a

[0042] vaccine, wherein the vaccine comprises an immunologically active *Enterovirus* VP0 polypeptide, such a

Claim

1. A vaccine comprising a virus-like particle, the virus-like particle comprising an Enterovirus polypeptide, the Enterovirus polypeptide comprising any combination of a P1 polypeptide, a VP0 polypeptide, a VP1 polypeptide, a VP2 polypeptide, a VP3 polypeptide, a VP4 polypeptide, or immunologically active fragments thereof, wherein the vaccine elicits a protective and/or neutralizing immune response directed against a human Enterovirus.

2. The vaccine according to Claim 1, wherein the Enterovirus polypeptide comprises an immunologically active Enterovirus P1 polypeptide.

3. The vaccine according to Claim 1, wherein the Enterovirus polypeptide comprises an immunologically active Enterovirus VP0 polypeptide.

4. The vaccine according to Claim 1, wherein the Enterovirus polypeptide comprises an immunologically active Enterovirus VP4-VP2-VP3 fusion polypeptide.

5. The vaccine according to Claim 1, wherein the Enterovirus polypeptide comprises an immunologically active Enterovirus VP2-VP3-VP1 fusion polypeptide.

6. The vaccine according to any one of Claims 1-5, wherein the Enterovirus polypeptide is from an Enterovirus A, an Enterovirus B, an Enterovirus C, an Enterovirus D, or any combination thereof.

7. The vaccine according to Claim 6, wherein the Enterovirus A is from a Human Enterovirus 71 or a Coxsackievirus A16.

8. The vaccine according to Claim 6, wherein the Enterovirus B is from a Coxsackievirus B or an Echovirus.

9. The vaccine according to Claim 6, wherein the Enterovirus C is from a Human poliovirus 1, a Human poliovirus 2 or a Human poliovirus 3.

10. The vaccine according to Claim 6, wherein the Human Enterovirus D is from an EV 68.

11. The vaccine according to any one of Claims 1-10, wherein the virus-like particle is in the form of a capsomer, a complex and/or an aggregate.

12. An expression cassette comprising a promoter operably linked to a nucleic acid encoding a virus-like particle, the virus-like particle comprising an Enterovirus P1 polypeptide, an Internal Ribosome Entry Site (IRES), and an Enterovirus 3CD protease.

13. The expression cassette according to Claim 12, wherein the Enterovirus P1 polypeptide comprises any combination of a P1 polypeptide, a VP0 polypeptide, a VP1 polypeptide, a VP2 polypeptide, a VP3 polypeptide, a VP4 polypeptide, or immunologically active fragments thereof.

14. The expression cassette according to Claim 12 or Claim 13, wherein the IRES is derived from Encephalomyocarditis virus (EMCV) or an Enterovirus.

15. The expression cassette according to Claim 12 or Claim 13, wherein the virus-like particle comprises a Human Enterovirus A P1 polypeptide, an EMCV IRES, and a Human Enterovirus A 3CD protease.

17. The expression cassette according to Claim 12 or Claim 13, wherein the virus-like particle comprises a Human Enterovirus A P1 polypeptide, an HEV71 IRES, and a Human Enterovirus A 3CD protease.

18. The expression cassette according to Claim 12 or Claim 13, wherein the virus-like particle comprises a Human Enterovirus A P1 polypeptide, a Human Enterovirus C IRES, and a Human Enterovirus A 3CD protease.

19. The expression cassette according to Claim 12 or Claim 13, wherein the virus-like particle comprises a Human Enterovirus C P1 polypeptide, a Human Enterovirus C IRES, and a Human Enterovirus C 3CD protease.

20. The expression cassette according to Claim 12 or Claim 13, wherein the virus-like particle comprises a Human Enterovirus C P1 polypeptide, an HEV71IRES, and a Human Enterovirus C 3CD protease.

21. The expression cassette according to Claim 12 or Claim 13, wherein the virus-like particle comprises a Human Enterovirus C P1 polypeptide, an EMCV IRES, and a Human Enterovirus C 3CD protease.

22. A method of making a vaccine comprising the step of culturing a host cell comprising an expression cassette as defined in any one of Claims 12-21 for a period of time sufficient to produce the Enterovirus P1 polypeptide and Enterovirus 3CD protease and form virus like particles, capsomers, complexes and/or aggregates.

23. The method according to claim 23, further comprising the step of recovering the virus like particles, capsomers, complexes and/or aggregates from the host cell and/or culture supernatant.

24. The method according to Claim 23, wherein the host cell is a eukaryotic cell.

25. A vaccine comprising a virus-like particle produced according to a method as defined by any one of Claims 22-24.

26. Use of a vaccine as defined in any one of Claims 1-11 or 25 in the manufacture of a medicament for vaccinating a subject against an Enterovirus infection.

27. Use of a vaccine as defined in any one of Claims 1-11 or 25 for vaccinating a subject against an Enterovirus infection.

CLAIMS

1. A vaccine comprising one or more immunologically active antigens comprising one or more human *Enterovirus* polypeptides selected from VP0, VP1, VP2, VP3, VP4, and immunologically active fragments thereof.

2. The vaccine of Claim 1, which elicits a protective and/or neutralizing immune response directed against a human *Enterovirus*.

3. The vaccine of Claim 1, wherein the human *Enterovirus* is selected from *Human enterovirus A*, *Human enterovirus B*, *Human enterovirus C* and *Human enterovirus D*.

4. The vaccine of Claim 3, wherein the *Human enterovirus A* is selected from *Human enterovirus* 71 (HEV71) and *Coxsackievirus* A16, wherein the *Human enterovirus B* is selected from *Coxsackievirus* B and echovirus, wherein the *Human enterovirus C* is selected from *Human poliovirus* 1, *Human poliovirus* 2 and *Human poliovirus* 3, and wherein the *Human enterovirus D* is *EV* 68.

5. The vaccine of Claim 1, wherein the vaccine comprises an immunologically active *Enterovirus* VP0 polypeptide.

6. The vaccine of Claim 1, wherein the vaccine comprises an immunologically active *Enterovirus* VP2 polypeptide.

7. The vaccine of Claim 1, wherein the vaccine comprises an immunologically active *Enterovirus* VP3 polypeptide.

8. The vaccine of Claim 1, wherein the vaccine comprises an immunologically active *Enterovirus* VP1 polypeptide.

9. The vaccine of Claim 1, wherein the vaccine comprises polypeptides from one or more *Enterovirus* species or serotype.

10. The vaccine of Claim 9, wherein the species or serotype may be *Human enterovirus A* selected from HEV71 and *Coxsackievirus* A16.

11. The vaccine of Claim 9, wherein the species or serotype may be *Human enterovirus C* selected from PV-1, PV-2 and PV-3.

12. The vaccine of Claim 1, wherein the one or more immunologically active antigens comprising one or more human *Enterovirus* polypeptides selected from VP0, VP1, VP2, VP3, VP4, and immunologically active fragments thereof, are in the form of a virus-like particle, capsomer, complex and/or aggregate.

13. The vaccine of Claim 12, wherein the virus-like particle comprises an immunologically active *Enterovirus* VP0 polypeptide.

14. The vaccine of Claim 12, wherein the virus-like particle comprises an immunologically active *Enterovirus* VP2 polypeptide.

15. The vaccine of Claim 12, wherein the virus-like particle comprises an immunologically active *Enterovirus* VP3 polypeptide.

16. The vaccine of Claim 12, wherein the virus-like particle comprises an immunologically active *Enterovirus* VP1 polypeptide.

17. A method of vaccinating a subject against an *Enterovirus* infection, comprising administering to the subject a vaccine comprising one or more

immunologically active antigens comprising one or more human *Enterovirus* polypeptides selected from VP0, VP1, VP2, VP3, VP4, and immunologically active fragments thereof, in an amount effective to elicit a protective and/or neutralizing immune response when administered to the subject.

18. The method of Claim 17, wherein the vaccine comprises an immunologically active *Enterovirus* VP0 polypeptide.

19. The method of Claim 17, wherein the vaccine comprises an immunologically active *Enterovirus* VP1 polypeptide.

20. The method of Claim 17, wherein the vaccine comprises an immunologically active *Enterovirus* VP3 polypeptide.

21. The method of Claim 17, wherein the vaccine comprises an immunologically active *Enterovirus* VP2 polypeptide.

22. The method of Claim 17, wherein the one or more immunologically active antigens comprising one or more human *Enterovirus* polypeptides selected from VP0, VP1, VP2, VP3, VP4, and immunologically active fragments thereof, are in the form of a virus-like particle, capsomer, complex and/or aggregate.

23. The method of Claim 22, wherein the virus-like particle comprises an immunologically active *Enterovirus* VP0 polypeptide.

24. The method of Claim 22, wherein the virus-like particle comprises an immunologically active *Enterovirus* VP2 polypeptide.

25. The method of Claim 22, wherein the virus-like particle comprises an immunologically active *Enterovirus* VP3 polypeptide.

26. The method of Claim 22, wherein the virus-like particle comprises an immunologically active *Enterovirus* VP1 polypeptide.

27. The method of Claim 17, wherein the vaccine comprises polypeptides from one or more *Enterovirus* species or serotype.

28. The method of Claim 27, wherein the *Enterovirus* species or serotype may be *Human enterovirus A* selected from HEV71 and *Coxsackievirus* A16.

29. The method of Claim 27, wherein the *Enterovirus* species or serotype may be *Human enterovirus C* selected from PV-1, PV-2 and PV-3.

30. An expression cassette comprising a promoter operably linked to a nucleic acid encoding a human *Enterovirus* P1 polypeptide, an Internal Ribosome Entry Site (IRES), and a nucleic acid encoding a human *Enterovirus* 3CD protease.

31. The expression cassette of Claim 30, wherein the polypeptide is human *Enterovirus* P1.

32. The expression cassette of Claim 30, wherein the human *Enterovirus* 3CD protease processes the human *Enterovirus* P1 polypeptide.

33. The expression cassette of Claim 32, wherein the processed human *Enterovirus* P1 polypeptides associate to form virus like particles, capsomers, complexes and/or aggregates.

34. The expression cassette of Claim 30, wherein the human *Enterovirus* P1 polypeptide comprises combinations of polypeptides selected fromVP0, VP1, VP2, VP3, VP4, and immunologically active fragments thereof.

35. The expression cassette of Claim 30, wherein the human *Enterovirus* is selected from *Human enterovirus A* and *Human enterovirus C*.

36. The expression cassette of Claim 35, wherein the *Human Enterovirus* A is selected from HEV71 and *Coxsackievirus* A16.

37. The expression cassette of Claim 35, wherein the *Human Enterovirus* C is selected from Human poliovirus 1 (PV-1), Human poliovirus 2 (PV-2) and Human poliovirus 3 (PV-3).

38. The expression cassette of Claim 30, wherein the IRES is derived from *Encephalomyocarditis virus* (EMCV).

39. The expression cassette of Claim 38, wherein the IRES derived from *Encephalomyocarditis virus* (EMCV) has been genetically modified to reduce IRES activity.

40. The expression cassette of Claim 39, wherein the IRES derived from EMCV has been genetically modified by adding one nucleotide (A7) to the A6 bifurcation loop in the JK segment.

41. The expression cassette of Claim 30, wherein the IRES is derived from a human *Enterovirus*.

42. The expression cassette of Claim 30, wherein the promoter is a eukaryotic promoter.

43. The expression cassette of Claim 42, wherein the eukaryotic promoter is a polyhedrin promoter.

44. The expression cassette of Claim 30, wherein the promoter is operably linked to a nucleic acid encoding a *Human enterovirus A* P1 polypeptide, an EMCV IRES, and a *Human enterovirus A* 3CD protease.

45. The expression cassette of Claim 44, wherein the EMCV IRES has been mutated to reduce IRES activity.

46. The expression cassette of Claim 30, wherein the promoter is operably linked to a nucleic acid encoding a *Human enterovirus A* P1 polypeptide, an HEV71 IRES, and a *Human enterovirus A* 3CD protease.

47. The expression cassette of Claim 30, wherein the promoter is operably linked to a nucleic acid encoding a *Human enterovirus A* P1 polypeptide, a *Human enterovirus C* IRES, and a *Human enterovirus A* 3CD protease.

48. The expression cassette of Claim 30, wherein the promoter is operably linked to a nucleic acid encoding a *Human enterovirus C* P1 polypeptide, a *Human enterovirus C* IRES, and a *Human enterovirus C* 3CD protease.

49. The expression cassette of Claim 30, wherein the promoter is operably linked to a nucleic acid encoding a *Human enterovirus C* P1 polypeptide, an HEV71IRES, and a *Human enterovirus C* 3CD protease.

50. The expression cassette of Claim 30, wherein the promoter is operably linked to a nucleic acid encoding a *Human enterovirus C* P1 polypeptide, an EMCV IRES, and a *Human enterovirus C* 3CD protease.

51. A method of making a vaccine comprising introducing the expression cassette of Claim 30 into a host cell, culturing the host cell for a period of time sufficient to produce the polypeptides of the expression cassette and recovering human *Enterovirus* polypeptides from the host cell and/or culture supernatant.

52. The method of Claim 51, wherein the host cell is a eukaryotic cell.

53. The method of Claim 52, wherein the eukaryotic cell is selected from insect cells, mammalian cell lines and yeast cells.

54. The method of Claim 53, wherein the insect cells are selected from *Spodoptera frugiperda*, *Trichoplusia ni*, drosophila, and mosquito cells derived from *Aedes albopictus*.

55. The method of Claim 53, wherein the mammalian cell lines are selected from CHO, HEK 293, COS-1, HeLa, Vero and NIH3T3.