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HEPATITIS A VIRUS VACCINES

5 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

10 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
15 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

20 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,
25 staff and adult contacts of children in day-care centers, male homosexuals, drug addicts, and travelers to endemic areas.

30 In developing countries, virtually the entire population is infected with hepatitis A virus at an early age. Much of this infection results in subclinical and inapparent infection, but, as countries improve their hygienic conditions, infection with hepatitis A virus

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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 vaccinee 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. Patent 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. Patent No. 4,620,978 describes a vaccine employing the

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HAV HM-175, triply cloned in AGMK cell culture and attenuated. U. S. Patent 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 disclosures 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 $10^{7.3}$ TCID₅₀ 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.

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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.

15 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.

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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.

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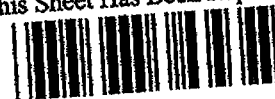


Fig. 2 is a graph plotting anti-HAV antibody production vs. time (weeks) vs. ALT and ICD levels for the chimpanzee studies with Virus 4380 and subsequent challenge with wild-type HAV, as described in Example 1. The conditions were the same as for Fig. 1.

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

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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.

5 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
10 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
15 above-cited and incorporated art [Cohen et al., J. Virol., 61:50-59 (1987)]. 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
20 cloned three times in AGMK cells by serial dilution, then passaged 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).

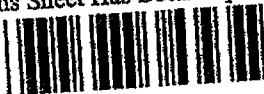
 The P-32 virus described above was passaged
25 three more times in AGMK, and molecularly cloned. The virus that was cloned was called P-35 and the full-length clone was referred to as pHAV/7. pHAV/7 is an infectious cDNA clone of the virus that can be maintained in a monoclonal state and amplified at will with diminished
30 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).

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U. S. Patent 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) and the nucleotide differences between HAV HM-175, Pass 35, clone pHAV/7 and the wild-type sequence. 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 from U. S. Patent 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. 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, Maryland under Accession No. 67495, deposited August 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 25-4-21. 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 April 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.

5 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

10 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

15 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

20 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

25 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

30 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

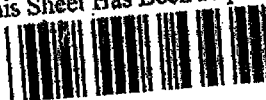
35 found to induce antibody production without adjuvant.



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. Patent 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 fourteen unique nucleotide differences between the pass-35 HM-175/7 virus and the MRC-5-adapted virus 4380, at least twelve of which mutations apparently occurred during the period of adaptation of the Pass 32 hepatitis A virus (HAV), strain HM-175 to growth in MRC-5 cells. Table I lists these twelve mutations by nucleotide differences and resulting amino acid (AA) differences, if any, acquired by the MRC-5-adapted virus HAV 4380. The other two mutations appear to be back-mutations to wild-type sequence, or more likely, to represent mutations that occurred between passages 32 and 35 in AGMK cells, and that therefore represent differences between the biologically characterized P-32 virus and the molecularly cloned P-35 virus. Note that the partial sequence of LSH/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 U represents uridine or thymine; Leu represents leucine, Phe represents phenylalanine, Ile represents isoleucine, Val represents valine and Ser represents serine.

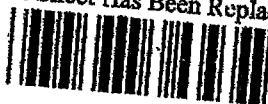


TABLE I

Nucleotide Changes Occurring During
Adaptation of HM-175 in MRC-5 Cells:
Comparison with sequence of HM-175/7 (P-35)

	<u>Nucleotide Change</u>	<u>Region of Genome</u>	<u>AA Change</u>
	591 A to G	5' nc	NA
	646 G to A	5' nc	NA
10	669 C to U	5' nc	NA
	687 U to G	5' nc	NA
	2864 U to A	VP1	No change
	3934 A to G	2B	No change
	4418 A to U	2C	Leu to Phe
15	4643 A to U	2C	No Change
	5145 A to G	3A	Ile to Val
	5745 A to U	3C	Thr to Ser
	6216 U to C	3D	No change
20	6908 U to C	3D	No change

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, 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.

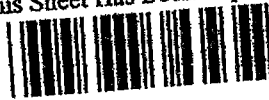
The mutagenic and genetic engineering techniques employed to construct chimeric or recombinant HAVs which incorporate one or more of these mutations are conventional and known to those of skill in the art [see, for example, Sambrook et al., Molecular Cloning. A Laboratory Manual, 2d edition, Cold Spring Harbor



Laboratory, Cold Spring Harbor, New York, (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.

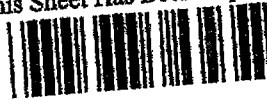


It is further anticipated that additional mutations may appear in a few regions of HAV that have yet to be sequenced. The mutations appearing in Table I may be incorporated in any combination, and/or with other mutations yet to be identified to construct a number of chimeric or recombinant HAVs with 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. Patent 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
5 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
10 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
15 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.

20 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
25 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
30 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 components 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-poly lactide/glycoside, pluronic polyois, 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
5 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.

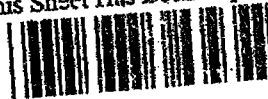
Similarly, suitable doses of the vaccine
10 composition of the invention can be readily determined by one of skill in the art. The dosage can be adjusted depending upon the human patient or the animal species being treated, i.e. its weight, age, and general health.

The following examples illustrate the preferred
15 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 HAV 4380 Vaccine in Marmosets and Chimpanzees

20 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
25 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
30 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 Saguinus 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 TCID₅₀ 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/1ml/I.V. Marmosets No. 541 and 578 received a diluent at a dose and route of 1ml/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, Abbott Laboratories, Chicago, IL). 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, Chimp 1309 and Chimp 1313, developed IgM anti-HAV on weeks ten and thirteen, respectively, when tested by the standard HAVAB-M (Abbott Laboratories, Chicago, IL) 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 utilizing 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 enzymes 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.

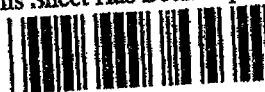


Table II
HISTOPATHOLOGY: CHIMPANZEES

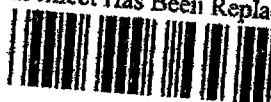
5	<u>Inoculum*</u>	<u># Inoc.</u>	<u># Infected</u>	<u>Liver Histopathology</u>	
				<u>#</u>	<u>Range of Severity**</u>
	W.T.	4	4	4	± - 3+
	P-21	6	6	1	0 - 3+
	P-32	6	6	0	0
10	MRC-5	4	3	0	0

* Dose: 10^3 - 10^5 ID₅₀ IV
 ** Scale of 0-3+

Table III
HISTOPATHOLOGY: MARMOSETS:

15	<u>Inoculum*</u>	<u># Inoc.</u>	<u># Infected</u>	<u>Liver Histopathology</u>	
				<u>#</u>	<u>Range of Severity**</u>
20	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

25 * Dose: 10^3 - 10^5 ID₅₀ 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.

5 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.
10 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
15 wild-type virus.

Table IV

VIRAL REPLICATION IN THE LIVER:
MARMOSETS (IMMUNOFLUORESCENCE)

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

* Dose: 10^3 - 10^5 ID₅₀ I.V.



Table V

VIRAL REPLICATION IN THE LIVER:
CHIMPANZEES (IMMUNOFLUORESCENCE)

5	Inoculum*	# Inoc.	# Infected	Mean	Mean
				Peak	Duration (wks)
	W.T.	4	4	1+	1.8
	P-21	6	6	<1+	0.5
	P-32	6	6	<1+	0.6
10	MRC-5	4	3	<1+	0.3
Dose: 10^3 - 10^5 ID ₅₀ I.V.					

15 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 animal were challenged with approximately 10^3 chimpanzee infectious doses of wild-type HM-175 strain HAV (human stool suspension), administered intravenously (Fig. 1 through 4).

20 All three previously infected chimpanzees were protected against type A hepatitis, as measured by persistently normal serum enzyme values (Figs. 1 through 25 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.

5 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
10 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.

15 In this clinical trial, volunteers received increasing titers of the live attenuated hepatitis A vaccine 4380, volunteer pool 87J19, which was previously tested in chimpanzees and marmosets as described in
20 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, Fort Detrick, Maryland. Eight
25 volunteers received the live attenuated hepatitis A vaccine (1ml) by the oral route in the following manner: two received a 10^4 TCID₅₀ dose, two a 10^5 TCID₅₀ dose, two a 10^6 TCID₅₀ dose, and two a 10^7 TCID₅₀ dose. Six volunteers received the vaccine by the intramuscular route in the
30 deltoid area in the following manner: 2 received a 10^5 TCID₅₀ dose, 2 a 10^6 TCID₅₀ dose and 2 a 10^7 TCID₅₀ 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, NY); 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, IL). 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 $\geq 1:10$ 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, IL).

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 TCID₅₀ dose by the intramuscular route. The four volunteers who received higher doses of vaccine (10^6 TCID₅₀ or 10^7 TCID₅₀) 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 TCID₅₀ 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 TCID₅₀ 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 TCID₅₀. 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
5 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
10 anti-HAV in volunteers who received 10^7 TCID₅₀ without evidence of hepatitis is suggestive of asymptomatic replication of the virus.

Example 3: Construction of Chimeric Viruses

Several exemplary chimeric viruses were
15 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
20 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
25 "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
30 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.

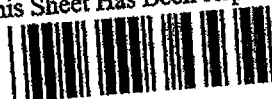


TABLE VI

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

5	Nucleotide Differences from P-35 HM-175	Method Mutation Introduced	Growth of Mutated Virus in Cell Cultures	
			<u>FRhK-4</u>	<u>MRC-5</u>
10	Virus #1 (MRC-5-adapted)	Cell Culture	+	+
	591 A to G			
	646 G to A			
	669 C to T			
15	687 T to G			
	2864 T to A			
	3196 G to A			
	3934 A to G			
	4418 A to T			
20	4643 A to T			
	5145 A to G			
	5745 A to T			
	6216 T to C			
	6908 T to C			
25	7032 C to T			
	Virus #2	Chimera	+	+
	591 A to G			
	646 G to A			
	669 C to T			
30	687 T to G			
	Virus #3	Chimera	+	+
	124 C to T			
	131 d to T			
	132 d to T			
35	133 d to T			
	134 d to G			
	152 G to A			
	203-207 d to T			
	591 A to G			
40	646 G to A			
	669 C to T			
	687 T to G			
45	d = Base at this position deleted in P-35 compared to wild-type			



TABLE VI (cont)

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 Culture	
			<u>FRhK-4</u>	<u>MRC-5</u>
5	Virus #4	Chimera	+	+
10	591 A to G 646 G to A 669 C to T 687 T to G 4418 A to T			
15	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			
20	591 A to G 646 G to A 669 C to T 687 T to G 4418 A to T 4643 A to T			
25				
30	Virus #6	Chimera	+	-
	4418 A to T 4643 A to T			
	Virus #7	Chimera Mutagenesis	+	ND
35	591 A to G 4418 A to T 4643 A to T			

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

TABLE VI (cont)

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 Culture	
			<u>FRhK-4</u>	<u>MRC-5</u>
5				
10	Virus #8 646 G to A 4418 A to T 4643 A to T	Chimera Mutagenesis	+	ND
15	Virus #9 669 C to T 4418 A to T 4643 A to T	Chimera Mutagenesis	+	ND
20	Virus #10 687 T to G 4418 A to T 4643 A to T	Chimera Mutagenesis	+	ND



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 two mutations, at nucleotides 4418 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 mutations in the 5' noncoding region and the two mutations in the 2C region of the genome of the MRC-5 cell-adapted virus appear to act synergistically in the adaptation of this virus to efficient growth in MRC-5 cells. Introduction of only the two mutations in the 2C 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 [32p]-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.



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International Application No: PCT/

MICROORGANISMSOptional Sheet in connection with the microorganism referred to on page 8, line 17-20 of the description *

A. IDENTIFICATION OF DEPOSIT : Hepatitis A Virus HAV/HM-175/7 cDNA
 Further deposits are identified on an additional sheet ☒ *

Name of depositary institution *

American Type Culture Collection

Address of depositary institution (including postal code and country) *

12301 Parklawn Drive
 Rockville, Maryland 20853 USA

Date of deposit *

07 August 1987

Accession Number *

67495

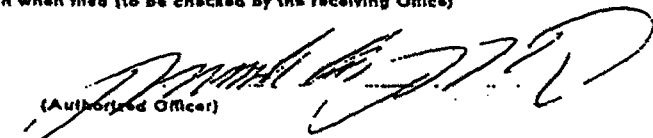
B. ADDITIONAL INDICATIONS * (leave blank if not applicable). This information is continued on a separate attached sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only to an independent expert, in accordance with the relevant patent legislation e.g EPC Rule 28(4), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (if the indications are not for all designated States)**D. SEPARATE FURNISHING OF INDICATIONS** * (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")

☒ This sheet was received with the international application when filed (to be checked by the receiving Office)

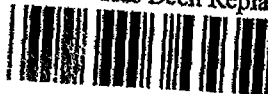

 (Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau is:

was

(Authorized Officer)

This Sheet Has Been Replaced



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PCT/US93/08610

36

International Application No: PCT/

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 9, line 1-4 of the description

A. IDENTIFICATION OF DEPOSIT: live attenuated Hepatitis A virus
Further deposits are identified on an additional sheet ☒ HAV 4380

Name of depositary institution *

Collection Nationale de Cultures de Microorganismes

Address of depositary institution (including postal code and country) *

Institut Pasteur, 25
rue du Docteur Roux
75724, Paris, France CEDEX 15

Date of deposit *

04 April 1990

Accession Number *

I-936

B. ADDITIONAL INDICATIONS * (leave blank if not applicable). This information is continued on a separate attached sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only to an independent expert, in accordance with the relevant patent legislation e.g. EPC Rule 28(4), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (If the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS * (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")

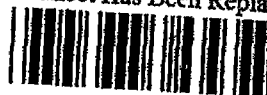
E. ☒ This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau is

was

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WHAT IS CLAIMED IS:

1. A live hepatitis A virus adapted to growth in MRC-5 cells.
2. The virus according to claim 1 which is attenuated.
3. The virus according to claim 1 which is attenuated for primates without inactivation.
4. The virus according to claim 1 which is HAV 4380.
5. The virus according to claim 1 which is characterized by a nucleotide sequence which contains a nucleotide in a nucleotide position homologous to that of the genomic nucleotide sequence of HAV strain HM-175, Pass 35, selected from the group consisting of
 - (a) at nucleotide position 591 in the 5' noncoding region of the virus, a G;
 - (b) at nucleotide position 646 in the 5' noncoding region of the virus, an A;
 - (c) at nucleotide position 669 in the 5' noncoding region of the virus, a U or T;
 - (d) at nucleotide position 687 in the 5' noncoding region of the virus, a G;
 - (e) at nucleotide position 2864 in the VP1 region of the virus, an A;
 - (f) at nucleotide position 3934 in the 2B region of the virus, a G;





(g) at nucleotide position 4418 in the 2C region of the virus, a U or T, resulting in the codon at that position encoding Phe;

(h) at nucleotide position 4643 in the 2C region of the virus, a U or T;

(i) at nucleotide position 5145 in the 3A region of the virus, a G, resulting in the codon at that position encoding Val;

(j) at nucleotide position 5745 in the 3C region of the virus, a U or T, resulting in the codon at that position encoding Ser;

(k) at nucleotide position 6216 in the 3D region of the virus, a C;

(l) at nucleotide position 6908 in the 3D region of the virus, a C;

(m) at nucleotide position 3196, an A;

(n) at nucleotide position 7032, a U or T; and

(o) any combination of one or more of the nucleotides (a) through (n).

6. The virus according to claim 5 wherein when the nucleotide at position 5145 is G, at least one additional nucleotide from (a) through (h), (j) through (l) is present.

7. The virus according to claim 5 comprising the nucleotides (a), (b), (c) and (d).

8. The virus according to claim 7 further containing at least one of the nucleotides (g) and (h).

9. The virus according to claim 5 comprising the nucleotides (a) through (d), (g) and (h).



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10. The virus according to claim 5 comprising the nucleotides (g) and (h).

11. The virus according to claim 10 further comprising a nucleotide selected from the group consisting of (a), (b), (c) and (d).

12. A vaccine composition useful for protecting primates against hepatitis A infection and disease comprising at least one live hepatitis A virus of claims 1 through 11.

13. The use of a virus according to claims 1 through 11 in the preparation of a vaccine composition for protecting a human or primate against hepatitis A virus infection.

14. A method for engineering a live attenuated hepatitis A virus strain HAV 4380 adapted to growth in MRC-5 cells comprising the step of modifying an HAV strain by incorporating into the nucleotide sequence of the HAV strain a nucleotide at a nucleotide position homologous to the nucleotide position in the genomic sequence of HM-175, Pass 35, selected from the group consisting of

(a) at nucleotide position 591 in the 5' noncoding region of the virus, a G;

(b) at nucleotide position 646 in the 5' noncoding region of the virus, an A;

(c) at nucleotide position 669 in the 5' noncoding region of the virus, a U or T;

(d) at nucleotide position 687 in the 5' noncoding region of the virus, a G;



- (e) at nucleotide position 2864 in the VP1 region of the virus, an A;
- (f) at nucleotide position 3934 in the 2B region of the virus, a G;
- (g) at nucleotide position 4418 in the 2C region of the virus, a U or T, resulting in the codon at that position encoding Phe;
- (h) at nucleotide position 4643 in the 2C region of the virus, a U or T;
- (i) at nucleotide position 5145 in the 3A region of the virus, a G, resulting in the codon at that position encoding Val;
- (j) at nucleotide position 5745 in the 3C region of the virus, a U or T, resulting in the codon at that position encoding Ser;
- (k) at nucleotide position 6216 in the 3D region of the virus, a C;
- (l) at nucleotide position 6908 in the 3D region of the virus, a C;
- (m) at nucleotide position 3196, an A;
- (n) at nucleotide position 7032, a U or T; and
- (o) any combination of two or more of the nucleotides (a) through (n).

15. The method according to claim 14 wherein said nucleotide is introduced by recombinant genetic engineering techniques.

16. A polynucleotide sequence encoding a live hepatitis A virus of claims 1 through 11.

17. The sequence according to claim 16 which is a cDNA.