(54) HUMAN HOOKWORM VACCINE

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(58) Field of Classification Search: None

(65) Prior Publication Data

(60) Provisional application No. 60/862,916, filed on Oct. 25, 2006.

(51) Int. Cl. A61K 39/00 A61K 39/002 (2006.01)

(52) U.S. Cl. 424/191.1; 424/184.1; 424/185.1; 530/350

(56) References Cited

U.S. PATENT DOCUMENTS

FOREIGN PATENT DOCUMENTS

* cited by examiner

Primary Examiner — Oluwatosi Ogunbiyi

ABSTRACT

A vaccine for human hookworm is provided. The vaccine comprises at least one L3 larval stage antigen (e.g. Na-ASP-2 or Na-SAA-2) and at least one adult stage human hookworm antigen (e.g. Na-APR-1, Na-CP-2, Na-CP-3, Na-CP-4, Na-CP-5, or Na-GST-1) and adjuvants.

1 Claim, 21 Drawing Sheets
A.

gaaatgctca atgatgcg ccgctcct gcgcctcac ccgccgcctcgcc 
tcctgcgct cacgcgctg ccgctcct gcgcctcac ccgccgcctcgcc 
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agagtaggctgcctgcgct gcgcctcac ccgccgcctcgcc 
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agagtaggctgcctgcgct gcgcctcac ccgccgcctcgcc 
agagtaggctgcctgcgct gcgcctc...
SEQ ID NO: 7

SEQ ID NO: 8
A.

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TCTGTTCCAGAAGAGATTTTCTACTAGAGAACACGGTTACTTCCAGTTTCCC
TTTTAGCAGAAACGCTATCTTTGGAGAGGAGGTATGAGTTGAGCCTCGCTCACTGCTAGGACAGCTAC
CAAAACAAGAAGATCTTCAACAGAAAGAAGATTTTCTGCTAGCTAGCGCTAAATAAGACTGCT
CTACAGTTTCCCACTTGCTAAGCAGAATGAGAATGAGTTGGGAAATTATATGAGATGCTCAA
ATTTATAGAGCTATTTCAAAATTTGGAAACACCGACTCAAAATTTACGTATTTTTGTGACACT
GGATCTCTCAGACTTGGGAGTCTTCT/GCAAGAAATGCTCCTTCTATGAGATAGCTGATGATG
TTCGACCAAGAGATACACGCCTGGAGGCTCTCTCCACAGTCAAAGAAGAGTTGGGAGAAAGATG
GCTATACACAAATGTTGGACAGCTGCATTCGAGAGATTCAATTCCGCAAGAGAATGTTAT
GGAGTGAACACCTGTTTTCCACACCTTTATATTGAGCACAAGAAGAGGTCCCATCACAGGTITT
GCTTTTTGGTTGAATATTTCATGGAACTGACTGAGATGGGAGAAATTTCTTGGTGGAG
GTTGAATACGAGAAGAGATATGTGAACCTATAGTCCTGGACACCTGGTTACAAAGAAGAGGTAT
TGGCAATGCAAATAAGGATAGGTGCTTGATAGCTGGATCTCTTACTTACGTGCTCTTACAGGGT
GTTCAAGCTATTCCAGACTGAACTCCCTTGATTGGCTGCTCAAAAGGCTCAAGTTTCA
GCTATACCAAAAGATATTTCATTGCTGAGGCAGATGAAAGGTAATACAGTGTCCAGTG
GAAAAAGCTCTCCTCCTTTCCAGACCTTTTTCTATTATGAGGAAAACACTTTTACTTTG
AAAGGAGAGGACTACGTGTTTAAAGCTGCTGAAAGCTATTGGTTGGCTGCT
TTTTGCGATCGATTTTCCGAAAAAGATAGGGAGAATGGTGGMTTTGGGAGAGTTTTCTC
ATTGTAATAATCTATATGTCTGTAGTGGCAGACTGAGCTTGGCTGCTCAAGCT
AAATCTGAGAGATGGTTTCCCAGATGTGGACACTGCTAGTTAGAATCTTTAGAACAATTCGAAAGA
GATTCGATCTACGAGAAGATGACCGTTTTTACCTTT
(SEQ ID NO: 9)
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B.

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SVHRRLFHQARHVTSVSLRSQPTLRLERLIASGSWEDYQKQRYHRYKRIAIKYAAKAS
KLQSANEIDBLLRNMYDNAQYGVQ1GTPAQFNTTVIFDGTSSNLWPSROKCPFY1ACML
LHHRVDSQASSTYKEDGRKMAIQ7TGSMG1VPMKDI1ICGAEQFPAEASPGL
TFIAKAFDGCILGMAPEIAVLGTVVPTFPHEEQKGBKTPVFAFWLNRNPESETGGREITFG
GGVDIRYVPEITFPVTIRYQRMMCMVQGGSISIAFNCQCAIAITJSTLIALAGPKA
QVRAIYKGAEP1MCGYMP1PVDPSPLPVPIILGKTFMLKSEDYVLTVRKAMGKSCI
LSSGNGMDPSPEKIGELWILGDFPIQKYTTYVFVDVGQARVGPAQKSEDFGPVFVPRTF
RQLQEDDSDFDSDVFT
(SEQ ID NO: 10)
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A.

TCCTTTTCCAGAAAGAAAGATTTTCTATCAAGCTGAGACAGACACCTTTCTTTCCCTAGGAGATTTTTGCTAGATCATCTCTGCTCCTTGGAGGAAGCTAC
CAAAAAACAGATATCTTATGGAAGAGCAGGTTTTGCTAGATCAAGCTGCTAAAATAGCT
TCCTAACTGGTAACTGCTGAAGCTGAATCTGAAATCTTGTGAGAAAATTATATCATCTCTCAA
TATTATGGAGTTATTCCAAATTGGACACCCAGCTCTCAAAAATTCTATTTATGTATTTTCTAG
AAGGGTTCTCCCTCTTTGCTCTTCTCAAGAAATGTTGCTTTCTCAAGACTTACAT
CTGACACCACAGATAAGACAATTGAACTCTTCTGAGGACTCCATATAAAAGAGATGAGAAGAGCT
GCTATTGCAAATATGTGACAGTGCTCTCAATGAAAGTTCATTCTCTTCACTTCAGAGATGAT
TTACTGTCTCTGCCTGCTGAGAAACATCTTTTGCTGAGCTATTCAAGCCAGAGGATTGACT
TCTATCTGCTGCTAAAGTTTGGAAATTGTTGGAATTTCTGGGAGTGTTTCTCTCGGCTG
GGATTAGCACCCCTTTTTTTGTTCCACACTTTTATTGGACAAAGAAGGTTGTTGCTACACCCAGAT
GCTTTTTTGGTGAATAGAAATCTCAGGTGGAGAGATTTTTTGCTGAGTGAAGTTTCTACTACAT
GTTACTAGAAGATGATTGGAAACATAGACTTGGGACAGCTGTTTACAAAAGAGATGATAT
TGAGCAATACAAAAGTGAATGTGCTCAAGGGAGTCTGCTTCTCTACTATGGCTGGTGCCTACGCTG
GTGAGCTATGCTGCAACTGGGAACTTTCTTGGATTGTCGTGCTTCCAGAAAGCTCAAGGTGA
GCTATGCAAAAGTTATATTGGCTGAGGGCCATTGGAGAAGTCCACTCAGGGT
GNNAGATGACAAGCTTCTCTTGGCAAGCACTTTTATTGGACAAAGAAGGTTGTTGCTACACCCAGAT
GCTTTTTTGGTGAATAGAAATCTCAGGTGGAGAGATTTTTTGCTGAGTGAAGTTTCTACTACAT
GTTACTAGAAGATGATTGGAAACATAGACTTGGGACAGCTGTTTACAAAAGAGATGATAT
TGAGCAATACAAAAGTGAATGTGCTCAAGGGAGTCTGCTTCTCTACTATGGCTGGTGCCTACGCTG
TTTATGAGCTATGCAAAATTTTTCTGGGAAATTGTTGGAATTTCTGGGAGTGTTTCTCTCGGCTG
GGATTAGCACCCCTTTTTTTGTTCCACACTTTTATTGGACAAAGAAGGTTGTTGCTACACCCAGAT
GCTTTTTTGGTGAATAGAAATCTCAGGTGGAGAGATTTTTTGCTGAGTGAAGTTTCTACTACAT
GTTACTAGAAGATGATTGGAAACATAGACTTGGGACAGCTGTTTACAAAAGAGATGATAT
TGAGCAATACAAAAGTGAATGTGCTCAAGGGAGTCTGCTTCTCTACTATGGCTGGTGCCTACGCTG

(SEQ ID NO: 11)

B.

SVHRRFLHQRHVTSSLVSLSRQPITLRELIAAGSWEDYQKRYHRYRKILAKAYANKAS
KLQSANEIDEULLRNMDAQYYGVIQICTPAQNFVTIPDTRGSNVLWPSRKPFPVYIDACM
LHRHRYSGASSTYKEDGRKMAIQYTGMSKPISKDIYCIAGICAEBQPFPARATSRPGL
TFIAAKEDGILMGAFPEIATLAVGTVPTFEFTFIIQKKVPSVPAFHLWNRNPEBHDICEFIT
GCVDTRRYVEPTTWTPTVRTRGWQKMDMVQGGSSSIAAYNCQAIAGTSTLIAGPKA
QEVAIQKYIGAEFLNMEYMYPCDKVSPSLPDSFIDGIKTFFLGEDYVLTVEKAAGKSI
CLSLGRMDPEPKIGELWILGDVFICGYYTVPDVGQARSVGFAQAUESDGPFPVPTKFT
RQILQEDSDSDEDVDVT

(SEQ ID NO: 14)
A.

TCTGTTCAAGAGATGTTCATGACAGTGAGACCTAATTTACTCTGTCTCTGATTGGTTAGGAAGTGACTAC
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TCGAACTTGGAATCTGCTACAGAGTTAGTTGAAATATATGTTGAGCTCAAT
ATATGGAGTATATTACAAAATTGGAGACCCGCTTCGAAATTTCACGGTTTTCCGACGACT
GGGATCTGCAAATTTGTTGCTGCTGAGGAAAGCAAGCTTGTTGAGCCTTTTCTGCTGAGGCTACTCTGCAAGCCAGATTGAC
TTCATTCTGCTACTAGAAGGATAAGGATTTTGGGTAATTGCTCTCTCGAATAATTTGTGTTCTTGGA
GAGTCACACCTGTTTACCTACACTTTATGGAAGCAAAAGAGGGTTCCATTCAACACCTGGTTT
GCTITTTGGTTGAAATAGAAATGGTCAACTGAGATTGGAAGAGATTTACTCTTGTTGTTGGA
GGAGATCTGAAAGATATGTTGAAACCTATTACTTTTGAAACACCTTGTTACAAAGAGAGTTAT
GCTGCAATACAAAATGGAATATGCTCAGAGAAGTCATTCTTCTGATTGGCTGCTCAAAAGGCTCAAAGTTGAA
GCTATTACAAATATATTGCTGCTACGCTAGAAGGAAAGGATACATGATTTCTTGTA
GAAAAGATCTCTCCTTCTTGGCAGACTCTTTTATTATGGAAGCAAAACCTTTTACTTT
AAAGAGGAGGAGACTACCTTTGTTAAAGCTCTCGGAAGATCTATTTTTGTTCTGCTGG
TTTAGAGTTATGAGTTTGGCAGAAAGATTTGGAAGATTTGGAATTTTGGGAGATTGTTTTCG
ATTAGGAAATACAACTATCTTTTGTTGGTCTGCAAGCAGAGTTGTTTCTGCTCAAGCT
AATGCTGAAAGGATTCTCTCCAGGACTTGTTGAGCAACCTGTCTTTTACACATGAAAGAAGGATGCT
GACTCGTACGAGCCAGATGACTTTTACTTTT

B.

SVHRRLFHQARPRHVTUAVSLRQPTELRELIASGSGSMEDYQKQKYHRFKKILAKYANKAS
KLQSANIDELERNYMDAQYYGYIQIGTPAQNTVTFPAATGSSNLWVPSRKCPYDIACM
LHRRYDGSASSTYKEDGRKMAIQYGGSMSKFGISKDVICIAGICAEPQPAEBASEPSG
LFIAAIAKFDGTLGAMPEAFAEIVGTYVFHPFLQKKVPSVVPFVWNLNNRPSEPSEGIEITFT
GGVDTTRYVYPEITWTTVPTVRQRGYWQFKMDNQVGQGSSSIAACPNCCQAIAGTSILAAGPKA
QVEAIAIYGAEMLKGEMYPCDKVPSLPDVDIPSIDGKPTLKGEDYVLTVKAAKSI
CLSGFGMMDPEKIGELNLLGDFVICQKYTVVDPVGQARVFGPAQKSEDEGFPGTPVRTFRGLOEDSDIDEFFVT

(SEQ ID NO: 15)

Figure 8A and B

(SEQ ID NO: 16)
A.

AAATGATGATGCTTATAGGCTATACCTACTGCTACGCTAGTGGAAGGATGCAGCAGATGCA
AGACAGATCCTCTGACCTCTGACGGTAGCGGATTGCGTGGTGACACAGAGAGCA
GCACGACAATTTGGGATACGAGCCGACTGAGCTGCAATGGCAGAAGCCTTAGG
TGAGAAGCTCAAGAAAGAAGAGATGGTATCTCTGCTGCTGCGATGAAATTCTCCGAGTATC
ACTAATCCCTTAAAGAAGCCCTTCTCTGTTTCTCGGCTGAGCTACCTGACGGCTG
GGACCTCTTGTCCTGGGAGCAATGCTACTCATTAGCTTACGTTCTTCTTGA
AAGCTATATCTGGAAGTAAAGAGGACATGGAAAGAGATCGAGCGATTCGGGAAACCTGAG
AAATGGATGCAAAACCCAGACAGACATCTGCTAATCCAGTGATGTTATCTACTT
GTTACGATCTATTTGAGTTAATCTTCTAGGTCAGAAGAATAATCATATTAGTTCTTCCATTAA
ATAGCCTTTATTCAGGCACTTTGAAATCCACTAGTTATTTCCTTCCATAAGCTACATTCT
CAGATGATGATGAGGATAAAA
(SEQ ID NO: 17)

B.

MVYKLYFAIRGAGECARQIFALADQEPFEDVRLDEKEQFAKYKPDLPFGQVPVLEVDGK
QLAQSLACRYLRQFEGAGKSTFPEAVDSLADQYSYDVEIKSFYTTYVIGMREGDVE
QLKKEVLLPRDKFPQFIKPLKSPSGILGDSLTVWDDLLEHSVHATMLTFVEFPLEG
YEPVEHEKIERAIPTKLKKIETRPFETLF^*
(SEQ ID NO: 18)

Figure 9A and B
A.

GATGTTCAATTACAAGCTCACTTACTTTCACTTGCGGCGCCGTGGGACTTTGTGCTGAACCTATTTGCC
AGATTTTGCCTTTGCTGTGCAAAATAATGAGATGTTGTTTACCTTTCAGGAATGG
CCCAAACAAGGAGTGAATGCTCAAGTTGTAAGCTTGGAGAGCTATGCAAGAGTTTGA
ACAACCTAGGCAATCATTCAGGCATGCTACGGGTATCAGGAGAGTTGCTGGCC
GAAAAACTCTTCTTGAGAAGAGCTTTAGTGACTCGGCTGGTGCTGACCAATACAGGACTAC
ATCAATGAGATTCGCTCAATACCTCAGGGGTCGTTGCAGGAGTCAGGAGGAGATCCCGA
GAAGCCTTTAAGGAAACTGCTCCTCTCCAGCAGCTCGTGAGAAAATTCTCTCGGGTTTTCAGAAAA
AAATTCCCTGGAGAAGGCAAAATCTGTTTACCTGCGTTGATTCGCTGGCACATACGCTGAC
TTGTCCTTACGGCGACACCACTCTGATCCTGCTGCAAGTGGCCCGCTATCGGATTTGG
TTTCCTGGAGATCAAAGCTCATGCGGAAAAAGGTTCGACGATACCGGGCTCTGGAAAAAT
GGATTTGAACCTCGACCAGCACTAAAATTCTAAATTTTTCCCGAGTTTGTACATATCGT
TCAGAGGGAGTTATAAAATAAGGGTTTTTTTCTTTTTAAAAAATGCTCTTTCTTCGAT
TGCAGGCTACTTTCAAAGCGGTGTG
(SEQ ID NO: 19)

B.

MVHYKLYFAAGRIAEPIQRIFALAGQKYEDVRYTPQESWPKHDEMPPFGQIPVLEEDGK
QLAGSFIAJARYSRKFPACTTFPEEAI.VDSVADQYKDYINEIRPYLRVAGVQDGPE
KLFKELLLPAREKFKGFMMPKFESKSSYVLDSVTVYALCLAEHTSIGAAKPSIYDG
FPEIKAHAEKVRSIPALKKWIETRPETKF*
(SEQ ID NO: 20)

Figure 10A and B
A.

GAAAGTGTTTAAATTACCACCAAGTCTGCTACTACAAGCTAACTATCTTCGACGG
ACGCGGTGCCGCTGAAGATTATTCGCTAGATTTTTGTCTCTTCCTGCTGCCAAATGAATCAGGG
ATATCCGCTTTAGTCACGACGATGGCAGCAAGTTACAAAG AACATGCTCCTGGCTATCAAC
TTGCCAGTGCTTTGGAAGTGCACGCGCAAAAGGACATTGCAAAATCTTTCTCGATACCGCGTT
CGTGCCCAAAATAATCCTGGTTTGCTGGAAAGATGCTGGCTCGTTTGGAAGAGGCTCTGGGTGACT
CGATCACCAGCAGAACATTACAGAGACTTCATCAATGAGATCCGCATCCATTTCTACAGTCTG
ATGGGTTTCGCCAGAGGGAGCTCTGGAGAAAGCTCACGAAAGCTACTTCTGCGCGCTCG
TGAAAGGTTCTTCCGGAATATGCGAAACATACCTTTCTCCATCAAGGAGGCAGAGTCTGCTGTATCTCG
TTGGTGATTCATTGACGGTCAGCAGACCTGACTAGTGCAATGCGCAATGCCATATCGCT
AAGAAAACCTCCGCAAATCTCGACGAGATGCCAAGAAATCAGACCCCATGCCGAAAGAGT
TCCGCAGAAACAGACTCTCAAGAAATGGATGAAAACCGACACAGAAAATCAAATCTCAAAT
TCCTCGCAACTACCTTGTTATTTCCATCGATTCGCCGTCGAATAAAAATAATTGTCTCTCAA
AAAAAAAAAAA

(SEQ ID NO: 21)

B.

MVYKLTDFDRGAAEIIIRQIFVLAGQHVEIDILSHDEHPKYKNFMPFGQLPVLEVDGK
KLAQSFALRARVAKKFGAKCPFEEALVDSITDQYKDFINEIRPFLRVAMCPAECDLE
KLISVFLPAREKFGFMNFLKESKSYLVGDSLTFADLYLABCASEFAKKTPTITFDG
FPEIKHAABKVIORSNPALKWETRPETKFI

(SEQ ID NO: 22)

Figure 11A and B
A.

GTAAAAGCCGCTGTAAGCAACAGGGTTCTTGTATTTAACCTCGTGCACCTCTTGAT
TCTCTGTTCGCCGTTGATGCCAAGGCATTTGAAGTTTCTTCTGTGCTCAAGACGAGCTG
CATATGCCTAGAAGACCTACAGGCGAGGCCCTGTTAGCTACGTCAATTCCGACCAAATCT
CTTCAAACAGCAGGCAAATATTTTACACAGGATGCAAGAATGAAATCTACGATTTA
TTGGGTTCATGATGAGTATTGCGAGATTGCAGAGAAGGATGGGAGCTGCTGCTGAGGTA
TAGATCTCCGTTTTCTTTACCTGGAAGTTTTCGAGCTCGTTGAAAATGCGGAGATAT
CTCTCTCAGTATATTATTCGCCGCTCAGTCCGCTGCGGAGATTTGGCAGTCTCTC
AGCAGAGGCTGATAGCCGAGGATCTGATACACATCAATGCAAAAAGCAGCTGATAT
TTCCGAAACGGGATATCCTTATCTAGTCTCTGAGCAACAGTGCCGGTACGGGTGTACCTCA
GGTCTGACGCTACGGTTTCTCAACTATGCAATTGCTCAGAAGGTTTTACAAGTGAGGGACC
ATATGGAACGAGGGTTTGGAAACCTATCTCTTCTATCATTGCGGCTATACATGCTC
ATCTGCACTATATATACGGTACGTTCGATTAGTTGTGCTAGGCTACCCACACATGCGAAAG
GCATGTCAATCCGACTATACGTTCCGTAACAAAGGATGACAGGATGCTCCGCGACAAAC
TATTGTCTTTCAGGCGAGGAAATTAAAGCAGAGATTTTCAATAAACGCCACATGGG
TAGCCACGTATACAGTTACGAGAGTTTCGCTTATTACACAGATGGAATTACATGACT
GGTCCTCGGTAGACAGGCACAGGCGCAGCAGTCAAAATTATTTGCTGGGTGGAAGGAAAA
TGGAGTCGAACTATTTCGTGGAGACCTGATGGGAGAATGCCGTCTTTCCGATGAAAAA
AGCTTTAAGGTGTAAGCTGATCCGAAAACAGGATATTGTTGCAACAAAACTTCTGCGCTATT

B.

MLTALALLISVLSVLVPTGIQGEFLAQMPAFAYARRRIITTGQALIDYNVNSHISLYKACKYSDDQ
ERMKSCRTMDI.SFMVDAEMMBEENDQEQCDLIDIALVPESFDAREKWPBCCPSIGILIRDQAS
GGCGCAVSAVEVMTRCIIQNSGTTKQVYVSETIDILSCGQRCGSGCTSGVPRQAPNYAI
RRGVCSCGGYPYGTKVCKYPYFPYPCGYHAFGHIFYGFCDGMWFPTCCEKACQSEDYTVFYPN
DDRTPGSKTVILTGEKIKREIFNPGLVATVTYVYEDFARYYNKIGYMTGLGRATGAHAV
KIIGWGEENGVKYWLIESWNTDWGENGFPRMLRGTINLCDIELSAATGGTFK*  

(SEQ ID NO: 23)

Figure 12A and B

(SEQ ID NO: 24)
A.

ttaattctta ttgctcttgtt ggtgacgggcc ttggctcaac aagcggcttttc
actaagggag tatctggaac aggctgaaacc ggagagggca gagaacgtttt
ccggagaagc gtgattgcggag ttctctgaaca aacgcaaatc gttgcttacgc
qctaagtcga cggcaaatgc tttaaatcatt ctttaaatgtc gttgtgatgga
atcgagatct ctcgaccaagtt taagaggttaa aagtctttaaa tattacgaga
ccgataaagg ctagatttaca actatttaca actatttgcag catttttgcag
tggttgggca atttgtggtgc tggatgtggag gaggaccaca caattcgacg
gttgggaatatt tttaagaaca cagcgcctttg cactgccggaa ctatatggga
caaaaggtttc ctgcaaaaaaac tatgctttttt atctatgtaa agacagaaagt
taagaaaaatt gccacaaaggatt tttttttttca acaccaaaaaactgcaaaaaatt
tttgtcagattatatcag cagagataacg cgaagcacaata tctacgacgc
attacgcata tcgaaatccca cagagagatec gttggatcaaa atttgagatc
atggagaacgg gcccttgagac agatcatttc aggtttttag cggatgtttgg
gtttcacgaa aaggagtttt atgtgactttt cagcgggagaag gagctagctttg
ggctacggtat taaatcatt ggtgagggaa cggaaagaatg aaacggaact
caagtgtacctactggttgtat tcgaactctct tgcctgtttacctcgcagttaaa
aaaaa aaaaaaaa aaaaaaa

(SEQ ID NO: 25)

B.

LILIALVVTL LAQQPLSLKE YLEQEPFEKAA ENLGRGAPAR PLMRKQSFPT AKTPKNAVIM
LMMEVQESRF LDNEEGEMLX EEDMDPQERI FYSDARDKW PCTTSIOFIR DQSICGSCWA
VSSATMSIDR LCVOSNNTIIE VLLSSTDDLCA CPGNCQACGC CQHTIRWEEY FKTQVGCTGG
LGYTKDSCKP YAPYTPCKDES YGKCPKDSFPP TKPCRKIQQY KYSKKYADDK YYAKSAAYRP
QNETMIIISI MRGQPTYASPG KYPDCFOFYE KYVYVTQSGK ELOQHAIIKK GWPTKVKYGT
DBLYWLIANS WTDWGSNG YPRILQCNHH COIIEQKVIAP MIVKQIPKSA GPPLQPNPSS

(SEQ ID NO: 26)

Figure 13A and B
A.

tcgttgaggc gttatttca a gttcttcg cctcgatttc agatctcca
atgttttcag tgaatcgtgg aacagtaaat ctcacttttg tggagtcaca
tgaagcctaa tttgcgttg gtcgtgcgctc ttctgcaaat aaccagttta
tatgeaatag acgctgccta ccaacagacg tccgaacacg gaccttagtg
cccaagcgctc gttgacttct ttaattgcga ccaaatcact tccaaaccag
aatattcggc aaccaatgga catttggta aagcccggtat aatggacata
aagatatgca cttgaggctag ccaacacatat ccaagaaggg gcattaactct
gaacggtgcaaa cttccttgaaa ggttgtgcgc acgtgaaagaa tggccacatt
ggctccctcat cggtctcatt ccgctacact cttgcttgccg ctgctgtgtag
ctgttcatcgg csgcgccgctg tttgctgcat cagactctgta tcccagacgaa
ccgcaaccag ccaagatcct tttgcctgac ggacatctctt gcgtgtttgtg
ggagaagcttg tgggccgagga tggagaggcc gttatccgat tcagggcgtac
ttcacactct taaaactctgg aagtatgtag ggaggagagt atccggaaga
aattgtatgc aaaaccatat ctttttatcc gttgtgacgga aactatggac
catgccccaa ggaggttcgc tttgacactc ccaagtgtcg gaaaaatgtg
cagttccgat atctctgttcc atacgagagga gataaagtgt tttggaaaaa
ttcaccacatc ctctctgcaag acaacgcaggc aagaatcaga caggaattt
tcataaaccgg aacagtggga gctaatatat acgttttcga aagactttata
cactcaacgg aagggattta taagcagaca tataagggaat ggtatgaggt
acatgcacatc aataattttagggttggcacg aagaaaatgga acaagattat
ggttgggctgc ttaactcgtac aactcagact gggagagaga tggcacccttc
cgttatttccc gtggaactaa taactcttggg atagaatcac aagtgatcgc
aagggagatg atgtatggaa tggcttaatga acgatttggtgc gcatggcgat
tctctgaagta aataagtgtaa atcagaaaaa a

(SEQ ID NO: 27)

B.

MKANFLVVLVLLAINTQLYADELLHKLQEBHGLSQQALVDYVNSH
QLFKTEYSPTNBQPVTARIMDIKYMTASIIKYPKGRINLVEFLSPRFDSREKWPHTCA
SGLRIEDHSAAGCWAVSAASVM1DR5CQTGNTQXKIXSSADILACCCGEDCGSCEG
GYPQIAFYLBNFVGCGBEYKBNVCKFYPYFPYCDONCGPCFKEAGAFDTPKCRKICQ
FRYPFVYBEDKVPQKNSHILQDNEAIRQEIPIFVPCANYFYVFEDFHYKEQ1YKQ
TYGKWLGVHAIKLIQGVTENGTDYWGTVANVSYDNLOGTFRILRGTNHCLIRSQVIA
TBMIV

(SEQ ID NO: 28)

Figure 14A and B
A.

tagataataa tctttttgcg ccgctcagagaa ttcttttgat aaaccacaaa
ttaacacatc tcagccgctgt caacagcgtgc aaaaactactc gtctatatct
ctctcccttc cccacaacaa ccaccactc agagagcgcg ctttaaccactc
attaccccatc ttgctttgagc ttctacagt gaaagctacta aagtgggagga
gtactttggcc gcacgagctgc cggaaatgct cacaatgatct cacaggaacg
ccctagttga gtcttgtaa ggctacagat ccacaatctta caatcgtatg
ctctcgcgttg gtctgcttagc ctctacagt gaaagctacta aagtgggagga
gcagagcgtcc gaaaccacctg aaatacttc ccgtgacgccactt actttctttc
tacaatagcc ttatatatg ccgttgctgc ccaaaaccac aacacatattc
ggacattgc acatggtggc cagggaaactgc ccaaaattc ccagttctttg
tcgatctcctg ccctctattc ccagttctttg
tgacatctg cccctccctt aatttattttt aagtgggagga
ggcagagcgtcc gaaaccacctg aaatacttc ccgtgacgccactt actttctttc
tcataagga gggtgtaattg ccgtagtgag aattcctgagc aattttcctc
tgatctcctg ccctctattc ccagttctttg
tgacatctg cccctccctt aatttattttt aagtgggagga
ggcagagcgtcc gaaaccacctg aaatacttc ccgtgacgccactt actttctttc
tcataagga gggtgtaattg ccgtagtgag aattcctgagc aattttcctc
(at seq id no: 29)

B.

MITITLTLIATISLTVKSLTVEEYLARPVPPSYATKLTGTQAYVGYVN
QHSQFVKAYQAKVAVMRSEFRMTKPNQNYVVKDEVLNNILPETFARREKWPNC
TSRTRDQSNCSCHVAASASVMRDLCIQSNGTQSWASDDTDLSCCCWWNGKGC
DQRPAFFAIYNDGCTGPPFRENVPCKPYAPFCGHQQNYKFGPCPKELMTPPKCR
KMCQLYKYNVAKDRLYGNADVSLPNNETRMQEFINTNPVGSFSVFADFAIYKGV
YVSNQIQNGCAHAVIKIIGGWGVQDGLKYWLINKSNNDWQDEGYVRFLRCDNHC
CIESR VVTGTMKV
(at seq id no: 30)

Figure 15A and B
A.

acttcaagcg atggtocgct tgtctactgc gtcctttcta ttggtggecg
cgctcagcac atttgctggga tttttcagtt gatggggag gtttaccagtt
tgctaaagat caagatactc ttgagaagaa tcatcacttg gtgaaggtac
ttttgattgc cattaaggag aagcttaaga tgcgtgaaacc gatgggaaca
gagctcagga aagagacatt aggccaggttg gacaaactatc tcagtaagtt
tcacaagttc ggccacaggg taccgcaagga gggtagtaagcg aatattgagg
agaacaaagg gaaatggcag caaatgttga acgtatatctt cgagaaggtt
gacctggacca gcgtgatgaa ttggctcaat ctgaagttcgc gcgtgtggctg
cagttagcc gctgcaacctg tcgctccggt tgggtctcgcg cttcatcctgt
aatcactttc taccgcgccc gactactgtg tttacccttg tgcctgtgtg
tgataagttgg atttggctgat gatgtgtatc tattgttttg gattttatatttt
ttttgtgtac tttccagata tcagtctcgg tatcctgaga cggaccacaca
tcccgcaagt actttttttgt atggttatca tcacgtaaat cctgtgacgt
gcgtaaaatg tttgatttttc cgataaatata aatttgccaa aaaaaaaaaa

(SEQ ID NO: 31)

B.

MFRPATAVLLLSASSTPAGPPDDVGLPLPSGVGDPFTKQFNNV
DLFAKDQDITLKNNLVKLWLWIAKIKAMLEPMANBQKKTQLGFQVQNLNEVQQQFD
QVAKEGSTKFEINKGKWQQMLNDFEKGGSDLVMKLNLKSGGRCIALLAVAPVVLAL
LIR

(SEQ ID NO: 32)

Figure 16A and B
A.

AAAAGGCTCCATAGTCATGCTCAAGCTCGTTGCACTCGTTTGCCTGGTTGCAAATCCTGCTCGCTCAGGAACACAAAGGCCCTCCCTGCTCTGCAAAGTGGCTCCAGCCGCTGTTCAAACAGACTTGCAAGCTCTTTCTGCTCAAATTGCTGCTCCAGACTGATGCGAGAAATCGACAAATAAGCTCCAGAATTGTGTTGGCAAAACCAAAGATGCATCCATCAAGACCGCATCCTGATCGTGTCGTGTCGAGAAGTAAGCGCTCCAGGGCGGTGAAGCTGGCGCTTATCCGCAAGTTCTACGGCAGAGGCCCAGGCGGCTGATGCACAGCTGAGCGCAATTGCGAAGCGCAGAGACAAACGCGCAAAAGGGAGCTGAGATCGACTCGTGACTCAAGGGACTTCTCCTCCAAATGTCGCGACAGACAGTGCAGAAAAGCCATGAAAGATAAGTCCTCTATTTTGATATATGAAACCCGATAAATATGCAACATAA

(SEQ ID NO: 33)

B.

MLKVALVCLVAICFAQGFPQPFLLFSAPAAAQVDPSVKLFVANAGSDAESDKMVQDWVGKQDASIKTAFAFVKEVEKAAQAQGEEAAHQAIAKFSAEAKAADAKLSIAANDRSTNQKGBAKDSVLKGLNPVREIENAMK *

(SEQ ID NO: 34)

Figure 17A and B
<table>
<thead>
<tr>
<th>Antigen</th>
<th>In vitro larval inhibition (0-2)</th>
<th>Fecal egg reduction (0-2)</th>
<th>Blood loss reduction (0-2)</th>
<th>Immunoeopathology Feasibility of expression (0-2)</th>
<th>Orthologous protective antigens (0-1)</th>
<th>Total Score (x10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead Candidates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASP-2</td>
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<td>2</td>
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<td>TBD</td>
<td>TBD</td>
<td>90</td>
</tr>
<tr>
<td>SAA-2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>TBD</td>
<td>TBD</td>
<td>90</td>
</tr>
<tr>
<td>Back-up Candidates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAA-1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ASP-3</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>MTP-1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>40</td>
</tr>
</tbody>
</table>

*Figure 20*
<table>
<thead>
<tr>
<th>Antigen</th>
<th>Total Score (x10)</th>
<th>Immunoepidemiology (0-2)</th>
<th>Feasibility of expression (0-2)</th>
<th>Orthologous protective antigens (0-1)</th>
<th>Blood loss reduction (0-2)</th>
<th>Fecal egg count reduction (0-2)</th>
<th>Warm Burden Reduction (0-2)</th>
<th>Kaoen mechanism (0-2)</th>
<th>Lead Candidates</th>
<th>Back-up Candidates</th>
</tr>
</thead>
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<tr>
<td>CP-213</td>
<td>2</td>
<td>TBD</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>APR-1</td>
<td>Cys</td>
</tr>
<tr>
<td>MEP-1</td>
<td>1</td>
<td>TBD</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>GST-1</td>
<td>CP-213</td>
</tr>
<tr>
<td>TMP-1</td>
<td>0</td>
<td>TBD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>MEP-1</td>
<td>MEP-1</td>
</tr>
<tr>
<td>FAA-1</td>
<td>1</td>
<td>TBD</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<td>Cys</td>
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<tr>
<td>ASP-46</td>
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<td>TBD</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>ASP-46</td>
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<tr>
<td>C-Lectin</td>
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<td>TBD</td>
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<td>0</td>
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<td>C-Lectin</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>AP-1</td>
<td>AP-1</td>
</tr>
</tbody>
</table>
HUMAN HOOKWORM VACCINE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of and is a continuation of U.S. patent application Ser. No. 11/863,912 filed Sep. 28, 2007 now abandoned, the complete contents of which is hereby incorporated by reference. This application also claims benefit of U.S. patent application Ser. No. 10/825,692, filed Apr. 16, 2004, now issued U.S. Pat. No. 7,303,752 and to U.S. provisional patent application 60/862,916, filed Oct. 25, 2006, the complete contents of both of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention generally relates to a vaccine for human hookworm. In particular, the invention provides a human hookworm vaccine comprising an L3 larval stage antigen (e.g. Na-ASP-2 or Na-SAA-A2) and at least one adult stage human hookworm antigen (e.g. Na-APR-1, Na-CP-2, Na-CP-3, Na-CP-4, Na-CP-5, or Na-GST-1) and two or more adjuvants, one of which is an aluminum-based adjuvant such as Alhydrogel®.

2. Background of the Invention

Hookworms are gastrointestinal nematodes that infect approximately 600 million people in developing countries (Hotez et al., 2006a). Adult hookworms bury their heads beneath the mucosa of the human intestine and feed on blood. Moderate to heavy infections result in iron deficiency anemia, the major pathologic sequela of hookworm disease, as well as protein malnutrition. The resulting hookworm disease and anemia has a serious deleterious impact on many aspects of the health of infected individuals, including childhood growth retardation and cognitive development, and impaired fetal development during pregnancy (Hotez et al., 2004). The global disease burden resulting from chronic hookworm infection in childhood and pregnancy is enormous, possibly as high as 22 million disability-adjusted life years annually (Chan, 1997), making hookworm the second most important parasitic infection of humans after malaria (Hotez et al., 2005). In addition, the chronic immune suppression induced by hookworms and other helminths also has enormous impact on the ability of people to respond in a competent fashion to other infections (including malaria and HIV/AIDS) and vaccines (Elliott et al., 2005; Su et al., 2005; Cooper et al., 2001; Cooper et al., 1999; Hotez et al., 2006b).

Unlike many other human helminth infections, clear-cut immunity against hookworms does not develop in the majority of infected individuals (Loukas et al., 2005). Indeed, the oldest people living in an endemic community sometimes have the heaviest worm burdens (Bethony et al., 2002). While anthelmintic drugs of the benzimidazole class are highly effective at eliminating existing hookworm infections, they do not protect against rapid re-infection (Hotez et al., 2006a). In areas of high transmission, hookworm re-infection will occur within 4–12 months (Albonico et al., 1995), leading to concerns about the long-term sustainability of such practices (Kremer 2004). In addition, newer data indicates that the efficacy of benzimidazole drugs decreases with frequent use (Albonico et al., 2003), leading to concerns about the possibility that anthelmintic drug resistance has developed (Albonico et al., 2004; Bethony et al., 2006). These observations have led to calls by the World Health Organization and other international agencies to develop new tools for the control of hookworm, including a hookworm vaccine (WHO, 2005).

Therefore, an anthelmintic vaccine that induces immunological protection to minimize pathology and interrupt hookworm transmission is a highly desirable goal.

While regional economic growth (and with it, improvements in sanitation and clean water) in some parts of North America, Japan, South Korea, and China have translated into substantial reductions in endemic hookworm (Hotez et al., 2006a), estimated prevalence rates for the world’s poorest and least developed regions remain high. For example, infection rates in sub-Saharan Africa (SSA) are equivalent to those first estimated more than 60 years ago (DeSilver et al., 2003), where an estimated 198 million cases occur (DeSilver et al., 2003). High hookworm infestation rates are principally in poverty-stricken rural areas where access to medical care is severely limited. Widespread use of a hookworm vaccine would lead to significant improvement in global health and in economic development (Hotez et al., 2006; Hotez and Ferris, 2006). Therefore, an ideal vaccine hookworm vaccine would also be relatively easy and inexpensive to produce, and would be effective without the need for constant boosting.

The prior art has thus far failed to provide such a vaccine against human hookworm.

SUMMARY OF THE INVENTION

It is an object of this invention to provide a bivalent human hookworm vaccine. The vaccine is effective at inducing an immune response in individuals to whom it is administered, and administration results in a reduction in symptoms of hookworm disease.

The vaccine comprises: one or more L3 larval stage antigens (e.g. Na-ASP-2 and/or Na-SAA-A2) and at least one adult stage human hookworm antigen (e.g. Na-APR-1, Na-CP-2, Na-CP-3, Na-CP-4, Na-CP-5, or Na-GST-1) and one or more adjuvants. In some embodiments, the vaccine composition includes two or more adjuvants, one of which is an aluminum-based adjuvant such as Alhydrogel®.

The present invention provides a hookworm vaccine comprising a hookworm larval stage antigen, a hookworm adult stage antigen, and one or more adjuvants. In one embodiment of the invention, the vaccine includes at least one larval-stage hookworm antigen, at least one adult-stage hookworm antigen, an aluminum-based adjuvant, and a second adjuvant. In one embodiment of the invention, the larval-stage hookworm antigen is Na-ASP-2 or Na-SAA-2, or both. Further, the larval-stage hookworm antigen may be antigenic fragments of Na-ASP-2 or Na-SAA-2, or both. In one embodiment of the invention, the adult-stage hookworm antigen is Na-APR-1, Na-GST, Na-CP-2, Na-CP-3, Na-CP-4, Na-CP-5, or antigenic fragments thereof, or a combination of several of these antigens. In one embodiment of the invention, the Na-APR-1 that is utilized is P. chabaudi optimized Na-APR-1, or an antigenic fragment thereof. In some embodiments, the aluminum-based adjuvant is Alhydrogel® and the second adjuvant is CpG or Synthetic lipid A. In some embodiments of the invention, the aluminum-based adjuvant and the second adjuvant are combined together.

The invention also includes a method for vaccinating a patient in need thereof against hookworm infections. The method comprises the step of administering to the patient a hookworm vaccine comprising a hookworm larval stage antigen, a hookworm adult stage antigen, and one or more adjuvants. In one embodiment of the invention, the vaccine includes at least one larval-stage hookworm antigen, at least one adult-stage hookworm antigen, an aluminum-based adjuvant, and a second adjuvant. In one embodiment of the inven-
tion, the larval-stage hookworm antigen is Na-ASP-2 or Na-SAA-2, or both. Further, the larval-stage hookworm antigen may be antigenic fragments of Na-ASP-2 or Na-SAA-2, or both. In one embodiment of the invention, the adult-stage hookworm antigen is Na-APR-1, Na-GST, Na-CP-2, Na-CP-3, Na-CP-4, Na-CP-5, or antigenic fragments thereof, or a combination of several of these antigens. In one embodiment of the invention, the Na-APR-1 that is utilized is Pichia optimized Na-APR-1, or an antigenic fragment thereof. In some embodiments, the aluminum-based adjuvant is Alhydrogel® and the second adjuvant is CpG or Synthetic lipid A. In some embodiments of the invention, the aluminum-based adjuvant and the second adjuvant are combined together. In one embodiment, the method further comprises the step of administering a deworming agent to said patient.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1A and B. A, nucleotide sequence (SEQ ID NO: 1); and B, amino acid sequence (SEQ ID NO: 2) encoded by nucleotide sequence for Na-ASP-2.

Fig. 2A and B. A, cDNA nucleotide sequence (SEQ ID NO: 3, partial sequence 62-1351bp); and B, amino acid sequence (SEQ ID NO: 4) of Na-APR-1, Shanghai strain.

FIG. 3A and B. A, cDNA nucleotide sequence (SEQ ID NO: 5); and B, amino acid sequence (SEQ ID NO: 6) of Na-APR-1, Australian strain.

FIG. 4. Amino acid sequence (without signal sequence) alignment between Shanghai (SEQ ID NO: 7) and Australian (SEQ ID NO: 8) strains of Na-APR-1.

Fig. 5A and B. A, cDNA nucleotide sequence (SEQ ID NO: 9); and B, amino acid sequence (SEQ ID NO: 10) of Pichia optimized Na-APR-1 (Na-APR-1-O), based on Australian strain; the sequence is identical to residues 17-446 of Na-APR-1 Australian strain.

Fig. 6A and B. A, cDNA nucleotide sequence (SEQ ID NO: 11); and B, amino acid sequence (SEQ ID NO: 12) encoded by nucleotide sequence for Pichia optimized Na-APR-1 (Australian strain) with Asp97 mutated to Ala97 (shown in bold and underlined).

Fig. 7A and B. A, cDNA nucleotide sequence (SEQ ID NO: 13); and B, amino acid sequence (SEQ ID NO: 14) encoded by nucleotide sequence for Pichia optimized Na-APR-1 (Australian strain) with Asp284 mutated to Ala284.

Fig. 8A and B. A, cDNA nucleotide sequence (SEQ ID NO: 15); and B, amino acid sequence (SEQ ID NO: 16) encoded by nucleotide sequence for Pichia optimized Na-APR-1 (Australian strain) with both Asp97 mutated to Ala97 and Asp284 mutated to Ala284.

Fig. 9A and B. A, cDNA nucleotide sequence (SEQ ID NO: 17); and B, amino acid sequence (SEQ ID NO: 18) encoded by nucleotide sequence for Ng-GST-1.

Fig. 10A and B. A, cDNA nucleotide sequence (SEQ ID NO: 19); and B, amino acid sequence (SEQ ID NO: 20) encoded by nucleotide sequence for Ng-GST-2.

Fig. 11A and B. A, cDNA nucleotide sequence (SEQ ID NO: 21); and B, amino acid sequence (SEQ ID NO: 22) encoded by nucleotide sequence for Ng-GST-3.

Fig. 12A and B. A, cDNA nucleotide sequence (SEQ ID NO: 23); and B, amino acid sequence (SEQ ID NO: 24) encoded by nucleotide sequence for Ng-CP-2.

Fig. 13A and B. A, nucleotide sequence (SEQ ID NO: 25); and B, amino acid sequence (SEQ ID NO: 26) encoded by nucleotide sequence for Ng-CP-3.

Fig. 14A and B. A, nucleotide sequence (SEQ ID NO: 27); and B, amino acid sequence (SEQ ID NO: 28) encoded by nucleotide sequence for Ng-CP-4.

**FIG. 15A and B. A, nucleotide sequence (SEQ ID NO: 29); and B, amino acid sequence (SEQ ID NO: 30) encoded by nucleotide sequence for Na-CP-5.**

**FIG. 16A and B. A, nucleotide sequence (SEQ ID NO: 31); and B, amino acid sequence (SEQ ID NO: 32) encoded by nucleotide sequence for Na-SAA-1.**

**FIG. 17A and B. A, nucleotide sequence (SEQ ID NO: 33); and B, amino acid sequence (SEQ ID NO: 34) encoded by nucleotide sequence for Na-SAA-2.**

**FIG. 18A and B. Individual titers of BALB/c mice given the indicated doses of Na-ASP-2/Alhydrogel® (80 mcg Alhydrogel®) with and without 5 mg ODN 2006 in 50 mcL i.m. at days 0 and 20, with terminal bleeds at day 30 (log scale). A, arithmetic mean; B, geometric mean.**

**FIG. 19A and B. Anti-Na-ASP-2 Specific IgG antibody responses in humans immunized with Na-ASP-2, as determined by ELISA (undetectable titers were arbitrarily assigned a titer of 50); B, proliferative response of peripheral blood mononuclear cells from humans immunized with Na-ASP-2, after in vitro stimulation with Na-ASP-2.**

**FIG. 20. Ranking criteria for larval antigens for the human hookworm vaccine.**

**FIG. 21. Ranking criteria for adult antigens for the human hookworm vaccine.**

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION**

It is an object of this invention to provide a bivalent human hookworm vaccine. The vaccine is effective at inducing an immune response in individuals to whom it is administered, and administration results in a reduction in symptoms of hookworm disease, e.g., worm burden, blood loss, etc.

The vaccine comprises: one or more L3 larval stage antigens and at least one adult stage human hookworm antigen [e.g. Na-APR-1 (example sequences for which include SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 12, SEQ ID NO: 14, and SEQ ID NO: 16], Na-CP-2 (SEQ ID NO: 24), Na-CP-3 (SEQ ID NO: 26), Na-CP-4 (SEQ ID NO: 28), Na-CP-5 (SEQ ID NO: 30), or Na-GST-1 (SEQ ID NO: 18)] and one or more adjuvants. In some embodiments, the vaccine composition includes two or more adjuvants, one of which may be an aluminum-based adjuvant such as Alhydrogel®.

In a preferred embodiment of the invention, the antigens are Necator americanus antigens.

With respect to the one or more larval stage antigens that are used in the vaccine, exemplary antigens are Na-ASP-2 (SEQ ID NO: 2), Na-SAA-1 (SEQ ID NO: 32), and Na-SAA-2 (SEQ ID NO: 34), the sequences of which are found in FIGS. 1, 16 and 17, respectively.

With respect to the one or more adult stage antigens that may be used in the vaccine composition, the following exemplary sequences are contemplated: Na-APR-1 Shanghai strain (SEQ ID NO: 4, partial sequence 62-1351bp) as depicted in FIG. 2; Na-APR-1 Australia strain (SEQ ID NO: 6) as depicted in FIG. 3; Na-APR-1 amino acid sequence (without signal) alignment between Shanghai (SEQ ID NO: 7) and Australia (SEQ ID NO: 8) strains as depicted in FIG. 4; Pichia Optimized Na-APR-1 (Na-APR-1-O, SEQ ID NO: 10) Sequence (based on Australia strain) as depicted in FIG. 5; Pichia Optimized Na-APR-1 (Na-APR-1-O) with Asp97 mutated to Ala97 (SEQ ID NO: 12) as depicted in FIG. 6; Pichia Optimized Na-APR-1 (Na-APR-1-O) with Asp284 mutated to Ala284 (SEQ ID NO: 14) as depicted in FIG. 7; Pichia Optimized Na-APR-1 (Na-APR-1-O) with both Asp97/Asp284 mutated to Asp97/Ala284 (SEQ ID NO: 16) as depicted in FIG. 8; Na-GST-1 as depicted in FIG. 9; Na-APR-1 as depicted in FIG. 10; Na-APR-1 as depicted in FIG. 11; Na-APR-1 as depicted in FIG. 12; Na-APR-1 as depicted in FIG. 13; Na-APR-1 as depicted in FIG. 14; and Na-APR-1 as depicted in FIG. 15.
GST-2 (SEQ ID NO: 20) as depicted in FIG. 10; Na-GST-3 (SEQ ID NO: 22) as depicted in FIG. 11; Na-CP-2 (SEQ ID NO: 24) as depicted in FIG. 12; Na-CP-3 (SEQ ID NO: 26) as depicted in FIG. 13; Na-CP-4 (SEQ ID NO: 28) as depicted in FIG. 14; and, Na-CP-5 (SEQ ID NO: 30) as depicted in FIG. 15.

“Larval stage antigen” or “L3 larval stage antigen” refers to antigens that are expressed during the L3 larval stage of the hookworm life cycle. In some cases, such antigens may also be expressed during other stages of the life cycle, i.e. the antigen may not be expressed exclusively in the larval stage. However, a “larval stage antigen” is expressed at least in the L3 larval stage.

In preferred embodiments, Pichia optimized Na-APR-1 sequences are used, as described in numbers 4-7 above. Codon optimization enhances the efficiency of DNA expression vectors used in DNA vaccination by increasing protein expression. The codon frequency of the foreign (i.e. hookworm) DNA embedded into the yeast expression vector may not be optimal for adequate protein expression in the host resulting in low level protein expression. A potential solution for the codon bias is to optimize the codon sequences of a gene to suit the requirements of the host without altering the original amino acid sequence of the protein See, for example, Jareborg N, Durbin R, ‘Alfresco—a workbench for comparative genomic sequence analysis’, Genome Res 2000 August; 10(8):1148-57, 16; and Kim C H, Oh Y, Lee T H: Codon optimization for high level expression of human erythropoietin (EPO) in mammalian cells. Gene 199:293-301 (1997).

With respect to the adult stage GST antigen, three exemplary Na-GST amino acid sequences, Na-GST-1, Na-GST-2, and Na-GST-3, are represented in FIGS. 9B, 10B and 11B, respectively, and nucleotide sequences that encode these antigens are represented in FIGS. 9A, 10A and 10B, respectively.

With respect to the adult stage Na-CP antigens that are used in the vaccine, exemplary amino acid sequences of this antigen are represented in FIGS. 12B (Na-CP-2), 13B (Na-CP-3), 14B (Na-CP-4), and 15B (Na-CP-5) and exemplary nucleotide sequences that encode these antigens are represented in FIGS. 12A, 13A, 14A and 15A, respectively.

With respect to the larval stage SAA antigens, exemplary nucleic acid sequences and encoded amino acid sequences of Na-SAA-1 and Na-SAA-2 are given in FIGS. 17 and 18, respectively.

Examples of antigens, their amino acid primary sequences, and nucleic acid sequences which encode them are given herein, and any combination of the antigens depicted herein may be used in the practice of the invention. However, those of skill in the art will recognize that many variants of the sequences presented herein may exist or be constructed which would also function as antigens in the practice of the present invention. For example, with respect to amino acid sequences, variants may exist or be constructed which display: conservative amino acid substitutions; non-conservative amino acid substitutions; truncation by, for example, deletion of amino acids at the amino or carboxy terminus, or internally within the molecule; or by addition of amino acids at the amino or carboxy terminus, or internally within the molecule (e.g. the addition of a histidine tag for purposes of facilitating protein isolation, the substitution of residues to alter solubility properties, the replacement of residues which comprise protease cleavage sites to eliminate cleavage and increase stability, the addition or elimination of glycosylation sites, and the like, or for any other reason). Such variants may be naturally occurring (e.g. as a result of natural variations between species or between individuals), or they may be purposefully introduced (e.g. in a laboratory setting using genetic engineering techniques). All such variants of the sequences disclosed herein are intended to be encompassed by the teaching of the present invention, provided the variant antigen displays sufficient identity to the described sequences. Preferably, identity will be in the range of about 50 to 100%, or in the range of about 75 to 100%, or in the range of about 80 to 100%, or 85% to 100%, or 90% to 100%, or about 95% to 100% of the disclosed sequences. The identity is with reference to the portion of the amino acid sequence that corresponds to the original antigen sequence, i.e. not including additional elements that might be added, such as those described below for chimeric antigens.

The invention also encompasses chimeric antigens, for example, antigens comprised of the presently described amino acid sequences plus additional sequences which were not necessarily associated with the disclosed sequences when isolated but the addition of which conveys some additional benefit. For example, such benefit may be utility in isolation and purification of the protein, (e.g. histidine tag, GST, and maltose binding protein); in directing the protein to a particular intracellular location (e.g. yeast secretory protein), in increasing the antigenicity of the protein (e.g. KHL, haptenes). All such chimeric constructs are intended to be encompassed by the present invention, provided the portion of the construct that is based on the sequences disclosed herein is present in at least the indicated level of homology.

Those of skill in the art will recognize that it may not be necessary to utilize the entire primary sequence of a protein or polypeptide in order to elicit an adequate antigenic response to the parasite from which the antigen originates. In some cases, a fragment of the protein is adequate to confer immunization. Thus, the present invention also encompasses antigenic fragments of the sequences disclosed herein, and their use in vaccine preparations. In general, such a fragment will be at least about 10-13 amino acids in length. Those of skill in the art will recognize that suitable sequences are often hydrophilic in nature, and are frequently surface accessible.

Likewise, with respect to the nucleic acid sequences disclosed herein, those of skill in the art will recognize that many variants of the sequences may exist or be constructed which would still function to provide the encoded antigens or desired portions thereof. For example, due to the redundancy of the genetic code, more than one codon may be used to code for an amino acid. Further, as described above, changes in the primary sequence of the antigen may be desired, and this would necessitate changes in the encoding nucleic acid sequences. In addition, those of skill in the art will recognize that many variations of the nucleic acid sequences may be constructed for purposes related to cloning strategy, (e.g. for ease of manipulation of a sequence for insertion into a vector, such as the introduction of restriction enzyme cleavage sites, etc.), for purposes of modifying transcription (e.g. the introduction of promoter or enhancer sequences, and the like), or for any other suitable purpose. All such variants of the nucleic acid sequences disclosed herein are intended to be encompassed by the present invention, provided the sequences display about 50 to 100% identity to the original sequence and preferably, about 75 to 100% identity, and most preferably, about 80 to 100% identity. The identity is with reference to the portion of the nucleic acid sequence that corresponds to the original sequence, and is not intended to cover additional elements such as promoters, vector-derived sequences, restriction enzyme cleavage sites, etc. derived from other sources.

In a preferred embodiment, the vaccine of the present invention includes an aluminum-based adjuvant such as the aluminum hydroxide adjuvant Alhydrogel® (available from
Superfos and Brenntag Biosector) or the aluminum-containing adjuvant AS04 (available from GlaxoSmithKline). In addition, at least one additional adjuvant is also a component of the vaccine. Exemplary additional or second adjuvants include but are not limited to the following:

1. AS03, a proprietary formulation manufactured by Glaxo Smith Kline that contains an oil-in-water emulsion;
2. AS02A, a proprietary formulation manufactured by Glaxo Smith Kline that contains the same oil-in-water emulsion as in AS03, plus two immunostimulants “3D-MPL” and “QS-21”;
3. AS03 and AS02A are described (under their original designations SBAS3 and SBAS2, respectively) in Stout et al NEJM 1997 336:86-91. It is noted that, AS02A and AS03 are designed to be used with the aluminum based adjuvant AS04, also available from GlaxoSmithKline.

A synthetic oligodeoxynucleotide adjuvant containing cytosine-guanine dinucleotides in particular base contexts or CpG motifs, (CpG ODN). This adjuvant is an immunomodulatory molecule and is available from Coley.

Various lipid A derivatives, Lipid A is the portion of lipopolysaccharide that is known to be the primary component with regard to adjuvanticity and toxicity. Derivatives of lipid A have been produced in an attempt to retain the immunostimulatory activity of Lipid A without reducing the toxicity. One such derivative, monophosphoryl lipid A (MPL, available from Chiron), has been shown to exhibit strong Th1 adjuvant activity with a considerably reduced toxicity compared to LPS. MPL has adjuvant activity whether used alone, or in combination with other immunostimulants, such as CpG ODN, or aluminum hydroxide. Another synthetic lipid A derivative that is very similar to the lipopolysaccharide derivative lipid A monophosphoryl (MPL) by Chiron is available from the Infectious Disease Research Institute, Seattle, Wash.

A publication by McCluskie and Weeratna (Infectious Disorders, 2001, 1, 263-271) gives examples of several different adjuvant systems, each of which may be employed in the practice of the present invention.

Examples of other suitable adjuvants include but are not limited to Seppic, Quil A, etc. Preferred adjuvants combinations are: Alhydrogel®+CpG 10103 and Alhydrogel®+synthetic lipid A.

The present invention provides compositions for use in eliciting an immune response against hookworm. The compositions may be utilized as a vaccine against hookworm. By “eliciting an immune response” we mean that an antigen stimulates synthesis of specific antibodies at a titer of about >1 to about 1x10^10 or greater. Preferably, the titer is from about 10,000 to about 1x10^10 or more, as measured by enzyme linked immunosorbent assay (ELISA) or greater than 1,000 antibody units as defined previously (Malkin et al., 2005a; 2005b). By “vaccine” we mean an antigen or antigen preparation that elicits an immune response that results in a decrease in hookworm burden of at least about 30% in an organism in relation to a non-vaccinated (e.g. adjuvant alone) control organism. This work burden reduction has been calculated to restore a child’s daily iron requirements that would otherwise be lost from a moderate (i.e. infections with between 2,000 and 4,000 hookworm eggs per gram of feces) infection with hookworm. Preferably, however, the level of the decrease in hookworm burden would approach 50%, or more.

The present invention provides compositions for use in eliciting an immune response which may be utilized as a vaccine against hookworm. The compositions include a substantially purified recombinant hookworm antigen or variant thereof as described herein, and a pharmaceutically suitable carrier. The preparation of such compositions for use as vaccines is well known to those of skill in the art. Typically, such compositions are prepared either as liquid solutions or suspensions, however solid forms such as tablets, pills, powders, and the like are also contemplated. Solid forms suitable for solution in, or suspension in, liquids prior to administration may also be prepared. The preparation may also be emulsified. The active ingredients may be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredients. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol and the like, or combinations thereof. In addition, the composition may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and the like. In addition, the composition may contain other adjuvants. If it is desired to administer an oral form of the composition, various thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders and the like may be added. The composition of the present invention may contain any such additional ingredients so as to provide the composition in a form suitable for administration. The final amount of hookworm antigen in the formulations may vary. However, in general, the amount in the formulations will be from about 1-99%.

The present invention also provides methods of eliciting an immune response to hookworm and methods of vaccinating a mammal against hookworm. The methods generally involve identifying a suitable vaccine recipient, and administering a composition comprising the hookworm antigens and adjuvants described herein in a pharmaceutically acceptable carrier to the recipient. The vaccine preparations of the present invention may be administered by any of the many suitable means which are well known to those of skill in the art, including but not limited to by injection, orally, intranasally, by ingestion of a food product containing the antigens, etc. In preferred embodiments, the mode of administration is subcutaneous or intramuscular. Patients with an existing worm burden may be treated with a de-worming agent such as benzimazole, and then be provided with the vaccine.

The present invention provides methods to elicit an immune response to hookworm in mammals. In one embodiment, the mammal is a human.

Those of skill in the art will recognize that, in general, in order to vaccinate (or elicit an immune response in) a species of interest (e.g. humans) against hookworm, the antigen which is utilized will be derived from a species of hookworm which parasites the species of interest. For example, in general, antigens from Necator americanus may be preferred for the immunization of humans, and antigens from Ancylostoma caninum may be preferred for the immunization of dogs. However, this may not always be the case. For example, Ancylostoma caninum is known to parasites humans as well as its primary canine host. Further, cross-species hookworm antigens may sometimes be highly effective in eliciting an immune response in a non-host animal, i.e. in an animal that does not typically serve as host for the parasite from which the antigen is derived. Rather, the measure of an antigen’s suitability for use in an immune-stimulating or vaccine preparation is dependent on its ability to confer protection against invasion and parasitization by the parasite as evidenced by, for example, hookworm burden reduction or inhibition of hookworm associated blood loss (e.g. as measured by hematocrit and/or hemoglobin concentration. For example, for use in a vaccine preparation, an antigen upon administration results in a reduction in worm burden of at least about 30%, preferably at least about 50%, and most preferably about 60 to about 70%.
EXAMPLES

Example 1

Scoring System for Determining an Efficacious Human Hookworm Vaccine

A scoring system that incorporates essential criteria for determining an efficacious human hookworm vaccine has been developed (Table 1). The criteria include endpoints that focus on pathology (blood loss, worm burdens), transmission (faecal egg counts), ease of process development (known function/structure of protein) and immunoepidemiology (associations between immune responses and infection intensities in naturally exposed/infected cohorts). Once produced in soluble form, recombinant versions of the major L3 ES products were tested for vaccine efficacy in the canine and hamster models of infection.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tbody>
<tr>
<td></td>
<td>Adult worm reduction (dog)</td>
<td>Adult worm reduction (hamster)</td>
<td>Reduced blood loss</td>
<td>EPG reduction</td>
<td>Known function/structure</td>
<td>Human immunoepidemiology</td>
<td>Protective homology</td>
<td>Final Score</td>
</tr>
<tr>
<td>ASP-2</td>
<td>2</td>
<td>3</td>
<td>0-4</td>
<td>3</td>
<td>2</td>
<td>0-3</td>
<td>2</td>
<td>16/25</td>
</tr>
<tr>
<td>APR-1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>ND⁴</td>
<td>1</td>
<td>14/22</td>
</tr>
<tr>
<td>CP-2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>ND</td>
<td>1</td>
<td>9/22</td>
</tr>
<tr>
<td>GST-1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>ND</td>
<td>1</td>
<td>9/22</td>
</tr>
</tbody>
</table>

1 Reflects quantiles of reduction in worm burdens in dogs compared to controls.
2 Reflects quantiles of reduction in worm burdens in hamsters compared to controls.
3 Each grade reflects an increase of 0.5 g/dL of hemoglobin above control group.
4 Reflects tertiles of egg reduction compared to controls.
5 Function or structure known in hookworm (grade of 2) or in a related helminth (grade of 1) enables biochemical assay development.
6 Association between antibody response and reduced egg in people (number = strength of association).
7 Protective homologues in other nematodes (grade of 2) or infectious agents (grade of 1).
8 Tally of scores from each category.
ND—Not Determined.

Based on this ranking system, recombinant antigens ASP-2, Ac-APR-1, GST and CP-2 were selected as a lead vaccine candidates for further process development, cGMP manufacture and clinical testing.

Evidence that ASP-2 is a protective antigen in dogs (Ac-ASP-2) and hamsters (Ay-ASP-2) was published by the inventors in Bethony et al (2005) and Gould et al (2004); Mendez et al (2005), respectively. Human immunoepidemiological evidence pointing to the protective effect of ASP-2 antibodies was published in Bethony et al (2005). Evidence that Na-ASP-2 is protective in hamsters is unpublished, while evidence that anti-Na-ASP-2 antibodies inhibit hookworm larval penetration in vitro was published by Gould et al (2005). Evidence that APR-1 is a protective antigen in dogs (Ac-APR-1) was published by the inventors in Loukas et al (2005). Evidence that Na-APR-1 is protective is also available, but unpublished. Evidence that GST is a protective antigen in dogs (Ac-GST-1) was published by the inventors in Loukas et al (2005).

Within each dose cohort, three subjects were randomized to receive saline placebo and nine subjects were randomized to receive one of three doses of the Na-ASP-2 hookworm vaccine. Those randomized to receive vaccine were given 10, 50, or 100 µg of Na-ASP-2 in the first, second and third dose cohorts, respectively. Higher dose concentrations or additional (second or third) injections were not administered until the effects of the preceding dose concentration and injection had been evaluated. Subjects were evaluated for adverse events, vital signs, blood chemistry, hematology, and urinalysis.

The cumulative safety data from this trial has demonstrated that the vaccine is both safe and immunogenic in healthy, hookworm-infected adults, with mild to moderate injection-site tenderness, erythema, swelling and pruritus being the most commonly observed vaccine-related adverse events. Induration and warmth at the injection site occurred less frequently. All injection site reactions were considered mild or moderate in severity and were typical of those observed with aluminum-adjuvanted vaccines administered intramus-
cularly. The frequency of injection site reactions was not dose-dependent, and did not increase with successive vaccinations. Unusual injection site reactions were observed in one male participant in the 10 µg dose group and in three female subjects in the 50 µg dose group after the second injection. These reactions were delayed erythematous reactions ranging in size from 5 to 12 cm in diameter that started approximately 10 days after the injection and lasted for 1 to 4 days, resolving without incident. Several vaccinated individuals also experienced mild to moderate systemic adverse events including fever, headache and nausea. No vaccine-related serious adverse events occurred during the study, and no clinically-significant alterations in clinical laboratory parameters were observed.

The Na-ASP-2 hookworm vaccine induced a significant antigen-specific IgG antibody response in a dose-dependent manner (Fig. 10). There was a statistically significant difference between the placebo and vaccine groups starting as early as 14 days after the second injection which remained through the 8 month follow-up time point after the third injection. By day 30, a statistically significant difference in IgG1 levels was seen between the placebo and vaccine groups. No appreciable antigen-specific IgM, IgA or IgE responses were detected. Finally, significant antigen-specific cellular immune responses were also observed, with increasing responses seen after successive injections of vaccine (Fig. 11).

While the invention has been described in terms of its preferred embodiments, those skilled in the art will recognize that the invention can be practiced with modification within the spirit and scope of the appended claims. Accordingly, the present invention should not be limited to the embodiments as described above, but should further include all modifications and equivalents thereof within the spirit and scope of the description provided herein.

Example 3

Comparative Immunogenicity of Na-ASP-2/Alhydrogel® with and without ODN 2006 in BALB/c Mice

For each adjuvant tested, compatibility and stability studies were undertaken to ensure that all individual components (antigen, adjuvant 1, adjuvant 2, etc.) were compatible and that adequate stability was achieved upon formulation. For Alhydrogel® based vaccine formulations to which other adjuvants were added, this involved assays that test antigen binding, conformation, and integrity over various periods of time and at different temperatures.

With reference to FIGS. 13 A and B, Groups of 10 female BALB/c mice were given the indicated doses of Na-ASP-2/Alhydrogel® with or without 5 micrograms ODN 2006, as indicated. Total antigen-specific IgG was measured in the sera of each animal by indirect ELISA.

FIGS. 14 A and B show the geometric and arithmetic means, respectively, of the results. As can be seen, a comparative immunogenicity study of Na-ASP-2/Alhydrogel® with and without ODN 2006 in BALB/c mice was performed. The results showed the ODN2006 boosts the immune response in BALB/c mice over that achieved with Na-ASP-2/Alhydrogel® alone, as determined by indirect ELISA that measure total antigen specific IgG (antibody).

References for Background and Examples 1-3


Hookworm Vaccine Antigens Screening with *Necator americanus*-hamster Model

1. Introduction

Among the three major soil-transmitted nematodes, *Acaris lumbricoides*, *Ancylostoma duodenale/*Necator americanus* (hookworms), and *Trichuris trichuria*, hookworms are the most pathogenic because of their blood feeding behavior that directly causes blood loss and iron deficiency anemia (de Silva et al., 2003; Bethony et al., 2006). More seriously for children and women who have low iron stores, hookworm infection can cause retardation of physical and intellectual development (Bundy et al., 1995; Brooker et al., 1999; Hotez et al., 2006, 2004a).

More than 700 million people living in the developing countries of tropical and subtropical regions are estimated to be infected with hookworms. Hookworm infection causes more DALY's lost (1.8 million) than any other helminthiasis with the exception of lymphatic filariasis (Hotez et al., 2006, 2004a, Bethony et al., 2006). Mass chemotherapy remains a mainstay of hookworm control strategies (WHO 2002; Allen et al., 2002; Hotez et al., 2002). Indeed, repeated chemotherapy at regular intervals in high-risk groups is useful to keep a low morbidity, and will frequently result in immediate improvement in child health and development (Bharagava et al., 2003; Stephenson et al., 1989) although continued use of anthelmintics is perhaps contributing to the development of anthelmintic resistance (Albonico et al., 2004). Unfortunately, the treated people, particularly, in highly endemic areas, soon become reinfected as early as 4-12 months after drug treatment. Therefore, preventive vaccine against hookworm infection becomes an attractive alternative for hookworm control.

The major obstacle for developing human hookworm vaccine is the absence of a suitable laboratory animal host to complete human hookworm’s life cycle (Hotez et al., 2003a, b, 2004b). Several laboratories have tried to infect *N. americanus* in mice, dogs, guinea pigs, rabbits and hamsters (Timothy and Behnke, 1993, 1997; Nagahana et al., 1962; Yoshiida et al., 1960; Yoshiida and Fukutome 1967; Sen, 1970; Sen and Seth, 1970; Sen and Deb, 1973). However, the efforts were not successful either due to inconsistent maintenance of the organism within the laboratory animals, the requirement for cortisone-immuno-suppression, or use of infant animals. However, great progress was made by Xue and her colleagues (Xue et al., 2003a, b) in the Institute of Parasitic Diseases (IPD), Chinese Center of Disease Control and Prevention (CCDCP) who successfully adapted *N. americanus* to the Chinese golden hamster *Mesocricetus auratus* without the requirement for exogenous steroids or other immunosuppression, or the requirement to infect infant hamsters. Infection with the human hookworm *N. americanus*, originally obtained from an infected patient living in Human Province, China, has been established in the golden hamster *Mesocricetus auratus* for more than 100 generations over a period of 26 years with no need of steroids (Xue et al., 2003). This model has been successfully used for testing anthelmintic drugs (Xue et al., 2005).

Several hookworm vaccine antigens have been tested with an *Ancylostoma caninum*-dog model or *Ancylostoma ceylanicum*-hamster model and some of them exhibited certain degrees of protection against *A. caninum* L3 challenge with reduction of either adult worm burden or blood loss (Hotez et al., 2003a; Goud et al., 2004; Mendez et al., 2005; Loukas et al., 2005, Bethony et al., 2005, Fujiwara (in press)). Among the vaccines tested, Na-ASP-2 is a leading antigen (Bethony et al,
2005; Goud et al, 2005). However, these animal models are used to test vaccine antigens from animal hookworms such as *A. caninum* or *A. ceylanicum*. The effect of such vaccines can be used to deduce or mimic the effect of human hookworm homologues, but can not reflect completely the real pattern of human hookworm. The *Necator americanus*-hamster model currently is the only animal model for maintaining the species of human hookworms. This human hookworm model was thus used to test various hookworm vaccine candidates. The results showed that some of the antigens conferred protective against symptoms of hookworm infection.

2. Materials and Methods

2.1 Hamsters

Male Chinese golden hamsters *Mesocricetus auratus* with an age of 7-8 weeks were supplied by either Shanghai Institute of Biological Products of the Chinese Ministry of Health or Shanghai Animal Center, Chinese Academy of Sciences (SCSKK (Hu) 2003-0003). The hamsters were housed in groups of 10 in plastic cages. All animals had free access to water and commercial rodent food purchased from Shanghai Shiling Biological and Scientific Technique Corporation.

2.2 Vaccine Antigen and Adjuvant

Ten recombinant hookworm proteins derived either from *N. americanus* or *A. caninum* were used to test vaccine effect with the *N. americanus*-hamster model performed in the IPD. CCDCP. Na-ASP-2, a major Ancylostoma-secreted protein-2 secreted by stimulated infective larvae of *N. americanus*, is a leading hookworm vaccine antigen. The recombinant Na-ASP-2 either with his-tag at C-terminal or without tag were expressed in the *Pichia pastoris* X-33 and purified by chromatography (Goud et al, 2005; Hawdon et al, 1999, Mendez et al, 2005). Na-ASP-1 is another Ancylostoma-secreted protein secreted by stimulated infective larvae of *N. americanus* (Hawdon et al, 1996, Goud et al, 2004). Ac-GST-1, a novel glutathione S-transferase produced by *A. caninum* adult worms, is a heme binding protein that is believed to be involved in the detoxification of heme derived from blood feeding (Zhan et al, 2005). Ac-CP-2 is a cathepsin-B cysteine protease from *A. caninum* involved in hemoglobin digestion of parasite (Harrop et al, 1995, Loukas et al, 2004). Na-CP-2 and Na-CP-4 are homologues of Ac-CP-2 cloned by screening cDNA library of *N. americanus* with partial Ac-CP-2 cDNA (unpublished). Ac-APR-1 is a cathepsin D-like aspartic protease from *A. caninum* (Williamson et al, 2002, 2003; Loukas et al, 2005). Ac-MTP is an astacin-like metalloprotease secreted by the stimulated infective larvae of *A. caninum* (Zhan et al, 2002, Williamson et al, 2006, Mendez et al, 2005). Na-CT-1 is a C-type Lectin of *N. americanus* (Daub et al, 2000). Na-SAA-1 is a *N. americanus* orthologue of Ac-SAA-1, an immunodominant surface-associated antigen from *A. caninum* (Zhan et al, 2004). All recombinant proteins were expressed in *Pichia pastoris* as soluble secretory proteins and purified with chromatography except for Na-SAA-1 and Na-CP-2 that were expressed in *E. coli*. Recombinant Na-SAA-1 was soluble and Na-CP-2 was insoluble and denatured in the 0.1% SDS.

The hookworm recombinant proteins were formulated with adjuvants of either Freund’s, ASO3 or Alhydrogel®. Complete and incomplete Freund’s adjuvants were obtained from Sigma (Saint Louis, Mo.). Twenty-five μg of recombinant protein was emulsified with 100 μl of complete Freund’s for each hamster for the first immunization and with complete Freund’s for the boost. ASO3 is a water-oil adjuvant (Stoute et al, 1997) kindly provided by GlaxoSmithKline (Rixensart, Belgium). Total volume of 100 μl of ASO3 was formulated with 25 μg of recombinant protein for each hamster by mixing for 30 minutes at room temperature. Formulation of antigen with Alhydrogel® was performed by mixing 25 μg of the recombinant protein with 25 μl of 2% Alhydrogel® in a total volume of 200 μl for each hamster.

2.3 Vaccination

The dose of each vaccine given to each hamster was 25 μg recombinant protein formulated with different adjuvants (Freund’s, ASO3 and Alhydrogel®) in a total volume of 200 μl. The vaccine was administrated subcutaneously and boosted twice with two weeks interval. Total of 10-26 hamsters were immunized with one vaccine, the same number of hamsters were injected with the same volume of adjuvant alone on the same immunization schedule as a control group.

For Freund’s adjuvant, complete Freund’s adjuvant was used in the initial immunization, followed by two boosts with incomplete Freund’s adjuvant.

2.4 Challenge with the Third-Stage Infective Larvae of *N. americanus*

The third stage infective larvae of *N. americanus* were collected from coprocultures of feces from hamsters infected with *N. americanus* larvae (Xue, 2003a, b). One week after the last immunization, the hamsters (vaccine and adjuvant control groups) were infected with fresh 150 infective larvae subcutaneously under the skin of central abdomen.

2.5. Necropsy and Evaluation of Vaccine Effect

Twenty-five to twenty-eight days post challenge, all hamsters with vaccinated or control groups were sacrificed and the hookworms located in the small intestine were collected and counted. The mean worm burden in each group was calculated. The differences between each vaccinated group and the control group were analyzed by using Student t-test.

3. Results

3.1 Protective Immunity of rNa-ASP-2

In the first trial, the ASO3 was used as adjuvant. In rNa-ASP-2 (with his-tag) group, 26 hamsters were used and the mean worm burden was 11.7±9.2, while 20.0±15.0 worms were found in the adjuvant control group. The difference between the two groups was statistically significant (P<0.05) (Table 2).

In the second trial, the protective effects of rNa-ASP-2 with his-tag and without his-tag were compared. However, the adjuvant was changed to Anhydrogel instead of SO3. In this trial, the mean worm burden of adjuvant group was 37.7±13.6, while those of rNa-ASP-2 (with his-tag) and rNa-ASP-2 (without his-tag) were 26.4±17.2 and 27.1±28.3, respectively. The difference of mean worm burdens between the rNa-ASP-2 (with his-tag) group and control group was statistically significant with a worm reduction rate of 30.0%. No significant difference was seen in mean worm burdens between the rNa-ASP-2 without his-tag and control group because of large standard deviation appeared in the vaccine group (Table 2).

Overall, the mean worm reduction rate combining the three trials is 31.8%, a statistically significant result when compared with the adjuvant only group.
TABLE 2  
Protective immunity elicited by immunizing recombinant rNa-ASP-2 in hamsters challenged with N. americanus L3.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Vaccine antigen</th>
<th>Vaccine mean worm ± SD (hamster#)</th>
<th>Control mean worm ± SD (hamster#)</th>
<th>Worm reduction rate (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rNa-ASP-2 (with his-tag)</td>
<td>11.7 ± 9.2 (26)</td>
<td>20.0 ± 15.0 (26)</td>
<td>41.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2</td>
<td>rNa-ASP-2 (with his-tag)</td>
<td>26.4 ± 17.2 (20)</td>
<td>37.7 ± 13.6 (20)</td>
<td>30.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3</td>
<td>rNa-ASP-2 (w/o his-tag)</td>
<td>27.1 ± 28.3 (20)</td>
<td>37.7 ± 13.6 (20)</td>
<td>28.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>21.7 ± 18.2 (66)</td>
<td>31.8 ± 14.0 (66)</td>
<td>31.8</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

3.2 Protective Immunity of rAc-GST-1
rAc-GST-1, the glutathione S-transferase-1 of A. cantinum, was formulated with the adjuvant Alhydrogel® for immunization of hamsters. The dosage of rAc-GST-1 used for immunization was 25 μg/hamster. In the first test, the mean worm burden in the rAc-GST-1 immunization group was 15.7±9.8 which was less than that of 33.9±15.0 in the Alhydrogel® group, with a worm reduction of 53.7%. In the second test, the mean worm burden in the rAc-GST-1 group was 16.7±6.6 which was similar to that of 20.2±8.1 in the adjuvant group, with a worm reduction rate of 17.3%. Therefore, a third test was performed. The mean worm burden in the rAc-GST-1 immunization group was significantly lower than that in the nonimmunized group with a worm reduction rate of 71.3%. When the results from the three tests were combined together for calculation, the mean worm burden in the immunization group was also lower than that of the adjuvant group, with a worm reduction rate of 48.4% (Table 3).

TABLE 3  
Protective immunity elicited by immunizing recombinant rAc-GST-1 in hamsters challenged with N. americanus L3.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Vaccine antigen</th>
<th>Vaccine mean worm ± SD (hamster#)</th>
<th>Control mean worm ± SD (hamster#)</th>
<th>Worm reduction rate (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rAc-GST-1</td>
<td>15.7 ± 9.8 (18)</td>
<td>33.9 ± 15.0 (20)</td>
<td>53.7</td>
<td>&lt;0.05</td>
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<tr>
<td>2</td>
<td>rAc-GST-1</td>
<td>16.7 ± 6.6 (19)</td>
<td>20.2 ± 8.1 (19)</td>
<td>17.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3</td>
<td>rAc-GST-1</td>
<td>7.1 ± 7.8 (20)</td>
<td>24.6 ± 10.5 (21)</td>
<td>71.3</td>
<td>&lt;0.01</td>
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<tr>
<td>Total</td>
<td></td>
<td>13.0 ± 9.0 (57)</td>
<td>25.2 ± 13.0 (60)</td>
<td>48.4</td>
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3.3 Protective Immunity of rNa-CP-2
Na-CP-2, cysteine protease-2 of N. americanus, was cloned by screening N. americanus L3 cDNA library with Ac-CP-2. The recombinant protein was expressed in E. coli. In the first trial, the mean worm burden in hamsters immunized with rNa-CP-2 was significantly lower than that in adjuvant group with worm reduction rate of 42%. In the repeat test, the difference of mean worm burdens between rNa-CP-2 group and adjuvant group was not significant. When the results of the two tests were combined together, the mean worm burden in rNa-CP-2 group was significantly lower than that in the adjuvant group (Table 4).

TABLE 4  
Protective immunity elicited by immunizing recombinant rNa-CP-2 in hamsters challenged with N. americanus L3.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Vaccine antigen</th>
<th>Vaccine mean worm ± SD (hamster#)</th>
<th>Control mean worm ± SD (hamster#)</th>
<th>Worm reduction rate (%)</th>
<th>P value</th>
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<tbody>
<tr>
<td>1</td>
<td>rNa-CP-2</td>
<td>26.6 ± 23.1 (20)</td>
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<td>42.0</td>
<td>&lt;0.05</td>
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<td>2</td>
<td>rNa-CP-2</td>
<td>31.8 ± 15.0 (20)</td>
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<tr>
<td>Total</td>
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<td>29.2 ± 19.1 (40)</td>
<td>42.4 ± 26.7 (33)</td>
<td>31.1</td>
<td>&lt;0.05</td>
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</table>

3.4 Protective Immunity of rAc-APR-1
Ac-APR-1, aspartic protease-1 secreted by A. cantinum adult worm, is a hemoglobinase for worm to digest host blood hemoglobin as resource of nutrition, therefore a good target for developing vaccine. Each hamster was immunized with 25 μg of recombinant Ac-APR-1 precipitated with 25 μl of 2% Alhydrogel®. After being boosted twice with the same formulation of recombinant Ac-APR-1, hamsters of vaccine and adjuvant group were challenged with 150 N. americanus L3. The mean worm burden of vaccinated group is 20.4±11.4 that is significantly lower than that from adjuvant control group (36.7±25.6) (Table 5).

TABLE 5  
Protective immunity elicited by immunizing recombinant rAc-APR-1 in hamsters challenged with N. americanus L3.

<table>
<thead>
<tr>
<th>Vaccine antigen</th>
<th>Vaccine mean worm ± SD (hamster#)</th>
<th>Adjuvant mean worm ± SD (hamster#)</th>
<th>Worm reduction rate (%)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>rAc-APR-1</td>
<td>20.4 ± 11.4 (16)</td>
<td>36.7 ± 25.6 (12)</td>
<td>44.4</td>
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</table>
Conclusion: These results show that vaccination with recombinant Ac-APR-1 resulted in a marked decrease in worm burden after L3 challenge. Ac-APR-1 thus affords protection against challenge with hookworm larvae to vaccinated hamsters.

3.5 Other Hookworm Vaccine Trials

Other 6 hookworm antigens (rNa-ASP-1, rAc-CP-2, rNa-CTL, rAc-MTP, rNa-CP-4, and rNa-SAA-1) were tested for their protective immunity in the N. americanus-hamster model. The result showed no protective effect for all antigens listed above for hamsters to resist infection with N. americanus L3 (Table 6).

<table>
<thead>
<tr>
<th>Vaccine antigen</th>
<th>Adjuvant</th>
<th>Vaccine mean worm SD (hamster)</th>
<th>Adjuvant mean worm SD (hamster)</th>
<th>Worm Reduction rate (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
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<td>rNa-ASP-1</td>
<td>Freund's</td>
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<td>18.4 ± 14.5 (8)</td>
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<td>—</td>
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<tr>
<td>rAc-CP-2</td>
<td>Freund's</td>
<td>35.7 ± 19.1 (9)</td>
<td>18.4 ± 14.5 (8)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>rNa-CTL</td>
<td>Freund's</td>
<td>25.4 ± 15.5 (8)</td>
<td>18.4 ± 14.5 (8)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>rAc-MTP</td>
<td>Alhydrogel®</td>
<td>32.4 ± 24.4 (20)</td>
<td>45.9 ± 27.9 (21)</td>
<td>29.4 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>rNa-CP-4</td>
<td>Alhydrogel®</td>
<td>20.5 ± 11.9 (4)</td>
<td>17.4 ± 13 (20)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>rNa-SAA-1</td>
<td>Alhydrogel®</td>
<td>19.2 ± 15.2 (2)</td>
<td>17.4 ± 13 (20)</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

References For Example 4


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Example 5

Further Evaluations

An innovative scoring system has been used to select larval antigens for use in the practice of the invention. The criteria were based on five criteria, including an evaluation of the antigen in preclinical studies to 1) reduce host worm burdens, 2) reduce host blood loss, 3) reduce fecal egg counts, and 4) for antibody to inhibit larval invasion in vitro. The fifth criterion was 5) whether there are known orthologues that protect in veterinary vaccines and the sixth criterion was 6) the feasibility and ease of expression, yield and stability. Other factors under consideration included a known function and mechanism of action, association with reductions in risk of acquiring heavy hookworm infection in endemic setting, and immunoeoepidemiology. The results are presented in tabular form in FIGS. 20 and 21. By these rankings, ASP-2 (a L3 secreted antigen) and SAA-2 (a L3 surface antigen) emerged as the two lead candidate larval antigens and APR-1 and GST-1 emerged as the lead candidate adult antigens, with CP-2/3 (cysteine protease) and Cys (cystatin) as viable back-up antigens.
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Phe Leu Glu Leu His Asn Ser Leu Arg Ser Val Ala Leu Gly Gln
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Ala Lys Asp Gly Ala Gly Gly Ala Pro Lys Ala Ala Lys Met Lys
50   55   60
Thr Met Ala Tyr Asp Cys Glu Val Glu Lys Thr Ala Met Asn Asn Ala
65   70   75   80
Lys Gln Cys Val Phe Lys His Ser Gln Pro Asn Gln Arg Lys Gln Leu
95   99  100
Gly Glu Asn Ile Phe Met Ser Ser Asp Ser Gly Lys Ala Lys Ala Ala
100  105  110
Glu Gln Ala Ser Lys Ala Trp Phe Gly Leu Ala Glu Lys Gly Val
115  120  125
Gly Gln Asn Leu Lys Leu Thr Gly Leu Phe Ser Arg Gly Val Gly
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His Tyr Thr Gln Met Val Trp Gln Thr Val Lys Leu Gly Cys Tyr
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  35   40   45
Ile Leu Ala Lys Tyr Ala Ala Arg Lys Leu Gln Ser Ala
  50   55   60
Aasn Glu Ile Arg Glu Leu Arg Asn Tyr Met Asp Ala Glu Tyr Tyr
  65   70   75   80
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Gly Ser Met Lys Gly Phe Ile Ser Lys Asp Ile Val Cys Ile Ala Gly
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Leu Thr Phe Ile Ala Ala Ala Lys Phe Asp Gly Ile Leu Gly Met Ala Phe
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195 200 205
Glu Gln Lys Lys Val Pro Ser Pro Val Phe Ala Phe Trp Pro Asn Arg
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Asn Pro Glu Ser Glu Ile Gly Glu Ile Thr Phe Gly Gly Val Asp
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Thr Arg Arg Tyr Val Glu Pro Ile Thr Trp Thr Pro Val Thr Arg Arg
245 250 255
Gly Tyr Trp Gln Phe Lys Met Asp Met Val Glu Gly Gly Ser Ser Ser
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Ile Ala Cys Pro Asn Gly Cys Gln Ala Ile Ala Asp Thr Gly Thr Ser
275 280 285
Leu Ile Ala Gly Pro Lys Ala Glu Ala Ile Glu Lys Tyr Ile
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Gly Ala Glu Pro Leu Met Lys Gly Tyr Met Ile Pro Cys Asp Lys
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<td>Val Pro Ser Leu Pro Asp Val Ser Phe Ile Ile Asp Gly Lys Thr Phe</td>
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<td>Thr Leu Lys Gly Asp Tyr Val Leu Thr Val Lys Ala Ala Gly Lys</td>
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<td>Ser Ile Cys Leu Ser Gly Phe Met Gly Met Asp Phe Pro Glu Lys Ile</td>
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<td>Gly Glu Leu Trp Ile Leu Gly Asp Val Phe Ile Gly Lys Tyr Tyr Thr</td>
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<td>Val Phe Asp Val Gly Glu Ala Arg Val Gly Phe Ala Glu Ala Lys Ser</td>
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<td>Gln Gly Asp Ser Asp Ser Asp Glu Asp Val Phe Thr Phe</td>
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<210> SEQ ID NO 7
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 7
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Val Ser Leu Ser Arg Glu Pro Thr Leu Arg Glu Arg Leu Ile Ala Ser 20 25 30
Gly Ser Trp Glu Asp Tyr Glu Lys Glu Arg Tyr His Tyr Arg Lys Lys 35 40 45
Ile Leu Ala Lys Tyr Ala Asn Lys Ala Ser Lys Leu Glu Ser Ala 50 55 60
Asn Glu Ile Asp Glu Leu Leu Arg Asn Tyr Met Asp Ala Glu Tyr Tyr 65 70 75 80
Gly Val Ile Glu Ile Gly Thr Pro Ala Glu Asn Phe Thr Val Ile Phe
Asp Thr Gly Ser Ser Asn Leu Trp Val Pro Ser Arg Lys Cys Pro Phe
100 105 110
Tyr Asp Ile Ala Cys Met Leu His Hist Arg Tyr Asp Gly Ala Ser
115 120 125
Ser Thr Tyr Lys Glu Asp Gly Arg Lys Met Ala Ile Gln Tyr Gly Thr
130 135 140
Gly Ser Met Lys Gly Phe Ile Ser Lys Asp Ile Val Cys Ile Ala Gly
145 150 155 160
Ile Cys Ala Glu Glu Gln Pro Phe Ala Glu Ala Thr Ser Glu Pro Gly
165 170 175
Leu Thr Phe Ile Ala Ala Ala Phe Asp Gly Ile Leu Gly Met Ala Phe
180 185 190
Pro Glu Ile Ala Val Leu Val Gly Thr Pro Val Phe His Thr Phe Ile
195 200 205
Glu Gln Lys Tyr Val Pro Ser Pro Pro Val Phe Ala Phe Trp Leu Asn Arg
210 215 220
Asn Pro Glu Ser Glu Ile Gly Gly Ile Thr Phe Gly Gly Val Asp
225 230 235 240
Thr Arg Arg Tyr Val Glu Pro Ile Thr Trp Thr Pro Val Thr Arg Arg
245 250 255
Gly Tyr Trp Gln Phe Lys Met Asp Met Val Gin Gly Gly Ser Ser Ser
260 265 270
Ile Ala Cys Pro Asn Gly Cys Gin Ala Ile Ala Asp Thr Gly Thr Ser
275 280 285
Leu Ile Ala Gly Pro Lys Ala Gin Val Glu Ala Ile Gln Lys Tyr Ile
290 295 300
Gly Ala Glu Pro Leu Met Lys Gly Glu Tyr Met Ile Pro Cys Asp Lys
305 310 315 320
Val Pro Ser Leu Pro Asp Val Ser Phe Ile Ile Asp Gly Lys Thr Phe
325 330 335
Thr Leu Lys Gly Glu Asp Tyr Val Leu Thr Val Lys Ala Ala Gly Lys
340 345 350
Ser Ile Cys Leu Ser Gly Phe Met Gly Met Asp Phe Pro Glu Lys Ile
355 360 365
Gly Glu Leu Trp Ile Leu Gly Asp Val Phe Ile Gly Lys Tyr Tyr Thr
370 375 380
Val Phe Asp Val Gly Gin Ala Arg Val Gly Phe Ala Gln Ala Lys Ser
385 390 395 400
Glu Asp Gly Phe Pro Val Gly Thr Pro Val Arg Thr Phe Arg Gin Leu
405 410 415
Gln Glu Asp Ser Asp Ser Asp Gin Gin Gin Asp Tyr Gin Val Gin Lys Lys
420 425 430

<210> SEQ ID NO 8
<211> LENGTH: 430
<212> TYPE: PRT
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 8
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Gly Ser Trp Glu Asp Tyr Gin Gin Arg Tyr His Tyr Gin Gin Lys Lys
Ile Leu Ala Lys Tyr Ala Ala Asn Lys Ala Ser Lys Leu Gln Ser Ala 50 55 60
Asn Glu Ile Asp Glu Leu Leu Arg Asn Tyr Met Asp Ala Gln Tyr Tyr 65 70 75 80
Gly Val Ile Gln Ile Gly Thr Pro Ala Gln Asn Phe Thr Val Ile Phe 85 90 95
Asp Thr Gly Ser Ser Asn Leu Trp Val Pro Ser Arg Lys Cys Pro Phe 100 105 110
Tyr Asp Ile Ala Cys Met Leu His His Arg Tyr Asp Ser Gly Ala Ser 115 120 125
Ser Thr Cys Lys Glu Asp Gly Arg Lys Met Ala Ile Gln Tyr Gly Thr 130 135 140
Gly Ser Met Lys Gly Phe Ile Ser Lys Asp Ile Val Cys Ile Ala Gly 145 150 155 160
Ile Cys Ala Glu Glu Gln Pro Phe Ala Gln Ala Thr Ser Glu Pro Gly 165 170 175
Leu Thr Phe Ile Ala Ala Lys Phe Asp Gly Ile Leu Gly Met Ala Phe 180 185 190
Pro Glu Ile Ala Val Leu Gly Val Thr Pro Val Phe His Thr Phe Ile 195 200 205
Glu Gln Lys Lys Val Pro Ser Pro Val Phe Ala Phe Trp Pro Asn Arg 210 215 220
Asn Pro Glu Ser Glu Ile Gly Glu Ile Thr Phe Gly Gly Val Asp 225 230 235 240
Thr Arg Arg Tyr Val Gln Pro Ile Thr Trp Thr Pro Val Thr Arg Arg 245 250 255
Gly Tyr Trp Gln Phe Lys Met Asp Met Val Gln Gly Gly Ser Ser Ser 260 265 270
Ile Ala Cys Pro Asn Gly Cys Gln Ala Ile Ala Asp Thr Gly Thr Ser 275 280 285
Leu Ile Ala Gly Pro Lys Ala Gln Val Gln Ala Ile Gln Lys Tyr Ile 290 295 300
Gly Ala Glu Pro Leu Met Lys Gly Tyr Met Ile Pro Cys Asp Lys 305 310 315 320
Val Pro Ser Leu Pro Asp Val Ser Phe Ile Ile Asp Gly Lys Thr Phe 325 330 335
Thr Leu Lys Gly Glu Asp Tyr Val Leu Thr Val Lys Ala Ala Gly Lys 340 345 350
Ser Ile Cys Leu Ser Gly Phe Met Gly Met Asp Phe Pro Glu Lys Ile 355 360 365
Gly Glu Leu Trp Ile Leu Gly Asp Val Phe Ile Gly Lys Tyr Thr 370 375 380
Val Phe Asp Val Gly Gln Ala Arg Val Gly Phe Ala Gln Ala Lys Ser 385 390 395 400
Glu Asp Gly Phe Pro Val Gly Thr Pro Val Arg Thr Phe Arg Gln Leu 405 410 415
Gln Glu Asp Ser Asp Ser Asp Glu Asp Asp Val Phe Thr Phe 420 425 430

<210> SEQ ID NO 9
<211> LENGTH: 1290
<212> TYPE: DNA
<213> ORGANISM: Necator americanus
<400>  SEQUENCE: 9

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cacagatatg attacagaaaa gaagagttttg gtaagagac gttgcctataaa ggtgccctaaag
ttgcaacttg ctatagagttt tttgaaaaatt atagagagttg tcaatattat
ggagttttc aataagttcag aacacctgaa aattttactgt tttatttgcg cactggtcct
	cacaattctg gggtccttc acagaaaaatg ccttctatag acatgcttgt tattggtgac

cacagatacg accttgagagc ttcctctcaca tacaagagaag attgagaaaga gattgtatt

catatggta cagcatcatg gaagagtttc atttccccag aacattgttg tattggtgga
atgtgttgt agagaacacg ttttgcttcag gtcaattcag agccaggtatg cattcttaatt
gctgtcaatt ttgagagatt tttggtcaggg tttctctcttt gttgagctatt
ttgtgata caaattactg gtttgagatt gaggagaaac tttctctttg gttgagctttt
actagagact atgtgaccc tattaccttg acacottgtta caataagagc atttcttcaaa
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gctgtcttg accttgagac ttcctctggt gttgctcagtt tgtgctcagtt gttgctcagtt

cacagatacg tttgctcag gcattctgag aaaggtgatt atcattgctaa
gttctcttc tggcctagc ttcctctatt attgcctgaa aacatcttttc tttggaagga
ggagctaca tcgcttctgt taaagttctgc ggaaccgcccta tttggttctg gtttcatcttg

ggtagtggatt ttcctctagaa gattgagaaatt tttggtagttg ttctttctttg
aaatctata cttttcttttt gttgctcgc agtacttttgc agtactttttgc

ggagctaggtt cttcctctttt gcacacacgcg aacaacctgtttag cgggatgtg

gattgctcag gcctctttttt

<210> SEQ ID NO: 10
<211> LENGTH: 429
<212> TYPE: PRT
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 10

Ser Val His Arg Arg Leu Phe His Gln Ala Arg Arg His Val Thr Ser
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Val Ser Leu Ser Arg Gln Pro Thr Leu Arg Glu Arg Leu Ile Ala Ser
20       25       30

Gly Ser Trp Glu Asp Tyr Gln Lys Gln Arg Tyr His Tyr Arg Lys Lys
35      40      45

Ile Leu Ala Lys Tyr Ala Ala Asn Lys Ala Ser Lys Leu Gln Ser Ala
50      55      60

Asn Glu Ile Asp Glu Leu Arg Leu Arg Tyr Met Asp Ala Glu Tyr Tyr
65      70      75      80

Gly Val Ile Gln Ile Gly Thr Pro Ala Glu Asn Phe Thr Val Ile Phe
95      100     105

Asp Thr Gly Ser Ser Asn Leu Thr Val Pro Ser Arg Lys Cys Pro Phe
110     115     120     125

Tyr Asp Ile Ala Cys Met Leu His His Arg Tyr Ser Asp Gly Ala Ser
130     135     140
Gly Ser Met Lys Gly Phe Ile Ser Lys Asp Ile Val Cys Ile Ala Gly
145 150 155 160
Ile Cys Ala Glu Glu Gln Pro Phe Ala Glu Ala Thr Ser Glu Pro Gly
165 170 175
Leu Thr Phe Ile Ala Ala Lys Phe Asp Gly Ile Leu Gly Met Ala Phe
180 185 190
Pro Glu Ile Ala Val Leu Gly Val Thr Pro Val Phe His Thr Phe Ile
195 200 205
Glu Glu Lys Lys Val Pro Ser Pro Val Phe Ala Phe Trp Leu Asn Arg
210 215 220
Asn Pro Glu Ser Glu Ile Gly Glu Ile Thr Phe Gly Gly Val Asp
225 230 235 240
Thr Arg Arg Tyr Val Glu Pro Ile Thr Trp Thr Pro Val Thr Arg Arg
245 250 255
Gly Tyr Trp Glu Phe Lys Met Asp Met Val Gin Gly Gly Ser Ser Ser
260 265 270
Ile Ala Cys Pro Asn Gly Cys Gin Ala Ile Ala Asp Thr Gly Thr Ser
275 280 285
Leu Ile Ala Gly Pro Lys Ala Ala Val Glu Ala Ile Gin Lys Tyr Ile
290 295 300
Gly Ala Glu Pro Leu Met Lys Gly Tyr Met Ile Pro Cys Asp Lys
305 310 315 320
Val Pro Ser Leu Pro Asp Val Ser Phe Ile Ile Asp Gly Lys Thr Phe
325 330 335
Thr Leu Lys Gly Asp Tyr Val Leu Thr Val Lys Ala Ala Gly Lys
340 345 350
Ser Ile Cys Leu Ser Gly Phe Met Gin Met Asp Phe Pro Glu Lys Ile
355 360 365
Gly Glu Leu Trp Ile Leu Asp Val Phe Ile Gly Tyr Tyr Thr
370 375 380
Val Phe Asp Val Gly Gin Ala Arg Val Gly Phe Ala Gin Ala Lys Ser
385 390 395 400
Glu Asp Gly Phe Pro Val Gly Thr Pro Val Arg Thr Phe Gin Gin Leu
405 410 415
Gln Glu Asp Ser Asp Gin Gin Asp Gin Gin Val Phe Thr
420 425

<210> SEQ ID NO 11
<211> LENGTH: 1290
<212> TYPE: DNA
<213> ORGANISM: Necator americanus

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caaagatctt attacagaaa gaagattttg gtaagtaagc tctgtaaatt ggctttcag 180
ttgccatctg ctacaggtat tgtgaattt tgtgaaattt atataagtt ctaaatattat 240
gggtttacct caatgggaac accagctcaa aattttactg ttattttgag cactgggtac 300
tcaacactgt gggtctctcc aagaaagatt cctttctatt agacttcatg tatgggtcacc 360
cagagatacg acctgtgaac ttctttcaca tacaagaaa atggaagaa gatggttatt 420
caatgatgca cagagtaatc gaagaggttc atttccaaag acatgtattg tattgggtga 480
atggtgctgg agaagacacc tttgtgtgag gctacttcag agccaggtgg gactttcatt 540
gctgctaagt ttgatggaat ttcctgtaaaggt gctggtcCaa aaaggtgaat attgacggaa ggaaagttctg ttgtggattit gctagagttg agaacttitta

<210> SEQ ID NO 12
<211> LENGTH: 428
<212> TYPE: PRT
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 12

Ser Val His Arg Arg Leu Phe His Gln Ala Arg Arg His Val Thr Ser
1  5  10  15
Val Ser Leu Ser Arg Gln Pro Thr Leu Arg Glu Arg Leu Ile Ala Ser
20  25  30
Gly Ser Trp Glu Asp Tyr Gln Lys Gln Arg Tyr His Tyr Arg Lys Lys
35  40  45
Ile Leu Ala Lys Tyr Ala Ala Asn Leu Lys Ala Ser Lys Leu Gln Ser Ala
50  55  60
Aam Glu Ile Asp Glu Leu Arg Asn Tyr Met Asp Ala Gln Tyr Tyr
65  70  75  80
Gly Val Ile Gln Ile Gly Thr Pro Ala Gln Asn Phe Thr Val Ile Phe
95  99 100
Ala Thr Gly Ser Ser Asn Leu Thr Val Pro Ser Arg Lys Cys Pro Phe
105 110
Tyr Asp Ile Ala Cys Leu His His Arg Tyr Asp Ser Gly Ala Ser Ser
115 120 125
Thr Tyr Lys Glu Asp Gly Arg Lys Met Ala Ile Gln Tyr Gly Thr Gly
130 135 140
Ser Met Lys Gly Phe Ile Ser Lys Asp Ile Val Cys Ile Ala Gly Ile
145 150 155 160
Cys Ala Glu Gln Pro Phe Ala Glu Ala Thr Ser Glu Pro Gly Leu
165 170 175
Thr Phe Ile Ala Lys Phe Asp Gly Ile Leu Gly Met Ala Phe Pro
180 185
Glu Ile Ala Val Leu Gly Val Thr Pro Val Phe His Thr Phe Ile Glu
195 200 205
Gln Lys Lys Val Pro Ser Pro Val Phe Ala Phe Trp Leu Asn Arg Aam
210 215 220
Pro Glu Ser Glu Ile Gly Gly Glu Ile Thr Phe Gly Gly Val Asp Thr
225 230 235 240
<210> SEQ ID NO 13
<211> LENGTH: 1290
<212> TYPE: DNA
<213> ORGANISM: Necator americanus

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caaagatattc attacagaaaa gaagattttt tgcataatgag tgccttctaac  180
tttgcaatctc taaagaggaat tggagaattttagatgagttc tctaatatat  240
ggagttacct aataggagcaac accagctcaac aatatatcctg ttatattcag cactggagatc 300
tccaaaattg ggttttcttc aagaaatgttt ctttctaatg acatgctttg tagttgac  360
cacagatgagactctggag ttcotttcccc acoaaagaaact taggaaagaatg gtttcttttatt 420
cataactgta cagatccagc aaagattcct aatcctccag aatcgcttttggattacc  480
attgttgtttt aataagccacct tttgtcctgag gcactctttgc agccaggttaga gcttttcttt  540
gctctagtt ttgatggaactctggtact ctctggattcttt ggtgctcttgctttttttttttttttttt 600
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aaatactata ctgcttccgt cgtgctcag gctagaggtg gttcgctca agctaacatct
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gaggtatgtt tccaggtgg aacagctgga agaactttta gacaatgca agaagattot
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1260
</210> SEQ ID NO: 14
</211> LENGTH: 429
</212> TYPE: PRO
</213> ORGANISM: Necator americanus
</214> SEQUENCE: 14

Val Val His Arg Arg Leu Phe His Gln Ala Arg Arg His Val Thr Ser
1  5  10  15
Val Ser Leu Ser Arg Gln Pro Thr Leu Arg Glu Arg Leu Ile Ala Ser
20  25  30  35
Gly Ser Trp Glu Asp Tyr Gln Gln Arg Tyr His Tyr Arg Lys Lys
35  40  45
Ile Leu Ala Lys Tyr Ala Ala Asn Lys Ala Ser Lys Leu Gln Ser Ala
50  55  60
Asn Glu Ile Asp Glu Leu Leu Arg Asn Tyr Met Asp Ala Gln Tyr Tyr
65  70  75  80
Gly Val Ile Gln Ile Gly Thr Pro Ala Gln Asn Phe Thr Val Ile Phe
85  90  95
Asp Thr Gly Ser Ser Asn Leu Thr Trp Val Pro Ser Arg Lys Cys Pro Phe
100 105 110
Tyr Asp Ile Ala Cys Met Leu His His Arg Tyr Asp Ser Gly Ala Ser
115 120 125
Ser Thr Tyr Lys Glu Asp Gly Arg Lys Met Ala Ile Gln Tyr Gly Thr
130 135 140
Gly Ser Met Lys Gly Phe Ile Ser Lys Asp Ile Val Cys Ile Ala Gly
145 150 155 160
Ile Cys Ala Glu Gln Pro Phe Ala Glu Ala Thr Ser Glu Pro Gly
165 170 175
Leu Thr Phe Ile Ala Ala Lys Phe Asp Gly Ile Leu Gly Met Ala Phe
180 185 190
Pro Glu Ile Ala Val Leu Gly Val Thr Pro Val Phe His Thr Phe Ile
195 200 205
Glu Gln Lys Lys Val Pro Ser Ser Pro Val Phe Ala Phe Trp Leu Asn Arg
210 215 220
Asn Pro Glu Ser Glu Ile Gly Gly Glu Ile Thr Phe Gly Gly Val Asp
225 230 235 240
Thr Arg Arg Tyr Val Glu Pro Ile Thr Trp Thr Pro Val Thr Arg Arg
245 250 255
Gly Tyr Trp Gln Phe Lys Met Asp Met Val Glu Gly Gly Ser Ser Ser Ser
260 265 270
Ile Ala Cys Pro Asn Gly Cys Glu Ala Ile Ala Ala Thr Gly Thr Ser
275 280 285
Leu Ile Ala Gly Pro Lys Ala Glu Val Glu Ala Ile Gln Lys Tyr Ile
290 295 300
Gly Ala Glu Pro Leu Met Lys Gly Gly Tyr Met Ile Pro Cys Asp Lys
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<td>Thr</td>
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<210> SEQ ID NO 15
<211> LENGTH: 1290
<212> TYPE: DNA
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 15

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agacaaaccct ctttagaga gaggaggttt gcccttagtt cagccagagaa gactacaac 120
cagagatctc aacagagaa gaggattttg gttatgacta aaggttta gtoctcttcag 180
tgtgaaatcc ctaagctga atgaaatg tttgagaaat atatggatgt tcaaacattat 240
ggagatttc aacatagcc aaccacctaa aatatttaat cttatattcg cactggatcc 300
tcaacgtgct ggttctcttc aagagatgt aacttccatg acaggttgg cacgttggcc 360
cagagatctc acctctcagag ttttctccat ctaaagaaag tataagata gtaggccatt 420
catagtacg cagagagctt aataacctaa acaggtttgtt tattgtggga 480
attgtgctg aagagcaacccht tttttctttg gatctccag accaagctgt gatttccttcg 540
gctgtaagg tggaggaatt gcttctccttg aaatcccatg ttttggga 600
aacatgcttc ttcatcactt catcagccaa aagagatgtt ctcctcggcc tttttctttc 660
tgtgaaataa gaaactgcga gcctgatgtt ggaggagaaatatcttgg gttgagttgt 720
aactagatc atgtgatgtc tattaccttg acactgata caagagagaa ttagtggca 780
tcttccata atatccgtca acgttcaatat tttttctctt tgtttctcga 840
gctttgttg caacctcgaa tctcttctgt cgccttctca aagatgatgtag 900
cagagatctc tttgcattgt gatggatagg aagagatgtt cagcagatcc 960
gttcttcttc tcgccagctt tcttttattt taggacggaa aacacttatacc ttgtagaag 1020
gagacacagtt cttgatcggt ttaagctgat ggaagatctta tttggtttgt ggtttttttg 1080
ggagattgt cttctcagag agttccagaa tttgggtttt ctggagatgc ttgatattttg 1140
aatttactac gttctttgg gttgtgtcga aagtattatt tgtttttttt 1200
gagatggtct ccccttggag aacctgttctg gaaccttgtt caagactttt gcaacagatttctt 1260
gatcagagc aagagctggt tttttttttct 1290

<210> SEQ ID NO 16
<211> LENGTH: 429
<212> TYPE: PRT
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 16

Ser Val His Arg Arg Leu Phe His Gln Ala Arg Arg His Val Thr Ser 1 5 10 15
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DNA ORGANISM: Necator americanus

SEQUENCE: 17

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tcgcaaaatg gaaactgctg gcttgtcctg gaccttgca gctgttcgga gaaagtgcc
agcaacctgg tcaatccctt gcatgtgccc gctatctggc caggaagttc cgcttccag
ccgtactc ggctcttgca gaccttgcag accttggcag cagcagtaa gctgttccag
gcgtcagat caagtggttc tgtactacgt tctatgggat gcatgaggtc gatgtaggc
aacccaaaaa agaaggtgta cttcctgctc gcgtataaatt cttctgatttc actactaaat
tcttaagaa aagcctctct ggtttccttg tccgctgaac ggtgactttt gaccataa

tgcggtcctg gacgctatg ctcacagttt acgtagttgg cgggttagtgc gccaagtggt
cctcaacttgc gcgtacattt tggacgctgc ggtgcgctct cagctctgtc cggcgtttc
gacaggtatt cctggtctgg ccttctctgat ccaaacttcc gatgtagctgc ctagtcatc
ggctcagat caagtggttc tgtactacgt tctatgggat gcatgaggtc gatgtaggc
aacccaaaaa agaaggtgta cttcctgctc gcgtataaatt cttctgatttc actactaaat
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PRT ORGANISM: Necator americanus

SEQUENCE: 18

Met Val His Tyr Lys Leu Thr Tyr Phe Ala Ile Arg Gly Ala Gly Glu 1 5 10 15
Cys Ala Arg Gin Ile Phe Ala Leu Ala Asp Gin Glu Phe Glu Asp Val 20 25 30
Arg Leu Asp Lys Glu Gin Phe Ala Lys Val Lys Pro Asp Leu Pro Phe 35 40 45
Gly Gin Val Pro Val Leu Glu Val Asp Gin Gly Lys Gin Leu Ala Gin Ser 50 55 60
Leu Ala Ile Cys Arg Tyr Leu Ala Arg Gin Phe Gly Phe Ala Gly Lys 65 70 75 80
Ser Thr Phe Asp Glu Ala Val Asp Ser Leu Ala Asp Gin Tyr Ser 85 90 95
Asp Tyr Arg Val Glu Ile Lys Ser Phe Phe Tyr Thr Val Ile Gly Met 100 105 110
Arg Glu Gly Asp Val Glu Gin Leu Lys Glu Val Leu Leu Pro Ala 115 120 125
Arg Asp Lys Phe Phe Gin Ile Thr Lys Phe Leu Lys Ser Pro 130 135 140
Ser Gly Phe Leu Val Gly Asp Ser Leu Thr Trp Val Asp Leu Leu Val 145 150 155 160
Ser Glu His Asn Ala Thr Met Leu Thr Phe Val Pro Glu Phe Leu Glu 165 170 175
Gly Tyr Pro Glu Val Lys Gly His Met Lys Glu Ile Arg Ala Ile Pro 180 185 190
Lys Leu Lys Lys Trp Ile Glu Thr Arg Pro Glu Thr Leu Phe 195 200 205
<210> SEQ ID NO 19
<211> LENGTH: 733
<212> TYPE: DNA
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 19

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caaacacaag gatgaagtgc cattgtgtca aatakacagtg tggaaagag atggaaaca 180
actagcgcac tcatctgctta tcttccaga aatcttggtt ttcgcggaaa 240
aatcttcgca gaaagacct tagttgac tgggtgac caatacaggg caatacatca 300
tgagatctg ccatctctca ggtcttggtc agaatctgat cagggagact cggagaagct 360
ttccaagga gctgcttcct cagcctgta gaaatctttc ggtctcatga aaaaatctct 420
tgagaaagcg aaatctggtt actctggttg tgattcytgg acatgyctgtg acttygctt 480
agcgcacgac acatgytggtc tgcgtgcgaa gttcccccagc atctatgatg gttcccccctg 540
gatcgaact ctgcgggaaa aggcgtcgtt ctggaaaaat ggtctgcgga 600
tgctgctcgg actacgcttt aatttttccg gttcggtta ccatcggtt cagaggttag 660	taaaaaaaag ggtttctct cctggagtgc cagctcgtt 720
caagccgctg tgt 733

<210> SEQ ID NO 20
<211> LENGTH: 206
<212> TYPE: PRT
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 20

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Pro Ile Arg Gin Ile Phe Ala Leu Ala Gly Gin Tyr Glu Asp Val
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Arg Tyr Thr Phe Gin Glu Trp Pro His Lys Asp Glu Met Pro Phe
35  40  45
Gly Gin Ile Pro Val Leu Glu Gly Asp Gly Lys Gin Leu Ala Gin Ser
50  55  60
Phe Ala Ile Ala Arg Tyr Leu Ser Arg Lys Phe Phe Ala Gly Lys
65  70  75  80
Thr Pro Phe Glu Ala Leu Val Asp Ser Val Ala Asp Gin Tyr Lye
95  100  105
Asp Tyr Ile Asn Glu Ile Arg Pro Tyr Leu Arg Val Ala Asp Val
110 115 120
Asp Gin Gly Asp Pro Glu Lys Leu Phe Lys Glu Leu Leu Leu Pro Ala
125 130 135
Arg Glu Lys Phe Phe Gly Phe Met Lys Lys Phe Glu Lys Ser Lye
140 145 150
Ser Gly Tyr Leu Val Asp Ser Val Thr Tyr Ala Asp Leu Cys Leu
155 160
Ala Glu His Thr Ser Gly Ile Ala Ala Lys Phe Pro Ser Ile Tyr Asp
165 170 175
Gly Phe Pro Glu Ile Lys Ala His Ala Glu Lys Val Arg Ser Ile Pro
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Ala Leu Lys Lys Trp Ile Glu Thr Arg Pro Glu Thr Lye Phe
<210> SEQ ID NO 21
<211> LENGTH: 724
<212> TYPE: DNA
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 21

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gccaaaaag tcggcctgcg cgtgctctcg gcgttttgaa aggtctcgtg tgactctcgcgc 300
cogatacaac acaagccatat cttacattct ccccctccag ttttctacag tttttcttcggt 360
ttgcacccag gacatttcgg gaaactcagc aacctgcct ttcgtcaagc cttcaagaggga 420
ttcctgcatt tccctccagc ggcagctcatt cgtttctcag ctgttttctcag ggtcctcgggg 480
tctgttgcct tcagctcgtc cttcctcagc gtccttctcg gctttctccag gcgttttctcg 540
cgacccagtct tcagctcgtc cccacagccc cccagccagc cccagccagc cccagccagc 600
cgagccttata cagatcttc gttgacccga cccagagccg cccagagccg cccagagccg 660
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<210> SEQ ID NO 22
<211> LENGTH: 206
<212> TYPE: PRT
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 22

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Ile Ile Arg Gin Ile Phe Val Leu Ala Gly Gin Glu Tyr Glu Asp Ile 20 25 30
Arg Leu Ser His Asp Glu Trp Pro Lys Tyr Lys Asn Glu Met Pro Phe 35 40 45
Gly Gin Leu Pro Val Leu Glu Val Asp Gly Lys Leu Ala Gin Ser 50 55 60
Phe Ala Ile Ala Arg Phe Val Ala Lys Phe Gly Phe Ala Gly Lys 65 70 75 80
Cys Pro Phe Glu Ala Leu Val Asp Ser Ile Thr Asp Gin Tyr Lys 85 90 95
Asp Phe Ile Asn Glu Ile Arg Pro Phe Leu Arg Val Ala Met Gly Phe 100 105 110
Ala Glu Gly Asp Leu Glu Lys Leu Ser Asn Glu Val Phe Leu Pro Ala 115 120 125
Arg Glu Lys Phe Phe Gly Phe Met Thr Asn Phe Leu Lys Glu Ser Lys 130 135 140
Ser Gly Tyr Leu Val Gly Asp Ser Leu Thr Phe Ala Asp Leu Tyr Leu 145 150 155 160
Ala Glu Cys Ala Ser Glu Phe Ala Lys Lys Thr Pro Thr Ile Phe Asp 165 170 175
Gly Phe Pro Glu Ile Lys Ala His Ala Glu Lys Val Arg Ser Asn Pro 180 185 190
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<211> LENGTH: 1134
<212> TYPE: DNA
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 23

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tacaagggca aatatccacc agatgtcctaa gaagcatgaa aatctgagaa taatgatttg 240
agtttcatgg ttgatgga agtgatagtg gaaagaaatgg accacgagga gatagtgatag 300
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ataagattaa tcaagtccgta gttgacgctgt gggagtctttt ggcagtaatc tcactacagag 420
gttgtagcag cacagatcct tgtacaatcga aatggaacaa acaggttgttga aagtaaatcctc 480
acgcgaatact tctcattccgt tggaacaagtc tgggtgaaggc ggtgtacctg atgggtgca 540
cgtcaaggttt ctaatgccct ggtgttgaac catggagacac atatggaaac 600
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gactctactg tttcgacaca ggggtgatcc atctcggcc tcaaaaaact tgtcttcagc 780
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ggaaacaacc tttcagctatt tgaacagcgc gcagctgagag gaagttcctaa atgtgtgccga 1080
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<210> SEQ ID NO 24
<211> LENGTH: 347
<212> TYPE: PRT
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 24

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20 25 30
Arg Leu Thr Gly Gln Ala Leu Val Asp Tyr Val Asn Ser His His Ser
35 40 45
Leu Tyr Lys Ala Lys Tyr Ser Pro Arg Ala Gln Glu Arg Met Lys Ser
50 55 60
Arg Ile Met Asp Leu Ser Phe Met Val Asp Ala Glu Val Met Met Glu
45 70 75 80
Glu Met Asp Gln Gln Glu Asp Ile Asp Leu Ala Val Ser Leu Pro Glu
85 90 95
Ser Phe Asp Ala Arg Glu Lys Trp Pro Glu Cys Pro Ser Ile Gly Leu
100 105 110
Ile Arg Asp Gln Ser Ala Gly Gly Gly Cys Trp Ala Val Ser Ser Ala
115 120 125
| Glu Val Met Thr Asp Arg Ile Cys Ile Gln Ser Asn Gly Thr Lys Glu |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 130  | 135  | 140  |
| Val Tyr Val Ser Glu Thr Asp Ile Leu Ser Cys Cys Gly Gln Arg Cys |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 145  | 150  | 155  | 160 |
| Gly Ser Gly Cys Thr Ser Gly Val Pro Arg Gln Ala Phe Asn Tyr Ala |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 165  | 170  | 175  |
| Ile Arg Lys Gly Val Cys Ser Gly Gly Pro Tyr Gly Thr Lys Gly Val |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 180  | 185  | 190  |
| Cys Lys Pro Tyr Pro Phe Tyr Pro Cys Gly Tyr His Ala His Leu Pro |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 195  | 200  | 205  |
| Tyr Tyr Gly Pro Cys Pro Asp Gly Met Trp Pro Thr Pro Thr Cys Glu |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 210  | 215  | 220  |
| Lys Ala Cys Gln Ser Asp Tyr Thr Val Pro Tyr Asn Asp Arg Ile |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 225  | 230  | 235  | 240 |
| Phe Gly Ser Lys Thr Ile Val Leu Thr Gly Glu Lys Ile Lys Arg |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 245  | 250  | 255  |
| Glu Ile Phe Asn Asn Gly Pro Val Leu Val Ala Thr Tyr Thr Val Tyr Glu |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 260  | 265  | 270  |
| Asp Phe Ala Tyr Tyr Lys Asn Gly Ile Tyr Met Thr Gly Leu Gly Arg |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 275  | 280  | 285  |
| Ala Thr Gly Ala His Ala Val Lys Ile Ile Gly Trp Gly Glu Glu Asn |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 290  | 295  | 300  |
| Gly Val Lys Tyr Trp Leu Ile Ala Asn Ser Trp Asn Thr Asp Trp Gly |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 305  | 310  | 315  | 320 |
| Glu Asn Gly Phe Phe Arg Met Leu Arg Gly Thr Leu Asn Cys Asp Ile |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 325  | 330  | 335  |
| Glu Leu Ser Ala Thr Gly Thr Phe Lys Val |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 340  | 345  |

<210> SEQ ID NO 25
<211> LENGTH: 1177
<212> TYPE: DNA
<213> ORGANISM: Necator americanus
<400> SEQUENCE: 25

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ttctgaca acagcacaat gttttttcag gtaaatcaca cgccaaatgc ttttacacatt 180
cctaaatgc gtgtgactgg atcgacattt ctggcataag aagaggytga aatgctaasa 240
gagggagaca tgtggctcag tgaagaaatt cctgttagtt tgtatgctctg agaacaattg 300
cccaatatca cccccatcag atttatcgcg gatcaactcag actgtgggcta actgtgggca 360
gtcggctcag caggaaagat gtcagatcga cttggtgtgcg ataacaacgg tacaatattg 420
gtaacctat cgtagatcga cttgctgccc tgtgcccggc atggatgtgc tggatgtgga 480
gagggccaca catttagaag gttggaatat tttagaaaca caggcttttg caagtggcga 540
cctataagaa caggaggttc ctcgcacaacca taagcttttc actcatgtaa agacgaaagt 600
tacggaaagtt gcggccagag ctttttttcca aacaacaaat gttgaaatat tttctcctgt 660
aatacatgta agaaagtcgg cagacacaa tactacgcca atcgccatca tgaatattcga 720
cagaatcaga cggatgtcga atggagacag atgagaagcc ggcctgtgag aagatcattc 780
aggatttacg cggatgtcga cttgcttcaag aaggagttt atgtgacttt aaggcaaggg 840
gaatagggc gcacggcatg taaaatcatt gcatggggag cggaaaaagt aacggaact 900
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335
Pro Leu Gln Pro Asn Pro Ser Ser
355
360

<210> SEQ ID NO 27
<211> LENGTH: 1181
<212> TYPE: DNA
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 27

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300
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360
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<210> SEQ ID NO 28
<211> LENGTH: 339
<212> TYPE: PRT
<213> ORGANISM: Necator americanus

<400> SEQUENCE: 28

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Leu Tyr Ala Asp Glu Leu Leu His Lys Gin Glu Ser Glu His Gly Leu
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Ser Gly Gin Ala Leu Val Asp Tyr Val Asn Ser His Gin Ser Leu Phe
35 40 45
Lys Thr Glu Tyr Ser Pro Thr Asn Gin Glu Phe Val Lys Ala Arg Ile
50 55 60
Met Asp Ile Lys Tyr Met Thr Glu Ala Ser His Lys Tyr Pro Arg Lys
65 70 75 80
Gly Ile Asn Leu Asn Val Glu Leu Pro Glu Arg Phe Asp Ala Arg Glu
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**ORGANISM:** Necator americanus
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<td>Necator americanus</td>
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Lys Leu Thr Gly Glm Ala Tyr Val Asp Tyr Val Asn Gln His Gln Ser
Phe Tyr Lys Glm Ala Glu Tyr Ser Pro Leu Val Glu Glu Tyr Ala Lys Al
Val Met Arg Ser Glu Phe Met Thr Lys Pro Asn Glm Asn Tyr Val Val
Lys Asp Val Asp Leu Asn Ile Asn Leu Pro Glu Thr Phe Asp Ala Arg
Glu Lys Trp Pro Asn Cys Thr Ser Ile Arg Thr Ile Arg Asp Glm Ser
Asn Cys Gly Ser Cys Trp Ala Val Ser Ala Ala Ser Val Met Ser Asp
Arg Leu Cys Ile Glm Ser Asn Gly Thr Ile Glm Ser Thr Ala Ser Asp
Thr Asp Ile Leu Ser Cys Trp Asn Cys Glm Met Gey Cys Asp Gly
Gly Arg Pro Phe Ala Ala Phe Phe Phe Ala Ile Asp Asn Gey Val Cys
Thr Gly Glm Pro Phe Arg Glu Pro Asn Val Cys Lys Pro Tyr Ala Phe
Tyr Pro Cys Gly Arg His Glm Asn Gey Lys Tyr Phe Gly Pro Cys Pro
Lys Glu Leu Trp Pro Thr Pro Lys Cys Arg Lys Met Cys Glm Leu Lys
Tyr Asn Val Ala Tyr Lys Asp Lys Ile Tyr Gly Asn Asp Ala Tyr
Ser Leu Pro Asn Asn Glu Thr Arg Ile Met Glm Glu Ile Phe Thr Asn
Gly Pro Val Val Gly Ser Phe Ser Val Phe Ala Asp Phe Ala Ile Tyr
Lys Lys Gly Val Tyr Val Ser Asn Gly Ile Gin Gin Asn Gly Ala His
Ala Val Lys Ile Ile Gly Trp Gly Val Gln Asp Gly Leu Lys Tyr Trp
290 295 300
Leu Ile Ala Asn Ser Trp Asn Asn Trp Gly Asp Gly Gly Tyr Val
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340

<210> SEQ ID NO 31
<211> LENGTH: 759
<212> TYPE: DNA
<213> ORGANISM: Necator american

<400> SEQUENCE: 31

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 180
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 240
gtcgccaacc gggcgtcaca aagacacatt agggggagtg gacaactata ctaaagtgaat
 300
tcaacagtc ggctatcagc tagcaagga ggttctcagc aaatttgagg gaacaaaggg
 360
gaatttcggc caaatttggc aagatatttt gcagggaaat ggtggagagc ggtggtgaa
 420
gtggctcaat cggatctcgg ggcctgctcg caagttgacg gtcgactgtcg tggccgctgt
 480
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 540
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 600
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 720
cgtttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
Gly Arg Cys Thr Leu Ala Ala Ala Val Ala Pro Val Val Leu Ala
145
Leu Ile Arg

SEQ ID NO 33
LENGTH: 527
TYPE: DNA
ORGANISM: Necator americanus

SEQ ID NO 34
LENGTH: 146
TYPE: ORGANISM: Necator americanus

Met Leu Lys Leu Val Ala Leu Val Cys Leu Val Ala Ile Cys Phe Ala
1 5 10 15
Gln Gly Pro Gln Gly Pro Pro Phe Leu Gln Ser Ala Pro Ala Ala
20 25 30
Val Gln Gln Asp Phe Asp Lys Leu Phe Val Asn Ala Gly Ser Thr
35 40 45
Asp Ala Glu Ile Asp Lys Met Val Gln Asp Trp Val Gly Lys Glu Asp
50 55 60
 Ala Ser Ile Lys Thr Ala Phe Asp Ala Phe Val Lys Glu Val Lys Ala
65 70 75 80
 Ala Gln Ala Gln Gly Glu Ala Ala His Gln Ala Ala Ile Ala Lys Phe
85 90 95
 Ser Ala Glu Ala Ala Asp Ala Lys Leu Ser Ala Ile Ala Asn
100 105 110
 Asp Arg Ser Lys Thr Asn Ala Gln Gly Ala Glu Ile Asp Ser Val
115 120 125
 Leu Lys Gly Leu Pro Pro Asn Val Arg Thr Glu Ile Glu Asn Ala Met
130 135 140
Lys Gly
145
We claim:

1. An immunogenic composition, comprising:
   isolated substantially purified recombinant hookworm antigens *Necator americans* aspartic protease 1 (Na-APR-1) and *Necator americans* glutathione S transferase 1 (Na-GST-1), wherein
   said Na-APR-1 hookworm antigen has the amino acid sequence as set forth in SEQ ID NO: 4 or SEQ ID NO: 6 or SEQ NO: 10 or SEQ NO: 12 or SEQ ID NO: 14 or SEQ ID NO: 16, and
   said Na-GST-1 hookworm antigen has the amino acid sequence as set forth in SEQ ID NO: 18.