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(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

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(54) **Title**: HIV-1 IMMUNOGENS

(57) **Abstract**: The present invention relates, in general, to HIV-1 and, in particular, to HIV-1 immunogens and to methods of using such immunogens to induce the production of broadly neutralizing HIV-1 antibodies in a subject (e.g., a human).

HIV-1 IMMUNOGENS

The present application claims benefit of U.S. Provisional Application No. 61/884,014 filed September 28, 2013, the entire contents of which is hereby incorporated herein by reference.

This invention was made with government support under Grants AI1067854 and AI100645 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

The present invention relates, in general, to HIV-1 and, in particular, to HIV-1 immunogens and to methods of using such immunogens to induce the production of broadly neutralizing HIV-1 antibodies in a subject (e.g., a human).

BACKGROUND

Induction of HIV-1 envelope (Env) broadly neutralizing antibodies (BnAbs) is a key goal of HIV-1 vaccine development. BnAbs can target conserved regions that include conformational glycans, the gp41 membrane proximal region, the V1/V2 region, glycans-associated C3/V3 on gp120, and the CD4 binding site (CD4bs) (Walker et al, Science 326:285-289 (2009), Walker et al, Nature 477:466-470 (2011), Burton et al, Science 337:183-186 (2012), Kwong and Mascola, Immunity 37:412-425 (2012), Wu et al, Science 329:856-861 (2010), Wu et al, Science 333:1593-1602 (2011), Zhou et al, Science 329:811-817 (2010), Sattentau and McMichael, F1000 Biol. Rep. 2:60 (2010), Stamatotos, Curr. Opin. Immunol. 24:316-323 (2012)). Most mature BnAbs have one or more unusual features (long heavy chain third complementarity determining regions [HCDR3s], polyreactivity for non-HIV-1 antigens, and high levels of somatic mutation) suggesting substantial barriers to their elicitation (Kwong and Mascola,

Immunity 37:412-425 (2012), Haynes et al, Science 308:1906-1908 (2005), Haynes et al, Nat. Biotechnol. 30:423-433 (2012), Mouquet and Nussenzweig, Cell Mol. Life Sci. 69:1435-1445 (2012), Scheid et al, Nature 458:636-640 (2009)). In particular, CD4bs BnAbs have extremely high levels of somatic mutation suggesting complex or prolonged maturation pathways (Kwong and Mascola, Immunity 37:412-425 (2012), Wu et al, Science 329:856-861 (2010), Wu et al, Science 333:1593-1602 (2011), Zhou et al, Science 329:811-817 (2010)). Moreover, it has been difficult to find Envs that bind with high affinity to BnAb germline or unmutated common ancestors (UCAs), a trait that would be desirable for candidate immunogens for induction of BnAbs (Zhou et al, Science 329:811-817 (2010), Chen et al, AIDS Res. Human Retrovirol. 23:11 (2008), Dimitrol, MAbs 2:347-356 (2010), Ma et al, PLoS Pathog. 7:e1002200 (2001), Pancera et al, J. Virol. 84:8098-8110 (2010), Xiao et al, Biochem. Biophys. Res. Commun. 390:404-409 (2009)). Whereas it has been found that Envs bind to UCAs of BnAbs targeting gp41 membrane proximal region (Ma et al, PLoS Pathog. 7:e1002200 (2001), Alam et al, J. Virol. 85:11725-11731 (2011)), and to UCAs of some V1/V2 BnAb (Bonsignori et al, J. Virol. 85:9998-10009 (2011)), to date, heterologous Envs have not been identified that bind the UCAs of CD4bs BnAb lineages (Zhou et al, Science 329:811-817 (2010), Xiao et al, Biochem. Biophys. Res. Commun. 390:404-409 (2009), Mouquet et al, Nature 467:591-595 (2010), Scheid et al, Science 333:1633-1637 (2011), Hoot et al, PLoS Pathog. 9:e1003106 (2013)), although Envs that bind CD4bs BnAb UCAs should exist (Hoot et al, PLoS Pathog. 9:e1003106 (2013)).

Eighty percent of heterosexual HIV-1 infections are established by one transmitted/founder (T/F) virus (Keele et al, Proc. Natl. Acad. Sci. USA 105:7552-7557 (2008)). The initial neutralizing antibody response to this virus arises approximately 3 months after transmission and is strain-specific (Richman et al, Proc. Natl. Acad. Sci. USA 100:4144-4149 (2003), Corti et al, PLoS One

5:e8805 (2010)). This antibody response to the T/F virus drives viral escape, such that virus mutants become resistant to neutralization by autologous plasma (Richman et al, Proc. Natl. Acad. Sci. USA 100:4144-4149 (2003), Corti et al, PLoS One 5:e8805 (2010)). This antibody-virus race leads to poor or restricted specificities of neutralizing antibodies in ~80% of patients; however in ~20% of patients, evolved variants of the T/F virus induce antibodies with considerable neutralization breadth, e.g. BnAbs (Walker et al, Nature 477:466-470 (2011), Bonsignori et al, J. Virol. 85:9998-10009 (2011), Corti et al, PLos One 5:e8805 (2010), Gray et al, J. Virol. 85:4828-4840 (2011), Klein et al, J. Exp. Med. 209:1469-1479 (2012), Lynch et al, J. Virol. 86:7588-7595 (2012), Moore et al, Curr. Opin. HIV AIDS 4:358-363 (2009), Moore et al, J. Virol. 85:3128-3141 (2011), Tomaras et al, J. Virol. 85:11502-11519 (2011)).

There are a number of potential molecular routes by which antibodies to HIV-1 may evolve and, indeed, types of antibodies with different neutralizing specificities may follow different routes (Wu et al, Science 333:1593-1602 (2011), Haynes et al, Nat. Biotechnol. 30:423-433 (2012), Dimitrol, MAbs 2:347-356 (2010), Liao et al, J. Exp. Med. 208:2237-2249 (2011)). Because the initial autologous neutralizing antibody response is specific for the T/F virus (Moore et al, Curr. Opin. HIV AIDS 4:358-363 (2009)), some T/F Envs might be predisposed to binding the germline or unmutated common ancestor (UCA) of the observed BnAb in those rare patients that make BnAbs. Thus, although neutralizing breadth generally is not observed until chronic infection, a precise understanding of the interplay between virus evolution and maturing BnAb lineages in early infection may provide insight into events that ultimately lead to BnAb development. BnAbs studied to date have only been isolated from individuals who were sampled during chronic infection (Walker et al, Science 326:285-289 (2009), Burton et al, Science 337:183-186 (2012), Kwong and Mascola, Immunity 37:412-425 (2012), Wu et al, Science 329:856-861 (2010),

Wu et al, Science 333:1593-1602 (2011), Zhou et al, Science 329:811-817 (2010), Bonsignori et al, J. Virol. 85:9998-10009 (2011), Corti et al, PLoS One 5:e8805 (2010), Klein et al, J. Exp. Med. 209:1469-1479 (2012)). Thus, the evolutionary trajectories of virus and antibody from the time of virus transmission through the development of broad neutralization remain unknown.

Vaccine strategies have been proposed that begin by targeting unmutated common ancestors (UCAs), the putative naïve B cell receptors of BnAbs, with relevant Env immunogens to trigger antibody lineages with potential ultimately to develop breadth (Wu et al, Science 333:1593-1602 (2011), Haynes et al, Nat. Biotechnol. 30:423-433 (2012), Scheid et al, Nature 458:636-640 (2009), Chen et al, AIDS Res. Human Retrovirol. 23:11 (2008), Dimitrol, MAbs 2:347-356 (2010), Ma et al, PLoS Pathog. 7:e1002200 (2001), Xiao et al, Biochem. Biophys. Res. Commun. 390:404-409 (2009), Alam et al, J. Virol. 85:11725-11731 (2011), Mouquet et al, Nature 467:591-595 (2010)). This would be followed by vaccination with Envs specifically selected to stimulate somatic mutation pathways that give rise to BnAbs. Both aspects of this strategy have proved challenging due to lack of knowledge of specific Envs capable of interacting with UCAs and early intermediate (I) antibodies of BnAbs.

The present invention results, at least in part, from studies that resulted in the isolation of HIV Envelope encoding sequences from an individual, CH1754, who developed plasma HIV-1 neutralization breadth over a two to three year period.

SUMMARY OF THE INVENTION

In general, the present invention relates to HIV-1. More specifically, the invention relates to HIV-1 immunogens and to methods of using such immunogens to induce the production of broadly neutralizing HIV-1 antibodies in a subject (e.g., a human).

Objects and advantages of the present invention will be clear from the description that follows.

BRIEF DESCRIPTION OF SEQUENCE LISTING

Figure 1 of the priority U.S. Application No. 61/884,014, filed September 28, 2013, incorporated herein by reference, provides Envelope gene sequences for CH1754.

The sequences of Figure 1 of the priority document are filed herewith as a part of the Sequence Listing, in IBM-PC machine format, as a file named "1579-2033_SL.txt" which is 3,849 KB and was created on September 26, 2014. Two (2) Compact Disc-Read Only Memory (CD-ROM) or Compact Disc-Recordable (CD-R) (labeled as "Copy 1" and "Copy 2" - in compliance with 37 CFR 1.52(e) – are being filed separately by hand carry on September 26, 2014, each disc containing a copy of the file named "1579-2033_SL.txt" which is 3,849 KB and was created on September 26, 2014, the entire contents of which file and Compact Discs are incorporated herein by reference. The two Compact Discs are identical.

DETAILED DESCRIPTION OF THE INVENTION

The present invention results, at least in part, from studies that resulted in the isolation of Envelope gene sequences from an individual, CH1754, who developed plasma HIV-1 neutralization breadth over a two to three year period. The invention relates the Env gene sequences shown in the Sequence Listing and the encoded amino acid sequences, and the use thereof as immunogens.

The envelopes to be used as immunogens in accordance with the invention can be expressed as full gp140, gp145 with transmembrane portions, gp120s, gp120 resurfaced core proteins, gp120 outer domain constructs, or other minimal gp120 constructs.

In accordance with the invention, immunization regimens can include sequential immunizations of Env constructs selected from those encoded by the sequences of the Sequence Listing, or can involve prime and boosts of combinations of Envs, or the administration of "swarms" of such sequences. Immunogenic fragments/subunits can also be used, as can encoding nucleic acid sequences. Alternatively, the transmitted founder virus Env constructs can be used as primes, followed by a boost with the transmitted founder Env and sequential additions of Envs from progressively later times after transmission in patient CH1754. Further, repetitive immunization can be effected with "swarms" of CH1754 Envs (for example, including various combinations of the nucleic acid sequences in the Sequence Listing and encoded proteins) ranging from, for example, 2 to 40 Envs.

In one embodiment, the present invention relates to a method of activating an appropriate naïve B cell response in a subject (e.g., a human) by administering the CH1754 T/F Env or Env subunits that can include the gp145 with a transmembrane portion, gp41 and gp120, an uncleaved gp140, a cleaved gp140, a gp120, a gp120 subunit such as a resurfaced core (Wu X, Science 329:856-61 (2010)), an outerdomain, or a minimum epitope expressing only contact points of broadly neutralizing antibodies (Bnabs) with Env (the minimal epitope to avoid dominant Env non-neutralizing epitopes), followed by boosting with representatives of subsequently evolved CH1754 Env variants (e.g., see the Sequence Listing) either given in combination to mimic the high diversity observed *in vivo* during affinity maturation, or in series, using vaccine immunogens specifically selected to trigger the appropriate maturation pathway by high affinity binding to UCA and antibody intermediates (Haynes et al, Nat. Biotechnol. 30:423-433 (2012)). DNA, RNA, protein or vectored immunogens can be used alone or in combination. In one embodiment of the invention, transmitted founder virus envelope is administered to the subject (e.g., human) as

the priming envelope and then one or more of the sequential envelopes disclosed herein is administered as a boost in an amount and under conditions such that BnAbs are produced in the subject (e.g., human). By way of example, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 envelopes (or subunits thereof) can be used with one prime and multiple boosts.

The present invention includes the specific envelope proteins disclosed herein (e.g., those encoded by the sequences in the Sequence Listing) and nucleic acids comprising nucleotide sequences encoding same (e.g., those in the Sequence Listing). The envelope proteins (and subunits thereof) can be expressed, for example, in 293T cells, 293F cells or CHO cells (Liao et al, Virology 353:268-82 (2006)). As indicated above, the envelope proteins can be expressed, for example, as gp120 or gp140 proteins and portions of the envelope proteins can be used as immunogens such as the resurfaced core protein design (RSC) (Wu et al, Science 329:856-861 (2010)); another possible design is an outer domain design (Lynch et al, J. Virol. 86:7588-95 (2012)). The invention includes immunogenic fragments/subunits of the envelope sequences disclosed herein, including fragments at least 6, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280 300, 320 or more amino acids in length, as well as nucleic acids comprising nucleotide sequences encoding such fragments and vectors containing same.

In other embodiments, the invention provides variants of the sequences encoded by the sequences in the Sequence Listing, including variants that comprise a mutation which repairs a trypsin cleavage site, thereby preventing protein clipping during Env protein production in a cell line, e.g., a CHO cell line.

The envelopes (immunogens) can be formulated with appropriate carriers using standard techniques to yield compositions suitable for administration. The compositions can include an adjuvant, such as, for example, alum, poly IC, MF-

59 or other squalene-based adjuvant, ASO1B or other liposomal based adjuvant suitable for protein immunization.

As indicated above, nucleic acid sequences (e.g., DNA sequences) encoding the immunogens can also be administered to a subject (e.g., a human) under conditions such that the immunogen is expressed *in vivo* and BnAbs are produced. The DNA can be present as an insert in a vector, such as a rAdenoviral (Barouch, et al. Nature Med. 16: 319-23 (2010), recombinant mycobacterial (i.e., BCG or M smegmatis) (Yu et al. Clinical Vaccine Immunol. 14: 886-093 (2007); ibid 13: 1204-11 (2006), or recombinant vaccinia type of vector (Santra S. Nature Med. 16: 324-8 (2010)).

Immunogens of the invention, and nucleic acids (e.g., DNAs) encoding same, are suitable for use in generating an immune response (e.g., BnAbs) in a patient (e.g., a human patient) to HIV-1. The mode of administration of the immunogen, or encoding sequence, can vary with the particular immunogen, the patient and the effect sought, similarly, the dose administered. Typically, the administration route is intramuscular or subcutaneous injection (intravenous and intraperitoneal can also be used). Additionally, the formulations can be administered via the intranasal route, or intrarectally or vaginally as a suppository-like vehicle. Optimum dosing regimens can be readily determined by one skilled in the art. The immunogens (and nucleic acids encoding same) are suitable for use prophylactically, however, their administration to infected individuals may reduce viral load.

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The entire contents of all documents and other information sources cited herein are hereby incorporated by reference, including, Provisional Appln. 61/613,222, filed March 20, 2012, Provisional Application No. 61/700,234, filed September 12, 2012, Provisional Application No. 61/700,252, filed September 12, 2012, Provisional Application No. 61/708,466, filed October 1, 2012, Provisional

Application No. 61/764,421, filed February 13, 2013, Provisional Application No. 61/542,469, filed October 3, 2011, Provisional Application No. 61/708,413, filed October 1, 2012, Provisional Application No. 61/708,503, filed October 1, 2012, Provisional Application No. 61/806,717, filed March 29, 2013, U.S. Application No. 13/314,712, filed December 8, 2011, PCT/US2012/000442, filed October 3, 2012, PCT/US2013/00210, filed September 12, 2013, Provisional Appln. No. 61/883,561, filed September 27, 2013 and PCT/US2013/059515, filed September 12, 2013.

WHAT IS CLAIMED IS:

1. A composition comprising an HIV-1 envelope protein encoded by a sequence set forth in the Sequence Listing, or immunogenic subunit thereof, and a carrier.

2. The composition according to claim 1 wherein said composition comprises the gp120 subunit of an HIV-1 envelope protein encoded by a sequence set forth in the Sequence Listing.

3. The composition according to claim 1 wherein said composition further comprises an adjuvant.

4. A construct comprising a nucleotide sequence encoding an HIV-1 envelope protein, as set forth in the Sequence Listing, or immunogenic subunit thereof, wherein said nucleotide sequence is present in a vector.

5. The construct according to claim 4 wherein said vector is a viral vector or mycobacterial vector.

6. The construct according to claim 5 wherein said vector is an adenoviral vector or a pox virus vector.

7. A composition comprising the construct according to claim 4 and a carrier.

8. A method of inducing an immune response comprising administering to a mammal in need thereof the composition according to claim 1 in an amount sufficient to effect said induction.

9. The method according to claim 8 wherein said composition is administered by injection.

10. The method according to claim 8 wherein said composition is administered intrarectally or vaginally.

11. A method of inducing an immune response comprising administering to a mammal in need thereof the construct according to claim 4 under conditions such that said nucleotide sequence is expressed, said HIV-1 envelope protein, or subunit thereof, is produced and said response is induced.

12. The method according to claim 8 or 11 wherein said mammal is a human.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/057708

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/16(2006.01)i, C12N 15/49(2006.01)i, C12N 15/63(2006.01)i, A61K 35/76(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K 14/16; none ; A61K 39/42; C12N 15/49; C12N 15/63; A61K 35/76

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: HIV-1 envelope protein, broadly neutralizing HIV-1 Ab

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013-006688 A2 (DUKE UNIVERSITY et al.) 10 January 2013 See abstract; claims 1-10; and page 16.	1-7
A	WO 2012-040562 A2 (INTERNATIONAL AIDS VACCINE INITIATIVE et al.) 29 March 2012 See abstract; and claims 1-13.	1-7
A	WALKER et al., 'Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target' Science, Vol.326, No.5950, pp.285-289 (2009) See the whole document.	1-7
A	HUSSAIN et al., 'Broadly neutralizing antibody responses in a subset of HIV-1-infected individuals in Chennai, India: potential avenues for vaccine design' Journal of the International Association of Providers of AIDS Care (JIAPAC), DOI:10.1177/1545109712467695 (internal pages 1-8) (e-pub. February 2013) See the whole document.	1-7

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 January 2015 (07.01.2015)	**08 January 2015 (08.01.2015)**

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/057708

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GRAY et al., 'The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection' Journal of Virology, Vol.85, No.10, pp.4828-4840 (2011) See the whole document.	1-7

INTERNATIONAL SEARCH REPORT

International application No.
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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8-12
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 8-12 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2014/057708

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013-006688 A2	10/01/2013	AU 2012-279018 A1	23/01/2014
		CA 2841376 A1	10/01/2013
		EP 2739300 A2	11/06/2014
		US 2014-0248311 A1	04/09/2014
		WO 2013-006688 A3	04/04/2013
WO 2012-040562 A2	29/03/2012	CA 2835522 A1	15/11/2012
		EP 2618843 A2	31/07/2013
		EP 2618843 A4	30/04/2014
		EP 2707388 A2	19/03/2014
		US 2013-0251726 A1	26/09/2013
		WO 2012-040562 A3	20/06/2013
		WO 2012-154311 A2	15/11/2012
		WO 2012-154311 A3	03/01/2013
		WO 2012-154312 A1	15/11/2012
		WO 2013-142324 A1	26/09/2013