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**Funkhouser et al.**

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(54) **ATTENUATED HEPATITIS A VIRUS VACCINE WHICH GROWS IN MRC-5 CELLS**

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(51) **Int. Cl.**[7] ..... **A61K 39/29**; C12N 7/08

(52) **U.S. Cl.** ..... **424/226.1**; 435/237

(58) **Field of Search** ..... 435/235.1, 236, 435/237; 424/93.1, 93.6, 226.1

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(74) *Attorney, Agent, or Firm*—Morgan & Finnegan, L.L.P.

(57) **ABSTRACT**

A live hepatitis A virus (HAV) adapted to grow in MRC-**5** cells is described, the HAV preferably characterized by suitable attenuation for effective vaccine administration to humans and animals without inactivation. Methods for adapting HAV to grow in MRC-**5** cells, vaccine compositions comprising the attenuated HAV, and methods of vaccinating humans against HAV infection are also described.

**6 Claims, 5 Drawing Sheets**

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FIG. 1

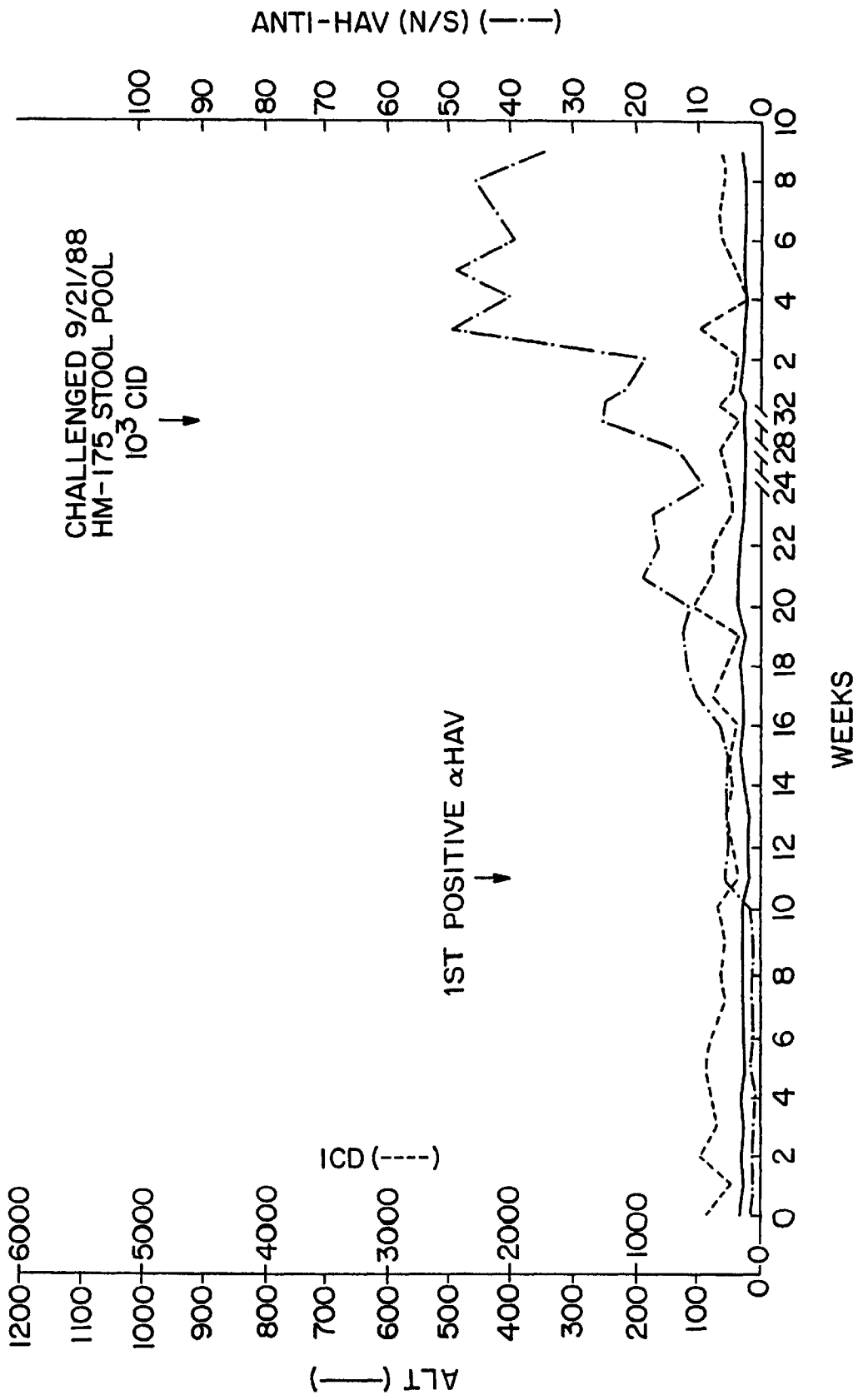
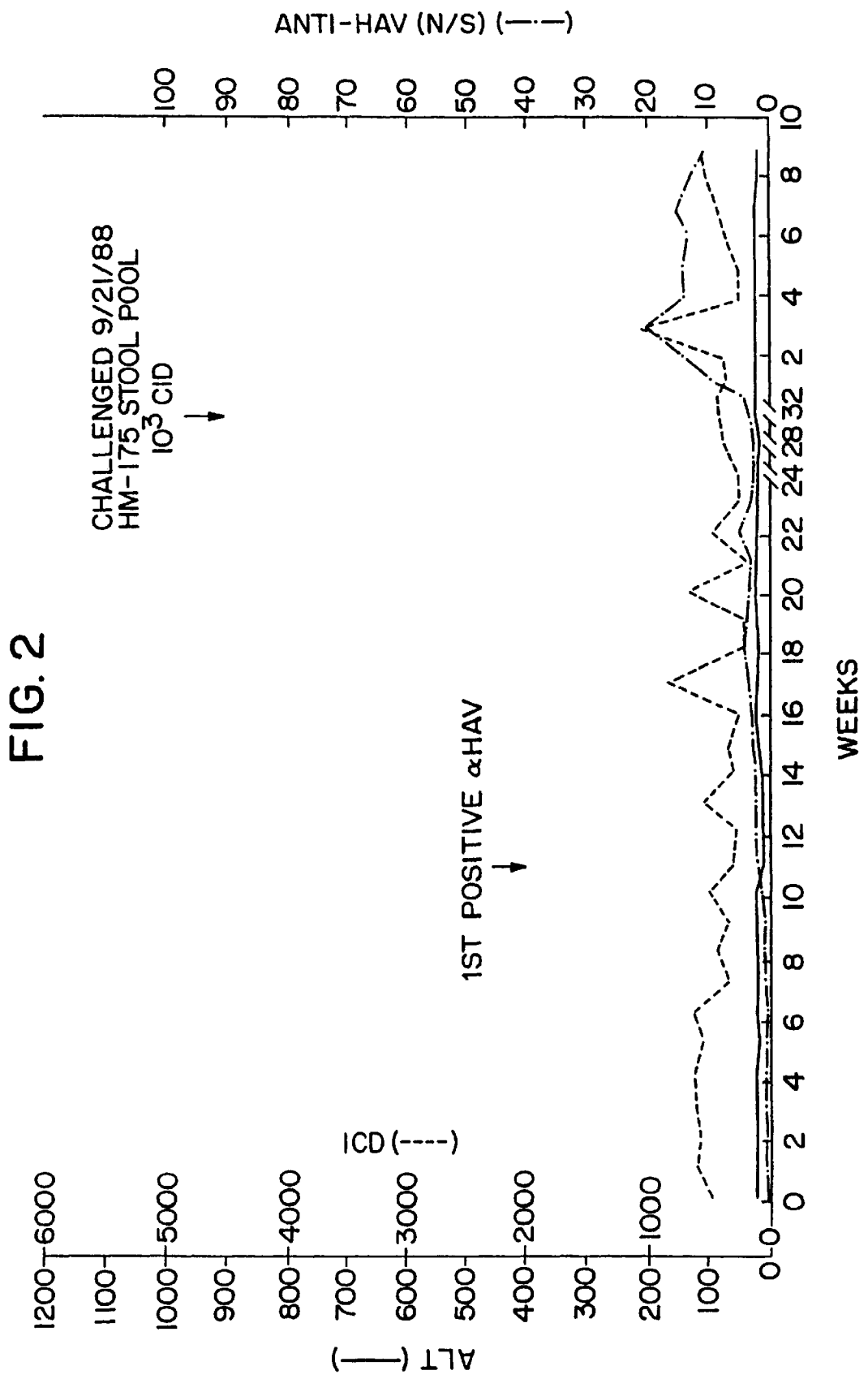

ANTI-HAV (N/S) (—·—)
100
90
80
70
60
50
40
30
20
10
0
CHALLENGED 9/21/88
HM-175 STOOL POOL
$10^3$ CID
1ST POSITIVE αHAV
0 2 4 6 8 10 12 14 16 18 20 22 24 28 32 2 4 6 8 10
WEEKS
ICD (----)
1200 1100 1000 900 800 700 600 500 400 300 200 100
6000 5000 4000 3000 2000 1000 0
ALT (——)

FIG. 2


ANTI-HAV (N/S) (—·—)
CHALLENGED 9/21/88
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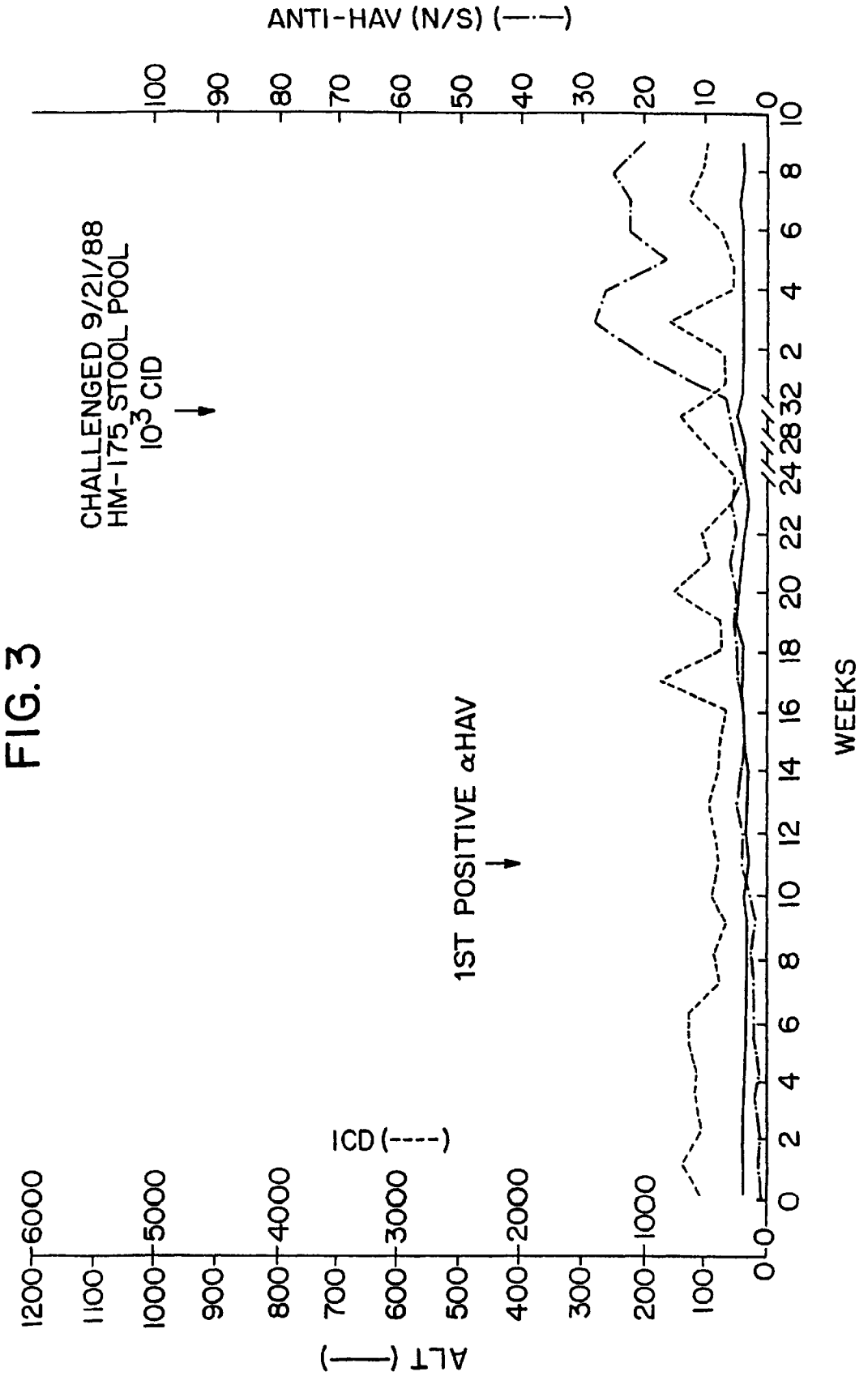
$10^3$ CID
1ST POSITIVE αHAV
ALT (——)
ICD (----)
WEEKS

FIG. 3


ANTI-HAV (N/S) (—·—)
CHALLENGED 9/21/88
HM-175 STOOL POOL
$10^3$ CID
1ST POSITIVE αHAV
ICD (----)
ALT (——)
WEEKS

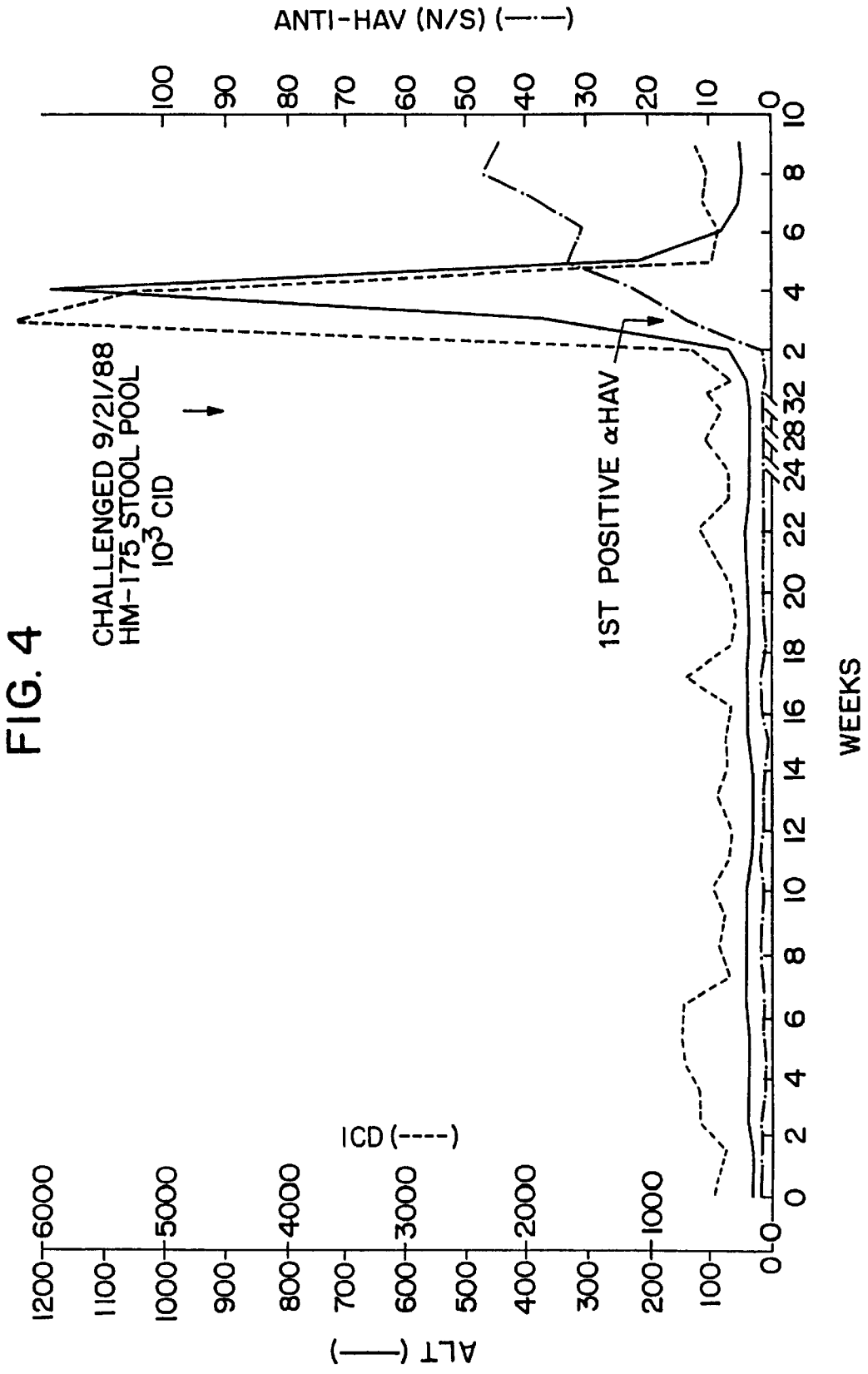
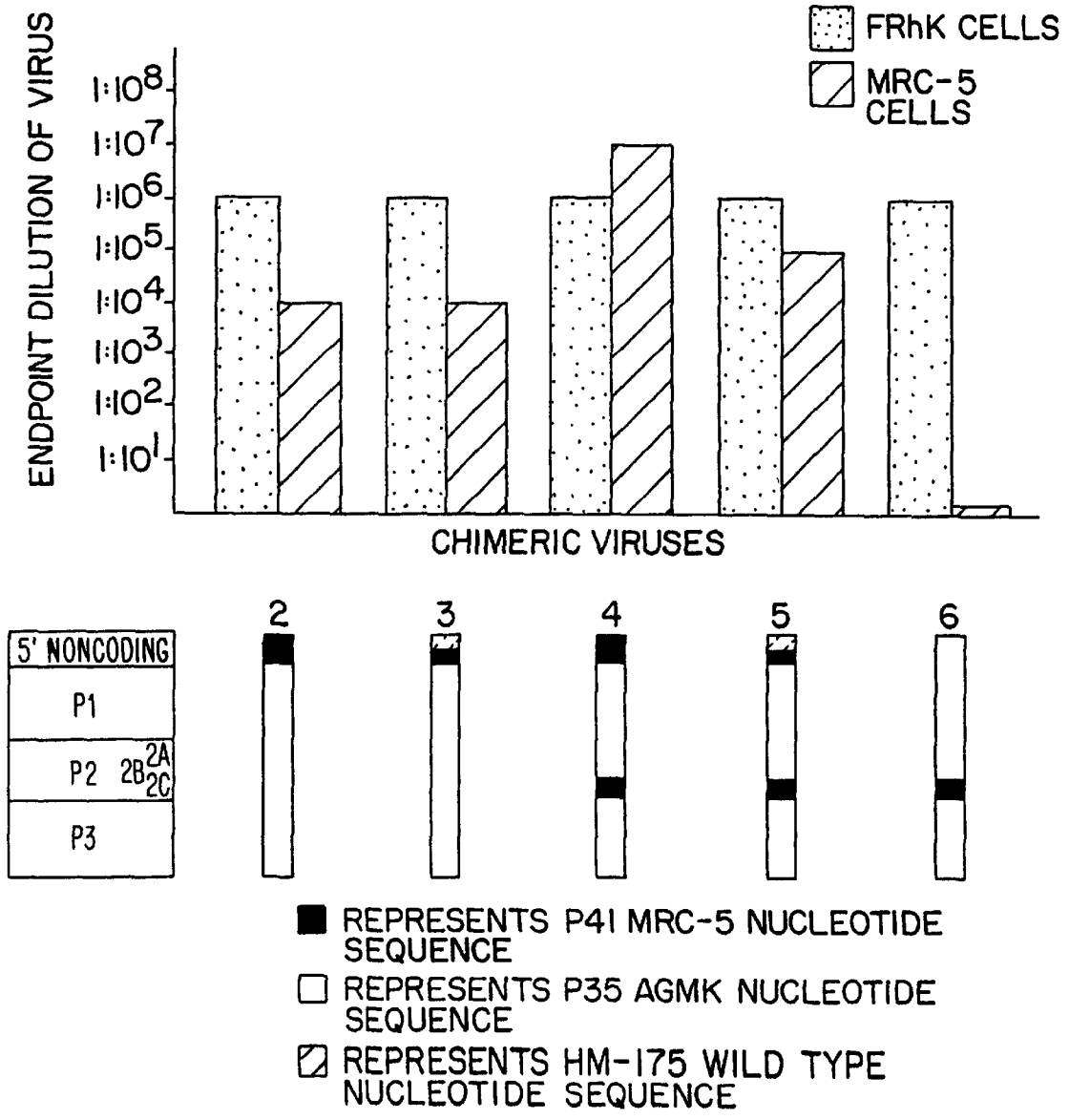

ANTI-HAV (N/S) (—·—)
100
90
80
70
60
50
40
30
20
10
0
CHALLENGED 9/21/88
HM-175 STOOL POOL
$10^3$ CID
1ST POSITIVE αHAV
0 2 4 6 8 10 12 14 16 18 20 22 24 28 32
WEEKS
ICD (----)
ALT (——)
1200 1100 1000 900 800 700 600 500 400 300 200 100
6000 5000 4000 3000 2000 1000
0

FIG. 4

FIG. 5


FRhK CELLS
MRC-5 CELLS
ENDPOINT DILUTION OF VIRUS
1:10^8
1:10^7
1:10^6
1:10^5
1:10^4
1:10^3
1:10^2
1:10^1
CHIMERIC VIRUSES
2
3
4
5
6
5' NONCODING
P1
P2 2B 2A 2C
P3
REPRESENTS P41 MRC-5 NUCLEOTIDE SEQUENCE
REPRESENTS P35 AGMK NUCLEOTIDE SEQUENCE
REPRESENTS HM-175 WILD TYPE NUCLEOTIDE SEQUENCE

# ATTENUATED HEPATITIS A VIRUS VACCINE WHICH GROWS IN MRC-5 CELLS

## CROSS-REFERENCE TO RELATED APPLICATIONS

This is a 371 national phase filing of PCT/US93/08610, filed Sep. 17, 1993, which was a continuation-in-part of U.S. patent application Ser. No. 07/947,338, filed Sep. 18, 1992, now abandoned.

This invention was made with government support under certain Collaborative Research and Development Agreements awarded by the Department of Health and Human Services. The government has certain rights in this invention.

## FIELD OF THE INVENTION

The present invention relates generally to the field of vaccinal compositions useful in the prophylaxis of hepatitis A. More specifically, the invention provides a novel live hepatitis A virus (HAV), and recombinant and chimeric HAVs, the genomes of which are modified from that of their parental strain HM-175 to provide them with the ability to propagate in MRC-5 cells and retain appropriate attenuation for use as live vaccines in humans and other primates.

## BACKGROUND OF THE INVENTION

In the United States, hepatitis A virus is the cause of approximately 25% of all clinical hepatitis cases, accounting for approximately 150,000 such cases. Populations at high risk of acquiring hepatitis A in industrialized countries include the socially disadvantaged, medical personnel, military personnel, staff and adult contacts of children in day-care centers, male homosexuals, drug addicts, and travelers to endemic areas.

In developing countries, virtually the entire population is infected with hepatitis A virus at an early age. Much of this invention results in subclinical and inapparent infection, but, as countries improve their hygienic conditions, infection with hepatitis A virus occurs at progressively older ages, resulting in a higher proportion of clinical disease. Thus, there is a paradoxical increase in clinical hepatitis A as the overall rate of infection diminishes. To successfully immunize against hepatitis A in the United States and in other industrialized countries as well as in developing countries, it will be necessary to vaccinate the entire pediatric population. There will be an increasing need for hepatitis A vaccines in such countries for the foreseeable future.

Research in HAV vaccines has focused on inactivated, or killed, viruses. However, in vaccine, therapy there are several advantages to a live vaccine, rather than an inactivated, vaccine. With a live vaccine, one can use a lower dosage and smaller number of doses, because a live vaccine replicates in the vaccines to produce more antigen and can stimulate the immune system of the vaccinee to make both IgA and IgM. Inactivated vaccines, such as the Salk polio vaccine, which stimulates production of IgG only in vaccinees, do not protect against infection by ingested virus, only against disease.

A major obstacle to the development of live, attenuated vaccine has been the difficulty in adapting HAV to a cell line that supports rapid viral growth and is licensed for vaccine production. Wild type hepatitis A virus (HAV) grows poorly in cell culture.

U.S. Pat. Nos. 4,532,215 and 4,636,469 describe, respectively, a strain of HAV designated HM-175 initially



isolated from human feces of a patient in Melbourne, Australia, and adapted to passage in vitro in African green monkey kidney (AGMK) culture cells and methods for obtaining same by serial passaging. U.S. Pat. No. 4,620,978 describes a vaccine employing the HAV HM-175, triply cloned in AGMK cell culture and attenuated. U.S. Pat. No. 4,894,228 describes HM-175 Pass 35, which differs from wild-type HM-175 by nucleotide changes in the genome, is attenuated for chimpanzees, elicits serum neutralizing antibodies, and is suitable for use as an attenuated HAV vaccine. It discloses the complete nucleotide sequence of HAV, strain HM-175/7. See, also, B. C. Ross et al., *J. Gen. Virol.,* 70:2805–2810 (1989); R. W. Jansen et al, *Virol.,* 163:299–307 (1988); and Tedeschi et al, *J. Med. Virol.,* (in press). The disclosure of these patents and articles are incorporated by reference herein.

N. Fineschi et al, *J. Hepatol.,* 13(4):S146–S151 (1991) describes an HAV isolate, LSH/S, which is a candidate for an inactivated vaccine. It was adapted to grow in human diploid MRC-5 cells, a preferred licensed cell for vaccine development. This document compares only a small part of its nucleotide sequence to that of wild-type HM-175.

Provost et al, *J. Med. Virol.,* 20:165–175 (1986) described the F and F' variants of the CR326 hepatitis A virus strain. While it is reported to be immunogenic in volunteer vaccinees, the F variant also caused abnormal serum ALT levels in a substantial proportion of individuals.

Another recent publication from this group of investigators has described further work with the F' variant [K. Midthun et al, *J. Infect. Dis.,* 163:735–739 (1991)]. They observed that the immunogenicity of the F' vaccine product is dose dependent, i.e., a $10^{7.3}$ $TCID_{50}$ evoked an antibody response in 100% of volunteers within 9 weeks after immunization whereas lower doses were immunogenic in a smaller percentage of volunteers, and anti-HAV was observed 4 to 6 months after immunization. Chinese investigators have recently described studies of a potential live attenuated hepatitis A vaccine prepared from the H2 strain of HAV [J. S. Mao et al, *J. Infect. Dis.,* 159:621–624 (1989)]. Twelve volunteers received the vaccine by the subcutaneous route.

A live attenuated hepatitis A vaccine could have a significant impact on the eradication of the disease. It could be anticipated that a live attenuated vaccine which requires minimal purification and no adjuvant would be less costly than presently available inactivated hepatitis A vaccines.

There is a need in the art for methods and compositions for effective vaccination of humans and animals against hepatitis A.

## SUMMARY OF THE INVENTION

In one aspect, the present invention provides a live hepatitis A virus adapted to growth in MRC-5 cells. This virus is preferably characterized by attenuation. The attenuated virus may be, recombinant or chimeric. More preferably, the HAV is characterized by suitable attenuation for effective vaccine administration to primates, preferably humans, without inactivation. The HAV may be characterized by containing one or more of fourteen specific nucleotides which differ from nucleotides in the same position in the genome of HAV HM-175, Pass 35.

In another aspect, the invention provides a vaccine useful for protecting humans or other primates against hepatitis A which vaccine contains at least one above-described HAV adapted to growth in MRC-5 cells. Preferably, the vaccine is effective in inducing a protective antibody response without adjuvant.

In still another aspect, the invention provides a method for protecting humans against hepatitis A virus infection which comprises administering to the human patient an effective amount of a vaccine composition of this invention.

In a further aspect, the invention provides a method for preparing a live HAV adapted to growth in MRC-5 cells by incorporating into a selected area of the genome of an HAV one or more of fourteen specific nucleotides. The HAV genome so modified is preferably HAV HM-175, Pass 35 or a related cell culture-adapted mutant.

In another embodiment, the HAV may be constructed using another HAV cDNA clone and inserting appropriate nucleotides into its genome. According to this method an attenuated, MRC-5-adapted HAV is provided without requiring further passaging in MRC-5 or other primate cell lines.

In still another aspect, the invention provides polynucleotide sequences encoding the recombinant or chimeric HAVs described above. Preferably these sequences are cDNAs useful as master seeds for vaccine preparation.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a graph plotting anti-HAV antibody production vs. time (weeks) vs. ALT and ICD levels for the chimpanzee studies with Virus 4380 and subsequent challenge with wild-type HAV, as described in Example 1. These results were obtained from a chimpanzee that was infected with the attenuated HAV at time 0 and challenged with virulent virus at week 28.

FIG. **2** is a graph plotting anti-HAV antibody production vs. time (weeks) vs. ALT and ICD levels for the chimpanzee studies with Virus 4380 and subsequent challenge with wild-type HAV, as described in Example 1. The conditions were the same as for FIG. **1**.

FIG. **3** is a graph plotting anti-HAV antibody production vs. time (weeks) vs. ALT and ICD levels for the chimpanzee studies with Virus 4380 and subsequent challenge with wild-type HAV, as described in Example 1. The conditions were the same as for FIG. **1**.

FIG. **4** is a graph plotting anti-HAV antibody production vs. time (weeks) vs. ALT and ICD levels for the chimpanzee studies with Virus 4380 and subsequent challenge with wild-type HAV, as described in Example 1. These results were obtained from the chimpanzee that was not infected with the attenuated HAV, and therefore developed hepatitis following challenge with the virulent virus. The conditions were the same as for FIG. **1**.

FIG. **5** is a bar graph of endpoint dilutions of several of the chimeric viruses, Viruses #2, 3, 4, 5, and 6, listed in Table VI.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides hepatitis A virus (HAV) adapted to growth in the human fibroblast-like cell line, MRC-5, a cell substrate suitable for commercial production and licensing of inactivated and live, attenuated hepatitis A vaccines. In addition to such adapted HAVs, the invention provides a method for adapting a selected HAV to growth in that human cell line and preparing an MRC-5-adapted, attenuated HAV without passaging in other primate cells. The HAV of this invention and the preparative method also preferably provides the HAV with sufficient attenuation to enable its efficacy as a vaccine for humans and animals.

Although the prior art discloses other candidate vaccine strains of hepatitis A virus which have been adapted to growth in human diploid fibroblasts, the genetic changes in the virus genome necessary and sufficient for such adaptation have not been characterized. Thus, these strains cannot be manipulated in vitro to assure a reproducible and fully-characterized vaccine product.

The present invention is based on the wild-type HAV, strain HM-175, which is described in detail in the above-cited and incorporated art [Cohen et al., *J. Virol.,* 61:50–59 (1987); SEQ ID NO:1 and 2]. Briefly described, the wild type, infectious HAV HM-175 virus was previously adapted to growth in primary African green monkey kidney (AGMK) cells at 37° C. After 26 passages in AGMK, the virus was cloned three times in AGMK cells by serial dilution, then passage three more times to provide passage 32 (P-32). P-32 was found to be attenuated as described in R. A. Karron et al, *J. Infec. Dis.,* 157:338–345 (1988).

The P-32 virus described above was passaged three more times in AGMK, and molecularly clones. The virus that was cloned was called P-35 and the full-length clone was referred to as pHAV/7. pHAV/7 is an infectious cDNA clone of the virus that can be maintained in a monoclonal state and amplified at will with diminished risk of spontaneous mutations. The resulting P-35 virus grew well in fetal rhesus monkey kidney (FRhK) cells and minimally in human fibroblastoid lung cells (MRC-5).

U.S. Pat. No. 4,894,228 and Cohen et al., *Proc. Natl. Acad. Sci., USA,* 84:2497–2501 (1987) provide the HAV nucleotide sequences of wild-type HAV strain HM-175 (see, FIG. **1** of the patent; SEQ ID NO:1 and 2) and the nucleotide differences between HAV HM-175, Pass 35, clone pHAV/7 and the wild-type sequence [SEQ ID NO:1]. Thus, these documents, incorporated by reference, provide the sequence of pHAV/7, P-35. The nucleotide numbers used herein to which the mutations of this invention correspond (Tables I and VI below) are the nucleotide numbers assigned to positions of the wild-type sequence of FIG. **1** [SEQ ID NO:1 and 2] from U.S. Pat. No. 4,894,228 containing the mutations for P-35. Note that the nucleotides deleted in P-35 are assigned the nucleotide position of the wild-type sequence [SEQ ID NO:1]. Thus, for example, nucleotide position 131 represents a nucleotide that was deleted between wild-type and P-35. The P-35 cDNA, i.e., HAV/HM-175/7, is on deposit at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. under Accession No. 67495, deposited Aug. 7, 1987. One of skill in the art can readily construct the nucleotide and amino acid sequences of P-35 by use of the above-cited art.

Thereafter the P-32 AGMK cell-adapted and attenuated virus was further manipulated to enable it to be adapted for growth in MRC-5 cells, so that it is available for large scale vaccine production. Passage 32 was double plaque cloned in MRC-5 to form Passage 37. Passage 37 was passaged once in MRC-5 of a selected clone 24-4-21. The resulting Passage 38 was passaged three times in MRC-5 cells, resulting in Passage 41, the master seed, designated 87J19. This master seed virus stock was also called virus 4380, and is referred to throughout this disclosure by the latter name.

Live attenuated virus HAV 4380, was deposited on Apr. 4, 1990 at the Collection Nationale de Cultures de Microorganismes, Institut Pasteur, 25, rue du Docteur Roux, 75724, Paris CEDEX 15 under Accession No. I-936. The deposited HAV 4380 virus has the nucleic acid sequence shown in SEQ ID NO:3.

TTCAAGAGGG GTCTCCGGGA ATTTCCGGAG TCCCTCTTGG AAGTCCATGG TGAGGGGACT 60

TGATACCTCA CCGCCGTTTG CCTAGGCTAT AGGCTAAATT TTCCCTTTCC CTTTTCCCTT 120

TCCCATTCCC TTTTGCTTGT AAATATTGAT TCCTGCAGGT TCAGGGTTCT TAAATCTGTT 180

TCTCTATAAG AACACTCATT TTCACGCTTT CTGTCTTCTT TCTTCCAGGG CTCTCCCCTT 240

GCCCTAGGCT CTGGCCGTTG CGCCCGGCGG GGTCAACTCC ATGATTAGCA TGGAGCTGTA 300

GGAGTCTAAA TTGGGGACAC AGATGTTTGG AACGTCACCT TGCAGTGTTA ACTTGGCTTT 360

CATGAATCTC TTTGATCTTC CACAAGGGGT AGGCTACGGG TGAAACCTCT TAGGCTAATA 420

CTTCTATGAA GAGATGCCTT GGATAGGGTA ACAGCGGCGG ATATTGGTGA GTTGTTAAGA 480

CAAAAACCAT TCAACGCCGG AGGACTGACT CTCATCCAGT GGATGCATTG AGTGGATTGA 540

CTGTCAGGGC TGTCTTTAGG CTTAATTCCA GACCTCTCTG TGCTTGGGGC AAACATCATT 600

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GGT CTT GAC CAC ATC CTG TCT TTG GCA GAC ATT GAG GAA GAG CAA ATG 819

ATT CAA TCA GTT GAT AGG ACT GCA GTG ACT GGT GCT TCT TAT TTT ACT 867

TCT GTG GAT CAA TCT TCA GTT CAT ACA GCT GAG GTT GGA TCA CAC CAG 915

GTT GAA CCT TTG AGA ACC TCT GTT GAT AAA CCC GGT TCA AAG AGG ACT 963

CAG GGA GAG AAA TTT TTC TTG ATT CAT TCT GCA GAT TGG CTT ACT ACA 1011

CAT GCT CTT TTC CAT GAA GTT GCA AAA TTG GAT GTG GTG AAA TTA TTA 1059

TAC AAT GAG CAG TTT GCT GTT CAA GGG TTG TTG AGA TAC CAT ACA TAT 1107

GCA AGA TTT GGC ATT GAA ATT CAA GTT CAG ATA AAC CCT ACA CCT TTC 1155

CAA CAG GGG GGA TTG ATC TGT GCT ATG GTT CCT GGT GAC CAG AGC TAT 1203

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TTA ACT CCT CTT TCT ACA CAA ATG ATG AGA AAT GAA TTT AGG GTC AGT 1491

ACT ACT GAG AAT GTG GTG AAT CTG TCA AAT TAT GAA GAT GCA AGA GCA 1539

AAG ATG TCT TTT GCT TTG GAT CAG GAA GAT TGG AAA TCT GAT CCG TCC 1587

CAG GGT GGT GGG ATC AAA ATT ACT CAT TTT ACT ACT TGG ACA TCT ATT 1635

CCA ACT TTG GCT GCT CAG TTT CCA TTT AAT GCT TCA GAC TCA GTT GGT 1683

CAA CAA ATT AAA GTT ATT CCA GTT GAC CCA TAT TTT TTC CAA ATG ACA 1731

AAT ACA AAT CCT GAC CAA AAA TGT ATA ACT GCT TTG GCT TCT ATT TGT 1779

CAG ATG TTT TGT TTT TGG AGA GGA GAT CTT GTC TTT GAT TTT CAA GTT 1827

TTT CCC ACC AAA TAT CAT TCA GGT AGA TTA CTG TTT TGT TTT GTT CCT 1875

GGC AAT GAG CTA ATA GAT GTT TCT GGA ATC ACA TTA AAG CAA GCA ACT 1923

ACT GCT CCT TGT GCA GTA ATG GAT ATT ACA GGA GTG CAG TCA ACT TTG 1971

AGA TTT CGT GTT CCC TGG ATT TCT GAC ACT CCT TAC AGA GTG AAC AGG 2019

TAT ACA AAG TCA GCA CAT CAG AAA GGT GAG TAC ACT GCC ATT GGG AAG 2067

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CTT ATT GTG TAT TGT TAT AAC AGA TTG ACC TCT CCT TCT AAC GTT GCT      2115

TCC CAT GTC AGA GTG AAT GTT TAT CTT TCA GCA ATT AAC TTG GAA TGT      2163

TTT GCT CCT CTT TAT CAT GCT ATG GAT GTT ACT ACA CAA GTT GGA GAT      2211

GAT TCT GGA GGT TTT TCA ACA ACA GTT TCT ACA GAA CAG AAT GTT CCA      2259

GAT CCC CAA GTT GGT ATA ACA ACC ATG AAA GAT TTG AAA GGA AAA GCT      2307

AAC AGA GGG AAA ATG GAT GTT TCA GGA GTA CAA GCA CCT GTG GGA GCT      2355

ATC ACA ACA ATT GAG GAT CCA GTT TTA GCA AAG AAA GTA CCT GAG ACA      2403

TTT CCT GAA TTG AAA CCT GGA GAA TCC AGA CAT ACA TCA GAT CAT ATG      2451

TCC ATC TAC AAG TTT ATG GGA AGG TCT CAT TTC TTG TGC ACT TTT ACA      2499

TTC AAT TCA AAT AAT AAA GAG TAC ACA TTT CCT ATA ACC TTG TCT TCA      2547

ACC TCT AAT CCT CCT CAT GGT TTG CCA TCA ACA CTG AGG TGG TTT TTC      2595

AAC TTG TTT CAG TTG TAT AGA GGG CCT TTA GAT CTG ACA ATT ATT ATT      2643

ACA GGA GCA ACT GAT GTA GAT GGC ATG GCC TGG TTC ACT CCA GTA GGT      2691

CTT GCC GTT GAT ACT CCT TGG GTA GAG AAG GAG TCA GCT TTG TCT ATT      2739

GAC TAT AAA ACT GCT CTT GGA GCT GTC AGA TTT AAC ACA AGG AGA ACA      2787

GGG AAC ATT CAG ATT AGA TTA CCA TGG TAT TCT TAT TTA TAT GCT GTG      2835

TCT GGA GCA CTG GAT GGT TTG GGA GAC AAG ACA GAT TCT ACA TTT GGA      2883

TTG GTT TCT ATT CAG ATT GCA AAT TAC AAT CAT TCT GAT GAA TAC TTG      2931

TCT TTT AGT TGT TAT TTG TCT GTC ACA GAA CAA TCA GAG TTT TAT TTT      2979

CCC AGA GCT CCA TTG AAC TCA AAT GCC ATG TTA TCC ACT GTA ACA ATG      3027

ATG AGC AGA ATT GCA GCT GGA GAC TTG GAG TCA TCA GTG GAT GAT CCT      3075

AGA TCA GAG GAA GAT AAA AGA TTT GAG AGT CAT ATA GAA TGC AGG AAG      3123

CCA TAT AAA GAA CTG AGA TTA GAA GTT GGG AAA CAA AGA CTC AAG TAT      3171

GCT CAG GAA GAA TTG TCA AAT GAA GTA CTT CCA CCC CCT AGG AAA ATG      3219

AAG GGA CTG TTT TCA CAA GCC AAA ATT TCT CTT TTT TAT ACT GAG GAG      3267

CAT GAA ATA ATG AAG TTT TCC TGG AGA GGT GTG ACT GCT GAT ACT AGA      3315

GCT TTA AGG AGG TTT GGA TTC TCT TTG GCC GCA GGC AGA AGT GTG TGG      3363

ACT CTT GAA ATG GAT GCT GGG GTT CTT ACT GGG AGA CTG ATT AGA TTG      3411

AAT GAT GAG AAA TGG ACA GAA ATG AAG GAT GAC AAG ATT GTT TCA TTG      3459

ATT GAA AAG TTT ACA AGT AAC AAA TAT TGG TCC AAA GTG AAT TTC CCA      3507

CAT GGG ATG TTG GAT CTT GAA GAA ATT GCT GCC AAT TCT AAG GAT TTT      3555

CCT AAC ATG TCT GAA ACG GAT TTG TGT TTC TTG CTG CAT TGG TTA AAT      3603

CCA AAG AAA ATT AAT TTA GCA GAT AGA ATG CTT GGA TTG TCT GGA GTT      3651

CAG GAA ATT AAA GAA CAA GGT GTT GGA TTA ATA GCA GAG TGT AGA ACT      3699

TTC TTA GAT TCT ATT GCT GGA ACT TTA AAA TCT ATG ATG TTT GGA TTT      3747

CAT CAT TCT GTG ACT GTT GAA ATT ATA AAC ACT GTG CTC TGT TTT GTT      3795

AAG AGT GGA ATT TTG CTT TAT GTA ATA CAA CAA TTG AAT CAG GAT GAA      3843

CAT TCT CAC ATA ATT GGT TTG TTG AGA GTC ATG AAT TAT GTA GAT ATT      3891

GGT TGT TCA GTT ATT TCA TGT GCC AAA GTT TTT TCC AGA ATG CTG GAA      3939

ACA GTC TTT AAT TGG CAA ATG GAC TCC AGA ATG ATG GAG TTA AGG ACT      3987
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CAG AGT TTT TCC AAC TGG TTA AGA GAT ATT TGT TCT GGG ATC ACC ATT      4035

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GAC TTT TAT GAA GTG AAT TAT GGC AAG AAG AAG GAC ATT TTA AAT ATT      4131

CTT AAA GAT AAC CAA CAA AAA ATA GAG AAA GCC ATT GAG GAA GCC GAT      4179

AAA TTT TGC ATT TTG CAA ATC CAA GAT GTG GAA AAA TCT GAA CAG TAT      4227

CAG AAA GGG GTT GAC TTG ATA CAA AAA TTG AGA ACT GTT CAT TCA ATG      4275

GCT CAG GTT GAT CCA AAT TTA ATG GTT CAT TTG TCA CCT TTG AGA GAT      4323

TGT ATA GCA AGA GTT CAT CAG AAA CTT AAA AAC CTT GGA TCT ATA AAT      4371

CAG GCA ATG GTA ACG AGA TGT GAG CCA GTT GTT TGT TAT TTT TAT GGC      4419

AAA AGA GGG GGA GGA AAG AGC TTA ACA TCA ATT GCA TTG GCA ACC AAA      4467

ATT TGT AAA CAT TAT GGT GTT GAG CCT GAA AAG AAT ATC TAT ACT AAA      4515

CCT GTG GCT TCA GAT TAC TGG GAT GGA TAT AGT GGA CAA TTA GTT TGC      4563

ATC ATT GAT GAT ATT GGC CAA AAC ACA ACA GAT GAG GAT TGG TCA GAT      4611

TTT TGT CAG TTA GTG TCA GGA TGT CCT ATG AGA TTA AAC ATG GCC TCT      4659

CTT GAG GAG AAG GGT AGG CAT TTT TCT TCT CCT TTT ATA ATA GCA ACT      4707

TCA AAT TGG TCA AAT CCA AGT CCA AAA ACA GTT TAT GTT AAG GAA GCA      4755

ATT GAC CGC AGA CTC CAT TTC AAG GTT GAA GTT AAA CCT GCT TCA TTT      4803

TTC AAA AAT CCT CAC AAT GAT ATG TTG AAT GTT AAT TTA GCT AAA ACA      4851

AAT GAT GCA ATC AAA GAT ATG TCT TGT GTT GAT TTG ATA ATG GAT GGA      4899

CAT AAT GTT TCA TTG ATG GAT TTG CTC AGT TCT TTA GTC ATG ACA GTT      4947

GAA ATT AGA AAA CAA AAC ATG ACT GAA TTC ATG GAG TTG TGG TCT CAG      4995

GGA ATT TCA GAT GAT GAT AAT GAT AGT GCA GTA GCT GAG TTT TTC CAG      5043

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CAA TCT GTT ACT AAT CAC AAG TGG GTT GCT GTG GGA GCT GCA GTT GGC      5139

GTT CTT GGA GTG CTC GTT GGA GGA TGG TTT GTG TAT AAG CAT TTC TCC      5187

CGC AAA GAG GAA GAA CCA ATC CCA GCT GAA GGG GTA TAT TAT GGT GTA      5235

ACT AAG CCC AAG CAA GTG ATT AAA TTA GAT GCA GAT CCA GTA GAA TCT      5283

CAG TCA ACT TTG GAA ATA GCA GGA CTG GTT AGG AAG AAC TTG GTT CAG      5331

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TTT GAG AAA GAT TAT GAA ATG ATG GAG TTT TAT TTT AAT AGA GGT GGA      5475

ACT TAC TAT TCA ATT TCA GCT GGT AAT GTT GTT ATT CAA TCT TTG GAT      5523

GTG GGA TTC CAG GAT GTT GTT CTG ATG AAG GTT CCT ACA ATT CCT AAG      5571

TTT AGA GAT ATT ACT CAG CAT TTT ATT AAG AAA GGG GAT GTG CCT AGA      5619

GCT TTG AAT CGC CTG GCA ACA TTA GTG ACA ACT GTA AAT GGA ACC CCT      5667

ATG TTA ATT TCT GAG GGC CCA CTA AAG ATG GAA GAG AAA GCT ACT TAT      5715

GTT CAT AAG AAA AAT GAT GGT ACA TCA GTT GAT TTA ACT GTG GAT CAG      5763

GCA TGG AGA GGA AAA GGC GAA GGT CTT CCT GGA ATG TGT GGT GGG GCC      5811

TTG GTT TCA TCG AAT CAA TCT ATA CAG AAT GCA ATC TTG GGC ATC CAT      5859

GTT GCT GGA GGA AAT TCA ATT CTT GTT GCA AAA TTG GTT ACT CAA GAA      5907
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ATG TTC CAA AAT ATT GAT AAG AAA ATT GAA AGT CAG AGA ATT ATG AAA      5955

GTG GAG TTT ACT CAG TGT TCA ATG AAT GTG GTC TCC AAA ACG CTT TTT      6003

AGA AAG AGT CCC ATT TAT CAT CAC ATT GAT AAA ACC ATG ATT AAT TTT      6051

CCT GCA GCT ATG CCC TTT TCT AAA GCT GAA ATT GAT CCA ATG GCT GTG      6099

ATG TTA TCT AAG TAT TCA TTA CCT ATT GTA GAA GAA CCA GAG AAT TAT      6147

AAA GAG GCT TCA ATT TTT TAT CAA AAT AAA ATA GTG GGT AAG ACT CAG      6195

TTA GTT GAT GAT TTT CTA GAT CTT GAT ATG GCC ATT ACA GGG GCC CCA      6243

GGA ATT GAT GCT ATC AAC ATG GAT TCA TCT CCT GGA TTT CCT TAT GTC      6291

CAG GAG AAG TTG ACC AAA AGA GAT TTA ATT TGG TTG GAT GAA AAT GGT      6339

TTA TTG CTG GGA GTT CAT CCA AGA TTG GCT CAG AGA ATC TTA TTC AAT      6387

ACT GTC ATG ATG GAA AAT TGT TCT GAT TTG GAT GTT GTT TTT ACA ACC      6435

TGT CCA AAA GAT GAA TTG AGA CCA TTA GAG AAA GTG TTG GAA TCA AAA      6483

ACA AGA GCT ATT GAT GCT TGT CCT CTG GAT TAC ACA ATT TTG TGC CGA      6531

ATG TAT TGG GGT CCA GCT ATT AGT TAT TTT CAT TTG AAT CCA GGT TTC      6579

CAT ACA GGT GTT GCT ATT GGC ATA GAT CCT GAT AGA CAG TGG GAT GAA      6627

TTA TTT AAA ACA ATG ATA AGA TTC GGA GAT GTT GGT CTT GAT TTA GAT      6675

TTC TCT GCT TTT GAT GCT AGT CTT AGT CCA TTT ATG ATT AGA GAA GCA      6723

GGT AGA ATC ATG AGT GAA CTA TCT GGA ACT CCA TCC CAT TTT GGC ACA      6771

GCT CTT ATC AAT ACT ATC ATT TAT TCC AAG CAT TTG CTG TAT AAC TGT      6819

TGT TAC CAT GTC TGT GGT TCA ATG CCC TCT GGG TCT CCT TGT ACA GCT      6867

TTG CTA AAT TCA ATT ATT AAT AAT GTC AAT TTG TAC TAT GTG TTT TCC      6915

AAG ATA TTT GGA AAG TCT CCA GTT TTC TTT TGT CAG GCT TTG AAG ATT      6963

CTC TGT TAT GGA GAT GAT GTT TTA ATA GTT TTC TCT CGA GAT GTT CAG      7011

ATT GAT AAT CTT GAT TTG ATT GGA CAA AAA ATT GTA GAT GAG TTT AAG      7059

AAA CTT GGC ATG ACA GCT ACT TCT GCT GAC AAG AAT GTA CCT CAG CTG      7107

AAA CCA GTT TCG GAA TTG ACT TTT CTC AAA AGA TCT TTC AAT TTG GTA      7155

GAG GAT AGA ATT AGA CCT GCA ATT TCG GAA AAA ACA ATT TGG TCT TTA      7203

ATA GCA TGG CAG AGA AGT AAC GCT GAG TTT GAG CAG AAT TTA GAA ATT      7251

GCT CAG TGG TTT GCT TTT ATG CAT GGC TAT GAG TTT TAT CAG AAA TTT      7299

TAT TAT TTT GTT CAG TCC TGT TTG GAG AAA GAG ATG ATA GAA TAC AGA      7347

CTT AAA TCT TAT GAT TGG TGG AGA ATG AGA TTT TAT GAC CAG TGT TTC      7395

ATT TGT GAC CTT TCA TGA TTTGTTTAAA CGAATTTTCT TAAAATTTCT            7443

GAGGTTTGTT TATTTCTTTT ATCAGTAAAT AAAAAAAAAA AAA                     7486
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This deposit was made under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms and has not been publicly disseminated. HAV 4380 is a cell culture-adapted and attenuated strain of hepatitis A virus strain HM-175, adapted to growth in a human fibroblast cell line (MRC-5) suitable for vaccine development by incubation at a reduced temperature of 32–35° C. Growth of the virus is determined by detection of viral antigen in a serological assay. The adapted virus is purified by plaque-purification, using an accepted method (radioimmunofocus assay).

After a total of nine passages in MRC-5 cells at reduced temperature, the resultant virus was characterized for its biological characteristics in cell culture and in two primate species that are considered to be surrogates for man, i.e., marmosets and chimpanzees. See, e.g., Example 1 below. The HAV 4380 virus was found to be temperature-sensitive (i.e., only grew at reduced temperatures) in MRC-5 cells but was still capable of growing at 37° C. in primary African green monkey kidney cells. The virus was further attenuated in virulence, compared to the parent virus HM-175, P-32, when tested in chimpanzees and marmoset monkeys, in

which species the virus replicated poorly or not at all. This reduced capacity for replication in primates was further confirmed in human volunteers, as described in Example 2.

A candidate inactivated hepatitis A vaccine was prepared from the HAV 4380 and demonstrated to be safe (i.e., it does not produce hepatitis or other serious adverse effects) and immunogenic in humans. It was also found to induce antibody production without adjuvant. HAV 4380, as it currently exists, grows well in a cell substrate suitable for commercial vaccine production. It also does not infect human beings when administered by the oral or intravenous route at doses of up to $10^7$ tissue culture infectious doses, even when not inactivated. HAV 4380 is suitable for use as a live HAV vaccine in humans. However, as indicated in Example 2, vaccine 4380 is believed to be somewhat over-attenuated, because it is not infectious, which characteristic reduces its efficiency when used as an attenuated vaccine.

In order to produce other vaccine candidates which are maximized for desirable levels of attenuation and good growth in MRC-5 cells, the inventors discovered genetic changes that occurred in the genome of the MRC-5-adapted HAV 4380 virus that altered its growth characteristics and made it more suitable for vaccine production than the related AGMK-adapted virus HM-175, Passage 35. The discovery of the following mutations in the nucleotide sequences in 4380, when compared to HM-175 Pass 35 [Cohen et al, cited above; and U.S. Pat. No. 4,894,228, FIG. 1; permit the manipulation of the HAV genome by genetic engineering techniques.

Thus, knowledge of the genomic differences between the AGMK-adapted passages of HM-175 and the more attenuated 4380 permit the construction of chimeric viruses having the improved growth characteristics, i.e., rapid and efficient growth in MRC-5 cell culture, but with a level of attenuation of virulence for primate species, including man, that will permit the virus to replicate efficiently without producing hepatitis or other untoward effects. This invention permits the design of a chimeric HAV that can achieve the optimum characteristics for a candidate live-attenuated hepatitis A vaccine. Such a virus will also permit the design of preferred inactivated vaccine candidates, if desired. The present invention identifies the mutations that are believed to have occurred during adaptation to growth of the HM-175 HAV, passage 32, strain in MRC-5 cells. One or a combination of these mutations are responsible for MRC-5 cell adaptation and overattenuation in HAV 4380. The nucleotide sequence of the MRC-5 cell-adapted virus HAV 4380 was compared with that of the AGMK-adapted, HM-175 virus, passage 35, clone 7. Nucleotide consensus sequences were determined directly from polymerase chain reaction products.

The inventors have discovered that there are at least sixteen unique nucleotide differences between the pass-35 HM-175/7 virus and the MRC-5-adapted virus 4380. Table I lists these sixteen mutations by nucleotide differences and resulting amino acid (AA) differences, if any, acquired by the MRC-5 adapted virus HAV 4380. Note that the partial sequence of LHS/S HAV of Fineschi et al., cited above, overlaps with only the mutation observed at position 5145.

In the Table, A represents adenine, G represents guanine, C represents cytosine, and T represents thymine; Leu represents leucine, Phe represents phenylalanine, Ile represents isoleucine, Val represents valine, Ser represents serine, Lys represents lysine, Asn represents asparagine and Thr represents threonine.

TABLE I

Difference in Nucleotide Sequence of MRC-5-Adapted Hepatitis A Virus: Comparison with Sequence of HM-175/7 (P-35)

| Nucleotide Change | Region of Genome | AA Change |
|---|---|---|
| 591 A to G | 5' nc | NA |
| 646 G to A | 5' nc | NA |
| 669 C to T | 5' nc | NA |
| 687 T to G | 5' nc | NA |
| 2750 C to T | VP1 | No change |
| 3027 T to A | 2A | Ser to Thr |
| 3196 G to A | 2A | Ser to Asn |
| 3934 A to G | 2B | Lys to Arg |
| 4418 A to T | 2C | Leu to Phe |
| 4563 A to G | 2C | Ile to Val |
| 4643 A to T | 2C | No change |
| 5145 A to G | 3A | Ile to Val |
| 5745 A to T | 3C | Thr to Ser |
| 6908 T to C | 3D | No change |
| 7032 C to T | 3D | No change |
| 7255 A to T | 3D | Asn to Ile |

New HAV vaccine candidates are designed by introducing one or more of these nucleotides into an HAV at a nucleotide position homologous to the nucleotide position in the genomic sequence of the AGMK-adapted virus HM-175, Pass 35. These nucleotides identified in Table I may be introduced at analogous and/or homologous nucleotide positions to those of P-35 in the genomic sequences of other HAV strains and variants to produce a recombinant or chimeric HAV of this invention. By the phrase "analogous or homologous nucleotide position" is meant a nucleotide in an HAV other than HAV HM-175, Pass 35 which is present in the same viral region, e.g., 2C, 3D and the like, at a position in that region similar to that of the nucleotide of Table I. In other words, the nucleotide position may differ in position number due to deletions in other regions of the virus; but one of skill in the art can readily determine its functional similarity to the nucleotide position in HM-175, Pass 35.

While such nucleotide positions may not have the identical nucleotide position numbers corresponding to the wild-type HM-175 [SEQ ID NO:1], it is anticipated that these analogous and/or homologous positions can be readily identified to enable HAVs other than strain HM-175 derivatives to be modified to create novel HAVs according to this invention.

Similarly, the inventors are able to manipulate the genome of a progenitor or intermediate of HAV 4380 with resort to this knowledge and can thereby 'reverse' certain mutations in 4380 to create new chimeric HAV viruses. One or more of these nucleotides, or varying combinations thereof, can be incorporated, by chimera formation or oligonucleotide-directed mutagenesis, into an HAV strain, most readily the cDNA clone HAV/HM-175/7, to produce new viable virus which has acquired the ability to grow in MRC-5 cells. Other HM-175 HAV derivatives are available from the American Type Culture Collection under ATCC designation numbers VR 2089, VR 2090, VR 2091, VR 2092, VR 2093, VR 2097, VR 2098, and VR 2099. These and other HAVs may be employed to derive desired HAVs of this invention. Since there are indications that the MRC-5-adapted virus 4380 may be over-attenuated for humans, it is important to be able to remove or introduce selected mutations into HM-175. The construction of nine exemplary chimeric viruses containing one or more such mutations is described in detail in Example 3 below.

The mutagenic and genetic engineering techniques employed to construct chimeric or recombinant HAVs which

incorporate one or more of these mutations are conventional and known to those of skill in the art [see, for example, Sambrook et al., *Molecular Cloning. A Laboratory Manual,* 2d edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1989)]. Other conventional techniques, including polymerase chain reactions and chemical synthetic techniques may also be used to design HAVs of this invention. Similarly, it is anticipated that homologous mutations may be made using other HM-175 passages. It also may be possible to adapt similar changes to HAV strains other than HM-175 by introducing these nucleotides into homologous regions.

Chimeric and recombinant viruses of this invention may be designed by application of similar techniques and selecting one or more different combinations of the nucleotides (mutations) appearing in Tables I and VI. For example, data from growth analyses of the chimeric viruses of Example 3 demonstrate that one or more of the four MRC-5 specific mutations in the 5' non-coding region (mutations at nucleotide positions 591, 646, 669, and 687 of HM-175/7) and one or both of the MRC-5 specific mutations in the 2C region (mutations at nucleotide positions 4418 and 4643 of HM-175/7) may be desirable for optimal growth of the virus in MRC-5 cells. Other mutations may also be involved. Specific exemplary chimeric HAVs of this invention are characterized by the mutations in the genome of HAV HM-175/7 that appear in viruses designated #2 through #10 in Table VI of Example 3 below.

HAVs of this invention may be characterized by the presence of one or more of these nucleotides of Tables I or VI in analogous genomic positions of HAV HM-175 derivatives or other HAV strains. HAVs of this invention may also be characterized by two or more such nucleotides, where one nucleotide in the HAV parent strain is a guanine (G) at position 5145 of pHAV/7 or the analogous position of another HAV strain.

It is further anticipated that additional mutations may appear in a few regions of HAV that have yet to be sequenced. The mutations appearing in Table I may be incorporated in any combination, and/or with other mutations yet to be identified to construct a number of chimeric or recombinant HAVs with desired characteristics for use as live HAV vaccines.

Additional chimeras and recombinant viruses constructed by oligonucleotide-directed mutagenesis may be designed and evaluated for assessment of the individual effects of the mutations and combinations thereof on viral growth in MRC-5 cells and on adaptation to growth in selected cell culture. The attenuation phenotype of these chimeric viruses may be evaluated in marmosets or chimpanzees by techniques such as described below in Example 1 for HAV 4380.

Also provided by this invention are the polynucleotide sequences encoding the HAVs of this invention. Such polynucleotide sequences are preferably cDNA sequences, which can form a master seed for the HAV vaccine. A cDNA sequence of this invention comprises a DNA sequence encoding a selected HAV genome characterized by the presence of one or more of the nucleotides identified as the twelve mutations in Table I in any desired combination which imparts desired characteristics to the novel HAV. Such cDNAs may be obtained by conventional techniques known to those of skill in the art. See, e.g., Sambrook et al, cited above, and U.S. Pat. No. 4,894,228.

Thus, the present invention provides a live vaccine composition useful in protecting against HAV infection and a prophylactic method entailing administering to a primate, preferably a human, an effective amount of such a composition. This vaccine composition may contain one or more of the HAVs of the invention, including HAV 4380, as well as the chimeric and recombinant HAVs described herein. The vaccine composition may also contain mixtures of two or more of the HAVs, if desired.

A vaccinal composition may be formulated to contain a carrier or diluent and one or more of the HAVs of the invention. Suitable pharmaceutically acceptable carriers facilitate administration of the viruses but are physiologically inert and/or nonharmful. Carriers may be selected by one of skill in the art. Exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextrin, agar, pectin, peanut oil, olive oil, sesame oil, and water. Additionally, the carrier or diluent may include a time delay material, such as glycerol monostearate or glycerol distearate alone or with a wax. In addition, slow release polymer formulations can be used.

Optionally, the vaccine composition may further contain preservatives, chemical stabilizers, other antigenic proteins, and conventional pharmaceutical ingredients. Suitable ingredients which may be used in a vaccinal composition in conjunction with the viruses include, for example, casamino acids, sucrose, gelatin, phenol red, N-Z amine, monopotassium diphosphate, lactose, lactalbumin hydrolysate, and dried milk. Typically, stabilizers, adjuvants, and preservatives are optimized to determine the best formulation for efficacy in the target human or animal. Suitable preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, parachlorophenol.

A vaccine composition of this invention is most preferably produced without an adjuvant. However, where necessary, one or more of the above described vaccine component may be admixed or adsorbed with a conventional adjuvant. The adjuvant is used as a non-specific irritant to attract leukocytes or enhance an immune response. Such adjuvants include, among others, mineral oil and water, aluminum hydroxide, Amphigen, Avridine, L121/squalene, D-lactide-polylactide/glycoside, pluronic plyois, muramyl dipeptide, killed Bordetella, saponins, and Quil A.

Alternatively, or in addition to the HAV of the invention, other agents useful in treating HAV infection, e.g., immunostimulatory agents, are expected to be useful in reducing and eliminating disease symptoms. The development of vaccine or therapeutic compositions containing these agents is within the skill of one of skill in the art in view of the teaching of this invention.

According to the method of the invention, a human or an animal may be vaccinated against HAV infection by administering an effective amount of a vaccine composition described above. An effective amount is defined as that amount of HAV vaccine capable of inducing protection in the vaccinee against HAV infection and/or against hepatitis. The vaccine may be administered by any suitable route. Such a composition may be administered parenterally, preferably intramuscularly or subcutaneously. However, it may also be formulated to be administered by any other suitable route, including orally.

Suitable effective amounts of the HAVs of this invention can be determined by one of skill in the art based upon the level of immune response desired. Such a composition may be administered once, and/or a booster may also be administered. However, suitable dosage adjustments may be made by the attending physician or veterinarian depending upon the age, sex, weight and general health of the human or animal patient.

Similarly, suitable doses of the vaccine composition of the invention can be readily determined by one of skill in the art.

The dosage can be adjusted depending upon the human patient or the animal species being treated, i.e. its weight, age, an general health.

The following examples illustrate the preferred methods for obtaining HAVs of the invention and using them as vaccine compositions. These examples are not intended to limit the scope thereof.

## EXAMPLE 1

### Test of MRC-5-Adapted HAB 4380 Vaccine in Marmosets and Chimpanzees

The attenuation of hepatitis A virus (HAV), strain HM-175, by serial passage in cell culture has previously been demonstrated. Following 32 passages in primary AGMK cells, the virus was completely attenuated for chimpanzees and almost completely attenuated for marmosets. Subsequently, according to this invention the virus was adapted to growth in MRC-5 cells and recloned by plaque purification.

HAV 4380 was prepared from Volunteer lot 87J19, passage level 41 of strain HM-175 HAV that was derived from previously characterized passage levels of the virus that have also been prepared as volunteer pools. Two such earlier passage pools were shown to be attenuated for chimpanzees and marmosets. However, neither was administered to volunteers because it was recognized that primary African green monkey kidney cells, the substrate for those volunteer pools, would not be available in sufficient quantities to produce an economically viable vaccine. Therefore, the virus was adapted to MRC-5 cells and further passaged to prepare volunteer lot 87J19 or HAV 4380.

The purpose of this experiment is to test the level of attenuation of this virus for marmosets and chimpanzees, prior to phase I trials in volunteers. Lot 87J19 was tested for safety and immunogenicity in four chimpanzees and four *Saquinus mystax* marmosets. Two additional marmosets served as uninoculated controls. The chimpanzees used in this study were bred and raised in captivity; the marmosets were wild-caught animals. An inoculum of $10^4$ $TCID_{50}$ of candidate vaccine lot 87J19 was administered intravenously to each animal. Residual inoculum was frozen and the titer reconfirmed subsequently in two different laboratories.

According to the experimental protocol, marmosets identified by the arbitrary ID numbers 570, 572, 566, and 575 and chimpanzees identified by the arbitrary ID numbers 1300, 1333, 1309 and 1313 received an inoculum of HAV 4380 from Clone 25-4-21, Lot 87 J 19575, 17/11/87 at a dose and route of administration of $10^{-3}$ dilution/1 ml/I.V. Marmosets No. 541 and 578 received a diluent at a dose and route of 1 ml/I.V.

A. Infection:

Three of four chimpanzees and one of four marmosets were infected, as determined by development of anti-HAV detectable by commercial radioimmunoassay (HAVAB, Abbot Laboratories, Chicago, Ill.). The chimpanzees seroconverted ten to eleven weeks following inoculation; the single marmoset seroconversion occurred eight weeks following inoculation. This marmoset subsequently died on week eleven of the study and another, noninfected, marmoset subsequently died on week fourteen, but neither death was attributable to the inoculum.

All three chimpanzees that seroconverted also developed IgM anti-Hav. Two of these, Chip 1309 and Chimp 1313, developed IgM anti-HAV on weeks ten and thirteen, respectively, when tested by the standard HAVAB-M (Abbott Laboratories, Chicago, Ill.) at a final serum dilution of 1:4,000. When sera were tested at a dilution of 1:40, Chimp 1313 and Chimp 1333 seroconverted at weeks nine and five, respectively. The HAVAB-M test is a capture assay utilization anti-human IgM and has not been standardized for use with sera from primates less closely related to man than to the chimpanzee. For this reason, the marmosets were not tested for IgM anti-HAV.

B. Biochemistry:

Biochemical evidence of hepatitis was monitored by weekly determinations of serum alanine amino transferase (ALT) and isocitric dehydrogenase (ICD). The former is the most reliable indirect means of diagnosing hepatitis in the chimpanzee and the latter is comparably sensitive for evaluating marmosets. None of the chimpanzees or marmosets had elevation of liver enzymers attributable to the inoculum. All values for chimpanzees were within normal limits. The only infected marmoset, number 582, had normal liver enzymes up to the time of its death. Marmosets 566, 570, and 578 each had one or more abnormal liver enzyme values, but the first two of these animals were not infected by the inoculum, as judged by failure to seroconvert, and the third was an uninoculated negative control.

Marmosets often have less stable liver enzyme values than chimpanzees, in part because they are, by nature, relatively fragile animals and because they are jungle-caught and therefore usually infected with a variety of endo- and ecto-parasites, including microfilaria.

C. Histology:

Histologic sections prepared from serial weekly liver biopsies obtained from the chimpanzees and marmosets were evaluated under code for histopathologic changes. Although some animals had a high base-line of histopathologic changes, none of the animals had evidence of histopathologic changes more severe than those seen in preinoculation biopsies. Equally important, there were no histologic changes that were temporally related to seroconversion in infected animals. The two marmosets that died were subjected to more extensive evaluation. Both animals had evidence of systemic disease that was probably etiologically related to their deaths, but histologic changes in the liver were diagnostic of chronic, not acute, disease and therefore not related to the inoculum.

A comparison of histopathologic changes observed in chimpanzees and marmosets with these various volunteer pools and wild-type virus was performed. See Tables II and III below.

TABLE II

HISTOPATHOLOGY: CHIMPANZEES

| Inoculum* | # Inoc. | # Infected | Liver Histopathology # | Liver Histopathology Range of Severity** |
|---|---|---|---|---|
| W.T. | 4 | 4 | 4 | ±–3+ |
| P-21 | 6 | 6 | 1 | 0–3+ |
| P-32 | 6 | 6 | 0 | 0 |
| MRC-5 | 4 | 3 | 0 | 0 |

*Dose: $10^3$–$10^5$ $ID_{50}$ IV
**Scale of 0–3+

TABLE III

HISTOPATHOLOGY: MARMOSETS

| Inoculum* | # Inoc. | # Infected | Liver Histopathology # | Liver Histopathology Range of Severity** |
|---|---|---|---|---|
| W.T. | 4 | 4 | 4 | 1+–2+ |
| P-21 | 8 | 8 | 8 | 1+–3+ |
| P-32 | 5 | 5 | 3 | 0–2+ |
| MRC-5 | 4 | 1 | 0 | 0 |

*Dose: $10^3$–$10^5$ $ID_{50}$ IV
**Scale of 0–3+

Lot 87J19 appears to be more attenuated than the other volunteer pools or wild-type virus, based upon infectivity and severity of histopathologic changes.

D. Immunofluorescence:

Serial snap-frozen liver biopsies obtained from infected animals were evaluated for expression of viral antigen by immunofluorescence. Only one animal, Chimp 1313, was definitely but weakly positive for intrahepatic viral antigen. This animal was positive for only one week. These results were compared with those obtained in the previous study of other volunteer pools and wild-type virus. As seen in Tables IV and V, intrahepatic replication was further diminished in both chimpanzees and marmosets when compared with the AGMK-grown virus and wild-type virus.

TABLE IV

VIRAL REPLICATION IN THE LIVER: MARMOSETS (IMMUNOFLUORESCENCE)

| Inoculum* | # Inoc. | # Infected | Mean Peak | Mean Duration (wks) |
|---|---|---|---|---|
| W.T. | 4 | 4 | 2.5+ | 12.2 |
| P-21 | 8 | 8 | 1+ | 3.5 |
| P-32 | 5 | 5 | <1+ | 2.4 |
| MRC-5 | 4 | 1 | 0 | 0 |

*Dose: $10^3$–$10^5$ $ID_{50}$ I.V.

TABLE V

VIRAL REPLICATION IN THE LIVER: CHIMPANZEES (IMMUNOFLUORESCENCE)

| Inoculum* | # Inoc. | # Infected | Mean Peak | Mean Duration (wks) |
|---|---|---|---|---|
| W.T. | 4 | 4 | 1+ | 1.8 |
| P-21 | 6 | 6 | <1+ | 0.5 |

TABLE V-continued

VIRAL REPLICATION IN THE LIVER: CHIMPANZEES (IMMUNOFLUORESCENCE)

| Inoculum* | # Inoc. | # Infected | Mean Peak | Mean Duration (wks) |
|---|---|---|---|---|
| P-32 | 6 | 6 | <1+ | 0.6 |
| MRC-5 | 4 | 3 | <1+ | 0.3 |

*Dose: $10^3$–$10^5$ $ID_{50}$ I.V.

E. Protection:

Although the single infected marmoset on study died, all four chimpanzees were available for challenge with wild-type parent HAV to determine if the levels of anti-HAV present in infected animals were protective. Consequently, the three infected and one uninfected animals were challenged with approximately $10^3$ chimpanzee infectious doses of wild-type HM-175 strain HAV (human stool suspension), administered intravenously (FIGS. **1** through **4**).

All three previously infected chimpanzees were protected against type A hepatitis, as measured by persistently normal serum enzyme values (FIGS. **1** through **3**). All three protected animals had an anamnestic antibody response to the challenge virus, suggesting that there was limited replication. In contrast, the previously uninfected chimpanzee developed high enzyme values diagnostic of hepatitis following challenge with wild-type virus (FIG. **4**). Thus, volunteer pool 87J19 produced an inapparent infection in chimpanzees that stimulated protection against subsequent challenge with virulent wild-type virus.

The results of these portions of the experiment demonstrate that volunteer pool 87J19 of HAV 4380, strain HM-175 (adapted to MRC-5 cells) was significantly more attenuated for chimpanzees and marmosets than its parent, HAV, strain HM-175 (AGMK, Pass-32). It is clear from these studies that HAV 4380, strain HM-175 volunteer pool 87J19, is highly attenuated for chimpanzees and marmosets which are accepted surrogates for man in the study of hepatitis A viruses.

## EXAMPLE 2

### Clinical Study of Volunteers

In this clinical trial, volunteers received increasing titers of the liver attenuated hepatitis A vaccine 4380, volunteer pool 87J19, which was previously tested in chimpanzees and marmosets as described in Example 1. These pre-clinical studies demonstrated that the vaccine was safe, immunogenic, and efficacious in experimental animal models.

Volunteers were admitted to a closed clinical ward at the United States Army Medical Research Institute of Infectious Diseases, For Detrick, Md. Eight volunteers received the live attenuated hepatitis A vaccine (1 ml) by the oral route in the following manner: two received a $10^4$ $TCID_{50}$ dose, tow a $10^5$ $TCID_{50}$ dose, two a $10^6$ $TCID_{50}$ dose, and two a $10^7$ $TCID_{50}$ dose. Six volunteers received the vaccine by the intramuscular route in the deltoid area in the following manner: 2 received a $10^5$ $TCID_{50}$ dose, 2 a $10^6$ $TCID_{50}$ dose and 2 a $10^7$ $TCID_{50}$ dose.

Each volunteer remained on the ward for three days after immunization. Local or systemic side effects were monitored during the admission period and for 12 weeks following the immunization. Volunteers were asked to return at 6 and 12 months for serological follow-up.

Sera were obtained prior to immunization and once a week for the next 12 weeks. In volunteers who completed the appropriate follow-up time, sera were also obtained at 6 and 12 months after initial administration of vaccine. Serum specimens were tested for alanine aminotransferase (ALT) and antibody to hepatitis A. ALT was tested with a Kodak EKTA Chem 700XR analyzer (Rochester, N.Y.); normal values were 0 to 50 IU/ml. Antibody to hepatitis A was tested by four different methods, including a commercial radioimmunoassay (HAVAB, Abbott Laboratories, N. Chicago, Ill.). Second, an enzyme-linked immunoassay developed by SmithKline Beecham (SKB-ELISA), which was more sensitive than the standard HAVAB, in which a level of ≧20 milli-International Units (mIU) was considered positive. Selected sera were tested by the RIFIT (radioimmunofocus) assay for neutralizing antibody to hepatitis A. With this test, a serum titer of 23 1:10 was considered positive [S. M. Lemon et al, *J. Infect. Dis.*, 148:1033–1039 (1983)]. Finally sera were tested for IgM anti-HAV by commercial radioimmunoassay (HAVAB-M, Abbott Laboratories, N. Chicago, Ill.).

Stools were collected from the volunteers two to three times per week for the first 12 weeks and were tested for the presence of hepatitis A virus by radioimmunoassay [R. H. Purcell et al, *J. Immunol.*, 116:349–356 (1976)] and molecular biology techniques [J. Ticehurst et al, *J. Clin. Microbiol.*, 25:1822–1829 (1987)].

All volunteers remained healthy during the follow-up period (14 weeks to one year). No systemic complaints were present immediately after immunization or during long-term follow-up. Serum alanine aminotransferase levels remained normal in all 14 individuals during the period of observation.

Antibody to hepatitis A was not observed in any of the eight volunteers who received the vaccine by the oral route or in the two volunteers who received the $10^5$ $TCID_{50}$ dose by the intramuscular route. The four volunteers who received higher doses of vaccine ($10^6$ $TCID_{50}$ or $10^7$ $TCID_{50}$) all had detectable antibody by the SKB ELISA as early as 3 weeks after immunization. Detectable levels persisted for the 12 weeks of observation. Selected sera tested for neutralizing antibody had titers ranging from 1:10 to 1:40 in a volunteer who received a $10^6$ dose and 1:40 to 1:2560 in a volunteer who received a $10^7$ $TCID_{50}$ dose. The commercial HAVAB assay detected anti-HAV in only one of the volunteers, who received the $10^7$ dose. IgM anti-HAV was not detected in any of the volunteers who received the vaccine orally. Sera from volunteers who received $10^7$ $TCID_{50}$ I.M. had detectable IgM anti-HAV.

Stools from all volunteers who received the oral vaccine were negative for hepatitis A virus, while those from volunteers who had received the vaccine by intramuscular route are in the process of being tested.

Although only a small number of volunteers received the vaccine orally, it appeared that the vaccine is not immunogenic by this route. This is likely due to over-attenuation of the virus, although other causes, such as inactivation in the gastrointestinal tract or too small an inoculum, should be considered. The vaccine was safe and immunogenic by the intramuscular route at doses of $10^6$ and $10^7$ $TCID_{50}$. The antibody response was prompt: anti-HAV was observed within 3 weeks of immunization, persisted during the period of observation, and did not diminish in titer. Such a response to one single inoculation of a preparation which lacked an adjuvant, is remarkable. If indeed, anti-HAV persists for a long time after one dose, the logistics of administration of this product would be much simpler and more successful than with present hepatitis A vaccines. The presence of IgM anti-HAV in volunteers who received $10^7$ $TCID_{50}$ without evidence of hepatitis is suggestive of asymptomatic replication of the virus.

## EXAMPLE 3

### Construction of Chimeric Viruses

Several exemplary chimeric viruses were generated to evaluate the effect of several of the mutations of Table I on host range and/or attenuation in primates. The sequence of the MRC-5-adapted virus 4380 was obtained using reverse-transcriptase:polymerase chain reaction (RT:PCR) to amplify regions of the virus as cDNA prior to sequencing (hence T instead of U in Table VI below). Numbers 2–10 in Table VI designate chimeric viruses made by inserting mutations found in the MRC-5-adapted virus 4380 into the cDNA clone of pHAV/7 encoding the attenuated HM-175 virus, Pass 35, of Cohen et al., *J. Virol.*, 61:3035–3039 (1987). Mutations introduced by "chimera" means a portion of the 4380 virus genome was amplified by RT:PCR, digested with specific restriction enzymes and the fragment used to replace the homologous fragment in the cDNA clone pHAV/7. Mutations introduced by mutagenesis were inserted by oligonucleotide-directed mutagenesis of the cDNA clone pHAV/7 using the Amersham mutagenesis protocol.

The chimeric cDNAs were transcribed into RNA in vitro and the nucleic acids (both RNA and DNA) transfected into FRhK-4 cells to generate chimeric viruses. Quantification of chimeric virus growth for the exemplary chimeras was performed by slot-blot assay.

Table VI reports the results of the construction and testing of nine chimeric viruses. As used in Table VI, the following terms are defined: Cell culture refers to virus containing indicated mutations selected by growth in MRC-5 cells. Mutagenesis refers to oligonucleotide-directed mutagenesis of P-35 or HM-175 cDNA clones. A chimeric viral genome refers to the construction of a chimeric viral genome using portions of P-35 cDNA clone and PCR-generated fragments of the MRC-5 cell-adapted virus 4380. ND means that this study has not yet been performed. The + symbol refers to virus that has some growth in that cell type. The – symbol refers to virus that has little or no growth in that cell type. The two cell types employed to test the growth of the chimeric viruses are the human lung fibroblast-like cell line MRC-5 and fetal rhesus monkey kidney epithelial-like cell line, FRhK-4. Note that Virus #1 in the Table refers to MRC-5-adapted HAV 4380. Viruses #2 through 10 are chimeric viruses of this invention.

TABLE VI

Differences in Nucleotide Sequence of MRC-5-Adapted Hepatitis A Virus: Comparison with P-35 HM-175 Virus

| Nucleotide Differences from P-35 HM-175 | Method Mutation Introduced | Growth of Mutated Virus in Cell Cultures: FRhK-4 | MRC-5 |
|---|---|---|---|
| Virus #1 (MRC-5-adapted) | Cell Culture | + | + |
| 591 A to G<br>646 G to A<br>669 C to T<br>687 T to G | | | |

TABLE VI-continued

| Differences in Nucleotide Sequence of MRC-5-Adapted Hepatitis A Virus: Comparison with P-35 HM-175 Virus | | | |
|---|---|---|---|
| Nucleotide Differences | Method Mutation | Growth of Mutated Virus in Cell Cultures | |
| from P-35 HM-175 | Introduced | FRhK-4 | MRC-5 |
| 2750 C to T | | | |
| 3027 T to A | | | |
| 3196 G to A | | | |
| 3934 A to G | | | |
| 4418 A to T | | | |
| 4563 A to G | | | |
| 4643 A to T | | | |
| 5145 A to G | | | |
| 5745 A to T | | | |
| 6908 T to C | | | |
| 7032 C to T | | | |
| 7255 A to T | | | |
| Virus #2 | Chimera | + | + |
| 591 A to G | | | |
| 646 G to A | | | |
| 669 C to T | | | |
| 687 T to G | | | |
| Virus #3 | Chimera | + | + |
| 124 C to T | | | |
| 131 d to T | | | |
| 132 d to T | | | |
| 133 d to T | | | |
| 134 d to G | | | |
| 152 G to A | | | |
| 203-207 d to T | | | |
| 591 A to G | | | |
| 646 G to A | | | |
| 669 C to T | | | |
| 687 T to G | | | |
| Virus #4 | Chimera | + | + |
| 591 A to G | | | |
| 646 G to A | | | |
| 669 C to T | | | |
| 687 T to G | | | |
| 4418 A to T | | | |
| 4563 A to G | | | |
| 4643 A to T | | | |
| Virus #5 | Chimera | + | + |
| 124 C to T | | | |
| 131 d to T | | | |
| 132 d to T | | | |
| 133 d to T | | | |
| 134 d to T | | | |
| 152 G to A | | | |
| 203-207 d to T | | | |
| 591 A to G | | | |
| 646 G to A | | | |
| 669 C to T | | | |
| 687 T to G | | | |
| 4418 A to T | | | |
| 4563 A to G | | | |
| 4643 A to T | | | |
| Virus #6 | Chimera | + | – |
| 4418 A to T | | | |
| 4563 A to G | | | |
| 4643 A to T | | | |
| Virus #7 | Chimera Mutagenesis | + | ND |
| 591 A to G | | | |
| 4418 A to T | | | |
| 4563 A to G | | | |
| 4643 A to T | | | |
| Virus #8 | Chimera Mutagenesis | + | ND |
| 646 G to A | | | |
| 4418 A to T | | | |
| 4563 A to G | | | |
| 4643 A to T | | | |
| Virus #9 | Chimera Mutagenesis | + | ND |
| 669 C to T | | | |
| 4418 A to T | | | |
| 4563 A to G | | | |
| 4643 A to T | | | |
| Virus #10 | Chimera Mutagenesis | + | ND |
| 687 T to G | | | |
| 4418 A to T | | | |
| 4563 A to G | | | |
| 4643 A to T | | | |

d = Base at this position deleted in P-35 compared to wild-type

Introduction of four mutations found in the 5' noncoding region, at nucleotide positions 591, 646, 669, and 687 of the P-35 genome, appear to be important for HAV host range in cell culture. They allow some growth of the transfected virus in MRC-5 cells, but do not account entirely for MRC-5 cell culture adaptation.

Introduction of three mutations, at nucleotides 4418, 4563 and 4643 in the 2C region of the MRC-5-adapted virus, with the 5' mutations allow full growth in MRC-5 cells. Thus the four mutation in the 5' noncoding region and the three mutations in the 2C region of the genome of the MRC-5 cell-adapted of this virus to efficient growth in MRC-5 cells. Introduction of only the three mutations in the 2 C region into the P-35 AGMK genome does not permit discernible growth of the transfected virus in MRC-5 cells.

## EXAMPLE 4

### Comparison of End Point Dilution of Chimeric Viruses in FRhK-4 Versus MRC-5 Cells

Chimeric viruses with the composition described in Table VI were serially diluted in ten-fold increments, and an equal aliquot of each dilution was plated onto FRhK-4 and MRC-5 cells. After 21 days incubation at 34.5° C. to permit virus growth, the cells were lysed by the addition of a buffer solution containing sodium dodecyl sulfate. The viral RNA was extracted with phenol and quantified by slot blot hybridization using a [$^{32}P$]-labeled riboprobe specific for hepatitis A virus. A radioautograph of the slot blot obtained from the FRhK-4 cells and from the MRC-5 cells illustrates that the endpoint dilution of the MRC-5-adapted virus was the same in both cell lines, indicating that this virus can grow in either cell line. In contrast, the P35 HM-175 virus had an endpoint dilution of $10^{-5}$ on FRhK-4 cells and $<10^{-1}$ on MRC-5 cells, demonstrating that this virus is unable to grow successfully on MRC-5 cells. As FIG. **5** illustrates, virus #6 was most like the pass 35 virus while virus #4 was most like the MRC-5 adapted virus and viruses #2, 3, and 5 were intermediate. These results show that certain mutations from the MRC-5-adapted virus can be introduced into the pHAV/7 cDNA clone to generate new viruses which also have acquired the ability to grow in MRC-5 cells.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 4

<210> SEQ ID NO 1
<211> LENGTH: 7493
<212> TYPE: DNA
<213> ORGANISM: WILD-TYPE HUMAN HEPATITIS A VIRUS, STRAIN HM-175
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (735)..(7415)

<400> SEQUENCE: 1

```
ttcaagaggg gtctccggga atttccggag tccctcttgg aagtccatgg tgaggggact     60

tgatacctca ccgccgtttg cctaggctat aggctaaatt ttccctttcc cttttccctt    120

tcctattccc tttgttttgc ttgtaaatat taattcctgc aggttcaggg ttcttaaatc    180

tgtttctcta taagaacact cattttcac gctttctgtc ttctttcttc cagggctctc    240

cccttgccct aggctctggc cgttgcgccc ggcggggtca actccatgat tagcatggag    300

ctgtaggagt ctaaattggg gacacagatg tttggaacgt caccttgcag tgttaacttg    360

gctttcatga atctctttga tcttccacaa ggggtaggct acgggtgaaa cctcttaggc    420

taatacttct atgaagagat gccttggata gggtaacagc ggcggatatt ggtgagttgt    480

taagacaaaa accattcaac gccggaggac tgactctcat ccagtggatg cattgagtgg    540

attgactgtc agggctgtct ttaggcttaa ttccagacct ctctgtgctt agggcaaaca    600

tcatttggcc ttaaatggga ttctgtgaga ggggatccct ccattgacag ctggactgtt    660

ctttggggcc ttatgtggtg tttgcctctg aggtactcag gggcatttag gtttttcctc    720

attcttaaat aata atg aac atg tct aga caa ggt att ttc cag act gtt      770
                Met Asn Met Ser Arg Gln Gly Ile Phe Gln Thr Val
                  1               5                  10
ggg agt ggt ctt gac cac atc ctg tct ttg gca gac att gag gaa gag      818
Gly Ser Gly Leu Asp His Ile Leu Ser Leu Ala Asp Ile Glu Glu Glu
         15                  20                  25

caa atg att caa tca gtt gat agg act gca gtg act ggt gct tct tat      866
Gln Met Ile Gln Ser Val Asp Arg Thr Ala Val Thr Gly Ala Ser Tyr
     30                  35                  40

ttt act tct gtg gat caa tct tca gtt cat aca gct gag gtt gga tca      914
Phe Thr Ser Val Asp Gln Ser Ser Val His Thr Ala Glu Val Gly Ser
 45                  50                  55                  60

cac cag gtt gaa cct ttg aga acc tct gtt gat aaa ccc ggt tca aag      962
His Gln Val Glu Pro Leu Arg Thr Ser Val Asp Lys Pro Gly Ser Lys
                 65                  70                  75

aag act cag gga gag aaa ttt ttc ttg att cat tct gca gat tgg ctt     1010
Lys Thr Gln Gly Glu Lys Phe Phe Leu Ile His Ser Ala Asp Trp Leu
             80                  85                  90

act aca cat gct ctt ttc cat gaa gtt gca aaa ttg gat gtg gtg aaa     1058
Thr Thr His Ala Leu Phe His Glu Val Ala Lys Leu Asp Val Val Lys
         95                 100                 105

tta tta tac aat gag cag ttt gct gtt caa ggg ttg ttg aga tac cat     1106
Leu Leu Tyr Asn Glu Gln Phe Ala Val Gln Gly Leu Leu Arg Tyr His
    110                 115                 120

aca tat gca aga ttt ggc att gaa att caa gtt cag ata aac cct aca     1154
Thr Tyr Ala Arg Phe Gly Ile Glu Ile Gln Val Gln Ile Asn Pro Thr
125                 130                 135                 140

cct ttc caa cag ggg gga ttg atc tgt gct atg gtt cct ggt gac cag     1202
Pro Phe Gln Gln Gly Gly Leu Ile Cys Ala Met Val Pro Gly Asp Gln
                145                 150                 155
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-continued

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agc tat ggt tct ata gca tca ttg act gtt tat cct cat ggt ttg tta     1250
Ser Tyr Gly Ser Ile Ala Ser Leu Thr Val Tyr Pro His Gly Leu Leu
            160                 165                 170

aat tgc aat att aac aat gtg gtt aga ata aag gtt cca ttt att tac     1298
Asn Cys Asn Ile Asn Asn Val Val Arg Ile Lys Val Pro Phe Ile Tyr
        175                 180                 185

aca aga ggt gct tac cac ttt aaa gat cca caa tac cca gtt tgg gaa     1346
Thr Arg Gly Ala Tyr His Phe Lys Asp Pro Gln Tyr Pro Val Trp Glu
    190                 195                 200

ttg aca att aga gtt tgg tca gaa tta aat att ggg aca gga act tca     1394
Leu Thr Ile Arg Val Trp Ser Glu Leu Asn Ile Gly Thr Gly Thr Ser
205                 210                 215                 220

gct tat act tca ctc aat gtt tta gct aga ttt aca gat ttg gag ttg     1442
Ala Tyr Thr Ser Leu Asn Val Leu Ala Arg Phe Thr Asp Leu Glu Leu
                225                 230                 235

cat gga tta act cct ctt tct aca caa atg atg aga aat gaa ttt agg     1490
His Gly Leu Thr Pro Leu Ser Thr Gln Met Met Arg Asn Glu Phe Arg
            240                 245                 250

gtc agt act act gag aat gtg gtg aat ctg tca aat tat gaa gat gca     1538
Val Ser Thr Thr Glu Asn Val Val Asn Leu Ser Asn Tyr Glu Asp Ala
        255                 260                 265

aga gca aag atg tct ttt gct ttg gat cag gaa gat tgg aaa tct gat     1586
Arg Ala Lys Met Ser Phe Ala Leu Asp Gln Glu Asp Trp Lys Ser Asp
    270                 275                 280

ccg tcc cag ggt ggt ggg atc aaa att act cat ttt act act tgg aca     1634
Pro Ser Gln Gly Gly Gly Ile Lys Ile Thr His Phe Thr Thr Trp Thr
285                 290                 295                 300

tct att cca act ttg gct gct cag ttt cca ttt aat gct tca gac tca     1682
Ser Ile Pro Thr Leu Ala Ala Gln Phe Pro Phe Asn Ala Ser Asp Ser
                305                 310                 315

gtt ggt caa caa att aaa gtt att cca gtt gac cca tat ttt ttc caa     1730
Val Gly Gln Gln Ile Lys Val Ile Pro Val Asp Pro Tyr Phe Phe Gln
            320                 325                 330

atg aca aat acg aat cct gac caa aaa tgt ata act gct ttg gct tct     1778
Met Thr Asn Thr Asn Pro Asp Gln Lys Cys Ile Thr Ala Leu Ala Ser
        335                 340                 345

att tgt cag atg ttt tgt ttt tgg aga gga gat ctt gtc ttt gat ttt     1826
Ile Cys Gln Met Phe Cys Phe Trp Arg Gly Asp Leu Val Phe Asp Phe
    350                 355                 360

caa gtt ttt ccc acc aaa tat cat tca ggt aga tta ctg ttt tgt ttt     1874
Gln Val Phe Pro Thr Lys Tyr His Ser Gly Arg Leu Leu Phe Cys Phe
365                 370                 375                 380

gtt cct ggc aat gag cta ata gat gtt tct gga atc aca tta aag caa     1922
Val Pro Gly Asn Glu Leu Ile Asp Val Ser Gly Ile Thr Leu Lys Gln
                385                 390                 395

gca act act gct cct tgt gca gta atg gat att aca gga gtg cag tca     1970
Ala Thr Thr Ala Pro Cys Ala Val Met Asp Ile Thr Gly Val Gln Ser
            400                 405                 410

act ttg aga ttt cgt gtt ccc tgg att tct gac act cct tac aga gtg     2018
Thr Leu Arg Phe Arg Val Pro Trp Ile Ser Asp Thr Pro Tyr Arg Val
        415                 420                 425

aac agg tat aca aag tca gca cat cag aaa ggt gag tac act gcc att     2066
Asn Arg Tyr Thr Lys Ser Ala His Gln Lys Gly Glu Tyr Thr Ala Ile
    430                 435                 440

ggg aag ctt att gtg tat tgt tat aac aga ttg acc tct cct tct aac     2114
Gly Lys Leu Ile Val Tyr Cys Tyr Asn Arg Leu Thr Ser Pro Ser Asn
445                 450                 455                 460

gtt gct tcc cat gtc aga gtg aat gtt tat ctt tca gca att aac ttg     2162
Val Ala Ser His Val Arg Val Asn Val Tyr Leu Ser Ala Ile Asn Leu
                465                 470                 475
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gaa tgt ttt gct cct ctt tat cat gct atg gat gtt act aca caa gtt     2210
Glu Cys Phe Ala Pro Leu Tyr His Ala Met Asp Val Thr Thr Gln Val
            480                 485                 490

gga gat gat tct gga ggt ttt tca aca aca gtt tct aca gaa cag aat     2258
Gly Asp Asp Ser Gly Gly Phe Ser Thr Thr Val Ser Thr Glu Gln Asn
        495                 500                 505

gtt cca gat ccc caa gtt ggt ata aca acc atg aaa gat ttg aaa gga     2306
Val Pro Asp Pro Gln Val Gly Ile Thr Thr Met Lys Asp Leu Lys Gly
    510                 515                 520

aaa gct aac aga ggg aaa atg gat gtt tca gga gta caa gca cct gtg     2354
Lys Ala Asn Arg Gly Lys Met Asp Val Ser Gly Val Gln Ala Pro Val
525                 530                 535                 540

gga gct atc aca aca att gag gat cca gtt tta gca aag aaa gta cct     2402
Gly Ala Ile Thr Thr Ile Glu Asp Pro Val Leu Ala Lys Lys Val Pro
                545                 550                 555

gag aca ttt cct gaa ttg aaa cct gga gaa tcc aga cat aca tca gat     2450
Glu Thr Phe Pro Glu Leu Lys Pro Gly Glu Ser Arg His Thr Ser Asp
            560                 565                 570

cat atg tcc atc tac aag ttt atg gga agg tct cat ttc ttg tgc act     2498
His Met Ser Ile Tyr Lys Phe Met Gly Arg Ser His Phe Leu Cys Thr
        575                 580                 585

ttt aca ttc aat tca aat aat aaa gag tac aca ttt cct ata acc ttg     2546
Phe Thr Phe Asn Ser Asn Asn Lys Glu Tyr Thr Phe Pro Ile Thr Leu
    590                 595                 600

tct tca acc tct aat cct cct cat ggt ttg cca tca aca ctg agg tgg     2594
Ser Ser Thr Ser Asn Pro Pro His Gly Leu Pro Ser Thr Leu Arg Trp
605                 610                 615                 620

ttt ttc aac ttg ttt cag ttg tat aga ggg cct tta gat ctg aca att     2642
Phe Phe Asn Leu Phe Gln Leu Tyr Arg Gly Pro Leu Asp Leu Thr Ile
                625                 630                 635

att att aca gga gca act gat gta gat ggc atg gcc tgg ttc act cca     2690
Ile Ile Thr Gly Ala Thr Asp Val Asp Gly Met Ala Trp Phe Thr Pro
            640                 645                 650

gta ggt ctt gcc gtt gat act cct tgg gta gag aag gag tca gct ttg     2738
Val Gly Leu Ala Val Asp Thr Pro Trp Val Glu Lys Glu Ser Ala Leu
        655                 660                 665

tct att gac tac aaa act gct ctt gga gct gtc aga ttt aac aca agg     2786
Ser Ile Asp Tyr Lys Thr Ala Leu Gly Ala Val Arg Phe Asn Thr Arg
    670                 675                 680

aga aca ggg aac att cag att aga tta cca tgg tat tct tat tta tat     2834
Arg Thr Gly Asn Ile Gln Ile Arg Leu Pro Trp Tyr Ser Tyr Leu Tyr
685                 690                 695                 700

gct gtg tct gga gca ctg gat ggt ttg ggt gac aag aca gat tct aca     2882
Ala Val Ser Gly Ala Leu Asp Gly Leu Gly Asp Lys Thr Asp Ser Thr
                705                 710                 715

ttt gga ttg gtt tct att cag att gca aat tac aat cat tct gat gaa     2930
Phe Gly Leu Val Ser Ile Gln Ile Ala Asn Tyr Asn His Ser Asp Glu
            720                 725                 730

tac ttg tct ttt agt tgt tat ttg tct gtc aca gaa caa tca gag ttt     2978
Tyr Leu Ser Phe Ser Cys Tyr Leu Ser Val Thr Glu Gln Ser Glu Phe
        735                 740                 745

tat ttt ccc aga gct cca ttg aac tca aat gcc atg tta tcc act gaa     3026
Tyr Phe Pro Arg Ala Pro Leu Asn Ser Asn Ala Met Leu Ser Thr Glu
    750                 755                 760

tca atg atg agc aga att gca gct gga gac ttg gag tca tca gtg gat     3074
Ser Met Met Ser Arg Ile Ala Ala Gly Asp Leu Glu Ser Ser Val Asp
765                 770                 775                 780

gat cct aga tca gag gaa gat aaa aga ttt gag agt cat ata gaa tgc     3122
Asp Pro Arg Ser Glu Glu Asp Lys Arg Phe Glu Ser His Ile Glu Cys
                785                 790                 795
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agg aag cca tat aaa gaa ctg aga tta gaa gtt ggg aaa caa aga ctc     3170
Arg Lys Pro Tyr Lys Glu Leu Arg Leu Glu Val Gly Lys Gln Arg Leu
            800                 805                 810

aag tat gct cag gaa gaa ttg tca aat gaa gta ctt cca ccc cct agg     3218
Lys Tyr Ala Gln Glu Glu Leu Ser Asn Glu Val Leu Pro Pro Pro Arg
        815                 820                 825

aaa atg aag gga ctg ttt tca caa gcc aaa att tct ctt ttt tat act     3266
Lys Met Lys Gly Leu Phe Ser Gln Ala Lys Ile Ser Leu Phe Tyr Thr
    830                 835                 840

gag gag cat gaa ata atg aag ttt tcc tgg aga ggt gtg act gct gat     3314
Glu Glu His Glu Ile Met Lys Phe Ser Trp Arg Gly Val Thr Ala Asp
845                 850                 855                 860

act aga gct tta agg agg ttt gga ttc tct ttg gcc gca ggc aga agt     3362
Thr Arg Ala Leu Arg Arg Phe Gly Phe Ser Leu Ala Ala Gly Arg Ser
                865                 870                 875

gtg tgg act ctt gaa atg gat gct ggg gtt ctt act ggg aga ctg att     3410
Val Trp Thr Leu Glu Met Asp Ala Gly Val Leu Thr Gly Arg Leu Ile
            880                 885                 890

aga ttg aat gat gag aaa tgg aca gaa atg aag gat gac aag att gtt     3458
Arg Leu Asn Asp Glu Lys Trp Thr Glu Met Lys Asp Asp Lys Ile Val
        895                 900                 905

tca ttg att gaa aag ttt aca agt aac aaa tat tgg tcc aaa gtg aat     3506
Ser Leu Ile Glu Lys Phe Thr Ser Asn Lys Tyr Trp Ser Lys Val Asn
    910                 915                 920

ttc cca cat ggg atg ttg gat ctt gaa gaa att gct gcc aat tct aag     3554
Phe Pro His Gly Met Leu Asp Leu Glu Glu Ile Ala Ala Asn Ser Lys
925                 930                 935                 940

gat ttt cct aac atg tct gaa acg gat ttg tgt ttc ttg ctg cat tgg     3602
Asp Phe Pro Asn Met Ser Glu Thr Asp Leu Cys Phe Leu Leu His Trp
                945                 950                 955

tta aat cca aag aaa att aat tta gca gat aga atg ctt gga ttg tct     3650
Leu Asn Pro Lys Lys Ile Asn Leu Ala Asp Arg Met Leu Gly Leu Ser
            960                 965                 970

gga gtt cag gaa att aaa gaa caa ggt gtt gga tta ata gca gag tgt     3698
Gly Val Gln Glu Ile Lys Glu Gln Gly Val Gly Leu Ile Ala Glu Cys
        975                 980                 985

aga act ttc tta gat tct att gct gga act tta aaa tct atg atg ttt     3746
Arg Thr Phe Leu Asp Ser Ile Ala Gly Thr Leu Lys Ser Met Met Phe
    990                 995                 1000

gga ttt cat cat tct gtg act gtt gaa att ata aac act gtg ctc tgt     3794
Gly Phe His His Ser Val Thr Val Glu Ile Ile Asn Thr Val Leu Cys
1005                1010                1015                1020

ttt gtt aag agt gga att ttg ctt tat gta ata caa caa ttg aat cag     3842
Phe Val Lys Ser Gly Ile Leu Leu Tyr Val Ile Gln Gln Leu Asn Gln
                1025                1030                1035

gat gaa cat tct cac ata att ggt ttg ttg aga gtc atg aat tat gca     3890
Asp Glu His Ser His Ile Ile Gly Leu Leu Arg Val Met Asn Tyr Ala
            1040                1045                1050

gat att ggt tgt tca gtt att tca tgt ggc aaa gtt ttt tcc aaa atg     3938
Asp Ile Gly Cys Ser Val Ile Ser Cys Gly Lys Val Phe Ser Lys Met
        1055                1060                1065

ctg gaa aca gtc ttt aat tgg caa atg gac tcc aga atg atg gag tta     3986
Leu Glu Thr Val Phe Asn Trp Gln Met Asp Ser Arg Met Met Glu Leu
    1070                1075                1080

agg act cag agt ttt tcc aac tgg tta aga gat att tgt tct ggg atc     4034
Arg Thr Gln Ser Phe Ser Asn Trp Leu Arg Asp Ile Cys Ser Gly Ile
1085                1090                1095                1100

acc att ttt aaa aac ttc aag gat gca att tat tgg ctt tat aca aaa     4082
Thr Ile Phe Lys Asn Phe Lys Asp Ala Ile Tyr Trp Leu Tyr Thr Lys
                1105                1110                1115
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tta aag gac ttt tat gaa gtg aat tat ggc aag aag aag gac att tta     4130
Leu Lys Asp Phe Tyr Glu Val Asn Tyr Gly Lys Lys Lys Asp Ile Leu
            1120                1125                1130

aat att ctt aaa gat aac caa caa aaa ata gag aaa gcc att gag gaa     4178
Asn Ile Leu Lys Asp Asn Gln Gln Lys Ile Glu Lys Ala Ile Glu Glu
        1135                1140                1145

gcc gat gaa ttt tgc att ttg caa atc caa gat gtg gaa aaa ttt gaa     4226
Ala Asp Glu Phe Cys Ile Leu Gln Ile Gln Asp Val Glu Lys Phe Glu
    1150                1155                1160

cag tat cag aaa ggg gtt gac ttg ata caa aaa ttg aga act gtt cat     4274
Gln Tyr Gln Lys Gly Val Asp Leu Ile Gln Lys Leu Arg Thr Val His
1165                1170                1175                1180

tca atg gct cag gtt gat cca aat tta atg gtt cat ttg tca cct ttg     4322
Ser Met Ala Gln Val Asp Pro Asn Leu Met Val His Leu Ser Pro Leu
                1185                1190                1195

aga gat tgt ata gca aga gtt cat cag aaa ctt aaa aac ctt gga tct     4370
Arg Asp Cys Ile Ala Arg Val His Gln Lys Leu Lys Asn Leu Gly Ser
            1200                1205                1210

ata aat cag gca atg gta acg aga tgt gag cca gtt gtt tgt tat tta     4418
Ile Asn Gln Ala Met Val Thr Arg Cys Glu Pro Val Val Cys Tyr Leu
        1215                1220                1225

tat ggc aaa aga ggg gga gga aag agc tta aca tca att gca ttg gca     4466
Tyr Gly Lys Arg Gly Gly Gly Lys Ser Leu Thr Ser Ile Ala Leu Ala
    1230                1235                1240

acc aaa att tgt aaa cat tat ggt gtt gag cct gaa aag aat atc tat     4514
Thr Lys Ile Cys Lys His Tyr Gly Val Glu Pro Glu Lys Asn Ile Tyr
1245                1250                1255                1260

act aaa cct gtg gct tca gat tac tgg gat gga tat agt gga caa tta     4562
Thr Lys Pro Val Ala Ser Asp Tyr Trp Asp Gly Tyr Ser Gly Gln Leu
                1265                1270                1275

gtt tgc atc att gat gat att ggc caa aac aca aca gat gag gat tgg     4610
Val Cys Ile Ile Asp Asp Ile Gly Gln Asn Thr Thr Asp Glu Asp Trp
            1280                1285                1290

tca gat ttt tgt cag tta gtg tca gga tgt cca atg aga tta aac atg     4658
Ser Asp Phe Cys Gln Leu Val Ser Gly Cys Pro Met Arg Leu Asn Met
        1295                1300                1305

gcc tct ctt gag gag aag ggt agg cat ttt tct tct cct ttt ata ata     4706
Ala Ser Leu Glu Glu Lys Gly Arg His Phe Ser Ser Pro Phe Ile Ile
    1310                1315                1320

gca act tca aat tgg tca aat cca agt cca aaa aca gtt tat gtt aag     4754
Ala Thr Ser Asn Trp Ser Asn Pro Ser Pro Lys Thr Val Tyr Val Lys
1325                1330                1335                1340

gaa gca att gac cgc aga ctc cat ttc aag gtt gaa gtt aaa cct gct     4802
Glu Ala Ile Asp Arg Arg Leu His Phe Lys Val Glu Val Lys Pro Ala
                1345                1350                1355

tca ttt ttc aaa aat cct cac aat gat atg ttg aat gtt aat tta gct     4850
Ser Phe Phe Lys Asn Pro His Asn Asp Met Leu Asn Val Asn Leu Ala
            1360                1365                1370

aaa aca aat gat gca atc aaa gat atg tct tgt gtt gat ttg ata atg     4898
Lys Thr Asn Asp Ala Ile Lys Asp Met Ser Cys Val Asp Leu Ile Met
        1375                1380                1385

gat gga cat aat gtt tca ttg atg gat ttg ctc agt tct tta gtc atg     4946
Asp Gly His Asn Val Ser Leu Met Asp Leu Leu Ser Ser Leu Val Met
    1390                1395                1400

aca gtt gaa att aga aaa caa aac atg act gaa ttc atg gag ttg tgg     4994
Thr Val Glu Ile Arg Lys Gln Asn Met Thr Glu Phe Met Glu Leu Trp
1405                1410                1415                1420

tct cag gga att tca gat gat gat aat gat agt gca gta gct gag ttt     5042
Ser Gln Gly Ile Ser Asp Asp Asp Asn Asp Ser Ala Val Ala Glu Phe
                1425                1430                1435
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ttc cag tct ttt cca tct ggt gaa cca tcg aac tct aaa tta tct ggc     5090
Phe Gln Ser Phe Pro Ser Gly Glu Pro Ser Asn Ser Lys Leu Ser Gly
            1440                1445                1450

ttt ttc caa tct gtt act aat cac aag tgg gtt gct gtg gga gct gca     5138
Phe Phe Gln Ser Val Thr Asn His Lys Trp Val Ala Val Gly Ala Ala
        1455                1460                1465

gtt ggc att ctt gga gtg ctc gtt gga gga tgg ttt gtg tat aag cat     5186
Val Gly Ile Leu Gly Val Leu Val Gly Gly Trp Phe Val Tyr Lys His
    1470                1475                1480

ttc tcc cgc aaa gag gag gaa cca atc cca gct gaa ggg gta tat cat     5234
Phe Ser Arg Lys Glu Glu Glu Pro Ile Pro Ala Glu Gly Val Tyr His
1485                1490                1495                1500

ggt gta act aag ccc aag caa gtg att aaa tta gat gca gat cca gta     5282
Gly Val Thr Lys Pro Lys Gln Val Ile Lys Leu Asp Ala Asp Pro Val
                1505                1510                1515

gaa tct cag tca act ttg gaa ata gca gga ctg gtt agg aag aac ttg     5330
Glu Ser Gln Ser Thr Leu Glu Ile Ala Gly Leu Val Arg Lys Asn Leu
            1520                1525                1530

gtt cag ttt gga gtt gga gag aag aat gga tgt gtg aga tgg gtt atg     5378
Val Gln Phe Gly Val Gly Glu Lys Asn Gly Cys Val Arg Trp Val Met
        1535                1540                1545

aat gcc ttg gga gtg aaa gat gat tgg ctg ctt gtg cct tcc cat gct     5426
Asn Ala Leu Gly Val Lys Asp Asp Trp Leu Leu Val Pro Ser His Ala
    1550                1555                1560

tat aaa ttt gag aaa gat tat gaa atg atg gag ttt tat ttt aat aga     5474
Tyr Lys Phe Glu Lys Asp Tyr Glu Met Met Glu Phe Tyr Phe Asn Arg
1565                1570                1575                1580

ggt gga act tac tat tca att tca gct ggt aat gtt gtt att caa tct     5522
Gly Gly Thr Tyr Tyr Ser Ile Ser Ala Gly Asn Val Val Ile Gln Ser
                1585                1590                1595

ttg gat gtg gga ttc cag gat gtt gtt ctg atg aag gtt cct aca att     5570
Leu Asp Val Gly Phe Gln Asp Val Val Leu Met Lys Val Pro Thr Ile
            1600                1605                1610

cct aag ttt aga gat att act cag cat ttt att aag aaa ggg gat gtg     5618
Pro Lys Phe Arg Asp Ile Thr Gln His Phe Ile Lys Lys Gly Asp Val
        1615                1620                1625

cct aga gct ttg aat cgc ctg gca aca tta gtg aca act gta aat gga     5666
Pro Arg Ala Leu Asn Arg Leu Ala Thr Leu Val Thr Thr Val Asn Gly
    1630                1635                1640

acc cct atg tta att tct gag ggc cca cta aag atg gaa gag aaa gct     5714
Thr Pro Met Leu Ile Ser Glu Gly Pro Leu Lys Met Glu Glu Lys Ala
1645                1650                1655                1660

act tat gtt cat aag aaa aat gat ggt aca aca gtt gat tta act gtg     5762
Thr Tyr Val His Lys Lys Asn Asp Gly Thr Thr Val Asp Leu Thr Val
                1665                1670                1675

gat cag gca tgg aga gga aaa ggc gaa ggt ctt cct gga atg tgt ggt     5810
Asp Gln Ala Trp Arg Gly Lys Gly Glu Gly Leu Pro Gly Met Cys Gly
            1680                1685                1690

ggg gcc ttg gtt tca tcg aat caa tct ata cag aat gca atc ttg ggc     5858
Gly Ala Leu Val Ser Ser Asn Gln Ser Ile Gln Asn Ala Ile Leu Gly
        1695                1700                1705

atc cat gtt gct gga gga aat tca att ctt gtt gca aaa ttg gtt act     5906
Ile His Val Ala Gly Gly Asn Ser Ile Leu Val Ala Lys Leu Val Thr
    1710                1715                1720

caa gaa atg ttc caa aat att gat aag aaa att gaa agt cag aga att     5954
Gln Glu Met Phe Gln Asn Ile Asp Lys Lys Ile Glu Ser Gln Arg Ile
1725                1730                1735                1740

atg aaa gtg gag ttt act cag tgt tca atg aat gtg gtc tcc aaa acg     6002
Met Lys Val Glu Phe Thr Gln Cys Ser Met Asn Val Val Ser Lys Thr
                1745                1750                1755
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ctt ttt aga aag agt ccc att tat cat cac att gat aaa acc atg att     6050
Leu Phe Arg Lys Ser Pro Ile Tyr His His Ile Asp Lys Thr Met Ile
            1760                1765                1770

aat ttt cct gca gct atg ccc ttt tct aaa gct gaa att gat cca atg     6098
Asn Phe Pro Ala Ala Met Pro Phe Ser Lys Ala Glu Ile Asp Pro Met
        1775                1780                1785

gct gtg atg tta tct aag tat tca tta cct att gta gaa gaa cca gag     6146
Ala Val Met Leu Ser Lys Tyr Ser Leu Pro Ile Val Glu Glu Pro Glu
    1790                1795                1800

gat tat aaa gag gct tca att ttt tat caa aat aaa ata gtg ggt aag     6194
Asp Tyr Lys Glu Ala Ser Ile Phe Tyr Gln Asn Lys Ile Val Gly Lys
1805                1810                1815                1820

act cag tta gtt gat gat ttt tta gat ctt gat atg gcc att aca ggg     6242
Thr Gln Leu Val Asp Asp Phe Leu Asp Leu Asp Met Ala Ile Thr Gly
                1825                1830                1835

gcc cca gga att gat gct atc aac atg gat tca tct cct gga ttt cct     6290
Ala Pro Gly Ile Asp Ala Ile Asn Met Asp Ser Ser Pro Gly Phe Pro
            1840                1845                1850

tat gtc cag gag aag ttg acc aaa aga gat tta att tgg ttg gat gaa     6338
Tyr Val Gln Glu Lys Leu Thr Lys Arg Asp Leu Ile Trp Leu Asp Glu
        1855                1860                1865

aat ggt tta ttg ctg gga gtt cat cca aga ttg gct cag aga atc tta     6386
Asn Gly Leu Leu Leu Gly Val His Pro Arg Leu Ala Gln Arg Ile Leu
    1870                1875                1880

ttc aat act gtc atg atg gaa aat tgt tct gat ttg gat gtt gtt ttt     6434
Phe Asn Thr Val Met Met Glu Asn Cys Ser Asp Leu Asp Val Val Phe
1885                1890                1895                1900

aca acc tgt cca aaa gat gaa ttg aga cca tta gag aaa gtg ttg gaa     6482
Thr Thr Cys Pro Lys Asp Glu Leu Arg Pro Leu Glu Lys Val Leu Glu
                1905                1910                1915

tca aaa aca aga gct att gat gct tgt cct ctg gat tac tca att ttg     6530
Ser Lys Thr Arg Ala Ile Asp Ala Cys Pro Leu Asp Tyr Ser Ile Leu
            1920                1925                1930

tgc cga atg tat tgg ggt cca gct att agt tat ttt cat ttg aat cca     6578
Cys Arg Met Tyr Trp Gly Pro Ala Ile Ser Tyr Phe His Leu Asn Pro
        1935                1940                1945

ggt ttc cat aca ggt gtt gct att ggc ata gat cct gat aga cag tgg     6626
Gly Phe His Thr Gly Val Ala Ile Gly Ile Asp Pro Asp Arg Gln Trp
    1950                1955                1960

gat gaa tta ttt aaa aca atg ata aga ttc gga gat gtt ggt ctt gat     6674
Asp Glu Leu Phe Lys Thr Met Ile Arg Phe Gly Asp Val Gly Leu Asp
1965                1970                1975                1980

tta gat ttc tct gct ttt gat gct agt ctt agt cca ttt atg att aga     6722
Leu Asp Phe Ser Ala Phe Asp Ala Ser Leu Ser Pro Phe Met Ile Arg
                1985                1990                1995

gaa gca ggt aga atc atg agt gaa cta tct gga act cca tcc cat ttt     6770
Glu Ala Gly Arg Ile Met Ser Glu Leu Ser Gly Thr Pro Ser His Phe
            2000                2005                2010

ggc aca gct ctt atc aat act atc att tat tcc aag cat ttg ctg tat     6818
Gly Thr Ala Leu Ile Asn Thr Ile Ile Tyr Ser Lys His Leu Leu Tyr
        2015                2020                2025

aac tgt tgt tac cat gtc tgt ggt tca atg ccc tct ggg tct cct tgt     6866
Asn Cys Cys Tyr His Val Cys Gly Ser Met Pro Ser Gly Ser Pro Cys
    2030                2035                2040

aca gct ttg cta aat tca att att aat aat gtc aat ttg tat tat gtg     6914
Thr Ala Leu Leu Asn Ser Ile Ile Asn Asn Val Asn Leu Tyr Tyr Val
2045                2050                2055                2060

ttt tcc aag ata ttt gga aag tct cca gtt ttc ttt tgt cag gct ttg     6962
Phe Ser Lys Ile Phe Gly Lys Ser Pro Val Phe Phe Cys Gln Ala Leu
                2065                2070                2075
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aag att ctc tgt tat gga gat gat gtt tta ata gtt ttc tct cga gat     7010
Lys Ile Leu Cys Tyr Gly Asp Asp Val Leu Ile Val Phe Ser Arg Asp
            2080                2085                2090

gtt cag att gat aat ctt gat ttg att gga caa aaa att gta gat gag     7058
Val Gln Ile Asp Asn Leu Asp Leu Ile Gly Gln Lys Ile Val Asp Glu
        2095                2100                2105

ttt aag aaa ctt ggc atg aca gct act tct gct gac aag aat gta cct     7106
Phe Lys Lys Leu Gly Met Thr Ala Thr Ser Ala Asp Lys Asn Val Pro
    2110                2115                2120

cag ctg aaa cca gtt tcg gaa ttg act ttt ctc aaa aga tct ttc aat     7154
Gln Leu Lys Pro Val Ser Glu Leu Thr Phe Leu Lys Arg Ser Phe Asn
2125                2130                2135                2140

ttg gta gag gat aga att aga cct gca att tcg gaa aaa aca att tgg     7202
Leu Val Glu Asp Arg Ile Arg Pro Ala Ile Ser Glu Lys Thr Ile Trp
                2145                2150                2155

tct tta ata gca tgg cag aga agt aac gct gag ttt gag cag aat tta     7250
Ser Leu Ile Ala Trp Gln Arg Ser Asn Ala Glu Phe Glu Gln Asn Leu
            2160                2165                2170

gaa aat gct cag tgg ttt gct ttt atg cat ggc tat gag ttt tat cag     7298
Glu Asn Ala Gln Trp Phe Ala Phe Met His Gly Tyr Glu Phe Tyr Gln
        2175                2180                2185

aaa ttt tat tat ttt gtt cag tcc tgt ttg gag aaa gag atg ata gaa     7346
Lys Phe Tyr Tyr Phe Val Gln Ser Cys Leu Glu Lys Glu Met Ile Glu
    2190                2195                2200

tac aga ctt aaa tct tat gat tgg tgg aga atg aga ttt tat gac cag     7394
Tyr Arg Leu Lys Ser Tyr Asp Trp Trp Arg Met Arg Phe Tyr Asp Gln
2205                2210                2215                2220

tgt ttc att tgt gac ctt tca tgatttgttt aaacaaattt tcttaaaatt       7445
Cys Phe Ile Cys Asp Leu Ser
                2225

tctgaggttt gtttatttct tttatcagta aataaaaaaa aaaaaaaa               7493


<210> SEQ ID NO 2
<211> LENGTH: 2227
<212> TYPE: PRT
<213> ORGANISM: WILD-TYPE HUMAN HEPATITIS A VIRUS, STRAIN HM-175

<400> SEQUENCE: 2

Met Asn Met Ser Arg Gln Gly Ile Phe Gln Thr Val Gly Ser Gly Leu
  1               5                  10                  15

Asp His Ile Leu Ser Leu Ala Asp Ile Glu Glu Glu Gln Met Ile Gln
             20                  25                  30

Ser Val Asp Arg Thr Ala Val Thr Gly Ala Ser Tyr Phe Thr Ser Val
         35                  40                  45

Asp Gln Ser Ser Val His Thr Ala Glu Val Gly Ser His Gln Val Glu
     50                  55                  60

Pro Leu Arg Thr Ser Val Asp Lys Pro Gly Ser Lys Lys Thr Gln Gly
 65                  70                  75                  80

Glu Lys Phe Phe Leu Ile His Ser Ala Asp Trp Leu Thr Thr His Ala
                 85                  90                  95

Leu Phe His Glu Val Ala Lys Leu Asp Val Val Lys Leu Leu Tyr Asn
            100                 105                 110

Glu Gln Phe Ala Val Gln Gly Leu Leu Arg Tyr His Thr Tyr Ala Arg
        115                 120                 125

Phe Gly Ile Glu Ile Gln Val Gln Ile Asn Pro Thr Pro Phe Gln Gln
    130                 135                 140

Gly Gly Leu Ile Cys Ala Met Val Pro Gly Asp Gln Ser Tyr Gly Ser
145                 150                 155                 160
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Ile Ala Ser Leu Thr Val Tyr Pro His Gly Leu Leu Asn Cys Asn Ile
                165                 170                 175

Asn Asn Val Val Arg Ile Lys Val Pro Phe Ile Tyr Thr Arg Gly Ala
            180                 185                 190

Tyr His Phe Lys Asp Pro Gln Tyr Pro Val Trp Glu Leu Thr Ile Arg
        195                 200                 205

Val Trp Ser Glu Leu Asn Ile Gly Thr Gly Thr Ser Ala Tyr Thr Ser
    210                 215                 220

Leu Asn Val Leu Ala Arg Phe Thr Asp Leu Glu Leu His Gly Leu Thr
225                 230                 235                 240

Pro Leu Ser Thr Gln Met Met Arg Asn Glu Phe Arg Val Ser Thr Thr
                245                 250                 255

Glu Asn Val Val Asn Leu Ser Asn Tyr Glu Asp Ala Arg Ala Lys Met
            260                 265                 270

Ser Phe Ala Leu Asp Gln Glu Asp Trp Lys Ser Asp Pro Ser Gln Gly
        275                 280                 285

Gly Gly Ile Lys Ile Thr His Phe Thr Thr Trp Thr Ser Ile Pro Thr
    290                 295                 300

Leu Ala Ala Gln Phe Pro Phe Asn Ala Ser Asp Ser Val Gly Gln Gln
305                 310                 315                 320

Ile Lys Val Ile Pro Val Asp Pro Tyr Phe Phe Gln Met Thr Asn Thr
                325                 330                 335

Asn Pro Asp Gln Lys Cys Ile Thr Ala Leu Ala Ser Ile Cys Gln Met
            340                 345                 350

Phe Cys Phe Trp Arg Gly Asp Leu Val Phe Asp Phe Gln Val Phe Pro
        355                 360                 365

Thr Lys Tyr His Ser Gly Arg Leu Leu Phe Cys Phe Val Pro Gly Asn
    370                 375                 380

Glu Leu Ile Asp Val Ser Gly Ile Thr Leu Lys Gln Ala Thr Thr Ala
385                 390                 395                 400

Pro Cys Ala Val Met Asp Ile Thr Gly Val Gln Ser Thr Leu Arg Phe
                405                 410                 415

Arg Val Pro Trp Ile Ser Asp Thr Pro Tyr Arg Val Asn Arg Tyr Thr
            420                 425                 430

Lys Ser Ala His Gln Lys Gly Glu Tyr Thr Ala Ile Gly Lys Leu Ile
        435                 440                 445

Val Tyr Cys Tyr Asn Arg Leu Thr Ser Pro Ser Asn Val Ala Ser His
    450                 455                 460

Val Arg Val Asn Val Tyr Leu Ser Ala Ile Asn Leu Glu Cys Phe Ala
465                 470                 475                 480

Pro Leu Tyr His Ala Met Asp Val Thr Thr Gln Val Gly Asp Asp Ser
                485                 490                 495

Gly Gly Phe Ser Thr Thr Val Ser Thr Glu Gln Asn Val Pro Asp Pro
            500                 505                 510

Gln Val Gly Ile Thr Thr Met Lys Asp Leu Lys Gly Lys Ala Asn Arg
        515                 520                 525

Gly Lys Met Asp Val Ser Gly Val Gln Ala Pro Val Gly Ala Ile Thr
    530                 535                 540

Thr Ile Glu Asp Pro Val Leu Ala Lys Lys Val Pro Glu Thr Phe Pro
545                 550                 555                 560

Glu Leu Lys Pro Gly Glu Ser Arg His Thr Ser Asp His Met Ser Ile
                565                 570                 575
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Tyr Lys Phe Met Gly Arg Ser His Phe Leu Cys Thr Phe Thr Phe Asn
            580                 585                 590

Ser Asn Asn Lys Glu Tyr Thr Phe Pro Ile Thr Leu Ser Ser Thr Ser
        595                 600                 605

Asn Pro Pro His Gly Leu Pro Ser Thr Leu Arg Trp Phe Phe Asn Leu
    610                 615                 620

Phe Gln Leu Tyr Arg Gly Pro Leu Asp Leu Thr Ile Ile Ile Thr Gly
625                 630                 635                 640

Ala Thr Asp Val Asp Gly Met Ala Trp Phe Thr Pro Val Gly Leu Ala
                645                 650                 655

Val Asp Thr Pro Trp Val Glu Lys Glu Ser Ala Leu Ser Ile Asp Tyr
            660                 665                 670

Lys Thr Ala Leu Gly Ala Val Arg Phe Asn Thr Arg Arg Thr Gly Asn
        675                 680                 685

Ile Gln Ile Arg Leu Pro Trp Tyr Ser Tyr Leu Tyr Ala Val Ser Gly
    690                 695                 700

Ala Leu Asp Gly Leu Gly Asp Lys Thr Asp Ser Thr Phe Gly Leu Val
705                 710                 715                 720

Ser Ile Gln Ile Ala Asn Tyr Asn His Ser Asp Glu Tyr Leu Ser Phe
                725                 730                 735

Ser Cys Tyr Leu Ser Val Thr Glu Gln Ser Glu Phe Tyr Phe Pro Arg
            740                 745                 750

Ala Pro Leu Asn Ser Asn Ala Met Leu Ser Thr Glu Ser Met Met Ser
        755                 760                 765

Arg Ile Ala Ala Gly Asp Leu Glu Ser Ser Val Asp Asp Pro Arg Ser
    770                 775                 780

Glu Glu Asp Lys Arg Phe Glu Ser His Ile Glu Cys Arg Lys Pro Tyr
785                 790                 795                 800

Lys Glu Leu Arg Leu Glu Val Gly Lys Gln Arg Leu Lys Tyr Ala Gln
                805                 810                 815

Glu Glu Leu Ser Asn Glu Val Leu Pro Pro Pro Arg Lys Met Lys Gly
            820                 825                 830

Leu Phe Ser Gln Ala Lys Ile Ser Leu Phe Tyr Thr Glu Glu His Glu
        835                 840                 845

Ile Met Lys Phe Ser Trp Arg Gly Val Thr Ala Asp Thr Arg Ala Leu
    850                 855                 860

Arg Arg Phe Gly Phe Ser Leu Ala Ala Gly Arg Ser Val Trp Thr Leu
865                 870                 875                 880

Glu Met Asp Ala Gly Val Leu Thr Gly Arg Leu Ile Arg Leu Asn Asp
                885                 890                 895

Glu Lys Trp Thr Glu Met Lys Asp Asp Lys Ile Val Ser Leu Ile Glu
            900                 905                 910

Lys Phe Thr Ser Asn Lys Tyr Trp Ser Lys Val Asn Phe Pro His Gly
        915                 920                 925

Met Leu Asp Leu Glu Glu Ile Ala Ala Asn Ser Lys Asp Phe Pro Asn
    930                 935                 940

Met Ser Glu Thr Asp Leu Cys Phe Leu Leu His Trp Leu Asn Pro Lys
945                 950                 955                 960

Lys Ile Asn Leu Ala Asp Arg Met Leu Gly Leu Ser Gly Val Gln Glu
                965                 970                 975

Ile Lys Glu Gln Gly Val Gly Leu Ile Ala Glu Cys Arg Thr Phe Leu
            980                 985                 990
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Asp Ser Ile Ala Gly Thr Leu Lys Ser Met Met Phe Gly Phe His His
        995                 1000                1005

Ser Val Thr Val Glu Ile Ile Asn Thr Val Leu Cys Phe Val Lys Ser
    1010                1015                1020

Gly Ile Leu Leu Tyr Val Ile Gln Gln Leu Asn Gln Asp Glu His Ser
1025                1030                1035                1040

His Ile Ile Gly Leu Leu Arg Val Met Asn Tyr Ala Asp Ile Gly Cys
                1045                1050                1055

Ser Val Ile Ser Cys Gly Lys Val Phe Ser Lys Met Leu Glu Thr Val
            1060                1065                1070

Phe Asn Trp Gln Met Asp Ser Arg Met Met Glu Leu Arg Thr Gln Ser
        1075                1080                1085

Phe Ser Asn Trp Leu Arg Asp Ile Cys Ser Gly Ile Thr Ile Phe Lys
    1090                1095                1100

Asn Phe Lys Asp Ala Ile Tyr Trp Leu Tyr Thr Lys Leu Lys Asp Phe
1105                1110                1115                1120

Tyr Glu Val Asn Tyr Gly Lys Lys Lys Asp Ile Leu Asn Ile Leu Lys
                1125                1130                1135

Asp Asn Gln Gln Lys Ile Glu Lys Ala Ile Glu Glu Ala Asp Glu Phe
            1140                1145                1150

Cys Ile Leu Gln Ile Gln Asp Val Glu Lys Phe Glu Gln Tyr Gln Lys
        1155                1160                1165

Gly Val Asp Leu Ile Gln Lys Leu Arg Thr Val His Ser Met Ala Gln
    1170                1175                1180

Val Asp Pro Asn Leu Met Val His Leu Ser Pro Leu Arg Asp Cys Ile
1185                1190                1195                1200

Ala Arg Val His Gln Lys Leu Lys Asn Leu Gly Ser Ile Asn Gln Ala
                1205                1210                1215

Met Val Thr Arg Cys Glu Pro Val Val Cys Tyr Leu Tyr Gly Lys Arg
            1220                1225                1230

Gly Gly Gly Lys Ser Leu Thr Ser Ile Ala Leu Ala Thr Lys Ile Cys
        1235                1240                1245

Lys His Tyr Gly Val Glu Pro Glu Lys Asn Ile Tyr Thr Lys Pro Val
    1250                1255                1260

Ala Ser Asp Tyr Trp Asp Gly Tyr Ser Gly Gln Leu Val Cys Ile Ile
1265                1270                1275                1280

Asp Asp Ile Gly Gln Asn Thr Thr Asp Glu Asp Trp Ser Asp Phe Cys
                1285                1290                1295

Gln Leu Val Ser Gly Cys Pro Met Arg Leu Asn Met Ala Ser Leu Glu
            1300                1305                1310

Glu Lys Gly Arg His Phe Ser Ser Pro Phe Ile Ile Ala Thr Ser Asn
        1315                1320                1325

Trp Ser Asn Pro Ser Pro Lys Thr Val Tyr Val Lys Glu Ala Ile Asp
    1330                1335                1340

Arg Arg Leu His Phe Lys Val Glu Val Lys Pro Ala Ser Phe Phe Lys
1345                1350                1355                1360

Asn Pro His Asn Asp Met Leu Asn Val Asn Leu Ala Lys Thr Asn Asp
                1365                1370                1375

Ala Ile Lys Asp Met Ser Cys Val Asp Leu Ile Met Asp Gly His Asn
            1380                1385                1390

Val Ser Leu Met Asp Leu Leu Ser Ser Leu Val Met Thr Val Glu Ile
        1395                1400                1405
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Arg Lys Gln Asn Met Thr Glu Phe Met Glu Leu Trp Ser Gln Gly Ile
    1410                1415                1420

Ser Asp Asp Asp Asn Asp Ser Ala Val Ala Glu Phe Phe Gln Ser Phe
1425                1430                1435                1440

Pro Ser Gly Glu Pro Ser Asn Ser Lys Leu Ser Gly Phe Phe Gln Ser
                1445                1450                1455

Val Thr Asn His Lys Trp Val Ala Val Gly Ala Ala Val Gly Ile Leu
            1460                1465                1470

Gly Val Leu Val Gly Gly Trp Phe Val Tyr Lys His Phe Ser Arg Lys
        1475                1480                1485

Glu Glu Glu Pro Ile Pro Ala Glu Gly Val Tyr His Gly Val Thr Lys
    1490                1495                1500

Pro Lys Gln Val Ile Lys Leu Asp Ala Asp Pro Val Glu Ser Gln Ser
1505                1510                1515                1520

Thr Leu Glu Ile Ala Gly Leu Val Arg Lys Asn Leu Val Gln Phe Gly
                1525                1530                1535

Val Gly Glu Lys Asn Gly Cys Val Arg Trp Val Met Asn Ala Leu Gly
            1540                1545                1550

Val Lys Asp Asp Trp Leu Leu Val Pro Ser His Ala Tyr Lys Phe Glu
        1555                1560                1565

Lys Asp Tyr Glu Met Met Glu Phe Tyr Phe Asn Arg Gly Gly Thr Tyr
    1570                1575                1580

Tyr Ser Ile Ser Ala Gly Asn Val Val Ile Gln Ser Leu Asp Val Gly
1585                1590                1595                1600

Phe Gln Asp Val Val Leu Met Lys Val Pro Thr Ile Pro Lys Phe Arg
                1605                1610                1615

Asp Ile Thr Gln His Phe Ile Lys Lys Gly Asp Val Pro Arg Ala Leu
            1620                1625                1630

Asn Arg Leu Ala Thr Leu Val Thr Thr Val Asn Gly Thr Pro Met Leu
        1635                1640                1645

Ile Ser Glu Gly Pro Leu Lys Met Glu Glu Lys Ala Thr Tyr Val His
    1650                1655                1660

Lys Lys Asn Asp Gly Thr Thr Val Asp Leu Thr Val Asp Gln Ala Trp
1665                1670                1675                1680

Arg Gly Lys Gly Glu Gly Leu Pro Gly Met Cys Gly Gly Ala Leu Val
                1685                1690                1695

Ser Ser Asn Gln Ser Ile Gln Asn Ala Ile Leu Gly Ile His Val Ala
            1700                1705                1710

Gly Gly Asn Ser Ile Leu Val Ala Lys Leu Val Thr Gln Glu Met Phe
        1715                1720                1725

Gln Asn Ile Asp Lys Lys Ile Glu Ser Gln Arg Ile Met Lys Val Glu
    1730                1735                1740

Phe Thr Gln Cys Ser Met Asn Val Val Ser Lys Thr Leu Phe Arg Lys
1745                1750                1755                1760

Ser Pro Ile Tyr His His Ile Asp Lys Thr Met Ile Asn Phe Pro Ala
                1765                1770                1775

Ala Met Pro Phe Ser Lys Ala Glu Ile Asp Pro Met Ala Val Met Leu
            1780                1785                1790

Ser Lys Tyr Ser Leu Pro Ile Val Glu Glu Pro Glu Asp Tyr Lys Glu
        1795                1800                1805

Ala Ser Ile Phe Tyr Gln Asn Lys Ile Val Gly Lys Thr Gln Leu Val
    1810                1815                1820
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Asp Asp Phe Leu Asp Leu Asp Met Ala Ile Thr Gly Ala Pro Gly Ile
1825                1830                1835                1840

Asp Ala Ile Asn Met Asp Ser Ser Pro Gly Phe Pro Tyr Val Gln Glu
                1845                1850                1855

Lys Leu Thr Lys Arg Asp Leu Ile Trp Leu Asp Glu Asn Gly Leu Leu
            1860                1865                1870

Leu Gly Val His Pro Arg Leu Ala Gln Arg Ile Leu Phe Asn Thr Val
        1875                1880                1885

Met Met Glu Asn Cys Ser Asp Leu Asp Val Val Phe Thr Thr Cys Pro
    1890                1895                1900

Lys Asp Glu Leu Arg Pro Leu Glu Lys Val Leu Glu Ser Lys Thr Arg
1905                1910                1915                1920

Ala Ile Asp Ala Cys Pro Leu Asp Tyr Ser Ile Leu Cys Arg Met Tyr
                1925                1930                1935

Trp Gly Pro Ala Ile Ser Tyr Phe His Leu Asn Pro Gly Phe His Thr
            1940                1945                1950

Gly Val Ala Ile Gly Ile Asp Pro Asp Arg Gln Trp Asp Glu Leu Phe
        1955                1960                1965

Lys Thr Met Ile Arg Phe Gly Asp Val Gly Leu Asp Leu Asp Phe Ser
    1970                1975                1980

Ala Phe Asp Ala Ser Leu Ser Pro Phe Met Ile Arg Glu Ala Gly Arg
1985                1990                1995                2000

Ile Met Ser Glu Leu Ser Gly Thr Pro Ser His Phe Gly Thr Ala Leu
                2005                2010                2015

Ile Asn Thr Ile Ile Tyr Ser Lys His Leu Leu Tyr Asn Cys Cys Tyr
            2020                2025                2030

His Val Cys Gly Ser Met Pro Ser Gly Ser Pro Cys Thr Ala Leu Leu
        2035                2040                2045

Asn Ser Ile Ile Asn Asn Val Asn Leu Tyr Tyr Val Phe Ser Lys Ile
    2050                2055                2060

Phe Gly Lys Ser Pro Val Phe Phe Cys Gln Ala Leu Lys Ile Leu Cys
1065                2070                2075                2080

Tyr Gly Asp Asp Val Leu Ile Val Phe Ser Arg Asp Val Gln Ile Asp
                2085                2090                2095

Asn Leu Asp Leu Ile Gly Gln Lys Ile Val Asp Glu Phe Lys Lys Leu
            2100                2105                2110

Gly Met Thr Ala Thr Ser Ala Asp Lys Asn Val Pro Gln Leu Lys Pro
        2115                2120                2125

Val Ser Glu Leu Thr Phe Leu Lys Arg Ser Phe Asn Leu Val Glu Asp
    2130                2135                2140

Arg Ile Arg Pro Ala Ile Ser Glu Lys Thr Ile Trp Ser Leu Ile Ala
2145                2150                2155                2160

Trp Gln Arg Ser Asn Ala Glu Phe Glu Gln Asn Leu Glu Asn Ala Gln
                2165                2170                2175

Trp Phe Ala Phe Met His Gly Tyr Glu Phe Tyr Gln Lys Phe Tyr Tyr
            2180                2185                2190

Phe Val Gln Ser Cys Leu Glu Lys Glu Met Ile Glu Tyr Arg Leu Lys
        2195                2200                2205

Ser Tyr Asp Trp Trp Arg Met Arg Phe Tyr Asp Gln Cys Phe Ile Cys
    2210                2215                2220

Asp Leu Ser
2225
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<211> LENGTH: 7486
<212> TYPE: DNA
<213> ORGANISM: Attenuated HAV (4380), strain HM-175
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (730)..(7410)

<400> SEQUENCE: 3

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tcccattccc ttttgcttgt aaatattgat tcctgcaggt tcagggttct taaatctgtt 180

tctctataag aacactcatt ttcacgcttt ctgtcttctt tcttccaggg ctctcccctt 240

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ggagtctaaa ttggggacac agatgtttgg aacgtcacct tgcagtgtta acttggcttt 360

catgaatctc tttgatcttc cacaaggggt aggctacggg tgaaacctct taggctaata 420

cttctatgaa gagatgcctt ggatagggta acagcggcgg atattggtga gttgttaaga 480

caaaaaccat tcaacgccgg aggactgact ctcatccagt ggatgcattg agtggattga 540

ctgtcagggc tgtctttagg cttaattcca gacctctctg tgcttggggc aaacatcatt 600

tggccttaaa tgggattctg tgagagggga tccctccatt aacagctgga ctgttctttg 660

gggtcttatg tggtgtttgc cgctgaggta ctcaggggca tttaggtttt tcctcattct 720

taaataata atg aac atg tct aga caa ggt att ttc cag act gtt ggg agt 771
Met Asn Met Ser Arg Gln Gly Ile Phe Gln Thr Val Gly Ser
1 5 10

ggt ctt gac cac atc ctg tct ttg gca gac att gag gaa gag caa atg 819
Gly Leu Asp His Ile Leu Ser Leu Ala Asp Ile Glu Glu Glu Gln Met
15 20 25 30

att caa tca gtt gat agg act gca gtg act ggt gct tct tat ttt act 867
Ile Gln Ser Val Asp Arg Thr Ala Val Thr Gly Ala Ser Tyr Phe Thr
35 40 45

tct gtg gat caa tct tca gtt cat aca gct gag gtt gga tca cac cag 915
Ser Val Asp Gln Ser Ser Val His Thr Ala Glu Val Gly Ser His Gln
50 55 60

gtt gaa cct ttg aga acc tct gtt gat aaa ccc ggt tca aag agg act 963
Val Glu Pro Leu Arg Thr Ser Val Asp Lys Pro Gly Ser Lys Arg Thr
65 70 75

cag gga gag aaa ttt ttc ttg att cat tct gca gat tgg ctt act aca 1011
Gln Gly Glu Lys Phe Phe Leu Ile His Ser Ala Asp Trp Leu Thr Thr
80 85 90

cat gct ctt ttc cat gaa gtt gca aaa ttg gat gtg gtg aaa tta tta 1059
His Ala Leu Phe His Glu Val Ala Lys Leu Asp Val Val Lys Leu Leu
95 100 105 110

tac aat gag cag ttt gct gtt caa ggg ttg ttg aga tac cat aca tat 1107
Tyr Asn Glu Gln Phe Ala Val Gln Gly Leu Leu Arg Tyr His Thr Tyr
115 120 125

gca aga ttt ggc att gaa att caa gtt cag ata aac cct aca cct ttc 1155
Ala Arg Phe Gly Ile Glu Ile Gln Val Gln Ile Asn Pro Thr Pro Phe
130 135 140

caa cag ggg gga ttg atc tgt gct atg gtt cct ggt gac cag agc tat 1203
Gln Gln Gly Gly Leu Ile Cys Ala Met Val Pro Gly Asp Gln Ser Tyr
145 150 155

ggt tct ata gca tca ttg act gtt tat cct cat ggt ttg tta aat tgc 1251
Gly Ser Ile Ala Ser Leu Thr Val Tyr Pro His Gly Leu Leu Asn Cys
160 165 170

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aat att aac aat gtg gtt aga ata aag gtt cca ttt att tac aca aga     1299
Asn Ile Asn Asn Val Val Arg Ile Lys Val Pro Phe Ile Tyr Thr Arg
175                 180                 185                 190

ggt gct tac cac ttt aaa gat cca caa tac cca gtt tgg gaa ttg aca     1347
Gly Ala Tyr His Phe Lys Asp Pro Gln Tyr Pro Val Trp Glu Leu Thr
                195                 200                 205

att aga gtt tgg tca gaa tta aat att ggg aca gga act tca gct tat     1395
Ile Arg Val Trp Ser Glu Leu Asn Ile Gly Thr Gly Thr Ser Ala Tyr
            210                 215                 220

act tca ctc aat gtt tta gct aga ttt aca gat ttg gag ttg cat gga     1443
Thr Ser Leu Asn Val Leu Ala Arg Phe Thr Asp Leu Glu Leu His Gly
        225                 230                 235

tta act cct ctt tct aca caa atg atg aga aat gaa ttt agg gtc agt     1491
Leu Thr Pro Leu Ser Thr Gln Met Met Arg Asn Glu Phe Arg Val Ser
    240                 245                 250

act act gag aat gtg gtg aat ctg tca aat tat gaa gat gca aga gca     1539
Thr Thr Glu Asn Val Val Asn Leu Ser Asn Tyr Glu Asp Ala Arg Ala
255                 260                 265                 270

aag atg tct ttt gct ttg gat cag gaa gat tgg aaa tct gat ccg tcc     1587
Lys Met Ser Phe Ala Leu Asp Gln Glu Asp Trp Lys Ser Asp Pro Ser
                275                 280                 285

cag ggt ggt ggg atc aaa att act cat ttt act act tgg aca tct att     1635
Gln Gly Gly Gly Ile Lys Ile Thr His Phe Thr Thr Trp Thr Ser Ile
            290                 295                 300

cca act ttg gct gct cag ttt cca ttt aat gct tca gac tca gtt ggt     1683
Pro Thr Leu Ala Ala Gln Phe Pro Phe Asn Ala Ser Asp Ser Val Gly
        305                 310                 315

caa caa att aaa gtt att cca gtt gac cca tat ttt ttc caa atg aca     1731
Gln Gln Ile Lys Val Ile Pro Val Asp Pro Tyr Phe Phe Gln Met Thr
    320                 325                 330

aat aca aat cct gac caa aaa tgt ata act gct ttg gct tct att tgt     1779
Asn Thr Asn Pro Asp Gln Lys Cys Ile Thr Ala Leu Ala Ser Ile Cys
335                 340                 345                 350

cag atg ttt tgt ttt tgg aga gga gat ctt gtc ttt gat ttt caa gtt     1827
Gln Met Phe Cys Phe Trp Arg Gly Asp Leu Val Phe Asp Phe Gln Val
                355                 360                 365

ttt ccc acc aaa tat cat tca ggt aga tta ctg ttt tgt ttt gtt cct     1875
Phe Pro Thr Lys Tyr His Ser Gly Arg Leu Leu Phe Cys Phe Val Pro
            370                 375                 380

ggc aat gag cta ata gat gtt tct gga atc aca tta aag caa gca act     1923
Gly Asn Glu Leu Ile Asp Val Ser Gly Ile Thr Leu Lys Gln Ala Thr
        385                 390                 395

act gct cct tgt gca gta atg gat att aca gga gtg cag tca act ttg     1971
Thr Ala Pro Cys Ala Val Met Asp Ile Thr Gly Val Gln Ser Thr Leu
    400                 405                 410

aga ttt cgt gtt ccc tgg att tct gac act cct tac aga gtg aac agg     2019
Arg Phe Arg Val Pro Trp Ile Ser Asp Thr Pro Tyr Arg Val Asn Arg
415                 420                 425                 430

tat aca aag tca gca cat cag aaa ggt gag tac act gcc att ggg aag     2067
Tyr Thr Lys Ser Ala His Gln Lys Gly Glu Tyr Thr Ala Ile Gly Lys
                435                 440                 445

ctt att gtg tat tgt tat aac aga ttg acc tct cct tct aac gtt gct     2115
Leu Ile Val Tyr Cys Tyr Asn Arg Leu Thr Ser Pro Ser Asn Val Ala
            450                 455                 460

tcc cat gtc aga gtg aat gtt tat ctt tca gca att aac ttg gaa tgt     2163
Ser His Val Arg Val Asn Val Tyr Leu Ser Ala Ile Asn Leu Glu Cys
        465                 470                 475

ttt gct cct ctt tat cat gct atg gat gtt act aca caa gtt gga gat     2211
Phe Ala Pro Leu Tyr His Ala Met Asp Val Thr Thr Gln Val Gly Asp
    480                 485                 490
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gat tct gga ggt ttt tca aca aca gtt tct aca gaa cag aat gtt cca     2259
Asp Ser Gly Gly Phe Ser Thr Thr Val Ser Thr Glu Gln Asn Val Pro
495                 500                 505                 510

gat ccc caa gtt ggt ata aca acc atg aaa gat ttg aaa gga aaa gct     2307
Asp Pro Gln Val Gly Ile Thr Thr Met Lys Asp Leu Lys Gly Lys Ala
                515                 520                 525

aac aga ggg aaa atg gat gtt tca gga gta caa gca cct gtg gga gct     2355
Asn Arg Gly Lys Met Asp Val Ser Gly Val Gln Ala Pro Val Gly Ala
            530                 535                 540

atc aca aca att gag gat cca gtt tta gca aag aaa gta cct gag aca     2403
Ile Thr Thr Ile Glu Asp Pro Val Leu Ala Lys Lys Val Pro Glu Thr
        545                 550                 555

ttt cct gaa ttg aaa cct gga gaa tcc aga cat aca tca gat cat atg     2451
Phe Pro Glu Leu Lys Pro Gly Glu Ser Arg His Thr Ser Asp His Met
    560                 565                 570

tcc atc tac aag ttt atg gga agg tct cat ttc ttg tgc act ttt aca     2499
Ser Ile Tyr Lys Phe Met Gly Arg Ser His Phe Leu Cys Thr Phe Thr
575                 580                 585                 590

ttc aat tca aat aat aaa gag tac aca ttt cct ata acc ttg tct tca     2547
Phe Asn Ser Asn Asn Lys Glu Tyr Thr Phe Pro Ile Thr Leu Ser Ser
                595                 600                 605

acc tct aat cct cct cat ggt ttg cca tca aca ctg agg tgg ttt ttc     2595
Thr Ser Asn Pro Pro His Gly Leu Pro Ser Thr Leu Arg Trp Phe Phe
            610                 615                 620

aac ttg ttt cag ttg tat aga ggg cct tta gat ctg aca att att att     2643
Asn Leu Phe Gln Leu Tyr Arg Gly Pro Leu Asp Leu Thr Ile Ile Ile
        625                 630                 635

aca gga gca act gat gta gat ggc atg gcc tgg ttc act cca gta ggt     2691
Thr Gly Ala Thr Asp Val Asp Gly Met Ala Trp Phe Thr Pro Val Gly
    640                 645                 650

ctt gcc gtt gat act cct tgg gta gag aag gag tca gct ttg tct att     2739
Leu Ala Val Asp Thr Pro Trp Val Glu Lys Glu Ser Ala Leu Ser Ile
655                 660                 665                 670

gac tat aaa act gct ctt gga gct gtc aga ttt aac aca agg aga aca     2787
Asp Tyr Lys Thr Ala Leu Gly Ala Val Arg Phe Asn Thr Arg Arg Thr
                675                 680                 685

ggg aac att cag att aga tta cca tgg tat tct tat tta tat gct gtg     2835
Gly Asn Ile Gln Ile Arg Leu Pro Trp Tyr Ser Tyr Leu Tyr Ala Val
            690                 695                 700

tct gga gca ctg gat ggt ttg gga gac aag aca gat tct aca ttt gga     2883
Ser Gly Ala Leu Asp Gly Leu Gly Asp Lys Thr Asp Ser Thr Phe Gly
        705                 710                 715

ttg gtt tct att cag att gca aat tac aat cat tct gat gaa tac ttg     2931
Leu Val Ser Ile Gln Ile Ala Asn Tyr Asn His Ser Asp Glu Tyr Leu
    720                 725                 730

tct ttt agt tgt tat ttg tct gtc aca gaa caa tca gag ttt tat ttt     2979
Ser Phe Ser Cys Tyr Leu Ser Val Thr Glu Gln Ser Glu Phe Tyr Phe
735                 740                 745                 750

ccc aga gct cca ttg aac tca aat gcc atg tta tcc act gta aca atg     3027
Pro Arg Ala Pro Leu Asn Ser Asn Ala Met Leu Ser Thr Val Thr Met
                755                 760                 765

atg agc aga att gca gct gga gac ttg gag tca tca gtg gat gat cct     3075
Met Ser Arg Ile Ala Ala Gly Asp Leu Glu Ser Ser Val Asp Asp Pro
            770                 775                 780

aga tca gag gaa gat aaa aga ttt gag agt cat ata gaa tgc agg aag     3123
Arg Ser Glu Glu Asp Lys Arg Phe Glu Ser His Ile Glu Cys Arg Lys
        785                 790                 795

cca tat aaa gaa ctg aga tta gaa gtt ggg aaa caa aga ctc aag tat     3171
Pro Tyr Lys Glu Leu Arg Leu Glu Val Gly Lys Gln Arg Leu Lys Tyr
    800                 805                 810
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gct cag gaa gaa ttg tca aat gaa gta ctt cca ccc cct agg aaa atg     3219
Ala Gln Glu Glu Leu Ser Asn Glu Val Leu Pro Pro Pro Arg Lys Met
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aag gga ctg ttt tca caa gcc aaa att tct ctt ttt tat act gag gag     3267
Lys Gly Leu Phe Ser Gln Ala Lys Ile Ser Leu Phe Tyr Thr Glu Glu
                835                 840                 845

cat gaa ata atg aag ttt tcc tgg aga ggt gtg act gct gat act aga     3315
His Glu Ile Met Lys Phe Ser Trp Arg Gly Val Thr Ala Asp Thr Arg
            850                 855                 860

gct tta agg agg ttt gga ttc tct ttg gcc gca ggc aga agt gtg tgg     3363
Ala Leu Arg Arg Phe Gly Phe Ser Leu Ala Ala Gly Arg Ser Val Trp
        865                 870                 875

act ctt gaa atg gat gct ggg gtt ctt act ggg aga ctg att aga ttg     3411
Thr Leu Glu Met Asp Ala Gly Val Leu Thr Gly Arg Leu Ile Arg Leu
    880                 885                 890

aat gat gag aaa tgg aca gaa atg aag gat gac aag att gtt tca ttg     3459
Asn Asp Glu Lys Trp Thr Glu Met Lys Asp Asp Lys Ile Val Ser Leu
895                 900                 905                 910

att gaa aag ttt aca agt aac aaa tat tgg tcc aaa gtg aat ttc cca     3507
Ile Glu Lys Phe Thr Ser Asn Lys Tyr Trp Ser Lys Val Asn Phe Pro
                915                 920                 925

cat ggg atg ttg gat ctt gaa gaa att gct gcc aat tct aag gat ttt     3555
His Gly Met Leu Asp Leu Glu Glu Ile Ala Ala Asn Ser Lys Asp Phe
            930                 935                 940

cct aac atg tct gaa acg gat ttg tgt ttc ttg ctg cat tgg tta aat     3603
Pro Asn Met Ser Glu Thr Asp Leu Cys Phe Leu Leu His Trp Leu Asn
        945                 950                 955

cca aag aaa att aat tta gca gat aga atg ctt gga ttg tct gga gtt     3651
Pro Lys Lys Ile Asn Leu Ala Asp Arg Met Leu Gly Leu Ser Gly Val
    960                 965                 970

cag gaa att aaa gaa caa ggt gtt gga tta ata gca gag tgt aga act     3699
Gln Glu Ile Lys Glu Gln Gly Val Gly Leu Ile Ala Glu Cys Arg Thr
975                 980                 985                 990

ttc tta gat tct att gct gga act tta aaa tct atg atg ttt gga ttt     3747
Phe Leu Asp Ser Ile Ala Gly Thr Leu Lys Ser Met Met Phe Gly Phe
                995                 1000                1005

cat cat tct gtg act gtt gaa att ata aac act gtg ctc tgt ttt gtt     3795
His His Ser Val Thr Val Glu Ile Ile Asn Thr Val Leu Cys Phe Val
            1010                1015                1020

aag agt gga att ttg ctt tat gta ata caa caa ttg aat cag gat gaa     3843
Lys Ser Gly Ile Leu Leu Tyr Val Ile Gln Gln Leu Asn Gln Asp Glu
        1025                1030                1035

cat tct cac ata att ggt ttg ttg aga gtc atg aat tat gta gat att     3891
His Ser His Ile Ile Gly Leu Leu Arg Val Met Asn Tyr Val Asp Ile
    1040                1045                1050

ggt tgt tca gtt att tca tgt gcc aaa gtt ttt tcc aga atg ctg gaa     3939
Gly Cys Ser Val Ile Ser Cys Ala Lys Val Phe Ser Arg Met Leu Glu
1055                1060                1065                1070

aca gtc ttt aat tgg caa atg gac tcc aga atg atg gag tta agg act     3987
Thr Val Phe Asn Trp Gln Met Asp Ser Arg Met Met Glu Leu Arg Thr
                1075                1080                1085

cag agt ttt tcc aac tgg tta aga gat att tgt tct ggg atc acc att     4035
Gln Ser Phe Ser Asn Trp Leu Arg Asp Ile Cys Ser Gly Ile Thr Ile
            1090                1095                1100

ttc aaa aac ttc aag gat gca att tat tgg ctt tat aca aaa tta atg     4083
Phe Lys Asn Phe Lys Asp Ala Ile Tyr Trp Leu Tyr Thr Lys Leu Met
        1105                1110                1115

gac ttt tat gaa gtg aat tat ggc aag aag aag gac att tta aat att     4131
Asp Phe Tyr Glu Val Asn Tyr Gly Lys Lys Lys Asp Ile Leu Asn Ile
    1120                1125                1130
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ctt aaa gat aac caa caa aaa ata gag aaa gcc att gag gaa gcc gat     4179
Leu Lys Asp Asn Gln Gln Lys Ile Glu Lys Ala Ile Glu Glu Ala Asp
1135                1140                1145                1150

aaa ttt tgc att ttg caa atc caa gat gtg gaa aaa tct gaa cag tat     4227
Lys Phe Cys Ile Leu Gln Ile Gln Asp Val Glu Lys Ser Glu Gln Tyr
                1155                1160                1165

cag aaa ggg gtt gac ttg ata caa aaa ttg aga act gtt cat tca atg     4275
Gln Lys Gly Val Asp Leu Ile Gln Lys Leu Arg Thr Val His Ser Met
            1170                1175                1180

gct cag gtt gat cca aat tta atg gtt cat ttg tca cct ttg aga gat     4323
Ala Gln Val Asp Pro Asn Leu Met Val His Leu Ser Pro Leu Arg Asp
        1185                1190                1195

tgt ata gca aga gtt cat cag aaa ctt aaa aac ctt gga tct ata aat     4371
Cys Ile Ala Arg Val His Gln Lys Leu Lys Asn Leu Gly Ser Ile Asn
    1200                1205                1210

cag gca atg gta acg aga tgt gag cca gtt gtt tgt tat ttt tat ggc     4419
Gln Ala Met Val Thr Arg Cys Glu Pro Val Val Cys Tyr Phe Tyr Gly
1215                1220                1225                1230

aaa aga ggg gga gga aag agc tta aca tca att gca ttg gca acc aaa     4467
Lys Arg Gly Gly Gly Lys Ser Leu Thr Ser Ile Ala Leu Ala Thr Lys
                1235                1240                1245

att tgt aaa cat tat ggt gtt gag cct gaa aag aat atc tat act aaa     4515
Ile Cys Lys His Tyr Gly Val Glu Pro Glu Lys Asn Ile Tyr Thr Lys
            1250                1255                1260

cct gtg gct tca gat tac tgg gat gga tat agt gga caa tta gtt tgc     4563
Pro Val Ala Ser Asp Tyr Trp Asp Gly Tyr Ser Gly Gln Leu Val Cys
        1265                1270                1275

atc att gat gat att ggc caa aac aca aca gat gag gat tgg tca gat     4611
Ile Ile Asp Asp Ile Gly Gln Asn Thr Thr Asp Glu Asp Trp Ser Asp
    1280                1285                1290

ttt tgt cag tta gtg tca gga tgt cct atg aga tta aac atg gcc tct     4659
Phe Cys Gln Leu Val Ser Gly Cys Pro Met Arg Leu Asn Met Ala Ser
1295                1300                1305                1310

ctt gag gag aag ggt agg cat ttt tct tct cct ttt ata ata gca act     4707
Leu Glu Glu Lys Gly Arg His Phe Ser Ser Pro Phe Ile Ile Ala Thr
                1315                1320                1325

tca aat tgg tca aat cca agt cca aaa aca gtt tat gtt aag gaa gca     4755
Ser Asn Trp Ser Asn Pro Ser Pro Lys Thr Val Tyr Val Lys Glu Ala
            1330                1335                1340

att gac cgc aga ctc cat ttc aag gtt gaa gtt aaa cct gct tca ttt     4803
Ile Asp Arg Arg Leu His Phe Lys Val Glu Val Lys Pro Ala Ser Phe
        1345                1350                1355

ttc aaa aat cct cac aat gat atg ttg aat gtt aat tta gct aaa aca     4851
Phe Lys Asn Pro His Asn Asp Met Leu Asn Val Asn Leu Ala Lys Thr
    1360                1365                1370

aat gat gca atc aaa gat atg tct tgt gtt gat ttg ata atg gat gga     4899
Asn Asp Ala Ile Lys Asp Met Ser Cys Val Asp Leu Ile Met Asp Gly
1375                1380                1385                1390

cat aat gtt tca ttg atg gat ttg ctc agt tct tta gtc atg aca gtt     4947
His Asn Val Ser Leu Met Asp Leu Leu Ser Ser Leu Val Met Thr Val
                1395                1400                1405

gaa att aga aaa caa aac atg act gaa ttc atg gag ttg tgg tct cag     4995
Glu Ile Arg Lys Gln Asn Met Thr Glu Phe Met Glu Leu Trp Ser Gln
            1410                1415                1420

gga att tca gat gat gat aat gat agt gca gta gct gag ttt ttc cag     5043
Gly Ile Ser Asp Asp Asp Asn Asp Ser Ala Val Ala Glu Phe Phe Gln
        1425                1430                1435

tct ttt cca tct ggt gaa cca tcg aac tct aaa tta tct ggc ttt ttc     5091
Ser Phe Pro Ser Gly Glu Pro Ser Asn Ser Lys Leu Ser Gly Phe Phe
    1440                1445                1450
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caa tct gtt act aat cac aag tgg gtt gct gtg gga gct gca gtt ggc     5139
Gln Ser Val Thr Asn His Lys Trp Val Ala Val Gly Ala Ala Val Gly
1455                1460                1465                1470

gtt ctt gga gtg ctc gtt gga gga tgg ttt gtg tat aag cat ttc tcc     5187
Val Leu Gly Val Leu Val Gly Gly Trp Phe Val Tyr Lys His Phe Ser
                1475                1480                1485

cgc aaa gag gaa gaa cca atc cca gct gaa ggg gta tat tat ggt gta     5235
Arg Lys Glu Glu Glu Pro Ile Pro Ala Glu Gly Val Tyr Tyr Gly Val
            1490                1495                1500

act aag ccc aag caa gtg att aaa tta gat gca gat cca gta gaa tct     5283
Thr Lys Pro Lys Gln Val Ile Lys Leu Asp Ala Asp Pro Val Glu Ser
        1505                1510                1515

cag tca act ttg gaa ata gca gga ctg gtt agg aag aac ttg gtt cag     5331
Gln Ser Thr Leu Glu Ile Ala Gly Leu Val Arg Lys Asn Leu Val Gln
    1520                1525                1530

ttt gga gtt gga gag aag aat gga tgt gtg aga tgg gtt atg aat gcc     5379
Phe Gly Val Gly Glu Lys Asn Gly Cys Val Arg Trp Val Met Asn Ala
1535                1540                1545                1550

ttg gga gtg aaa gat gat tgg ctg ctt gtg cct tcc cat gct tat aaa     5427
Leu Gly Val Lys Asp Asp Trp Leu Leu Val Pro Ser His Ala Tyr Lys
                1555                1560                1565

ttt gag aaa gat tat gaa atg atg gag ttt tat ttt aat aga ggt gga     5475
Phe Glu Lys Asp Tyr Glu Met Met Glu Phe Tyr Phe Asn Arg Gly Gly
            1570                1575                1580

act tac tat tca att tca gct ggt aat gtt gtt att caa tct ttg gat     5523
Thr Tyr Tyr Ser Ile Ser Ala Gly Asn Val Val Ile Gln Ser Leu Asp
        1585                1590                1595

gtg gga ttc cag gat gtt gtt ctg atg aag gtt cct aca att cct aag     5571
Val Gly Phe Gln Asp Val Val Leu Met Lys Val Pro Thr Ile Pro Lys
    1600                1605                1610

ttt aga gat att act cag cat ttt att aag aaa ggg gat gtg cct aga     5619
Phe Arg Asp Ile Thr Gln His Phe Ile Lys Lys Gly Asp Val Pro Arg
1615                1620                1625                1630

gct ttg aat cgc ctg gca aca tta gtg aca act gta aat gga acc cct     5667
Ala Leu Asn Arg Leu Ala Thr Leu Val Thr Thr Val Asn Gly Thr Pro
                1635                1640                1645

atg tta att tct gag ggc cca cta aag atg gaa gag aaa gct act tat     5715
Met Leu Ile Ser Glu Gly Pro Leu Lys Met Glu Glu Lys Ala Thr Tyr
            1650                1655                1660

gtt cat aag aaa aat gat ggt aca tca gtt gat tta act gtg gat cag     5763
Val His Lys Lys Asn Asp Gly Thr Ser Val Asp Leu Thr Val Asp Gln
        1665                1670                1675

gca tgg aga gga aaa ggc gaa ggt ctt cct gga atg tgt ggt ggg gcc     5811
Ala Trp Arg Gly Lys Gly Glu Gly Leu Pro Gly Met Cys Gly Gly Ala
    1680                1685                1690

ttg gtt tca tcg aat caa tct ata cag aat gca atc ttg ggc atc cat     5859
Leu Val Ser Ser Asn Gln Ser Ile Gln Asn Ala Ile Leu Gly Ile His
1695                1700                1705                1710

gtt gct gga gga aat tca att ctt gtt gca aaa ttg gtt act caa gaa     5907
Val Ala Gly Gly Asn Ser Ile Leu Val Ala Lys Leu Val Thr Gln Glu
                1715                1720                1725

atg ttc caa aat att gat aag aaa att gaa agt cag aga att atg aaa     5955
Met Phe Gln Asn Ile Asp Lys Lys Ile Glu Ser Gln Arg Ile Met Lys
            1730                1735                1740

gtg gag ttt act cag tgt tca atg aat gtg gtc tcc aaa acg ctt ttt     6003
Val Glu Phe Thr Gln Cys Ser Met Asn Val Val Ser Lys Thr Leu Phe
        1745                1750                1755

aga aag agt ccc att tat cat cac att gat aaa acc atg att aat ttt     6051
Arg Lys Ser Pro Ile Tyr His His Ile Asp Lys Thr Met Ile Asn Phe
    1760                1765                1770
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cct gca gct atg ccc ttt tct aaa gct gaa att gat cca atg gct gtg     6099
Pro Ala Ala Met Pro Phe Ser Lys Ala Glu Ile Asp Pro Met Ala Val
1775                1780                1785                1790

atg tta tct aag tat tca tta cct att gta gaa gaa cca gag aat tat     6147
Met Leu Ser Lys Tyr Ser Leu Pro Ile Val Glu Glu Pro Glu Asn Tyr
                1795                1800                1805

aaa gag gct tca att ttt tat caa aat aaa ata gtg ggt aag act cag     6195
Lys Glu Ala Ser Ile Phe Tyr Gln Asn Lys Ile Val Gly Lys Thr Gln
            1810                1815                1820

tta gtt gat gat ttt cta gat ctt gat atg gcc att aca ggg gcc cca     6243
Leu Val Asp Asp Phe Leu Asp Leu Asp Met Ala Ile Thr Gly Ala Pro
        1825                1830                1835

gga att gat gct atc aac atg gat tca tct cct gga ttt cct tat gtc     6291
Gly Ile Asp Ala Ile Asn Met Asp Ser Ser Pro Gly Phe Pro Tyr Val
    1840                1845                1850

cag gag aag ttg acc aaa aga gat tta att tgg ttg gat gaa aat ggt     6339
Gln Glu Lys Leu Thr Lys Arg Asp Leu Ile Trp Leu Asp Glu Asn Gly
1855                1860                1865                1870

tta ttg ctg gga gtt cat cca aga ttg gct cag aga atc tta ttc aat     6387
Leu Leu Leu Gly Val His Pro Arg Leu Ala Gln Arg Ile Leu Phe Asn
                1875                1880                1885

act gtc atg atg gaa aat tgt tct gat ttg gat gtt gtt ttt aca acc     6435
Thr Val Met Met Glu Asn Cys Ser Asp Leu Asp Val Val Phe Thr Thr
            1890                1895                1900

tgt cca aaa gat gaa ttg aga cca tta gag aaa gtg ttg gaa tca aaa     6483
Cys Pro Lys Asp Glu Leu Arg Pro Leu Glu Lys Val Leu Glu Ser Lys
        1905                1910                1915

aca aga gct att gat gct tgt cct ctg gat tac aca att ttg tgc cga     6531
Thr Arg Ala Ile Asp Ala Cys Pro Leu Asp Tyr Thr Ile Leu Cys Arg
    1920                1925                1930

atg tat tgg ggt cca gct att agt tat ttt cat ttg aat cca ggt ttc     6579
Met Tyr Trp Gly Pro Ala Ile Ser Tyr Phe His Leu Asn Pro Gly Phe
1935                1940                1945                1950

cat aca ggt gtt gct att ggc ata gat cct gat aga cag tgg gat gaa     6627
His Thr Gly Val Ala Ile Gly Ile Asp Pro Asp Arg Gln Trp Asp Glu
                1955                1960                1965

tta ttt aaa aca atg ata aga ttc gga gat gtt ggt ctt gat tta gat     6675
Leu Phe Lys Thr Met Ile Arg Phe Gly Asp Val Gly Leu Asp Leu Asp
            1970                1975                1980

ttc tct gct ttt gat gct agt ctt agt cca ttt atg att aga gaa gca     6723
Phe Ser Ala Phe Asp Ala Ser Leu Ser Pro Phe Met Ile Arg Glu Ala
        1985                1990                1995

ggt aga atc atg agt gaa cta tct gga act cca tcc cat ttt ggc aca     6771
Gly Arg Ile Met Ser Glu Leu Ser Gly Thr Pro Ser His Phe Gly Thr
    2000                2005                2010

gct ctt atc aat act atc att tat tcc aag cat ttg ctg tat aac tgt     6819
Ala Leu Ile Asn Thr Ile Ile Tyr Ser Lys His Leu Leu Tyr Asn Cys
2015                2020                2025                2030

tgt tac cat gtc tgt ggt tca atg ccc tct ggg tct cct tgt aca gct     6867
Cys Tyr His Val Cys Gly Ser Met Pro Ser Gly Ser Pro Cys Thr Ala
                2035                2040                2045

ttg cta aat tca att att aat aat gtc aat ttg tac tat gtg ttt tcc     6915
Leu Leu Asn Ser Ile Ile Asn Asn Val Asn Leu Tyr Tyr Val Phe Ser
            2050                2055                2060

aag ata ttt gga aag tct cca gtt ttc ttt tgt cag gct ttg aag att     6963
Lys Ile Phe Gly Lys Ser Pro Val Phe Phe Cys Gln Ala Leu Lys Ile
        2065                2070                2075

ctc tgt tat gga gat gat gtt tta ata gtt ttc tct cga gat gtt cag     7011
Leu Cys Tyr Gly Asp Asp Val Leu Ile Val Phe Ser Arg Asp Val Gln
    2080                2085                2090
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att gat aat ctt gat ttg att gga caa aaa att gta gat gag ttt aag     7059
Ile Asp Asn Leu Asp Leu Ile Gly Gln Lys Ile Val Asp Glu Phe Lys
2095                2100                2105                2110

aaa ctt ggc atg aca gct act tct gct gac aag aat gta cct cag ctg     7107
Lys Leu Gly Met Thr Ala Thr Ser Ala Asp Lys Asn Val Pro Gln Leu
                2115                2120                2125

aaa cca gtt tcg gaa ttg act ttt ctc aaa aga tct ttc aat ttg gta     7155
Lys Pro Val Ser Glu Leu Thr Phe Leu Lys Arg Ser Phe Asn Leu Val
            2130                2135                2140

gag gat aga att aga cct gca att tcg gaa aaa aca att tgg tct tta     7203
Glu Asp Arg Ile Arg Pro Ala Ile Ser Glu Lys Thr Ile Trp Ser Leu
        2145                2150                2155

ata gca tgg cag aga agt aac gct gag ttt gag cag aat tta gaa att     7251
Ile Ala Trp Gln Arg Ser Asn Ala Glu Phe Glu Gln Asn Leu Glu Ile
    2160                2165                2170

gct cag tgg ttt gct ttt atg cat ggc tat gag ttt tat cag aaa ttt     7299
Ala Gln Trp Phe Ala Phe Met His Gly Tyr Glu Phe Tyr Gln Lys Phe
2175                2180                2185                2190

tat tat ttt gtt cag tcc tgt ttg gag aaa gag atg ata gaa tac aga     7347
Tyr Tyr Phe Val Gln Ser Cys Leu Glu Lys Glu Met Ile Glu Tyr Arg
                2195                2200                2205

ctt aaa tct tat gat tgg tgg aga atg aga ttt tat gac cag tgt ttc     7395
Leu Lys Ser Tyr Asp Trp Trp Arg Met Arg Phe Tyr Asp Gln Cys Phe
            2210                2215                2220

att tgt gac ctt tca tgatttgttt aaacgaattt tcttaaaatt tctgaggttt     7450
Ile Cys Asp Leu Ser
        2225

gtttatttct tttatcagta aataaaaaaa aaaaaa                             7486


<210> SEQ ID NO 4
<211> LENGTH: 2227
<212> TYPE: PRT
<213> ORGANISM: Attenuated (4380) HAV, strain HM-175

<400> SEQUENCE: 4

Met Asn Met Ser Arg Gln Gly Ile Phe Gln Thr Val Gly Ser Gly Leu
  1               5                  10                  15

Asp His Ile Leu Ser Leu Ala Asp Ile Glu Glu Glu Gln Met Ile Gln
             20                  25                  30

Ser Val Asp Arg Thr Ala Val Thr Gly Ala Ser Tyr Phe Thr Ser Val
         35                  40                  45

Asp Gln Ser Ser Val His Thr Ala Glu Val Gly Ser His Gln Val Glu
     50                  55                  60

Pro Leu Arg Thr Ser Val Asp Lys Pro Gly Ser Lys Arg Thr Gln Gly
 65                  70                  75                  80

Glu Lys Phe Phe Leu Ile His Ser Ala Asp Trp Leu Thr Thr His Ala
                 85                  90                  95

Leu Phe His Glu Val Ala Lys Leu Asp Val Val Lys Leu Leu Tyr Asn
            100                 105                 110

Glu Gln Phe Ala Val Gln Gly Leu Leu Arg Tyr His Thr Tyr Ala Arg
        115                 120                 125

Phe Gly Ile Glu Ile Gln Val Gln Ile Asn Pro Thr Pro Phe Gln Gln
    130                 135                 140

Gly Gly Leu Ile Cys Ala Met Val Pro Gly Asp Gln Ser Tyr Gly Ser
145                 150                 155                 160

Ile Ala Ser Leu Thr Val Tyr Pro His Gly Leu Leu Asn Cys Asn Ile
                165                 170                 175
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Asn Asn Val Val Arg Ile Lys Val Pro Phe Ile Tyr Thr Arg Gly Ala
            180                 185                 190

Tyr His Phe Lys Asp Pro Gln Tyr Pro Val Trp Glu Leu Thr Ile Arg
        195                 200                 205

Val Trp Ser Glu Leu Asn Ile Gly Thr Gly Thr Ser Ala Tyr Thr Ser
    210                 215                 220

Leu Asn Val Leu Ala Arg Phe Thr Asp Leu Glu Leu His Gly Leu Thr
225                 230                 235                 240

Pro Leu Ser Thr Gln Met Met Arg Asn Glu Phe Arg Val Ser Thr Thr
                245                 250                 255

Glu Asn Val Val Asn Leu Ser Asn Tyr Glu Asp Ala Arg Ala Lys Met
            260                 265                 270

Ser Phe Ala Leu Asp Gln Glu Asp Trp Lys Ser Asp Pro Ser Gln Gly
        275                 280                 285

Gly Gly Ile Lys Ile Thr His Phe Thr Thr Trp Thr Ser Ile Pro Thr
    290                 295                 300

Leu Ala Ala Gln Phe Pro Phe Asn Ala Ser Asp Ser Val Gly Gln Gln
305                 310                 315                 320

Ile Lys Val Ile Pro Val Asp Pro Tyr Phe Phe Gln Met Thr Asn Thr
                325                 330                 335

Asn Pro Asp Gln Lys Cys Ile Thr Ala Leu Ala Ser Ile Cys Gln Met
            340                 345                 350

Phe Cys Phe Trp Arg Gly Asp Leu Val Phe Asp Phe Gln Val Phe Pro
        355                 360                 365

Thr Lys Tyr His Ser Gly Arg Leu Leu Phe Cys Phe Val Pro Gly Asn
    370                 375                 380

Glu Leu Ile Asp Val Ser Gly Ile Thr Leu Lys Gln Ala Thr Thr Ala
385                 390                 395                 400

Pro Cys Ala Val Met Asp Ile Thr Gly Val Gln Ser Thr Leu Arg Phe
                405                 410                 415

Arg Val Pro Trp Ile Ser Asp Thr Pro Tyr Arg Val Asn Arg Tyr Thr
            420                 425                 430

Lys Ser Ala His Gln Lys Gly Glu Tyr Thr Ala Ile Gly Lys Leu Ile
        435                 440                 445

Val Tyr Cys Tyr Asn Arg Leu Thr Ser Pro Ser Asn Val Ala Ser His
    450                 455                 460

Val Arg Val Asn Val Tyr Leu Ser Ala Ile Asn Leu Glu Cys Phe Ala
465                 470                 475                 480

Pro Leu Tyr His Ala Met Asp Val Thr Thr Gln Val Gly Asp Asp Ser
                485                 490                 495

Gly Gly Phe Ser Thr Thr Val Ser Thr Glu Gln Asn Val Pro Asp Pro
            500                 505                 510

Gln Val Gly Ile Thr Thr Met Lys Asp Leu Lys Gly Lys Ala Asn Arg
        515                 520                 525

Gly Lys Met Asp Val Ser Gly Val Gln Ala Pro Val Gly Ala Ile Thr
    530                 535                 540

Thr Ile Glu Asp Pro Val Leu Ala Lys Lys Val Pro Glu Thr Phe Pro
545                 550                 555                 560

Glu Leu Lys Pro Gly Glu Ser Arg His Thr Ser Asp His Met Ser Ile
                565                 570                 575

Tyr Lys Phe Met Gly Arg Ser His Phe Leu Cys Thr Phe Thr Phe Asn
            580                 585                 590
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Ser Asn Asn Lys Glu Tyr Thr Phe Pro Ile Thr Leu Ser Ser Thr Ser
        595                 600                 605

Asn Pro Pro His Gly Leu Pro Ser Thr Leu Arg Trp Phe Phe Asn Leu
    610                 615                 620

Phe Gln Leu Tyr Arg Gly Pro Leu Asp Leu Thr Ile Ile Ile Thr Gly
625                 630                 635                 640

Ala Thr Asp Val Asp Gly Met Ala Trp Phe Thr Pro Val Gly Leu Ala
                645                 650                 655

Val Asp Thr Pro Trp Val Glu Lys Glu Ser Ala Leu Ser Ile Asp Tyr
            660                 665                 670

Lys Thr Ala Leu Gly Ala Val Arg Phe Asn Thr Arg Arg Thr Gly Asn
        675                 680                 685

Ile Gln Ile Arg Leu Pro Trp Tyr Ser Tyr Leu Tyr Ala Val Ser Gly
    690                 695                 700

Ala Leu Asp Gly Leu Gly Asp Lys Thr Asp Ser Thr Phe Gly Leu Val
705                 710                 715                 720

Ser Ile Gln Ile Ala Asn Tyr Asn His Ser Asp Glu Tyr Leu Ser Phe
                725                 730                 735

Ser Cys Tyr Leu Ser Val Thr Glu Gln Ser Glu Phe Tyr Phe Pro Arg
            740                 745                 750

Ala Pro Leu Asn Ser Asn Ala Met Leu Ser Thr Val Thr Met Met Ser
        755                 760                 765

Arg Ile Ala Ala Gly Asp Leu Glu Ser Ser Val Asp Asp Pro Arg Ser
    770                 775                 780

Glu Glu Asp Lys Arg Phe Glu Ser His Ile Glu Cys Arg Lys Pro Tyr
785                 790                 795                 800

Lys Glu Leu Arg Leu Glu Val Gly Lys Gln Arg Leu Lys Tyr Ala Gln
                805                 810                 815

Glu Glu Leu Ser Asn Glu Val Leu Pro Pro Pro Arg Lys Met Lys Gly
            820                 825                 830

Leu Phe Ser Gln Ala Lys Ile Ser Leu Phe Tyr Thr Glu Glu His Glu
        835                 840                 845

Ile Met Lys Phe Ser Trp Arg Gly Val Thr Ala Asp Thr Arg Ala Leu
    850                 855                 860

Arg Arg Phe Gly Phe Ser Leu Ala Ala Gly Arg Ser Val Trp Thr Leu
865                 870                 875                 880

Glu Met Asp Ala Gly Val Leu Thr Gly Arg Leu Ile Arg Leu Asn Asp
                885                 890                 895

Glu Lys Trp Thr Glu Met Lys Asp Asp Lys Ile Val Ser Leu Ile Glu
            900                 905                 910

Lys Phe Thr Ser Asn Lys Tyr Trp Ser Lys Val Asn Phe Pro His Gly
        915                 920                 925

Met Leu Asp Leu Glu Glu Ile Ala Ala Asn Ser Lys Asp Phe Pro Asn
    930                 935                 940

Met Ser Glu Thr Asp Leu Cys Phe Leu Leu His Trp Leu Asn Pro Lys
945                 950                 955                 960

Lys Ile Asn Leu Ala Asp Arg Met Leu Gly Leu Ser Gly Val Gln Glu
                965                 970                 975

Ile Lys Glu Gln Gly Val Gly Leu Ile Ala Glu Cys Arg Thr Phe Leu
            980                 985                 990

Asp Ser Ile Ala Gly Thr Leu Lys Ser Met Met Phe Gly Phe His His
        995                 1000                1005
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Ser Val Thr Val Glu Ile Ile Asn Thr Val Leu Cys Phe Val Lys Ser
    1010                1015                1020

Gly Ile Leu Leu Tyr Val Ile Gln Gln Leu Asn Gln Asp Glu His Ser
1025                1030                1035                1040

His Ile Ile Gly Leu Leu Arg Val Met Asn Tyr Val Asp Ile Gly Cys
                1045                1050                1055

Ser Val Ile Ser Cys Ala Lys Val Phe Ser Arg Met Leu Glu Thr Val
            1060                1065                1070

Phe Asn Trp Gln Met Asp Ser Arg Met Met Glu Leu Arg Thr Gln Ser
        1075                1080                1085

Phe Ser Asn Trp Leu Arg Asp Ile Cys Ser Gly Ile Thr Ile Phe Lys
    1090                1095                1100

Asn Phe Lys Asp Ala Ile Tyr Trp Leu Tyr Thr Lys Leu Met Asp Phe
1105                1110                1115                1120

Tyr Glu Val Asn Tyr Gly Lys Lys Lys Asp Ile Leu Asn Ile Leu Lys
                1125                1130                1135

Asp Asn Gln Gln Lys Ile Glu Lys Ala Ile Glu Glu Ala Asp Lys Phe
            1140                1145                1150

Cys Ile Leu Gln Ile Gln Asp Val Glu Lys Ser Glu Gln Tyr Gln Lys
        1155                1160                1165

Gly Val Asp Leu Ile Gln Lys Leu Arg Thr Val His Ser Met Ala Gln
    1170                1175                1180

Val Asp Pro Asn Leu Met Val His Leu Ser Pro Leu Arg Asp Cys Ile
1185                1190                1195                1200

Ala Arg Val His Gln Lys Leu Lys Asn Leu Gly Ser Ile Asn Gln Ala
                1205                1210                1215

Met Val Thr Arg Cys Glu Pro Val Val Cys Tyr Phe Tyr Gly Lys Arg
            1220                1225                1230

Gly Gly Gly Lys Ser Leu Thr Ser Ile Ala Leu Ala Thr Lys Ile Cys
        1235                1240                1245

Lys His Tyr Gly Val Glu Pro Glu Lys Asn Ile Tyr Thr Lys Pro Val
    1250                1255                1260

Ala Ser Asp Tyr Trp Asp Gly Tyr Ser Gly Gln Leu Val Cys Ile Ile
1265                1270                1275                1280

Asp Asp Ile Gly Gln Asn Thr Thr Asp Glu Asp Trp Ser Asp Phe Cys
                1285                1290                1295

Gln Leu Val Ser Gly Cys Pro Met Arg Leu Asn Met Ala Ser Leu Glu
            1300                1305                1310

Glu Lys Gly Arg His Phe Ser Ser Pro Phe Ile Ile Ala Thr Ser Asn
        1315                1320                1325

Trp Ser Asn Pro Ser Pro Lys Thr Val Tyr Val Lys Glu Ala Ile Asp
    1330                1335                1340

Arg Arg Leu His Phe Lys Val Glu Val Lys Pro Ala Ser Phe Phe Lys
1345                1350                1355                1360

Asn Pro His Asn Asp Met Leu Asn Val Asn Leu Ala Lys Thr Asn Asp
                1365                1370                1375

Ala Ile Lys Asp Met Ser Cys Val Asp Leu Ile Met Asp Gly His Asn
            1380                1385                1390

Val Ser Leu Met Asp Leu Leu Ser Ser Leu Val Met Thr Val Glu Ile
        1395                1400                1405

Arg Lys Gln Asn Met Thr Glu Phe Met Glu Leu Trp Ser Gln Gly Ile
    1410                1415                1420
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Ser Asp Asp Asp Asn Asp Ser Ala Val Ala Glu Phe Phe Gln Ser Phe
1425                1430                1435                1440

Pro Ser Gly Glu Pro Ser Asn Ser Lys Leu Ser Gly Phe Phe Gln Ser
                1445                1450                1455

Val Thr Asn His Lys Trp Val Ala Val Gly Ala Ala Val Gly Val Leu
            1460                1465                1470

Gly Val Leu Val Gly Gly Trp Phe Val Tyr Lys His Phe Ser Arg Lys
        1475                1480                1485

Glu Glu Glu Pro Ile Pro Ala Glu Gly Val Tyr Tyr Gly Val Thr Lys
    1490                1495                1500

Pro Lys Gln Val Ile Lys Leu Asp Ala Asp Pro Val Glu Ser Gln Ser
1505                1510                1515                1520

Thr Leu Glu Ile Ala Gly Leu Val Arg Lys Asn Leu Val Gln Phe Gly
                1525                1530                1535

Val Gly Glu Lys Asn Gly Cys Val Arg Trp Val Met Asn Ala Leu Gly
            1540                1545                1550

Val Lys Asp Asp Trp Leu Leu Val Pro Ser His Ala Tyr Lys Phe Glu
        1555                1560                1565

Lys Asp Tyr Glu Met Met Glu Phe Tyr Phe Asn Arg Gly Gly Thr Tyr
    1570                1575                1580

Tyr Ser Ile Ser Ala Gly Asn Val Val Ile Gln Ser Leu Asp Val Gly
1585                1590                1595                1600

Phe Gln Asp Val Val Leu Met Lys Val Pro Thr Ile Pro Lys Phe Arg
                1605                1610                1615

Asp Ile Thr Gln His Phe Ile Lys Lys Gly Asp Val Pro Arg Ala Leu
            1620                1625                1630

Asn Arg Leu Ala Thr Leu Val Thr Thr Val Asn Gly Thr Pro Met Leu
        1635                1640                1645

Ile Ser Glu Gly Pro Leu Lys Met Glu Glu Lys Ala Thr Tyr Val His
    1650                1655                1660

Lys Lys Asn Asp Gly Thr Ser Val Asp Leu Thr Val Asp Gln Ala Trp
1665                1670                1675                1680

Arg Gly Lys Gly Glu Gly Leu Pro Gly Met Cys Gly Gly Ala Leu Val
                1685                1690                1695

Ser Ser Asn Gln Ser Ile Gln Asn Ala Ile Leu Gly Ile His Val Ala
            1700                1705                1710

Gly Gly Asn Ser Ile Leu Val Ala Lys Leu Val Thr Gln Glu Met Phe
        1715                1720                1725

Gln Asn Ile Asp Lys Lys Ile Glu Ser Gln Arg Ile Met Lys Val Glu
    1730                1735                1740

Phe Thr Gln Cys Ser Met Asn Val Val Ser Lys Thr Leu Phe Arg Lys
1745                1750                1755                1760

Ser Pro Ile Tyr His His Ile Asp Lys Thr Met Ile Asn Phe Pro Ala
                1765                1770                1775

Ala Met Pro Phe Ser Lys Ala Glu Ile Asp Pro Met Ala Val Met Leu
            1780                1785                1790

Ser Lys Tyr Ser Leu Pro Ile Val Glu Glu Pro Glu Asn Tyr Lys Glu
        1795                1800                1805

Ala Ser Ile Phe Tyr Gln Asn Lys Ile Val Gly Lys Thr Gln Leu Val
    1810                1815                1820

Asp Asp Phe Leu Asp Leu Asp Met Ala Ile Thr Gly Ala Pro Gly Ile
1825                1830                1835                1840
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Asp Ala Ile Asn Met Asp Ser Ser Pro Gly Phe Pro Tyr Val Gln Glu
                1845                1850                1855

Lys Leu Thr Lys Arg Asp Leu Ile Trp Leu Asp Glu Asn Gly Leu Leu
            1860                1865                1870

Leu Gly Val His Pro Arg Leu Ala Gln Arg Ile Leu Phe Asn Thr Val
        1875                1880                1885

Met Met Glu Asn Cys Ser Asp Leu Asp Val Val Phe Thr Thr Cys Pro
    1890                1895                1900

Lys Asp Glu Leu Arg Pro Leu Glu Lys Val Leu Glu Ser Lys Thr Arg
1905                1910                1915                1920

Ala Ile Asp Ala Cys Pro Leu Asp Tyr Thr Ile Leu Cys Arg Met Tyr
                1925                1930                1935

Trp Gly Pro Ala Ile Ser Tyr Phe His Leu Asn Pro Gly Phe His Thr
            1940                1945                1950

Gly Val Ala Ile Gly Ile Asp Pro Asp Arg Gln Trp Asp Glu Leu Phe
        1955                1960                1965

Lys Thr Met Ile Arg Phe Gly Asp Val Gly Leu Asp Leu Asp Phe Ser
    1970                1975                1980

Ala Phe Asp Ala Ser Leu Ser Pro Phe Met Ile Arg Glu Ala Gly Arg
1985                1990                1995                2000

Ile Met Ser Glu Leu Ser Gly Thr Pro Ser His Phe Gly Thr Ala Leu
                2005                2010                2015

Ile Asn Thr Ile Ile Tyr Ser Lys His Leu Leu Tyr Asn Cys Cys Tyr
            2020                2025                2030

His Val Cys Gly Ser Met Pro Ser Gly Ser Pro Cys Thr Ala Leu Leu
        2035                2040                2045

Asn Ser Ile Ile Asn Asn Val Asn Leu Tyr Tyr Val Phe Ser Lys Ile
    2050                2055                2060

Phe Gly Lys Ser Pro Val Phe Phe Cys Gln Ala Leu Lys Ile Leu Cys
2065                2070                2075                2080

Tyr Gly Asp Asp Val Leu Ile Val Phe Ser Arg Asp Val Gln Ile Asp
                2085                2090                2095

Asn Leu Asp Leu Ile Gly Gln Lys Ile Val Asp Glu Phe Lys Lys Leu
            2100                2105                2110

Gly Met Thr Ala Thr Ser Ala Asp Lys Asn Val Pro Gln Leu Lys Pro
        2115                2120                2125

Val Ser Glu Leu Thr Phe Leu Lys Arg Ser Phe Asn Leu Val Glu Asp
    2130                2135                2140

Arg Ile Arg Pro Ala Ile Ser Glu Lys Thr Ile Trp Ser Leu Ile Ala
2145                2150                2155                2160

Trp Gln Arg Ser Asn Ala Glu Phe Glu Gln Asn Leu Glu Ile Ala Gln
                2165                2170                2175

Trp Phe Ala Phe Met His Gly Tyr Glu Phe Tyr Gln Lys Phe Tyr Tyr
            2180                2185                2190

Phe Val Gln Ser Cys Leu Glu Lys Glu Met Ile Glu Tyr Arg Leu Lys
        2195                2200                2205

Ser Tyr Asp Trp Trp Arg Met Arg Phe Tyr Asp Gln Cys Phe Ile Cys
    2210                2215                2220

Asp Leu Ser
2225
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What is claimed is:

1. A hepatitis A virus HAV 4380 having Institute Pasteur Accession No. I-936, where said virus is characterized by the ability to grow in MRC-5 cells.

2. A vaccine composition useful for protecting humans or primates against hepatitis A, said vaccine comprising the hepatitis A virus of claim 1.

3. A method for protecting a human or primate against hepatitis A, said method comprising administering the vaccine of claim 1 to said human or primate in an amount effective to protect said human or primate against hepatitis A.

4. A hepatitis A virus encoded by a nucleic acid molecule having the nucleic acid sequence according to SEQ ID NO:3.

5. A hepatitis A vaccine comprising the virus of claim 4.

6. A method for protecting a human or primate against hepatitis A, said method comprising administering the vaccine of claim 5 to said human or primate in an amount effective to protect said human or primate against hepatitis A.

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UNITED STATES PATENT AND TRADEMARK OFFICE

# CERTIFICATE OF CORRECTION

PATENT NO. : 6,180,110 B1 
DATED : January 30, 2001
INVENTOR(S) : Ann W. Funkhouser, Suzanne U. Emerson, Robert H. Purcell and Eric O. Horbt

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 77,
Line 2, delete "Institute" and insert -- Institut --.

Signed and Sealed this

Thirtieth Day of April, 2002

*Attest:*

*Attesting Officer*

JAMES E. ROGAN
*Director of the United States Patent and Trademark Office*