(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 1 476 468 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Description Paragraph(s) 20

(48) Corrigendum issued on:
08.04.2009 Bulletin 2009/15

(45) Date of publication and mention of the grant of the patent:
03.09.2008 Bulletin 2008/36

(21) Application number: **03705004.4**

(22) Date of filing: **29.01.2003**

(51) Int Cl.:
C07K 16/10 *(2006.01)* ***G01N 33/576*** *(2006.01)*
C07K 16/42 *(2006.01)*

(86) International application number:
PCT/IT2003/000032

(87) International publication number:
WO 2003/064473 (07.08.2003 Gazette 2003/32)

(54) **HUMAN MONOCLONAL ANTIBODY FAB FRAGMENTS DIRECTED AGAINST HCV E2 GLYCOPROTEIN AND ENDOWED WITH IN VITRO NEUTRALIZING ACTIVITY**

FAB-FRAGMENTE HUMANER MONOKLONALER ANTIKÖRPER MIT NEUTRALISIERENDER AKTIVITÄT GEGEN DAS HCV E2 GLYCOPROTEIN

FRAGMENTS FAB D'ANTICORPS MONOCLONAUX HUMAINS DIRIGES CONTRE LA GLYCOPROTEINE VHC/E2 ET POSSEDANT UNE ACTIVITE DE NEUTRALISATION IN VITRO

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PT SE SI SK TR
Designated Extension States:
AL LT LV MK RO

(30) Priority: **30.01.2002 IT RM20020049**

(43) Date of publication of application:
17.11.2004 Bulletin 2004/47

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- **ALLANDER T ET AL: "RECOMBINANT HUMAN MONOCLONAL ANTIBODIES AGAINST DIFFERENT CONFORMATIONAL EPITOPES OF THE E2 ENVELOPE GLYCOPROTEIN OF HEPATITIS C VIRUS THAT INHIBIT ITS INTERACTION WITH CD81" JOURNAL OF GENERAL VIROLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB, vol. 81, 2000, pages 2451-2459, XP002939743 ISSN: 0022-1317**
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EP 1 476 468 B9

Description

[0001] The invention concerns human monoclonal antibody Fab fragments directed against HCV E2 glycoprotein and endowed with *in vitro* neutralizing activity. Hepatitis C virus (VCV) infects about 4% of the world population (World Health Organization, 1999). Over 80% of subjects coming into contact with this pathogen develop a chronic infection as the host immune response is unable to eradicate the infection, with the risk of severe liver diseases such as chronic hepatitis, cirrhosis and liver cell carcinoma [1, 2].

[0002] Treatment of chronic infection is based on combined therapy with interferon and ribavirin, which is extremely costly causes major side effects and is moderately effective (only 1 patient in 4 obtains long-term results) [3, 4]. The viral infection does not provide immune protection. This fact, together with the virus's high variability in antigenic structure recognized by the immune system, has hindered the development of an effective serum therapy and vaccines to protect individuals against HCV infection. It is therefore clear that new antiviral strategies are strongly needed.

[0003] The author has cloned the genes coding for a large number of human Fabs antibody fragments directed against one of the HCV proteins, the external E2 glycoprotein, considered the most important target for immune protective response [5]. However, the evaluation of the biological activity of these antibody fragments is not simple, as no reliable *in vitro* systems are available to determine the neutralizing activity against HCV. Hence, the author has only evaluated and described the variable ability of different Fabs to inhibit the binding of protein E2 to the target cell, without demonstrating a correlation between this activity and the neutralizing activity of the sera [5].

[0004] In a previous work, Burioni et al. (2001) [6], showed that some anti-E2 antibodies produced by HCV-infected patients have a negative effect, rendering the virus less sensitive to host immune response, probably due to its binding to the E2 antigen and to modifications of its conformation [6]. This could explain why high anti-E2 antibody titers are not directly correlated with protection against HCV infection.

[0005] The international patent application WO 00/05266 and Allander et al., (J. of General Virol., (2000)81:2451-2459) disclose Recombinant human antibodies capable of neutralizing the binding of the HCV E2 glycoprotein onto susceptible cells or to CD81, respectively. WO 02/055560 discloses human monoclonal antibody that exhibits immunological binding affinity for HCV E2 antigen having specific amino acid sequences. Finally, Rosa et al., (PNAS (1996)93:1759-1763) describes a method to estimate neutralizing antibodies to the HCV by cytofluorimetric assessment of E2 binding to target cells.

[0006] Bugli et al., 2001 [7] generated a map of E2 protein epitopes that can bind *in vitro* the panel of anti-E2 human Fabs, showing four discrete regions against which immune response is directed (Fig. 2) [7]. The presence of antibodies directed against one or more of these regions in the serum of chronically infected patients could be associated with complications, reduced effectiveness of treatment and a different prognosis. It is therefore evident that there is a need for a method to determine antibodies in a biological fluid directed against different epitopes of the HCV E2 protein. An embodiment of the present invention provides this method.

[0007] The authors of the invention have also evaluated the neutralizing activity of various anti-E2 antibodies in a system of viral pseudotypes, i.e. viruses externally identical to HCV- but, after entering the target cells which are able to produce a protein that produces fluorescence [8]. By revealing the presence or absence of fluorescence in the cells, the method provides a direct measure of the *in vivo* neutralizing activity of anti-E2 antibodies directed against different epitopes.

[0008] Unexpectedly, the authors found that two of the assayed antibodies, e137 and e301, can neutralize the virus at concentrations obtainable with a single parenteral administration of an antibody preparation; two other antibodies had no neutralizing activity and one was even able to promote viral infection.

[0009] The development of the method of titering different antibody populations in a patient represents a valuable diagnostic and prognostic instrument with the potential to distinguish between affected subjects at risk for developing severe complications and those with a more favorable prognosis. In this latter group, this method would eliminate the need to administer a largely ineffective treatment that is also associated with severe side effects, while providing a considerable reduction in costs.

[0010] As the E2 epitopes, so identified, are not reproducible by synthesizing synthetic peptides [5], the method represents the only way to determine the amount of antibodies against the different parts of the protein E2, with correlated clinical and epidemiological data.

[0011] The identification of anti-E2 antibodies in the human Fabs format with a good neutralizing ability permits their large-scale production and use as a medication in anti-HCV treatment, or as a preventive agent in topical form to inhibit viral transmission to subjects at risk (couples with discordant HCV state, individuals subject to occupational exposure, etc.).

[0012] The antibodies of the invention can be advantageously used to evaluate *in vitro* candidate molecules for anti-HCV vaccines, i.e. able to stimulate neutralizing antibodies but not ineffective or negative antibodies.

[0013] The availability of neutralizing human antibodies able to recognize a broad spectrum of viruses could be crucial in the production of artificial vaccines. The neutralizing antibodies described in this document can be used as a template

for the development of vaccines (made from peptides or anti-idiotype antibodies) able to stimulate a neutralizing cross-reactive response.

[0014] The object of this invention is the use of a human antibody, or its functional fragments. characterized by having the following amino acid sequences of the variable parts of the heavy and light chains:

e 137 Heavy chain (HC)

LLEQSGSEVKVPGSSLKVSCKTSGGTFSTYTFSWVRQAPGQGLEWMG
GITPIIGIANYARNFQDRVTITADESTSTVYMEVRRLRSEDTAVYYCAKTS
EVTATRGRTFFYSAMDVWGQGT

e 137 Light chain (LC)

MAELTQSPSFLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYA
ASTLQSGVPSRFSGSGSWTEFTLTISRLQPEDFATYYCQHLNTYPWTFG
QGT

for the preparation of:

a) a medicament foranti-HCV treatment; or
b) as a preventive agent in topical form to inhibit viral transmission to a subject at risk.

[0015] Preferably the human antibody, or its functional fragments is the human monoclonal antibody Fab fragment e137 or a full-size human monoclonal antibody containing said Fab fragment.

[0016] Another object of the invention is the use of a human antibody or its functional fragment, characterized by having the following amino acid sequences of the variable parts of the heavy and light chains:

e 301 Heavy chain (HC)

LLEQSGSEVKKPGSSVRVSCTTSGGTLSDYGFNWLRQAPGQGPEWMG
GIIPLFRRTTYGQKFQGRLTITADESTGATYMELSSLRSDDTAVYYCARE
KVSVLTGGKSLHYFEYWGKGT

e 301 Light chain (LC)

MAELTQSPATLSVSPGERATLSCRASQSVSSRLAWYQQKRGQAPSLLIY
DTSSRATGVPARFSASGSGTQFTLTISSLQSEDFALYYCQQYNDWPSTF
GQGT

for the preparation of:

a) a medicament foranti-HCV treatment; or
b) as a preventive agent in topical form to inhibit viral transmission to a subject at risk.

[0017] Preferably the human antibody, or its functional fragments is the human monoclonal antibody Fab fragment e301 or a full-size human monoclonal antibody containing said Fab fragment.

[0018] Preferably the human antibodies of the invention are a full-size IgG1 molecule.

[0019] A further object of the invention is a composition for anti-HCV therapy comprising in a therapeutically effective amount at least one of the antibodies of the invention. Preferably, the composition is supplied in purified form for parenteral use or in another formulation for topical use as a gel, creme, ointment, ovule, with excipients known to experts in the field.

[0020] It is a further object of the invention a method for the determination of the presence of antibodies having HCV neutralizing activity in a biological fluid, comprising the steps of:

a) labeling the human monoclonal antibody Fabs e137 or e301, or of a full-size human monoclonal antibody containing at least one of said Fab fragments;
b) determining the presence of antibodies in said fluid able to inhibit the binding of said labeled human monoclonal antibody Fab, or of said full-size human monoclonal antibody to the E2 HCV protein.

the epitope recognized by the Fab in the serum. In panels B and C, the bound antibodies, as they compete with Fab, proportionately diminish the amount bound compared with panel A. In panels D and E, the presence of antibodies not directed against the specific epitope does not minimally influence Fab binding.

- Figure 1 FIT: THEORETICAL BASIS. Panel A shows the binding of a Fab-FLAG to its epitopes without competitors. Using the same concentration of Fab present in (A), preincubation of the antigen with the patient's serum permits quantitative analysis of antibodies directed against.
- Figure 2 A and B: Inhibition of binding between e8-FLAG (A) and e509-FLAG (B) to HCV/E2 by sera containing known concentrations of e8-IgG1 and e509-IgG1 (whole antibodies directed against the epitopes recognized by the Fab). It is clear that the inhibition of Fab binding can be observed only in the presence of the whole antibody having the same specificity and that this depends on antibody concentration.
- Figures 3A, B and C: Inhibition of infection of VSV/HCV and VSV/G pseudotypes by purified anti-HCV/E2 human recombinant Fabs at different concentrations. HepG2 cells infected with Fab-treated pseudotypes were incubated for 16 hr and the number of green fluorescent protein-expressing cells was determined by fluorescence microscopy. Data are presented as % of the infection detected in control wells (no Fabs added). The results shown are the average of three independent assays performed in double.
- Figure 4:Two-dimensional surface-like map of the human B cell epitopes present on the surface of HCV/E2 as recognized by the monoclonal antibodies used in this study. Overlapping circles indicate reciprocal inhibition. Fabs endowed with VSV/HCV pseudotype neutralizing activity are underlined. The putative region mediating the interaction of HCV/E2 with the cellular target is indicated by the dotted line. The putative region recognized by neutralizing antibodies is indicated by a solid black circle. Due to modifications that can be induced by antigen-antibody interactions, this diagram does not correspond to the actual physical map.

EXAMPLE 1

Materials and methods

Anti-HCV Fabs and full-size IgG1 production

[0021] Generation, purification and characterization of the anti-HCV/E2 Fabs have been described elsewhere [5]. FLAG-Fabs (Fabs labeled with a FLAG epitope fused at the carboxyterminal of the heavy chain fragment with a pentapeptide bridge) were constructed and purified as described elsewhere [6]. Validation and standardization of the assay were performed using Fab-coding genes to construct full-size human monoclonal antibodies (HuMabs), which were inserted in an appropriate eukaryotic vector for subsequent production in transfected cells [9]. The HuMabs present in the culture supernatant were purified by immunoaffinity as described [10] and purity-checked by PAGE. The amount of human antibody was assayed by a sandwich immunoassay. All antibodies and Fabs were stored at -70°C until use.

Sera and specimens

[0022] Sera obtained from healthy donors and HCV-positive patients were analyzed using commercial diagnostic kits (Ortho, Raritan, NJ) following standard procedures. For the preparation of mock specimens with known amounts of antibodies directed against a given epitope, HCV-negative sera were spiked with concentrated purified HuMabs in PBS and treated exactly like the positive and negative sera.

Design of Fab Inhibition Titer (FIT) assay

[0023] The purpose of this assay is to assess the ability of sera to inhibit the binding of a labeled Fab to its epitope,

thus obtaining an indirect measure of the amount of epitope-binding antibodies in sera (Fig.1).

[0024] FLAG-Fabs were purified [10] and assayed in a FLAG-Fab-specific ELISA to determine the correct concentration to be used in inhibition experiments. Briefly, FLAG-Fab preparations of known concentration were titered by ELISA [11], where antigen-coated plates were blocked for 1 h at 37°C with PBS/1%BSA. After removing the blocking solution, 50 μl of progressive dilutions of FLAG-Fab made in PBS/BSA 1% were added to the wells and incubated for 2 h at 37°C. Plates were washed 10 times with PBS/0.05% Tween-20 in an automated plate washer (DiaSorin, Saluggia, Italy) before adding 50 μl of a 10 μg/ml solution of anti-FLAG mouse monoclonal antibody M2 (Sigma, St. Louis, MO; 10 μg/ml in PBS) in PBS/BSA 1%. After 1 h incubation at 37°C, wells were washed 10 times with PBS/Tween-20 as above and mouse monoclonal antibody binding was revealed with horseradish peroxidase-conjugated goat anti-mouse IgG (Pierce; 1:8,000 in PBS). Substrate was added and plates were read for OD_{450} in an automated plate reader after 30 min incubation at room temperature in the dark. All assays were performed at least in double. A negative control antigen (BSA) was always included and the OD reading was subtracted as background.

[0025] For the determination of the Fab Inhibiting Titer (FIT) of sera, a concentration of purified FLAG-Fabs yielding in standard conditions an OD_{450} reading equal to 50% of maximum reading was used for further experiments of Fab inhibition ELISA. For these experiments, plates were coated and blocked as described above. Progressive 1:4 serum dilutions in PBS/BSA 1% were added in the amount of 50 μl per ELISA well. After 2 h of incubation at 37°C, purified FLAG-Fab was added directly to serum dilutions to reach the desired final concentration. Plates were incubated for additional 30 min and then processed as described above for FLAG-Fab ELISA. A positive control sample, containing a 20:1 excess of purified unlabeled Fab, corresponding to 100% inhibition, is included. A negative control sample, containing an excess of a control uncorrelated Fab [12] and corresponding to 0% inhibition, is also included. The final results are determined as % of inhibition with the formula: percent inhibition = 100 x (OD_{450} of probe FLAG-Fab alone - OD_{450} of probe FLAG-Fab with competing serum)/ OD_{450} of probe FLAG-Fab alone.

[0026] The highest serum dilution giving more than 70% inhibition of FLAG-Fab binding is considered as the Fab Inhibiting Titer (FIT) for that epitope and for that serum.

Results

[0027] The appropriate FLAG-Fab concentration to be employed in the assay is determined for each FLAG-Fab and ranges from 10 μg/ml (e8, e20, e137, e301, e509) to 0.1 μg/ml (e10-B). The amino acid sequences of the light and heavy chains of the various antibodies are given below:

e8 HC

LLEQSGAEVKMPGATVKVSCQSSRYTFTSYGIGWVRQAPGQGLEWMG
WISGYTHETKYAQSFQGRVTMTAETSTGTAYMELRSLRSDDTATYYCA
RDGGGRVVVPPTHLRAFDVWGQGT

e8 LC

MAELTQSPGTLSLSPGERATLSCRASHRVNNNFLAWYQQKPGQAPRLLI
SGASTRATGIPDRFSGSGSGTDFTLTISRLEPDDFAVYYCQQYGDSPLY
SFGQGT

e10 HC

LLESGPGLVKPSQTLSLTCTVSGVSISYGGRGVSYWGWVRQSPGKGLE
WIGHIYYFGDTFYNPSLNNRATISIDSSKNQFSLKLKSVTASDTALYFCAR
STLQYFDWLLTREAAYSIDFWGQGI

e10 LC

MAELTQSPSFLSASVGDRVTITCRASQGVTILLAWYQQKPGKPPKALIYA
ASSLQSGVPSRFSGSGSDTDFTLTISSLQPEDSATYYCQQLNTYPWTFG
QGT

e20 HC

LLEQSGAEVKKPGSSVKVSCKASGDHYGINWVRQAPGQGLEWMGGIIP
VFGTTTYAQKFQGRATITADDSTGTAFLELTRLTFDDTAVYFCATPHQLH
VLRGGKALSPWDYWGQGT

e20 LC

MAELTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKRGQAPSLLIY
GTSTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNDWPSTF
GQGT

e137 HC

LLEQSGSEVKVPGSSLKVSCKTSGGTFSTYTFSWVRQAPGQGLEWMG
GITPIIGIANYARNFQDRVTITADESTSTVYMEVRRLRSEDTAVYYCAKTS
EVTATRGRTFFYSAMDVWGQGT

e137 LC

MAELTQSPSFLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYA
ASTLQSGVPSRFSGSGSWTEFTLTISRLQPEDFATYYCQHLNTYPWTFG
QGT

e301 HC

LLEQSGSEVKKPGSSVRVSCTTSGGTLSDYGFNWLRQAPGQGPEWMG
GIIPLFRRTTYGQKFQGRLTITADESTGATYMELSSLRSDDTAVYYCARE
KVSVLTGGKSLHYFEYWGKGT

e301 LC

MAELTQSPATLSVSPGERATLSCRASQSVSSRLAWYQQKRGQAPSLLIY
DTSSRATGVPARFSASGSGTQFTLTISSLQSEDFALYYCQQYNDWPSTF
GQGT

e509 HC

LLEESGAEVKKPGSSVKVSCKTSGDTFRYGITWVRQAPGQGLEWMGQI
MPTFATATYAQRFQGRVTISADESTSTAYLEVRSLRSEDTAVYYCATPR
QVTILRGPKALSPWDYWGQGT

e509 LC

MAELTQSPATLSASPGERASLSCRASQSVSSNLAWYQQKPGQAPRLLIS
GASTRATGVPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPPH
FGQGT

[0028] The nucleotide sequences coding for the Fab fragments listed above are indicated as follows:

e8 HC

CTGCTCGAGCAGTCTGGAGCTGAGGTGAAGATGCCTGGGGCCACAG
TGAAGGTCTCCTGCCAGTCTTCCCGTTACACCTTCACCAGTTACGGT
ATCGGCTGGGTGCGACAGGCCCCTGGACAGGGGCTTGAGTGGATG
GGATGGATCAGCGGATACACCCATGAGACAAAATATGCACAGAGTTT
CCAGGGCAGAGTCACCATGACCGCAGAGACATCCACGGGCACAGCG
TATATGGAGTTGAGGAGCCTGCGGTCTGACGACACGGCCACATATTA
CTGCGCGAGAGATGGAGGAGGGAGGGTGGTAGTGCCGCCTACTCAT
CTACGTGCTTTTGATGTCTGGGGTCAAGGGACG

e8 LC

ATGGCCGAGCTCACCCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGG
GGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCACAGAGTCAATAACA
ACTTCTTAGCCTGGTATCAGCAGAAACCTGGCCAGGCTCCCAGGCTC
CTCATCTCTGGTGCATCTACCAGGGCCACTGGCATCCCAGACAGGTT
CAGTGGCAGTGGGTCTGGAACAGACTTCACTCTCACCATCAGCAGAC
TGGAGCCTGATGATTTTGCAGTTTATTATTGTCAGCAGTATGGTGACT
CACCTCTTTATTCTTTTGGCCAGGGGACC

e10 HC

CTGCTCGAGTCTGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGT
CCCTCACCTGCACCGTCTCCGGTGTCTCCATCAGTTACGGTGGTCGT
GGCGTTTCCTACTGGGGTTGGGTCCGCCAGTCCCCAGGGAAGGGCC
TGGAGTGGATTGGCCACATCTACTACTTTGGAGACACCTTCTACAAC
CCGTCCCTCAACAATCGAGCTACCATATCAATAGACTCATCCAAAAAC
CAGTTCTCCCTCAAGCTCAAGTCTGTGACTGCCTCAGACACGGCCCT
GTATTTCTGTGCCAGGAGCACCCTACAGTATTTTGACTGGTTATTGAC
ACGGGAGGCTGCCTACTCCATTGACTTCTGGGGCCAGGGAATA

e10 LC

ATGGCCGAGCTCACCCAGTCTCCATCCTTCCTGTCTGCATCTGTTGG
AGACCGAGTCACCATCACTTGCCGGGCCAGTCAGGGCGTCACCATT
CTTTTAGCCTGGTATCAGCAAAAGCCAGGGAAACCCCCTAAGGCCCT
GATTTATGCTGCATCGTCTTTGCAAAGTGGGGTCCCATCAAGGTTCA
GCGGCAGTGGTTCTGACACAGATTTCACTCTCACAATCAGCAGCCTA
CAGCCTGAAGATTCTGCAACTTATTACTGTCAACAACTTAACACTTAC
CCGTGGACGTTCGGCCAGGGGACC

e20 HC

CTGCTCGAGCAGTCAGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGG
TGAAGGTCTCCTGCAAGGCTTCTGGAGACCACTATGGTATCAACTGG
GTGCGACAGGCCCCTGGACAAGGGCTGGAGTGGATGGGCGGTATCA
TCCCTGTCTTTGGCACAACTACCTACGCACAGAAGTTCCAGGGCAGA
GCCACCATTACCGCGGACGACTCCACGGGGACGGCCTTTTTGGAGC
TGACCAGACTGACATTTGACGACACGGCCGTCTATTTCTGTGCGACA

CCTCACCAACTGCATGTCCTCCGGGGCGGTAAAGCCCTCTCCCCCT
GGGACTACTGGGGCCAGGGAACC

e20 LC

ATGGCCGAGCTCACCCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGG
GGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGT
AACTTAGCCTGGTACCAGCAGAAACGTGGCCAGGCTCCCAGTCTCCT
CATCTACGGAACATCTACCAGGGCCACTGGTATCCCAGCCAGGTTCA
GTGGCAGTGGGTCTGGGACAGAGTTCACTCTCACCATCAGCAGCCT
GCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATGATTG
GCCCTCCACCTTCGGCCAAGGGACA

e137 HC

CTGCTCGAGCAGTCTGGGTCTGAAGTAAAAGTGCCCGGGTCCTCGTT
GAAGGTCTCCTGCAAGACTTCTGGAGGCACCTTCAGCACCTATACTT
TCAGCTGGGTGCGACAGGCCCCTGGACAGGGACTTGAGTGGATGGG
GGGGATCACCCCTATCATTGGCATCGCAAACTACGCACGGAACTTCC
AGGACAGAGTCACCATCACCGCGGACGAATCCACGAGCACGGTCTA
CATGGAGGTGAGGAGGCTGAGATCTGAGGACACGGCCGTATATTATT
GTGCGAAAACTTCGGAAGTAACAGCCACTAGAGGGCGGACTTTCTTC
TACTCCGCTATGGACGTCTGGGGTCAAGGGACC

e137 LC

ATGGCCGAGCTCACCCAGTCTCCATCCTTCCTGTCTGCATCTGTAGG
AGACAGAGTCACCATCACTTGCCGGGCCAGTCAGGGCATAAGCAATT
ATTTAGCCTGGTATCAGCAAAAACCAGGGAAAGCCCCTAAGCTCCTG
ATCTATGCTGCATCCACTTTGCAAAGTGGGGTCCCATCGAGGTTCAG
CGGCAGTGGATCTTGGACAGAATTCACTCTCACAATCAGCCGCCTCC
AGCCTGAAGATTTTGCAACTTATTACTGTCAACACCTTAATACTTACCC
GTGGACGTTCGGCCAAGGGACC

e301 HC

CTGCTCGAGCAGTCTGGGTCTGAGGTGAAGAAACCTGGGTCCTCGG
TGAGGGTCTCGTGCACGACTTCTGGAGGCACCTTGAGCGACTATGGT
TTCAACTGGTTACGACAGGCCCCTGGACAAGGGCCTGAGTGGATGG
GAGGGATCATCCCTTTGTTTCGAAGAACAACCTACGGACAGAAGTTC

CAGGGCAGACTCACCATTACCGCGGACGAGTCCACGGGCGCAACCT
ACATGGAGCTGAGCAGCCTGAGATCTGACGACACGGCCGTCTATTAC
TGTGCGAGAGAGAAAGTTTCGGTCCTCACAGGCGGAAAGTCACTCCA
TTACTTTGAATATTGGGGCAAGGGAACC

e301 LC

ATGGCCGAGCTCACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAG
GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG
CAGGTTAGCCTGGTACCAGCAGAAACGTGGCCAGGCTCCCAGTCTC
CTCATCTATGACACATCTTCCAGGGCCACTGGTGTCCCAGCCAGGTT
CAGTGCCAGTGGGTCTGGGACGCAGTTCACTCTCACCATCAGCAGC
CTGCAGTCTGAAGATTTTGCACTTTATTACTGTCAGCAGTATAATGATT
GGCCCTCCACCTTCGGCCAAGGGACA

e509 HC

CTGCTCGAGGAGTCTGGGGCTGAGGTGAAGAAGCCAGGGTCCTCGG
TGAAGGTCTCCTGCAAGACTTCTGGAGACACCTTCAGATATGGTATC
ACGTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAC
AGATCATGCCTACGTTTGCGACAGCAACCTACGCACAGAGGTTCCAG
GGCAGAGTCACGATTTCCGCGGACGAATCCACGAGCACAGCCTACTT
GGAGGTGCGCAGCCTGAGATCTGAAGACACGGCCGTCTATTACTGT
GCGACACCTCGCCAAGTTACTATACTTCGGGGACCTAAAGCCCTCTC
CCCTTGGGACTACTGGGGCCAGGGAACC

e509 LC

ATGGCCGAGCTCACCCAGTCTCCAGCCACCCTGTCTGCGTCTCCAG
GGGAAAGAGCCTCCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGTAG
CAACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTC
CTCATCTCTGGTGCATCCACCAGGGCCACTGGTGTCCCGGCCAGGT
TCAGTGGCAGTGGGTCTGGGACAGAGTTCACTCTCACCATCAGTAGC
CTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATAAC
TGGCCTCCCCACTTTGGCCAGGGGACC

[0029] FLAG-Fab ELISA on purified labeled Fab molecules yields very specific and reproducible results. Determination of FIT is performed on 10 HCV-negative sera; the titer is consistently >1:20, the upper detection limit of our test, indicating that no inhibition occurs in the absence of specific anti-HCV antibodies.

[0030] To demonstrate that FIT effectively measures the antibodies directed against epitopes recognized by our FLAG-Fabs, the same analysis is performed on mock specimens prepared by mixing negative sera with human monoclonal antibodies of given specificity, obtaining false samples containing known amounts of IgG directed against the HCV E2 epitopes defined by our Fabs. Results (Figures 2A and B) show a good correlation between FIT and antibody amount, indicating that FIT can provide reliable information on the amount of epitope-specific antibodies in a patient's serum.

[0031] Finally, FIT is always positive in HCV-positive sera, with values encompassing a wide range of dilutions. FIT is very diverse for the different Fabs in the same serum sample, with considerable heterogeneity between patients.

EXAMPLE 2

Materials and Methods

Human antibody fragments

[0032] The human recombinant antibody fragments in this example are fully described in Bugli et al. (2001) [7] and correspond to those used in Example 1. Briefly, genes coding for the Fabs were obtained from a phage display combinatorial library containing the IgG1/kappa repertoire of a 58-year-old woman with chronic hepatitis with persistent presence in the blood of HCV RNA of genotype 1 b. The genes selected are inserted in an appropriate bacterial expression vector [13] and the transformed cells are then used as a source of recombinant Fabs, which are produced and purified as described [14]. Neutralization of E2 binding to cell (NOB) activity [5, 15] and the reciprocal interactions [7] of these molecules have been described. The presence of similar antibodies in the serum of HCV-infected patients is determined

by inhibition ELISA [7].

Pseudotypes and neutralization assay

[0033] The pseudotypes used here have been fully characterized and described in Matsuura et al., 2001 [8]. Briefly, the VSVΔG*/HCVE1-E2 pseudotype (VSV/HCV) consists of Vesicular Stomatitis Virus, where the G envelope protein is replaced with chimeric E1 and E2 HCV envelope glycoproteins consisting of the ectodomains of E1 and E2 proteins of type 1b HCV cDNA clone (NIH-J1) fused to the N-terminal signal sequences, with transmembrane and cytoplasmatic domains of VSV G protein [8]. The construction of plasmids [16], and eukaryotic expression vectors has been described [8, 17]. VSV/HCV is prepared by infecting CHO cells constitutively expressing chimeric E1 and E2 cDNA with a recombinant VSV in which the G protein-coding region has been replaced with the green fluorescent protein gene (GFP) [18]. The VSVΔG*/HCVE1-E2 (VSV/G) pseudotype used as control (and to produce the VSV/HCV pseudotype), is produced by infecting with VSVΔG* a cell line transiently expressing G protein. The neutralization assay is performed as described [8]. Dilutions of purified human recombinant Fabs are incubated with 2.4 X 10^3 Infection Units (IU) of the pseudotype VSV/HCV or VSV/G for 30 min at 37°C and inoculated into HepG2 cells ($4x10^4$ cells) prepared in a 96-well plate. After adsorption for 60 min at 37°C, the cells are washed 3 times with DMEM containing 10% FBS and incubated at 37°C for 16 hr.

[0034] The IU of the virus are determined by counting the number of GFP-expressing cells by fluorescence microscopy. Data are presented as percent of inhibition compared with control wells where no antibody was added. Data are the average of three experiments performed in double.

Results

Anti-HCV/E2 human monoclonal antibody panel generation and sequence characterization

[0035] The panel of human monoclonal antibody Fab fragments represents the anti-HCV/E2 immune repertoire of a patient with a persistent infection with HCV of genotype 1b [5, 19]. Antibody fragments, selected with purified recombinant HCV/E2 of 1a genotype (strain H)[20] expressed in CHO cells, have been fully characterized and correspond to clones present in the serum of chronically infected patients [7] with a shared equal affinity for HCV/E2. Each of the five antibodies represents one of the five families in which the whole anti-E2 antibody repertoire of this patient is grouped. Fabs belonging to the same family share similar biological activity and have strong homologies of DNA sequences [5]. Each of the five Fabs recognizes a different epitope on the surface of E2 [7]. Divergences from the relative germ-line sequences are typical of antigen-driven affinity maturation (Tables 1a and 1 b), suggesting a prolonged exposure to the antigen.

[0036] TABLES 1 A, B. Germlines and V gene mutations in variable regions of anti-HCVE2 human monoclonal antibodies.

[0037] Sequences are determined as described in Burioni et al., 1998 [5] and aligned with germline sequences in the IMGT database [21]. The percentage of nucleotide and amino acid mutations are calculated according to the Kabat and Wu alignment method [22], taking into account framework region (FR) 1, FR 2 and FR 3 for heavy and light chains, the complementarity determining region (CDR) 1 and CDR 2 for heavy chains, CDR 1, CDR 2 and CDR 3 for light chains.

Table 1 a - HEAVY CHAINS

		% of mutated nucleotides		% of mutated amino acids	
Antibody	V gene	FRs	CDRs	FRs	CDRs
e 8	VH1-18	9.5	22.2	14.9	33.3
e 20	VH1-69	9.4	16.9	19	38
e 137	VH1-69	11.5	15.3	14	41.7
e 301	VH1-69	8.9	19.4	15.6	45.8
e 509	VH1-69	5.2	15.9	10.9	33.3

Table 1 b - LIGHT CHAINS

Antibody	V gene	% of mutated nucleotides		% of mutated amino acids	
		FRs	CDRs	FRs	CDRs
e 8	KV 3-20	2.7	16	2.6	33.3
e 20	KV 1- 9	4.3	7.7	9.7	22.2
e 137	KV 1- 8	2.2	9	3.2	15.4
e 301	KV 3-15	3.8	14.3	9.7	23
e 509	KV 3-15	3.2	1.3	6.5	0

[0038] Neutralizing of binding (NOB) activity of each Fab was also determined [5], with some clones (e137 and e8) found to be unable to inhibit HCV/E2 binding to cells and others inhibiting HCV/E2 binding even at very low concentration (see below).

Neutralization of the pseudotype virus bv human recombinant Fabs

[0039] Two of the Fabs, e8 and e20, recognizing different epitopes on the surface of HCV/E2 [7] do not neutralize VSV/HCV pseudotype infection even at high concentrations (80 μg/ml). One of these two Fabs, e20, has strong NOB activity [5], confirming that even antibodies inhibiting E2 binding may fail to prevent viral infection.

[0040] Two other Fabs, e137 and e301, efficiently neutralize VSV/HCV at a concentration of 10 μg/ml, while VSV pseudotypes bearing the VSV G envelope protein (VSV/G pseudotypes) are not affected (Figures 3a and 3b). These data are congruent with previous findings indicating that these two clones compete for the same E2 region, probably recognized by human antibodies endowed with neutralizing activity, as indicated in a two-dimensional surface map of the human epitopes on HCV/E2 (Figure 4).

[0041] Fab 509 is currently the strongest available antibody in terms of NOB activity, and is able to inhibit binding between E2 and the cellular target at very low concentrations (Table 2). Incubation of VSV/HCV pseudotypes with this Fab enhance virus entry into hepatoma cells down to a concentration of 1μg/ml. No increase in infectivity is demonstrated when VSV/G pseudotypes are used, thus ruling out the possibility that a nonspecific interaction of this Fab with cellular membrane promotes viral entry into the cell (Fig.3C).

Table 2 - Anti-HCV/E2 antibodies characteristics

NOB activity is calculated as the concentration (in μg/ml) achieving 50% of neutralization of binding of a purified HCV/E2 preparation to cellular targets.		
Fab clone	50% NOB concentration (μg/ml)	Effect on VSV/HCV infection
e8	>40 (none)	none
e20	3 (high)	none
e137	40 (low)	inhibition
e301	3 (high)	strong inhibition
e509	<0.035 (highest)	enhancement

[0042] A control antibody [23] exerts no effect on the pseudotype system, as it fails to neutralize both VSV/HCV and VSV/G pseudotypes. The VSV/G pseudotype is duly neutralized by dilutions up to 1:1000 of a polyclonal anti-VSV antiserum used as neutralizing control in these experiments [8], which have no effect on the VSV/HCV. Polyclonal and monoclonal anti-E1 and anti- E2 antibodies raised in several hosts show no neutralizing effect on VSV/HCV pseudotypes.

[0043] The neutralizing activity of monovalent Fabs shows that HCV entry can be inhibited without the need for virion aggregation or cross-linking; furthermore, blocking of interaction between the virus and its cellular target seems unlikely to be a key factor in HCV neutralization. These data can explain at the molecular level the lack of correlation between NOB activity in the serum and protection from disease.

[0044] Some degree of cross-protection is provided by anti-HCV antibodies, as anti-E2 antibodies selected with E2 of 1a genotype are able to neutralize a pseudotype bearing E2 of 1 b genotype.

[0045] The results show that Fab 509 is able to enhance the infectivity of the VSV/HCV pseudotype virus, although

no effect on the VSV/G construct was apparent. A possible explanation for the ability of e509 to promote viral entry can be found in the observation that this antibody binds specifically and very efficiently to the region of E2 that binds to CD81, a cellular structure involved in viral attachment to the cell [24]. The binding of e509 to E2 could mimic the binding of E2 to one of its cellular targets and promote a modification of E2 conformation similar to the one induced by CD81. E2 is present in at least two conformational states and antibody binding to this protein can modify the sterical status of the protein by modulating the NOB activity of human Fabs without binding competition [6]. Hence, Fab 509 seems to be a key tool for the study of the interactions between HCV and the cell surface and could be used in *in vitro* models for the evaluation of molecules for vaccines.

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SEQUENCE LISTING

[0047]

<110> Burioni, Roberto

<120> HUMAN MONOCLONAL ANTIBODY FAB FRAGMENTS DIRECTED AGAINST HCV E2 GLYCOPROTEIN AND ENDOWED WITH IN VITRO NEUTRALIZING ACTIVITY

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caagggcctg agtggatggg agggatcatc cctttgtttc gaagaacaac ctacggacag 180
aagttccagg gcagactcac cattaccgcg gacgagtcca cgggcgcaac ctacatggag 240

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ggccaggctc ccagtctcct catctatgac acatcttcca gggccactgg tgtcccagcc 180
aggttcagtg ccagtgggtc tgggacgcag ttcactctca ccatcagcag cctgcagtct 240
gaagattttg cactttatta ctgtcagcag tataatgatt ggccctccac cttcggccaa 300
gggaca 306

<210> 23
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<212> DNA
<213> Homo sapiens

<400> 23

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gggcttgagt ggatgggaca gatcatgcct acgtttgcga cagcaaccta cgcacagagg 180
ttccagggca gagtcacgat ttccgcggac gaatccacga gcacagccta cttggaggtg 240
cgcagcctga gatctgaaga cacggccgtc tattactgtg cgacacctcg ccaagttact 300
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<212> DNA
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<400> 24

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ggccaggctc ccaggctcct catctctggt gcatccacca gggccactgg tgtcccggcc 180
aggttcagtg gcagtgggtc tgggacagag ttcactctca ccatcagtag cctgcagtct 240
gaagattttg cagtttatta ctgtcagcag tataataact ggcctcccca ctttggccag 300
gggacc 306

Claims

1. Use of a human antibody or its functional fragment, **characterized by** having the following sequences of variable parts of the heavy chain and of the light chain:

e 137 Heavy chain (HC)

LLEQSGSEVKVPGSSLKVSCKTSGGTFSTYTFSWVRQAPGQGLEWMG
GITPIIGIANYARNFQDRVTITADESTSTVYMEVRRLRSEDTAVYYCAKTS
EVTATRGRTFFYSAMDVWGQGT

e 137 Light chain (LC)

MAELTQSPSFLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYA
ASTLQSGVPSRFSGSGSWTEFTLTISRLQPEDFATYYCQHLNTYPWTFG
QGT,

for the preparation of :

a) a medicament for anti-HCV treatment; or
b) as a preventive agent in topical form to inhibit viral transmission to a subject at risk.

2. The use of claim 1, wherein the said antibody or its fragment is the human monoclonal antibody Fab fragment e137 or a full-size human monoclonal antibody containing said Fab fragment.

3. Use of a human antibody or its functional fragment, **characterized by** having the following sequences of variable parts of the heavy chain and of the light chain:

e 301 Heavy chain (HC)

LLEQSGSEVKKPGSSVRVSCTTSGGTLSDYGFNWLRQAPGQGPEWMG
GIIPLFRRTTYGQKFQGRLTITADESTGATYMELSSLRSDDTAVYYCARE
KVSVLTGGKSLHYFEYWGKGT

e 301 Light chain (LC)

MAELTQSPATLSVSPGERATLSCRASQSVSSRLAWYQQKRGQAPSLLIY
DTSSRATGVPARFSASGSGTQFTLTISSLQSEDFALYYCQQYNDWPSTF
GQGT,

for the preparation of ;

a) a medicament for anti-HCV treatment; or
b) as a preventive agent in topical form to inhibit viral transmission to a subject at risk.

4. The use of claim 3, wherein the said antibody or its fragment is the human monoclonal antibody Fab fragment e301 or a full-size human monoclonal antibody containing said Fab fragment.

5. The use of claims 1-4 wherein the human antibody is a full-size IgG1 molecule.

6. Composition for anti-HCV therapy comprising in a therapeutically effective amount at least a human antibody, or its functional fragments, said antibody or its fragment being the human monoclonal antibody Fab fragments e301 or e137, according to claims 1 or 3 or a full-size human monoclonal antibody containing any of said Fab fragments.

7. The composition according to claim 6 for parenteral or topical use.

8. A method for the determination of the presence of antibodies having HCV neutralizing activity in a biological fluid, comprising the steps of:

a) labeling the human monoclonal antibody Fabs e137 or e301, according to claims 1 or 3, or of a full-size human monoclonal antibody containing at least one of said Fab fragments;
b) determining the presence of antibodies in said fluid able to inhibit the binding of said labeled human monoclonal antibody Fab, or of said full-size human monoclonal antibody to the E2 HCV protein.

Patentansprüche

1. Verwendung eines menschlichen Antikörpers oder seines funktionalen Fragments, **dadurch gekennzeichnet, dass** er/es die nachfolgenden Sequenzen variabler Teile der schweren Kette und der leichten Kette aufweist:

e 137 schwere Kette (HC)

LLEQSGSEVKVPGSSLKVSCKTSGGTFSTYTFSWVRQAPGQGLEWMGGITPIIGI
ANYARNFQDRVTITADESTSTVYMEVRRLRSEDTAVYYCAKTSEVTATRGRTFF
YSAMDVWGQGT

e 137 leichte Kette (LC)

MAELTQSPSFLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYAASTLQ
SGVPSRFSGSGSWTEFTLTISRLQPEDFATYYCQHLNTYPWTFGQGT,

für die Zubereitung:

a) eines Medikaments für die Behandlung gegen HCV oder
b) als vorbeugendes Mittel in topischer Form zur Hemmung der Virusübertragung auf eine gefährdete Person.

2. Verwendung nach Anspruch 1, wobei der genannte Antikörper oder sein Fragment der menschliche monoklonale Fab-Fragment-Antikörper e137 oder ein vollständiger menschlicher monoklonaler Antikörper, der das genannte Fab-Fragment enthält, ist.

3. Verwendung eines menschlichen Antikörpers oder seines funktionalen Fragments, **dadurch gekennzeichnet, dass** er/es die nachfolgenden Sequenzen variabler Teile der schweren Kette und der leichten Kette aufweist:

e 301 schwere Kette (HC)

LLEQSGSEVKKPGSSVRVSCTTSGGTLSDYGFNWLRQAPGQGPEWMGGIIPLF
RRTTYGQKFQGRLTITADESTGATYMELSSLRSDDTAVYYCAREKVSVLTGGKS
LHYFEYWGKGT

e 301 leichte Kette (LC)

MAELTQSPATLSVSPGERATLSCRASQSVSSRLAWYQQKRGQAPSLLIYDTSSR
ATGVPARFSASGSGTQFTLTISSLQSEDFALYYCQQYNDWPSTFGQGT,

für die Zubereitung:

a) eines Medikaments für die Behandlung gegen HCV oder
b) als vorbeugendes Mittel in topischer Form zur Hemmung der Virusübertragung auf eine gefährdete Person.

4. Verwendung nach Anspruch 3, wobei der genannte Antikörper oder sein Fragment der menschliche monoklonale Fab-Fragment-Antikörper e301 oder ein vollständiger menschlicher monoklonaler Antikörper, der das genannte Fab-Fragment enthält, ist.

5. Verwendung nach den Ansprüchen 1-4, wobei der menschliche Antikörper ein vollständiges lgG1-Molekül ist.

6. Zusammensetzung für eine HCV-Therapie, die in einer therapeutisch wirkungsvollen Menge mindestens einen menschlichen Antikörper oder seine funktionalen Fragmente umfasst, wobei der genannte Antikörper oder seine Fragmente der menschliche monoklonale Fab-Fragment-Antikörper e301 oder e131 nach den Ansprüchen 1 oder 3 oder ein vollständiger menschlicher monoklonaler Antikörper sind, der irgendeines der genannten Fab-Fragmente enthält.

7. Zusammensetzung nach Anspruch 6 für parenterale oder topische Anwendung.

8. Eine Methode zur Feststellung von Antikörpern mit einer HCV-neutralisierenden Wirkung in einer biologischen Flüssigkeit, die folgende Schritte umfasst:

a) Markierung der menschlichen monoklonalen Fab-Antikörper e137 oder 301 nach den Ansprüchen 1 oder 3 oder eines vollständigen menschlichen monoklonalen Antikörpers, der mindestens eines der genannten Fab-Fragmente enthält;
b) Ermittlung von Antikörpern in genannter Flüssigkeit, die in der Lage sind, die Bindung genannter **gekennzeichnet**er menschlicher monoklonaler Fab-Antikörper oder des genannten vollständigen menschlichen monoklonalen Antikörpers mit dem HCV-E2-Protein zu verhindern.

Revendications

1. Utilisation d'un anticorps humain ou de son fragment fonctionnel, **caractérisé par** le fait d'avoir les séquences suivantes des parties variables de la chaîne lourde et de la chaîne légère:

chaîne lourde e 137 (HC)

LLEQSGSEVKVPGSSLKVSCKTSGGTFSTYTFSWVRQAPGQGLEWMGGITPIIGI
ANYARNFQDRVTITADESTSTVYMEVRRLRSEDTAVYYCAKTSEVTATRGRTFF
YSAMDVWGQGT

chaîne légère e 137 (LC)

MAELTQSPSFLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYAASTLQ
SGVPSRFSGSGSWTEFTLTISRLQPEDFATYYCQHLNTYPWTFGQGT,

pour la préparation de :

a) un médicament pour la thérapie anti-VHC ; ou
b) comme médicament préventif sous forme topique pour inhiber la transmission virale à un sujet à risque.

2. Utilisation de la revendication 1, dans laquelle ledit anticorps ou son fragment est le fragment Fab d'anticorps monoclonaux humains e137 ou un anticorps monoclonal humain complet comprenant ledit fragment Fab.

3. Utilisation de l'anticorps humain ou de son fragment fonctionnel, **caractérisé par** le fait d'avoir les séquences suivantes des parties variables de la chaîne lourde et de la chaîne légère :

chaîne lourde e 301 (HC)

LLEQSGSEVKKPGSSVRVSCTTSGGTLSDYGFNWLRQAPGQGPEWMGGIIPLF
RRTTYGQKFQGRLTITADESTGATYMELSSLRSDDTAVYYCAREKVSVLTGGKS
LHYFEYWGKGT

chaîne légère e 301 (HC)

MAELTQSPATLSVSPGERATLSCRASQSVSSRLAWYQQKRGQAPSLLIYDTSSR
ATGVPARFSASGSGTQFTLTISSLQSEDFALYYCQQYNDWPSTFGQGT,

pour la préparation de :

a) un médicament pour la thérapie anti-VHC ; ou
b) comme médicament préventif sous forme topique pour inhiber la transmission virale à un sujet à risque.

4. Utilisation de la revendication 3, dans laquelle ledit anticorps ou son fragment est le fragment Fab d'anticorps monoclonaux humains e301 ou un anticorps monoclonal humain complet comprenant ledit fragment Fab.

5. Utilisation des revendications 1-4, dans lesquelles l'anticorps humain est une molécule d'IgG1 complète.

6. Composition pour la thérapie anti-VHC comprenant en quantité thérapeutiquement efficace au moins un anticorps humain ou ses fragments fonctionnels ; ledit anticorps ou son fragment étant les fragments Fab d'anticorps monoclonaux humains e301 ou e137 selon l'une quelconque des revendications 1 ou 3, ou un anticorps monoclonal humain complet comprenant l'un ou l'autre desdits fragments Fab.

7. Composition selon la revendication 6 d'usage parentéral ou topique.

8. Méthode pour déterminer la présence d'anticorps ayant un effet d'activité de neutralisation du VHC dans un liquide biologique, comprenant les étapes :

a) marquage des Fab d'anticorps monoclonaux humains e137 ou e 301 selon l'une quelconque des revendications 1 ou 3 ; ou d'un anticorps monoclonal humain complet comprenant au moins l'un desdits fragments Fab ;
b) détermination de la présence d'anticorps dans ledit liquide en mesure d'inhiber la liaison dudit Fab d'anticorps monoclonaux humains marqué, ou dudit anticorps monoclonal humain complet, à la protéine E2 du VHC.

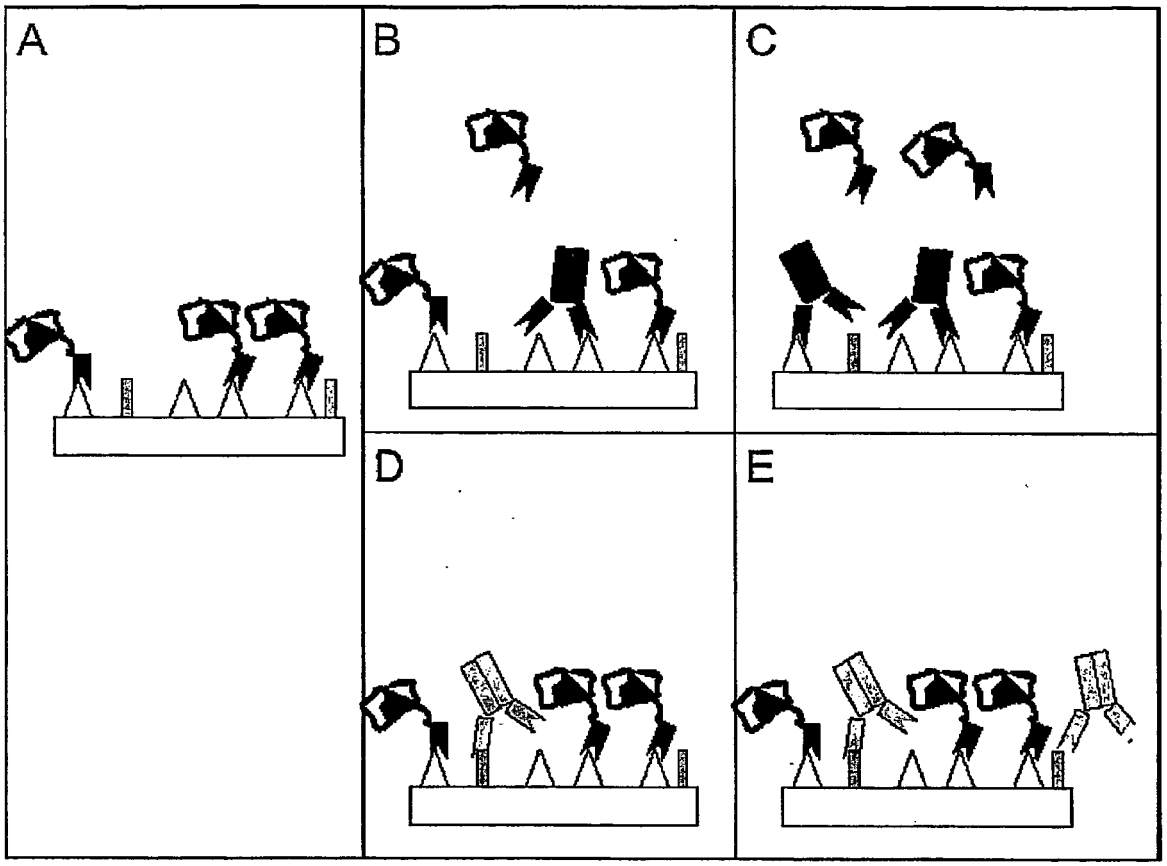

FIGURE 1

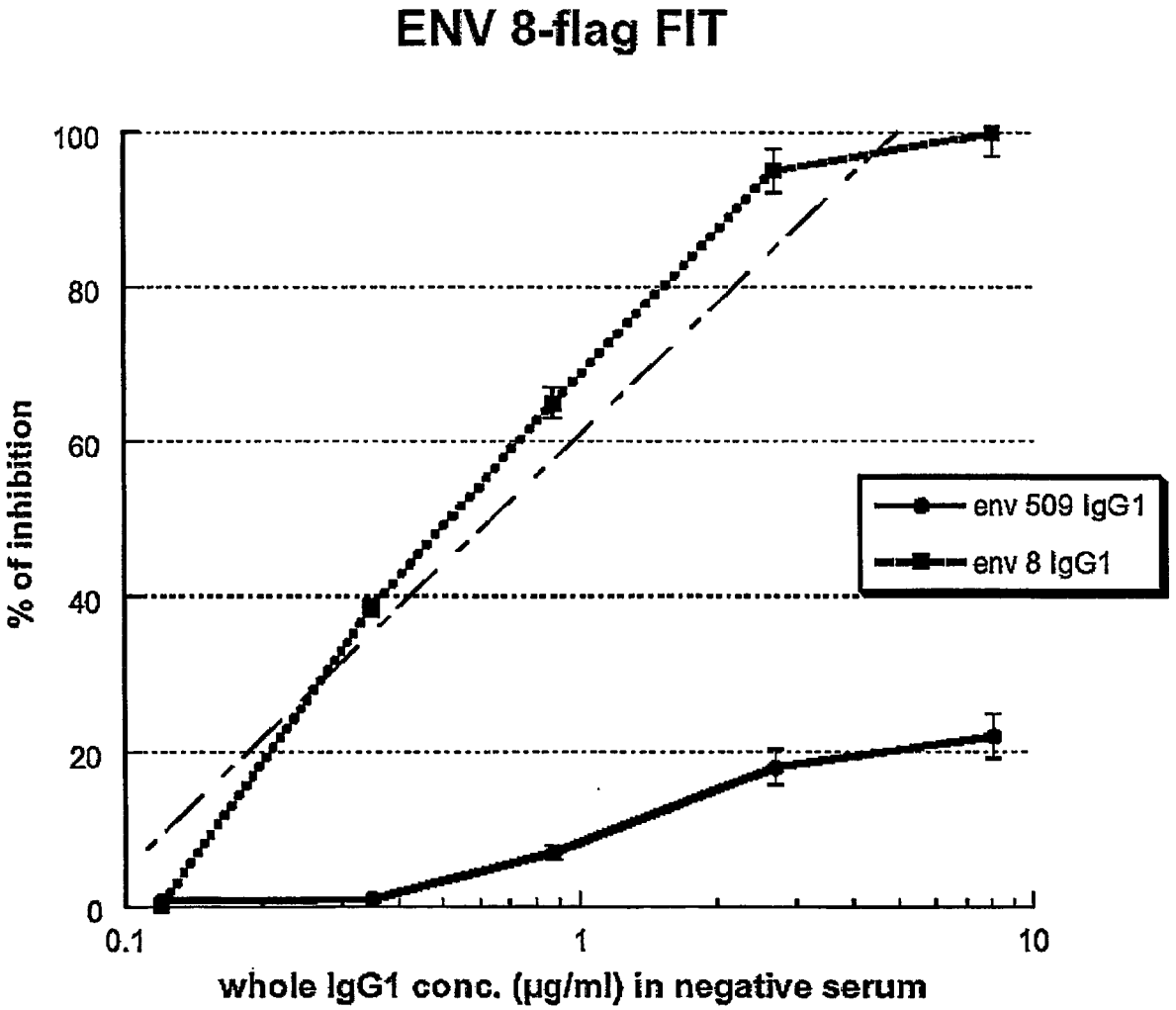
ENV 8-flag FIT
100
80
60
40
20
0
% of inhibition
0.1
1
10
whole IgG1 conc. (µg/ml) in negative serum
env 509 IgG1
env 8 IgG1

FIGURE 2A

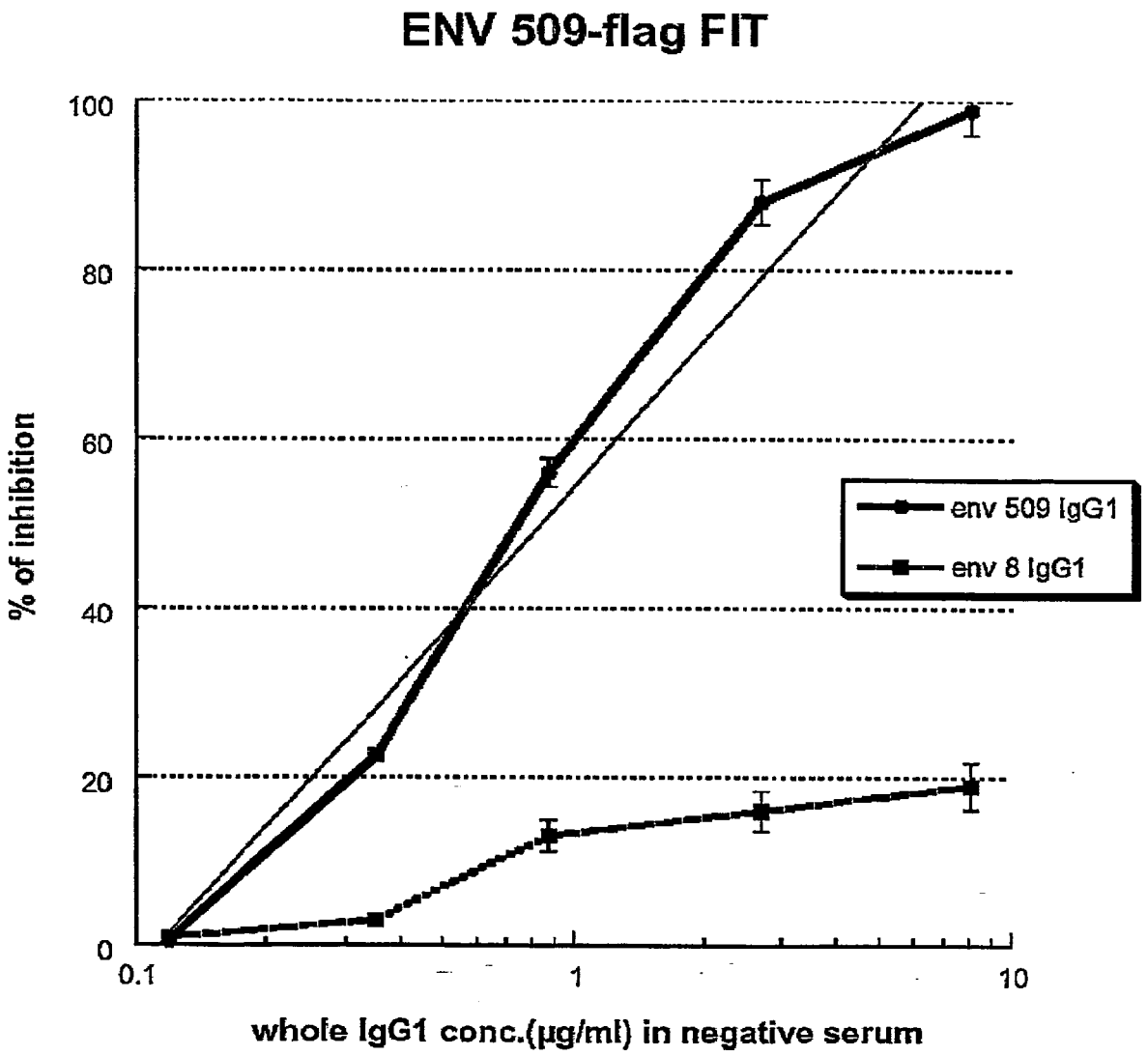
ENV 509-flag FIT
100
80
60
40
20
0
% of inhibition
0.1
1
10
whole IgG1 conc.(µg/ml) in negative serum
env 509 IgG1
env 8 IgG1

FIGURE 2B

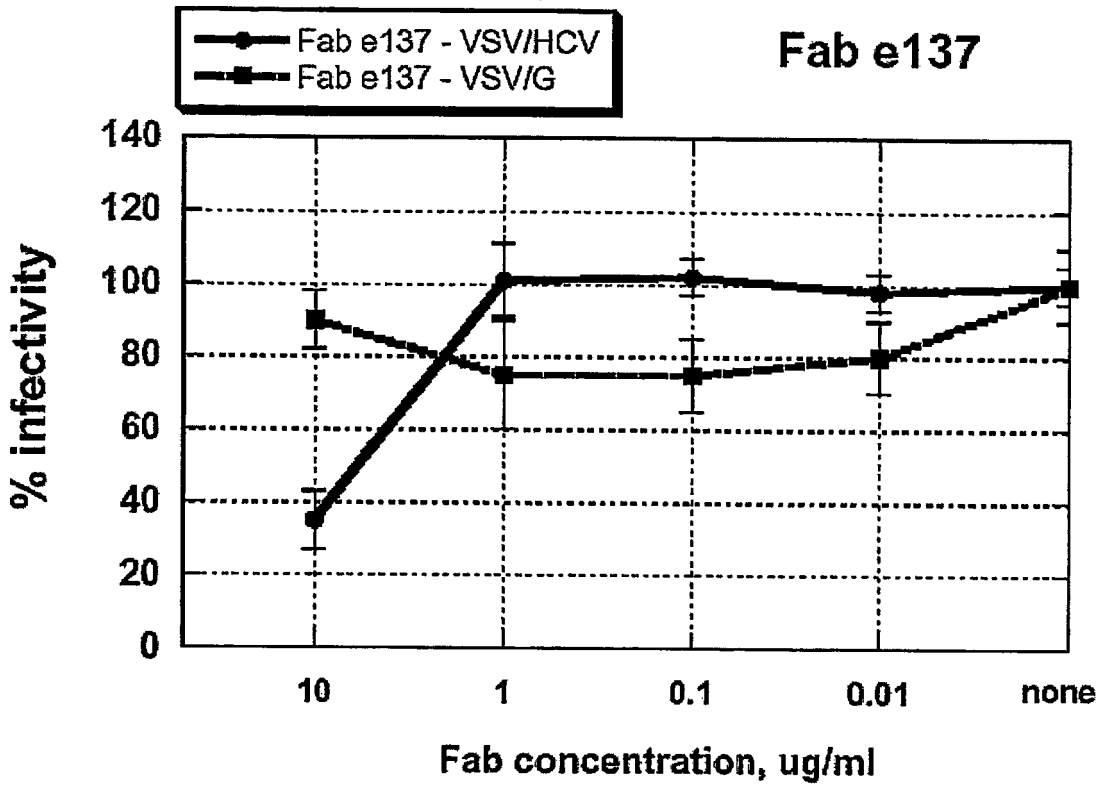
Fab e137 - VSV/HCV
Fab e137 - VSV/G
Fab e137
% infectivity
140
120
100
80
60
40
20
0
10
1
0.1
0.01
none
Fab concentration, ug/ml

FIGURE 3A

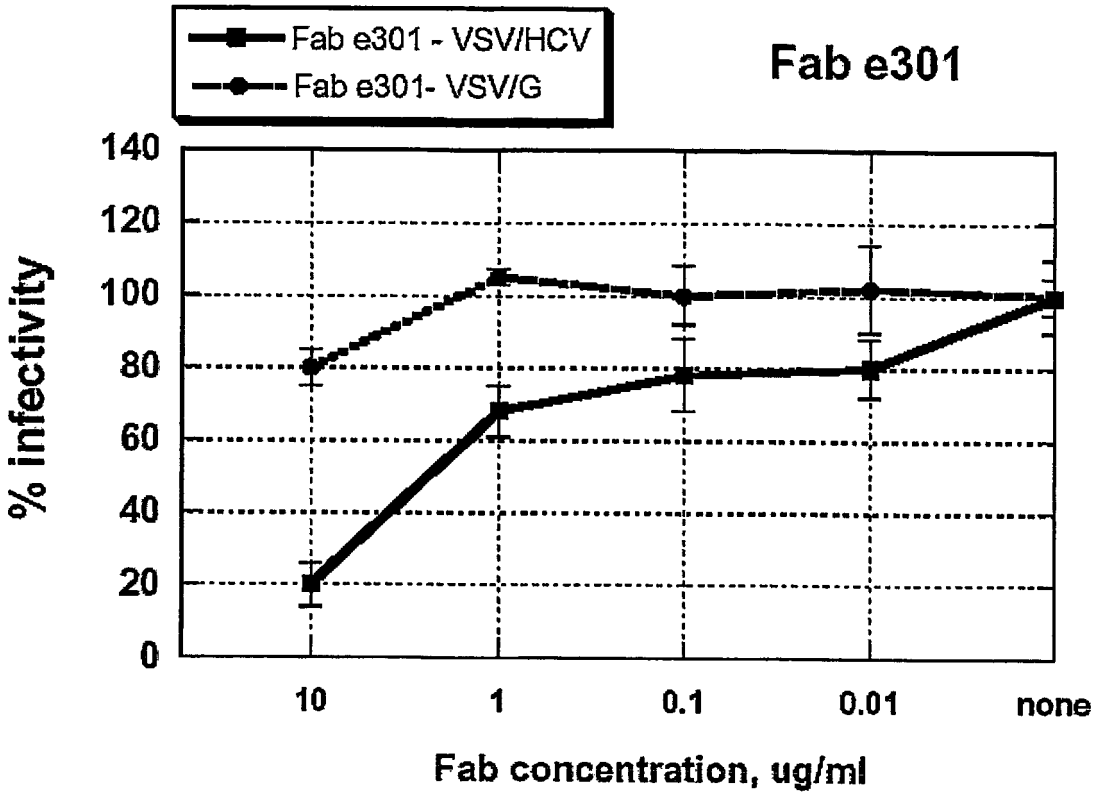
Fab e301 - VSV/HCV
Fab e301- VSV/G
Fab e301
% infectivity
140
120
100
80
60
40
20
0
10
1
0.1
0.01
none
Fab concentration, ug/ml

FIGURE 3B

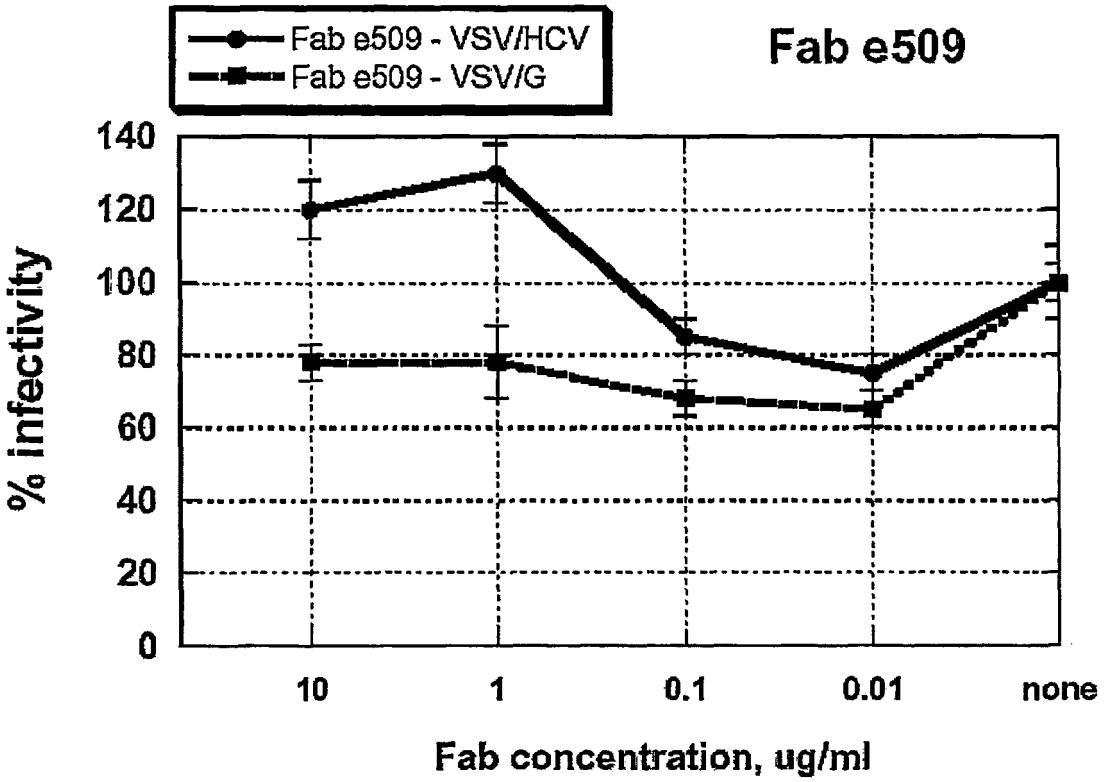
Fab e509 - VSV/HCV
Fab e509 - VSV/G
Fab e509
% infectivity
140
120
100
80
60
40
20
0
10
1
0.1
0.01
none
Fab concentration, ug/ml

FIGURE 3C

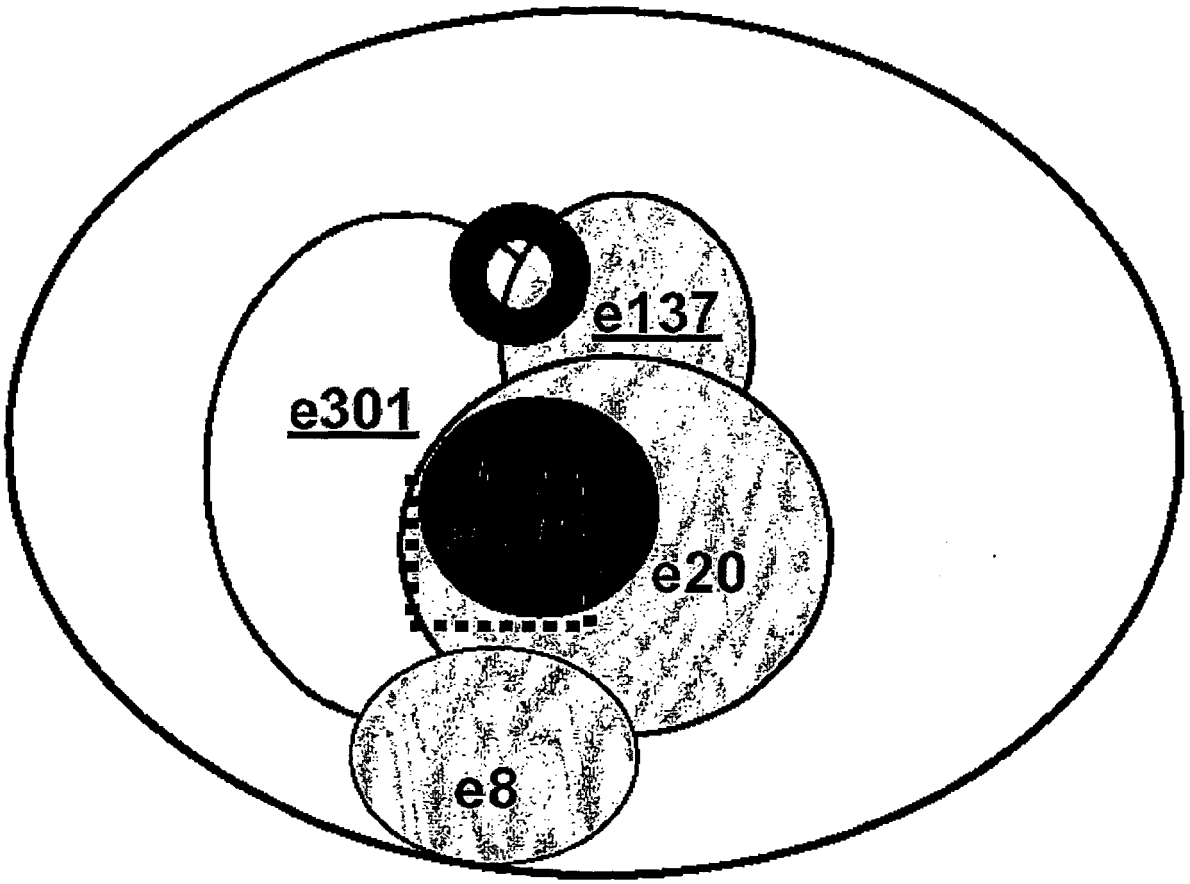

FIGURE 4

REFERENCES CITED IN THE DESCRIPTION

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