SOLE INVENTOR

~~113173~~
202874

APPLICATION FOR
UNITED STATES LETTERS PATENT

SPECIFICATION

TO ALL WHOM IT MAY CONCERN:

Be it known that I, FU-KUEN LIN

a citizen of the United States, residing at 438 Thunderhead Street, Thousand Oaks,

in the County of Ventura and State of California

have invented a new and useful "PRODUCTION OF ERYTHROPOIETIN"

of which the following is a specification.

WHAT IS CLAIMED IS:

1. A purified and isolated polypeptide having part or all of the primary structural conformation and one or more of the biological properties of naturally-occurring erythropoietin and characterized by being the product of procaryotic or eucaryotic expression of an exogenous DNA sequence.

2. A polypeptide according to claim 1 further characterized by being free of association with any mammalian protein.

3. A polypeptide according to claim 1 wherein the exogenous DNA sequence is a cDNA sequence.

4. A polypeptide according to claim 1 wherein the exogenous DNA sequence is a manufactured DNA sequence.

5. A polypeptide according to claim 1 wherein the exogenous DNA sequence is a genomic DNA sequence.

6. A polypeptide according to claim 1 wherein the exogenous DNA sequence is carried on an autonomously replicating circular DNA plasmid or viral vector.

7. A polypeptide according to claim 1 possessing part or all of the primary structural conformation of human erythropoietin as set forth in Table VI or any naturally occurring allelic variant thereof.

8. A polypeptide according to claim 1 possessing part or all of the primary structural conformation of monkey erythropoietin as set forth in Table V or any naturally occurring allelic variant thereof.

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9. A polypeptide according to claim 1 which has the immunological properties of naturally-occurring erythropoietin.

10. A polypeptide according to claim 1 which has the in vivo biological activity of naturally-occurring erythropoietin.

11. A polypeptide according to claim 1 which has the in vitro biological activity of naturally-occurring erythropoietin.

12. A polypeptide according to claim 1 further characterized by being covalently associated with a detectable label substance.

13. A polypeptide according to claim 12 wherein said detectable label is a radiolabel.

14. A DNA sequence for use in securing expression in a procaryotic or eucaryotic host cell of a polypeptide product having at least a part of the primary structural conformation and one or more of the biological properties of naturally-occurring erythropoietin, said DNA sequence selected from among:

(a) the DNA sequences set out in Tables V and VI or their complementary strands;

(b) DNA sequences which hybridize to the DNA sequences defined in (a) or fragments thereof; and

(c) DNA sequences which, but for the degeneracy of the genetic code, would hybridize to the DNA sequences defined in (a) and (b).

15. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according

to claim 14 in a manner allowing the host cell to express said polypeptide product.

16. A polypeptide product of the expression of a DNA sequence of claim 14 in a procaryotic or eucaryotic host.

17. A purified and isolated DNA sequence coding for procaryotic or eucaryotic host expression of a polypeptide having part or all of the primary structural conformation and one or more of the biological properties of erythropoietin.

18. A cDNA sequence according to claim 17.

19. A monkey species erythropoietin coding DNA sequence according to claim 18.

20. A DNA sequence according to claim 19 and including the protein coding region set forth in Table V.

21. A genomic DNA sequence according to claim 17.

22. A human species erythropoietin coding DNA sequence according to claim 21.

23. A DNA sequence according to claim 22 and including the protein coding region set forth in Table VI.

24. A manufactured DNA sequence according to claim 14.

25. A manufactured DNA sequence according to claim 24 and including one or more codons preferred for expression in E.coli cells.

26. A manufactured DNA sequence according to claim 25, coding for expression of human species erythropoietin.

27. A manufactured DNA sequence according to claim 26 including the protein coding region set forth in Table XIV.

28. A manufactured DNA sequence according to claim 24 and including one or more codons preferred for expression in yeast cells.

29. A manufactured DNA sequence according to claim 28, coding for expression of human species erythropoietin.

30. A manufactured DNA sequence according to claim 29 including the protein coding region set forth in Table XXI.

31. A DNA sequence according to claim 17 covalently associated with a detectable label substance.

32. A DNA sequence according to claim 31 wherein the detectable label is a radiolabel.

33. A single-strand DNA sequence according to claim 31.

34. A DNA sequence coding for a polypeptide fragment or polypeptide analog of naturally-occurring erythropoietin.

35. A DNA sequence coding for [Phe^{15}]hEPO, [Phe^{49}]hEPO, [Phe^{145}]hEPO, [His^{7}]hEPO, [Asp^{2} des-Pro^{2} through Ile^{6}]hEPO, [des-Thr^{163} through Arg^{166}]hEPO, or [Δ27-55]hEPO.

36. A DNA sequence according to claim 34 which is a manufactured sequence.

37. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to either of claims 14, 17, 34 or 35.

38. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to claim 37.

39. A polypeptide product of the expression in a procaryotic or eucaryotic host cell of a DNA sequence according to claims 17 or 34.

40. A glycoprotein product having a primary structural conformation sufficiently duplicative of that of a naturally-occurring erythropoietin to allow possession of one or more of the biological properties thereof and having an average carbohydrate composition which differs from that of naturally-occurring erythropoietin.

41. A glycoprotein product having a primary structural conformation sufficiently duplicative of that of a naturally-occurring human erythropoietin to allow possession of one or more of the biological properties thereof and having an average carbohydrate composition which differs from that of naturally-occurring human erythropoietin.

42. Vertebrate cells which can be propagated in vitro continuously and which upon growth in culture are capable of producing in the medium of their growth in excess of 100 U of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay.

43. Vertebrate cells according to claim 42 capable of producing in excess of 500 U erythropoietin per 10^6 cells in 48 hours.

44. Vertebrate cells according to claim 42 capable of producing in excess of 1,000 U erythropoietin per 10^6 cells in 48 hours.

45. Vertebrate cells according to claim 42 which are mammalian or avian cells.

46. Vertebrate cells according to claim 45 which are COS-1 cells or CHO cells.

47. A synthetic polypeptide having part or all of the amino acid sequence as set forth in Figure 5 ~~Table V~~ and having one or more of the in vivo or in vitro biological activities of naturally-occurring monkey erythropoietin.

48. A synthetic polypeptide having part or all of the amino acid sequence set forth in Figure 6 ~~Table VI~~, other than a sequence of residues entirely within the sequence numbered 1 through 20, and having a biological property of naturally-occurring human erythropoietin.

49. A synthetic polypeptide having part or all of the secondary conformation of part or all of the amino acid sequence set forth in Figure 6 ~~Table VI~~, other than a sequence of residues entirely within the sequence numbered 1 through 20, and having a biological property of naturally-occurring human erythropoietin.

50. A process for the production of a polypeptide having part or all of the primary structural conformation and one or more of the biological properties of naturally-occurring erythropoietin, said process comprising:

growing, under suitable nutrient conditions, procaryotic or eucaryotic host cells transformed or transfected with a DNA vector according to claim 37 and isolating desired polypeptide products of the expression of DNA sequences in said vector.

51. An antibody substance characterized by immunoreactivity with erythropoietin and with a synthetic polypeptide having a primary structural conformation substantially duplicative of a continuous sequence of amino acid residues extant in naturally-occurring erythropoietin except for any polypeptide comprising a sequence of amino acid residues entirely comprehended within sequence,
A-P-P-R-L-I-C-D-S-R-V-L-E-R-Y-L-L-E-A-K.

52. An antibody according to claim 51, which is a monoclonal antibody.

53. An antibody according to claim 51, which is a polyclonal antibody.

54. An antibody according to claim 51, which is immunoreactive with erythropoietin and a synthetic polypeptide having the sequence selected from the sequences:
V-P-D-T-K-V-N-F-Y-A-W-K-R-M-E-V-G,
K-E-A-I-S-P-P-D-A-A-S-A-A, and
V-Y-S-N-F-L-R-G-K-L-K-L-Y-T-G-E-A-C-R-T-G-D-R.

55. A pharmaceutical composition comprising an effective amount of a polypeptide according to claims 1, 16, 39, 40 or 41 and a pharmaceutically acceptable diluent, adjuvant or carrier.

56. A method for providing erythropoietin therapy to a mammal comprising administering an effective amount of a polypeptide according to claims 1, 16, 39, 40 or 41.

57. A method according to claim 56 wherein the therapy comprises enhancing hematocrit levels.

58. A purified and isolated DNA sequence as set out in Table V or VI or a fragment thereof or the complementary strand of such a sequence or fragment.

59. A polypeptide product of the expression of a DNA sequence according to claim 58 in a procaryotic or eucaryotic host cell.

60. An improvement in the method for detection of a specific single stranded polynucleotide of unknown sequence in a heterogeneous cellular or viral sample including multiple single-stranded polynucleotides wherein:

(a) a mixture of labelled single-stranded polynucleotide probes is prepared having uniformly varying sequences of bases, each of said probes being potentially specifically complementary to a sequence of bases which is putatively unique to the polynucleotide to be detected,

(b) the sample is fixed to a solid substrate;

(c) the substrate having the sample fixed thereto is treated to diminish further binding of polynucleotides thereto except by way of hybridization to polynucleotides in said sample,

(d) the treated substrate having the sample fixed thereto is transitorily contacted with said mixture of labelled probes under conditions facilitative of hybridization only between totally complementary polynucleotides, and,

(e) the specific polynucleotide is detected by monitoring for the presence of a hybridization reaction between it and a totally complementary probe within said mixture of labelled probes, as evidenced by the presence of a higher density of labelled material on the substrate at the locus of the specific polynucleotide in comparison to a background density of labelled material resulting from non-specific binding of labelled probes to the substrate,

said improvement comprising using in excess of 32 mixed probes and performance of one or more of the following:

(1) employing a nylon-based paper as said solid substrate;

(2) treating with a protease in step (c);

(3) employing individual labelled probe concentrations of approximately 0.025 picomoles; and

(4) employing as one of the hybridization conditions in step (d) stringent temperatures approaching to with 4°C away from the lowest calculated Td of any of the probes employed.

add C3

add m1

Add D', F', I'

add I2.5

add K'

add L'