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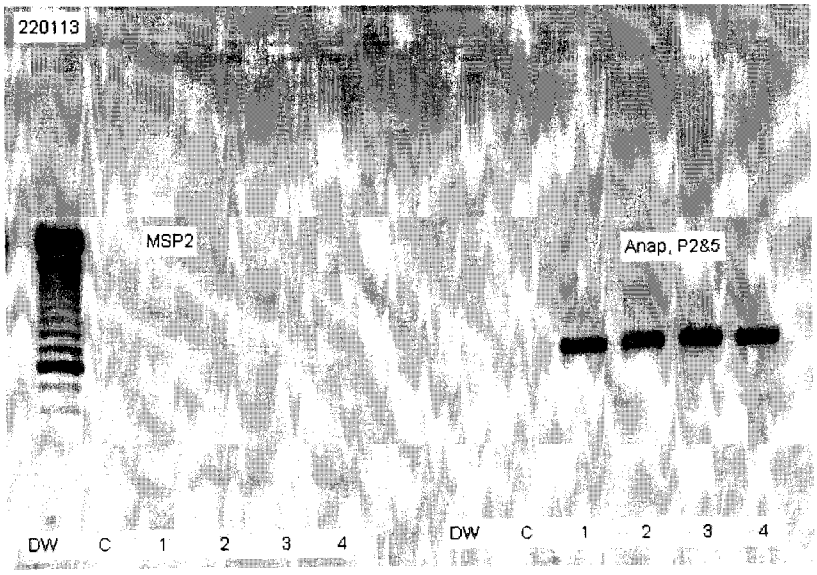
(54) Title: DETECTION OF DNA SEQUENCES AS RISK FACTORS FOR HIV INFECTION

Figure 1(A)



(57) Abstract: A method for identifying a risk factor for diseases, disorders or conditions, such as those caused by human immunodeficiency virus, using the polymerase chain reaction and specific primers. Methods for treating patients having these diseases, disorders or conditions by antimicrobial treatment of the risk factor by combined antiviral and antibacterial treatment or by sustaining or stimulating the subject's immune system. Methods for screening biological products including red blood cell preparations. Primers and methods for detecting nucleic acids or microbial agents associated with red blood cells, such as those associated with red blood cells in subjects infected with HIV and undergoing antiretroviral therapy.

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AMENDED CLAIMS
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1. An agent

(i) that is associated with red blood cells,

(ii) that passes through a 0.45 micron filter; and

(iii) that comprises DNA that is at least 95% identical to a DNA fragment on human chromosome 1 or on human chromosome 7 that is amplified from a sample of human red blood cells by (i) primer pairs 1 (SEQ ID NOS: 3 and 4) or 2 (SEQ ID NOS: 5 and 6), (ii) a pair of primers described by Appendix 3 (SEQ ID NOS: 7-14 or SEQ ID NOS: 15-23), or (iii) the pair primers described in Appendix 4 (SEQ ID NOS: 24 and 25).

2. The agent of claim 1 that is sensitive to antibiotics.

3. The agent of claim 1 that is sensitive to azithromycin or to a cyclin antibiotic.

4. The agent of claim 1 that comprises DNA that is at least 95% identical to a DNA fragment on human chromosome 1 or on human chromosome 7 that is amplified from a sample of human red blood cells from a subject infected with human immunodeficiency virus.

5. (Cancelled)

6. (Cancelled)

7. The agent of claim 1 that contains DNA that is amplified by a pair of primers described by Appendix 3 (SEQ ID NOS: 7-14 or SEQ ID NOS: 15-23).

8. The agent of claim 1 that contains DNA that is amplified by a pair of primers described by Appendix 4 (SEQ ID NOS: 24 and 25).

9. The agent of claim 1 that contains DNA that is amplified by primer pairs 1 (SEQ ID NOS: 3 and 4) or 2 (SEQ ID NOS: 5 and 6) and that is at least 95% identical to human DNA.

10. The agent of claim 1 that contains DNA that is amplified by primer pairs 1 (SEQ ID NOS: 3 and 4) or 2 (SEQ ID NOS: 5 and 6) and that is 99% identical to human DNA.

11. The agent of claim 1 that is found in the red blood cells of an HIV-infected patient, but not detectable in the white blood cells of said patient.

12. The agent of claim 1 that becomes detectable in the red blood cells of an HIV-infected patient within the first year after HIV infection or after initiation of anti-retroviral treatment.

13. The agent of claim 1 that is detectable in the red blood cells of an African subject who is HIV-negative or has lived in Africa.

14. (Cancelled)

15. The agent of claim 1 that is a microorganism.

16. The agent of claim 1 that is a bacterium.

17. An isolated red blood cell that comprises the agent of claim 1.

18. A supernatant that comprises the agent of claim 1, which supernatant is produced by freezing and thawing red blood cells after removing white blood cells and then removing material that pellets by centrifugation for 10 mins at 1,500 g.

19. An isolated red blood cell that contains DNA that is at least 95% identical to a DNA fragment of human chromosome 1 or human chromosome 7 that is amplified by (i) primer pairs 1 (SEQ ID NOS: 3 and 4) or 2 (SEQ ID NOS: 5 and 6), (ii) a pair of primers described by Appendix 3 (SEQ ID NOS: 7-14 or SEQ ID NOS: 15-23), or (iii), the pair of primers described in Appendix 4 (SEQ ID NOS: 24 and 25).

20. Isolated or purified DNA that is produced by amplifying DNA that is at least 95% identical to a DNA fragment on human chromosome 1 or human chromosome 7 from a human red blood cell using (i) primer pairs 1 (SEQ ID NOS: 3 and 4) or 2 (SEQ ID NOS: 5 and 6), (ii) primers described by Appendix 3 (SEQ ID NOS: 7-14 or SEQ ID NOS: 15-23), or (iii), the primers described in Appendix 4 (SEQ ID NOS: 24 and 25).

21. The isolated or purified DNA of claim 20 that comprises a DNA sequence or fragment thereof described by Appendix 5.

22. A vector containing the isolated or purified DNA of claim 20.

23. A host cell transformed with the isolated or purified DNA of claim 20.

24. A method for detecting the agent of claim 1 comprising:

contacting material from red blood cells of a subject with (i) primer pairs 1 (SEQ ID NOS: 3 and 4) or 2 (SEQ ID NOS: 5 and 6), (ii) primers described by Appendix 3 (SEQ ID NOS: 7-14 or SEQ ID NOS: 15-23), or (iii), the primers described in Appendix 4 (SEQ ID NOS: 24 and 25) under conditions suitable for amplification of DNA that is at least 95% identical to a DNA fragment of human chromosome 1 or human chromosome 7 by said primers, and

detecting said agent when DNA is amplified.

25. The method of claim 24, wherein said primers are selected from the group consisting of:

pair 1:

5' GCCTA CAGAT TAAAG GCT (SEQ ID NO: 3)
5' ATCAT ARTCA CCATC ACCTA (SEQ ID NO: 4);
pair 2:
5' CYTAC AGAGT GAAGG CT (SEQ ID NO: 5)
5' ATCAT ARTCA CCATC ACCTA (SEQ ID NO: 6).

26. The method of claim 24, wherein said primers are selected from the group consisting of primers 1-8 for human chromosome 1 (SEQ ID NOS: 7-14) described in Appendix 3 or from a pair of primers that amplify at least twenty consecutive nucleotides of the DNA amplified by said primers 1-8.

27. The method of claim 24, wherein said primers are selected from the group consisting of primers 1-9 for human chromosome 7 (SEQ ID NOS: 15-23) described in Appendix 3 or from a pair of primers that amplify at least twenty consecutive nucleotides of the DNA amplified by said primers 1-9.

28. The method of claim 24, wherein said primers are selected from the group consisting of the primers described in Appendix 4 (SEQ ID NOS: 24 and 25) or from a pair of primers that amplify at least twenty consecutive nucleotides of the DNA amplified by said primers.

29. The method of claim 24, wherein said biological sample is whole blood or a cellular component of whole blood.

30.: The method of claim 24, wherein said biological sample is isolated anucleated red blood cells.

31. The method of claim 24, wherein said biological sample comprises an isolated erythroblast or other precursor cell of an anucleated red blood cell.

32. The method of claim 24, wherein said biological sample comprises isolated

bone marrow cells.

33. The method of claim 24, wherein said biological sample is a red blood cell lysate.

34. The method of claim 24, wherein said biological sample is blood plasma or serum.

35. A method for treating or for reducing the severity of a disease, disorder, or condition associated with the agent of claim 1 comprising treating a patient with an agent that reduces the titer of said agent or that reduces the amount of DNA amplified from a cell associated with said agent.

36. The method of claim 35 comprising treating the patient with one or more antibiotics.

37. The method of claim 35 comprising treating the patient with an azithromycin or a cyclin antibiotic.

38. The method of claim 35 comprising treating the patient with one or more natural immunostimulants, active vaccines, passive vaccines, antioxidants or antibiotics.

39. The method of claim 35, wherein the patient is HIV-negative.

40. The method of claim 35, wherein the subject is African or has lived in Africa.

41. The method of claim 35, wherein the subject is Caucasian or has lived in Europe.

42. The method of claim 35, wherein the patient is HIV-positive.

43. The method of claim 35, wherein said patient is HIV-positive and has or is undergoing antiretroviral therapy or therapy to decrease human immunodeficiency virus infection.

44. A method for treating a disease, disorder or condition associated with the agent of claim 1 comprising contacting red blood cells with a substance that reduces the amount of DNA (i) that is at least 95% identical to a DNA fragment of human chromosome 1 or human chromosome 7 amplified from a red blood cell using Primer Pair 1 or 2, primers described by Appendix 3, primers described by Appendix 4 or primers that amplify a portion of the same DNA as said primers.

45. A method for treating a disease, disorder or condition associated with the agent of claim 1 comprising contacting red blood cells of a subject with a substance that reduces the transmission of said agent ~~to the~~ between red blood cells.

46. A method for treating a disease, disorder or condition associated with the agent of claim 1 comprising replacing the red blood cells in a subject with red blood cells that are not associated with said agent or by stimulating the development of new red blood cells in said subject.

47. A method for treating a disease, disorder or condition associated with the agent of claim 1 comprising treating red blood cells with an agent that degrades nucleic acids inside of or associated with a red blood cell.

48. A method for screening blood for red blood cells from which DNA can be amplified using (i) primer pairs 1 (SEQ ID NOS: 3 and 4) or 2 (SEQ ID NOS: 5 and 6), (ii) a pair of primers described by Appendix 3 (SEQ ID NOS: 7-14 or SEQ ID NOS: 15-23), (iii), the pair of primers described in Appendix 4 (SEQ ID NOS: 24 and 25); or a pair primers that amplify at least twenty consecutive nucleotides of the same DNA as said primers, comprising contacting a sample of red blood cells with said primers and detecting amplified DNA that is at least 95% identical to a DNA fragment of human

chromosome 1 or human chromosome 7 and selecting a blood sample from which said DNA was amplified or alternatively selecting a blood sample from which no DNA was amplified.

49. An isolated or purified DNA molecule comprising the about 700 bp polynucleotide amplified by the primer pair described by SEQ ID NOS: 24 and 25.

50. The isolated red blood cell of claim 19 that is obtained from a subject infected with human immunodeficiency virus.

51. The isolated or purified DNA of claim 20 that was amplified from the red blood cells of a subject infected with human immunodeficiency virus.

52. An agent

(i) that is associated with red blood cells,

(ii) that passes through a 0.45 micron filter; and

(iii) comprises DNA that is amplified by the primers described by SEQ ID NO: 1 and SEQ ID NO: 2.

53. A method for detecting an agent associated with risk of or progression of HIV infection or opportunistic infection associated with HIV

comprising:

contacting DNA isolated from a red blood cell fraction with the primers described by SEQ ID NO: 1 and SEQ ID NO: 2, and

detecting DNA amplified by these primers.