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(71) Applicant: THE UNITED STATES OF AMERICA, represented by THE SECRETARY, UNITED STATES DEPARTMENT OF COMMERCE [US/US]; Washington, DC 20231 (US).

(72) Inventors: PAPAS, Takis, S. ; 10700 Tulip Lane, Potomac, MD 20854 (US). ZUBER, Mohammed ; 4993 Robin Court, Frederick, MD 21701 (US). SAMUEL, Kenneth, P. ; 406 Torrington Place, Silver Spring, MD 20901 (US).

(74) Agents: OLIFF, James, A. et al.; Oliff & Berridge, P.O. Box 19928, Alexandria, VA 22320 (US).

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(57) Abstract

Five unique recombinant polypeptides, each encoded by DNA segments representing most of the HIV-2 (NIH-Z strain) *env* gene, were produced at relatively high-levels (5 % of total cell protein) as *c*II-fusion products in *Escherichia coli*. These recombinant polypeptides were characterized serologically by the Western blot assay against a pannel of HIV-2 and HIV-1 antibody positive sera, and with normal human sera (HIV-1 and HIV-2 antibody negative). Only those polypeptides from the N-terminal region of the transmembrane protein gp35 (amino acids 537 to 707) were immunoreactive. Three polypeptides (921, 996 and 997), each encoding this region of the HIV-2 gp35 reacted strongly and specifically with antibodies in sera from HIV-2 positive individuals, but not with HIV-1 positive or normal human sera. Thus, the N-terminal region of the HIV-2 gp35 contains a highly antigenic determinant which is strongly immunogenic in HIV-2 infected individuals. The gp35 recombinant *env* polypeptides may be used in diagnostic assays to specifically differentiate between HIV-2 and HIV-1 infections.

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ANTIGEN AND IMMUNOASSAY FOR HUMAN IMMUNODEFICIENCY VIRUS TYPE 2

BACKGROUND OF THE INVENTION

The present invention relates to a polypeptide comprising an amino acid sequence that displays at least one epitope for specifically binding an antibody that recognizes an antigen of human immunodeficiency virus type 2 (HIV-2), where the polypeptide is further characterized by the inability to bind to an antibody that recognizes an antigen of human immunodeficiency virus type 1 (HIV-1). The present invention also relates to methods for producing such HIV-2 polypeptide antigens involving expression of fragments of genes encoding viral envelope proteins in recombinant cells, for example in a bacterium such as *Escherichia coli (E. coli)*.

In this application, absent an express statement to the contrary, each use of the term "polypeptide" encompasses any polymer comprising two or more amino acids coupled by peptide linkages (i.e., dipeptides, oligopeptides, peptides, polypeptides) as well as proteins consisting of multiple polypeptide subunits.

The term "recognizes" as used herein means "reacts specifically with", within the context of the customary usage for such terms in relation to the concept of immunospecificity of antigen-antibody binding reactions. It will be understood by one of ordinary skill in the art of immunoassays that, for clinical diagnostic purposes, the definition of a positive specific reaction of an antibody with an antigen is a matter of practical consideration. For example, according to many widely used methods of immunoassay, the antibody species to be detected is only roughly quantitated (ultimately by an indicator dye or other label), and the antibody level in a sample is indicated by such relative terms as "negative" (meaning undetectable), "positive" (meaning detectable); the latter term may be modified, as in the case of "weak" or "strong" reactions, by comparison to the reactions of standard reference sera that exemplify the class of sera

which the immunoassay is intended to discriminate. Assays for antiviral antibodies in human serum, for instance, are typically standardized so that the limit of detection is commensurate with the threshold of reactivity that is comparable to at least the lower bound of the distribution found in a representative population of previously infected persons. The gross reactivity reflects, of course, both titer and avidity of all antibodies in the serum that is examined.

Antibodies that recognize HIV-2 proteins obtained from virus particles have been found in the sera of individuals from several West African countries who present clinical symptoms similar to the Advanced Immune Deficiency Syndrome (AIDS) or the AIDS-Related Complex of symptoms (ARC), as well as from healthy people and individuals from other disease categories. Antibodies to the virus are seroprevalent in individuals belonging to high risk groups for AIDS/ARC (prostitutes and individuals with sexually-transmitted diseases). These serum antibodies recognize the core antigens (group-specific antigens or gag proteins, encoded by the *gag* gene) or the envelope proteins (encoded by the *env* gene) of the virus.

A limiting factor in studies of the immune response to these viruses and their epidemiological significance has been the difficulty in isolating antigens that are specific for the HIV-2 virus in a pure form and in quantity. As with other retroviruses, the two major envelope glycoproteins of HIV-2 (with apparent molecular weights of about 120 kilodaltons (kDa) and 35 kDa, designated gp120 and gp35, respectively) are the most immunogenic products since they are the first viral antigens presented to the immune system of the host, as parts of the virions, before the immune system is disabled by the infection. Accordingly, these products should provide the most useful antigens for detection of anti-HIV-2 antibodies in sera. As yet, however, for reasons given below, there is no single specific diagnostic antigen available for reliably detecting antibodies to HIV-2 in human sera.

One problem with specific detection of HIV-2 is that this virus is related to, although quite distinct from, HIV-1, the known causative agent of AIDS/ARC. At present, immunodiagnostic assays comprising HIV-1 antigens are routinely used for screening for HIV-1 specific antibodies in essentially all donated blood in the U.S. and much of the rest of the world. These assays may take the form of various quantitative or qualitative immunoassay methods employing individual viral proteins or combinations thereof. These notably include enzyme-linked immunosorbent assays (ELISA), which by virtue of immobilization of the antigen and colorimetric detection methods offer rapid, automated screening of many samples and quantitation of antigen-bound antibody levels. For more positive verification of the antigenic specificity of antibodies that bind to an immobilized antigen, so-called "Western blot" assays are commonly used, in which antigen components of differing sizes are separated by electrophoresis and immobilized to a membrane prior to reaction with serum antibodies. Such ELISA and Western blot assays for antiviral antibodies, often in the form of diagnostic kits, are used extensively for testing of persons having sexually transmitted diseases or other factors associated with high risk of AIDS. For certain situations, such as in AIDS patients, it is also useful to monitor levels of HIV-1 proteins in the blood or other tissues as an indicator of current viral activity which cannot be determined from the level of antibodies to the virus. Such assays for viral antigens typically make use of animal antibodies raised against whole viral proteins or portions thereof, which may be produced, for example, by recombinant DNA means or, in principle, by synthetic peptide chemistry.

On the basis of the limited data on the seroepidemiology and pathology of HIV-2 presently available, mainly from research in Africa, it is clear that there is a need to specifically detect HIV-2 antibodies in human sera, even in the presence of contaminating HIV-1 antibodies. It is readily conceivable that the development of an

economical and reliable capability for such HIV-2-specific serodiagnosis would establish a sufficiently widespread prevalence to warrant extensive blood testing for HIV-2 on levels like current levels of HIV-1 testing.

At present, the only reliable test for antibodies against HIV-2 antigens requires the use of natural HIV-2 antigens isolated from HIV-2-infected cells or virions. For example, it has been shown by the Western blotting immunoassay technique (F. Barin et al., 1987, J. Virol. Methods 17:55-61) that certain human sera contain antibodies that bind to various HIV-2 polypeptides that have been isolated from purified HIV-2 virions and analytically separated by electrophoresis. For mass screening of sera, however, mass production of HIV-2 antigens with the necessary reproducible quality from infected cells or virus particles is not only difficult and expensive, but also potentially hazardous, since large quantities of infectious agent would be handled in concentrated form.

Furthermore, use of such natural HIV-2 antigens suffers from an additional problem related to specificity. Experiments have indicated that sera from a substantial number of individuals that contain antibodies reacting with HIV-2 proteins also contain antibodies reacting with HIV-1 proteins. In some cases, this coincident reactivity appears to be due to infection of some high-risk individuals to both viruses; alternatively, in other cases, apparently the same antibodies are able to recognize highly similar antibody binding sites of both viruses (P.J. Kanki et a, 1987, Science 236:827-831). Therefore, to provide serodiagnosis specific for antibodies that can only bind to HIV-2 antigens, even in the presence of HIV-1-reactive antibodies, it is clear that HIV-2 antigens must be found that bear antibody binding sites that are dissimilar to HIV-i antibody binding sites.

For some purposes, such as mass testing of blood supplies, for example, a diagnostic antigen that is proven to be capable of binding antibodies commonly raised in infected human beings against both HIV-2 and HIV-1 may be

useful for initial screening purposes. It is clear, however, that present assays for HIV-1 do not detect all human antibodies against HIV-2. Moreover, since the pathology and prognosis upon infection with these two viruses can differ, the ability to distinguish between infections due to one or the other virus or both can be expected to assume increasing importance in the expanding war on these insidious agents.

One approach to this problem has been attempted by using short synthetic peptides to serve as antigens for detecting antibodies against HIV-1 or HIV-2 (Gnann et al, 1987a, Science *237*:1346-1349). A partially conserved immunoreactive epitope was identified in the transmembrane glycoproteins of HIV-1 and HIV-2, and synthetic peptides were prepared that spanned this region [amino acid residues 598-609, inclusive, in the gp41] of HIV-1; and corresponding residues of the HIV-2 gp35, numbered 592-603 in the HIV-2_{ROD} strain (M. Guyader et al, 1987, Nature (London) *326*:662)]. Certain problems with such short synthetic peptide antigens are inherent in their use for detection of antibodies in human serum, as documented by the present inventors and associates (M. Zweig et al, 1988, AIDS Res. Hum. Retroviruses, *4*:487-492). In the case of HIV-1, for instance, direct comparisons were made among ELISAs employing viral proteins, a synthetic peptide consisting of 21 amino acids (R21S, spanning residues 586-606 of HIV-1 gp41; J.J.G. Wang et al, 1986, Proc. Nat. Acad. Sci. USA, *83*:6159-6163), and a bacterially expressed fragment of gp 41 developed by the present inventors (protein 566, covering residues 548-736; K.P. Samuel et al, 1988, Gene *64*:121-134).

In this comparison, the R21S peptide ELISA was the least sensitive in detecting antibodies to HIV-1, failing to detect such antibodies in any of eleven known positive reference sera diluted by 20,000-fold, and some known positive sera were not positively identified at a 1280-fold dilution. In this same study, a similar lack of sensitivity was observed in an ELISA based on closely

homologous short gp41 peptides described by Gnann et al, 1987b (J. Virol. *61*:2639-2641). In contrast, the larger bacterially expressed gp41 fragment, detected HIV-1 antibodies in all of these same sera at dilutions of at least 20,000-fold. The HIV-1 ELISAs using synthetic peptides also showed greater heterogeneity in detectable antibody levels in both HIV-1 positive and negative sera, thus making clearcut identification of certain known positive sera equivocal at best. Other laboratories have reported related problems with synthetic peptide antigens for diagnostic detection of HIV-1 antibodies (see, for example, U. Certa, et al, 1986, EMBO J. *5*:3051-3056). Finally, the small size of the synthetic HIV-1 peptides precludes their meaningful resolution by gel electrophoresis, and hence, their use in Western blot assays for qualitative verification of binding specificity to an antigen of the expected size.

Accordingly, there remains a readily apparent need for a practical and economical serodiagnostic antigen specific for HIV-2 antibodies, even in the presence of HIV-1 antibodies that would be more sensitive and reliable than any such antigen comprising a short synthetic peptide, including that described in Gnann et al, 1987a, *supra*.

The proviral DNA of the NIH-Z isolate of HIV-2 has been molecularly cloned and its complete nucleotide sequence has been determined (Zagury et al, 1988, Proc. Natl. Acad. Sci. USA *85*:5941, which in its entirety is hereby incorporated herein by reference). The HIV-2_{NIH-Z} genome is approximately 9.4 kilobases (kb) long with an *env* gene of about 2.7 kb which codes for an envelope glycoprotein precursor of 856 amino acids. This 160 kDa envelope protein precursor (gp160) is matured into the exterior glycoprotein (gp120) and the transmembrane protein (gp35). The gp120 and gp35 envelope proteins of HIV-2_{NIH-Z} share only about 32% and 39% amino acid sequence homology, respectively, with a representative strain of HIV-1 (e.g., the HTLV-IIIB strain). Thus most of the

sequence of the HIV-2 envelope proteins differ from the corresponding HIV-1 proteins, and the low level of homology between HIV-2 and HIV-1 is not localized to a single region of the *env* proteins. Further, the properties that confer superior serologic sensitivity and specificity on some polypeptides with a given size and amino acid composition are not predictable.

Accordingly, little direction in the search for a diagnostic antigen specific for HIV-2 infections is provided by comparison of HIV-2 sequences to those of HIV-1. To be useful as such an antigen, a polypeptide must meet several criteria besides the most obvious requirement to display at least one antibody binding site (i.e., an "epitope", also known as an "antigenic determinant") for an antibody that recognizes an antigen of HIV-2. To have wide applicability, the epitope(s) on the polypeptide must be recognized by the immune systems of the vast majority of people infected with HIV-2 [i.e., the epitope(s) must be immunogenic]. In addition, for sensitive HIV-2 specific diagnosis, a polypeptide must present the epitope in its natural conformation to allow antibodies that recognize HIV-2 virions to bind firmly to the diagnostic antigen. Finally, the polypeptide must not bind antibodies that recognize HIV-1 antigens that are homologous to HIV-2 counterparts.

Accordingly, finding a polypeptide that meets all of the requirements for an HIV-2-specific diagnostic antigen must necessarily involve experimental testing with human sera of polypeptides spanning limited portions of most, if not all, of the HIV-2 *env* protein sequences.

Various molecular cloning systems and methods are known for expressing complete or partial genes for polypeptides and testing for antigenic determinants that are displayed on the expressed amino acid sequences. In particular, the expression plasmid pWS50 (Sisk et al, Gene *48*:183-193, 1986) was derived from the pJL6 vector (Lautenberger et al, Gene *23*:75-84, 1983: U.S. Patent application entitled "Novel plasmid pJL6", Serial No.

511,108), which contains the 13 amino-terminal codons of the lambda *cII* gene placed under the transcriptional control of the λP_L promoter and the *lacZ* (*β*-galactosidase) gene for identifying open reading frames of interest as tripartite fusion proteins expressed in *E. coli*. These plasmids are known and have been successfully used to express HIV-1 (Samuel et al, 1988, Gene 64:121-134) and HTLV-I (Samuel et al, 1984, Science *226*:1094-1097; Sisk et al, 1986, Gene *48*:183-193) *env* proteins.

It is therefore an object of the present invention to isolate a polypeptide comprising a region of the HIV-2 gp35 envelope protein that is highly immunogenic in infected people.

It is a further object of the present invention to provide a plasmid for expressing the immunogenic protein fragment.

It is an additional object of the present invention to produce this antigenic polypeptide at high levels in bacteria.

An ultimate object of this invention is to provide a polypeptide which is serologically specific for identifying antisera containing antibodies to HIV-2 even when these sera contain antibodies that recognize HIV-1 antigens that share homology with HIV-2 antigens.

SUMMARY OF THE INVENTION

The present invention contemplates the application of methods of recombinant DNA technology to fulfill the above needs for a diagnostic antigen that displays at least one epitope for specifically binding an antibody that recognizes an antigen of HIV-2 while not binding antibodies that recognize an antigen of HIV-1. More specifically, a highly antigenic region of the HIV-2 gp35 envelope protein was identified by production of several related polypeptides in bacteria, and these polypeptides were shown to be serologically specific for identifying antibodies that recognize HIV-2 virion antigens, even in the presence of antibodies that recognize HIV-1 antigens.

The production of these antigenically important

fragments of human retroviral envelope proteins in cells, such as bacteria, by means of recombinant DNA technology according to the present invention has the advantages of reduced cost, greater abundance, high reliability, and safety considerations, with respect to obtaining such envelope antigens from purified virus or virus-infected cells. Utility in Western blot assays and greater sensitivity are advantages over short synthetic peptide antigens that are also afforded by the polypeptides of the present invention.

In a principal aspect, the present invention relates to a polypeptide comprising at least 22 amino acids displaying at least one epitope that specifically binds to an HIV-2-specific antibody. An HIV-2-specific antibody is one that recognizes an antigen of human immunodeficiency virus type 2 (HIV-2). This polypeptide of the present invention is unable to bind to an antibody which recognizes an antigen of human immunodeficiency virus type 1 (HIV-1).

In the present case, antibodies that recognize the HIV-2 epitope of the present invention are found, for example, in human sera from individuals who are believed to have been infected with HIV-2, as illustrated in the Examples below. According to the practice of the present invention, a panel of reference sera is established which comprises human antisera that react specifically with HIV-2 antigens derived, for instance, from HIV-2 virus particles or infected cells.

According to a preferred embodiment of this aspect of the present invention, the polypeptide described above comprises about 146 amino acids that display the HIV-2 epitope, although other amino acids may be included in the polypeptide (for example, amino acids encoded by an expression vector used to produce a fusion product containing the polypeptide according to this invention).

In this preferred embodiment (exemplified by a 16 kDa polypeptide produced by the recombinant DNA clone designated pMZ921), the polypeptide includes at least one

amino acid sequence found in residue 555 through 700, inclusive, of the envelope glycoprotein gp35 of HIV-2.

In another preferred embodiment (exemplified by a 20 kDa polypeptide produced by the recombinant DNA clone designated pMZ996), the polypeptide further includes at least one amino acid sequence found in residues adjacent to either end of the residues 555 through 700, inclusive, of the envelope glycoprotein gp35 of HIV-2, namely, in the additional residues 537 through 554, inclusive, or in residues 701 through 707, inclusive, of HIV-2 gp35.

The antigenic polypeptides of the present invention were identified and produced by using a bacterial expression vector (pWS50) that was designed to identify open reading frames (orf's) in DNA segments of interest. The construction of this vector is such that DNA segments are inserted between 5' sequences encoding a short amino-terminal fragment of a gene for a bacteriophage λ protein (the *cII* gene) and 3' sequences encoding the gene for amino terminus of the *E. coli β-galactosidase protein (i.e., the Lacz* gene). Thus insertion of a DNA segment with an open reading frame in the proper translational phase results in production of a hybrid protein that is fused at its amino terminus to the *cII* fragment and at the carboxyl terminus to the β-galactosidase fragment. This hybrid protein can readily be detected by a color reaction of the β-galactosidase enzyme in recombinant bacterial colonies, combined with detection of increased size compared to the corresponding protein produced by plasmids lacking an inserted orf in the proper reading frame. Several such recombinant plasmid clones, expressing different regions of genes for the HIV-2_{NIH-Z} gp120 and gp35 proteins, were isolated by this approach, as described below in Example 1.

Once identified as clones expressing desired fusion proteins, the hybrid genes were further modified by introduction of a translation termination codon near the junction between the *env* gene insert and the β-galactosidase gene (at a *BamHI* site in the β-galactosidase gene).

This termination codon eliminated β-galactosidase amino acids that might interfere with the antigenic performance of the polypeptides.

In the case of one particular clone, which produces a polypeptide that exemplifies a preferred embodiment of this aspect of the present invention, additional modifications to the inserted *env* gene fragment were made. The fragment in this case is the 510 bp *Hpa*II-*Hpa*II fragment from the gp35 region of the *env* gene. It should be noted that before cloning this isolated fragment, it was treated with an exonuclease (Bal-31) to remove enough base pairs from the 5' end of the 510 bp *Hpa*II-*Hpa*II fragment to align the correct orf in the gp35 sequence with the orf in the *λcII* gene of the vector. Although the Bal-31 treatment was designed, in principle, to remove only one or a few nucleotides from the ends of the 510 bp fragment, leaving perfectly "blunt" double-stranded ends, it is well known in the art of genetic engineering that under similar conditions this nuclease may remove additional bases from both DNA strands before producing blunt end. Here is evidence that some such additional digestion occurred during the process of cloning of *Hpa*II-*Hpa*II *env* gene fragment in the present constructs, as outlined below. In the absence of relevant sequence data from the affected clones (pMZ921, pMZ996 and pMZ997), the precise boundaries of the *env* gene fragments originally inserted into these clones can only be defined to include at most the 510 bp between the two *Hpa*II cleavage sites. These DNA sequences are sufficient to encode about 171 amino acids of the *env* protein gp35, from residue 537-707, inclusive, according to the conventional numbering system.

The outer bounds of the amino acid sequence of the polypeptide of this aspect of this invention are further defined by additional modifications to the cloned DNA segments and observations on the polypeptides that they express. In the case of clone pMZ921, the *env* gene insert (originally 510 bp before Bal-31 treatment) was truncated at the 3' end by cleavage at a unique *StuI* restriction

site within the *env* gene fragment, thereby removing the codons for the last 7 amino acids in the original fragment. The end so cleaved by *StuI* was then ligated directly to the end of a vector DNA segment, the terminal 3 bases of which encoded an in-frame translation stop codon. Accordingly, the amino acid residue on the carboxyl terminus of the *env* protein fragment produced by pMZ921 corresponds to residue 700 of the HIV-2 gp35 polypeptide, thus making the largest *env*-related amino acid sequence that could be encoded by pMZ921 correspond to the 164 residues between residues 537 through 700, inclusive, in the HIV-2 gp35 protein.

The amino terminal end of the polypeptide exemplifying the preferred embodiment of this aspect of the invention can be surmised from expression data on clone pMZ921. This clone expresses two polypeptides that react with HIV-2-specific human antibodies, as shown in Example 2, below. One is a 20 kDa species and the other, a 16 kDa species. Analysis of the DNA sequence of the entire 510 bp *Hpa*II-*Hpa*II gp35 DNA segment reveals an internal translation initiation codon (ATG) located 10 nucleotides downstream from a probable consensus ribosome binding site (AGAG). Accordingly, the 16 kDa polypeptide of plasmid pMZ921 is believed to be translated beginning at this internal ATG, while the 20 kDa polypeptide is initiated from the ATG upstream of the *env* gene fragment, in the vector. Thus the amino terminus of the 16 kDa species is thought to begin with amino acid residue number 555, and the entire sequence of that polypeptide is believed to comprise the 146 residues 555 through 700, inclusive, of the HIV-2 gp35 protein.

This embodiment of this aspect of the present invention is also exemplified by two other clones, pMZ996 and pMZ997, which were also derived from the 510 bp HpaII-HpaII HIV-2 gp35 env gene DNA segment. After termination of translation of the β-galactosidase sequences in the fusion proteins, by insertion of a stop codon at the BamHI site in the vector (not at the StuI site of the env gene

segment), however, these two clones express only single polypeptide species of about 20 kDa, as expected from the initiation and termination sites in the vector. The lack of a shorter product could be explained by removal of the potential ribosome binding signal (AGAG) from the env gene segment by the Bal-31 treatment. Accordingly, it is believed that the amino terminus of the polypeptide of this aspect of the present invention, as exemplified by the products of clones pMZ996 and pMZ997, begins at some point corresponding to a residue higher than number 555 of the HIV-2 gp35 protein.

It will be readily understood by one skilled in the art of peptide synthesis that chemically synthesized peptides may be used advantageously as polypeptides of the present invention, especially since the synthesis of such peptides comprising 50 or more amino acids can now be achieved on scales sufficient for diagnostic purposes (in batches of 1 gram or more, for example). By progressively shortening the known maximum sequence of the HIV-2 gp35 fragment of the present invention, while testing with reference antisera for retention of sensitivity and specificity of reaction with HIV-2-specific antisera, it is clear that any peptide embedded in this sequence, which retains utility as an HIV-2 diagnostic antigen while having a sequence shorter than the presently known maximum but longer than the 21 residues of the R21S peptide mentioned above, can be readily identified.

In the polypeptide of this aspect of this invention, the amino acid sequence which displays an epitope for an HIV-2 antibody may include all or part of the same amino acid sequence responsible for displaying the functionally identical epitope on a HIV-2 gp35 protein. In some cases, a single epitope for binding a given antibody comprises more than one contiguous amino acid sequence of a polypeptide (see discussion of "discontinuous epitope", below); accordingly, the present invention contemplates that the polypeptide may include at least one amino acid sequence of a HIV-2 gp 35 protein that displays a func-

tionally homologous epitope.

In other words, both the polypeptide of this invention and an HIV-2 antigen display epitopes which are functionally homologous in that they both are able to bind the same antibody. The fact that this polypeptide and an HIV-2 protein both display functionally homologous binding sites for the same antibody does *not* imply, however, that these binding sites are displayed by the same amino acid sequence in each instance, i.e., the polypeptides displaying the two epitopes are not necessary structurally homologous at the level of amino acid sequences displaying the epitopes.

In principle, an amino acid sequence displaying an epitope for anti-HIV-2 antibodies may be selected from all the sequences in the HIV-2 gp35 protein fragment of the present invention by using known HIV-2 positive antisera and any polypeptide containing the relevant sequence of the HIV-2 gp35. For example, such antisera may be used to isolate a peptide displaying its epitope from a proteolytic digest of an HIV-2 gp35 protein by means of affinity chromatography methods that are well known in the art.

In another embodiment of this aspect of the present invention, the amino acid sequence that displays the epitope for an HIV-2-specific antibody in the polypeptide of this invention may be an analog of the amino acid sequence for that epitope on the HIV-2 gp35 protein. Such an analog may contain, for instance, naturally occurring or man-made amino acids or derivatives thereof, for instance, which provide the necessary epitope(s) for HIV-2-specific antibody binding in a sufficiently functional form to be used as an HIV-2 specified diagnostic antigen according to the practice of the present invention.

In another aspect, the present invention further relates to a process for producing an antigenic protein fragment of the viral envelope proteins of HIV-2, comprising the steps of: isolating a DNA segment from the 5' region of the transmembrane gp35 coding sequence of the HIV-2 *env* gene; inserting the segment into an open reading

frame vector whereby a recombinant plasmid is produced; introducing the recombinant plasmid into a bacterial strain; culturing said bacterial strain under conditions that allow expression of the DNA segment; and isolating the antigenic protein fragment from the bacterial strain. This method is further described in Example 1, below.

The present invention also relates to a DNA segment comprising nucleotide sequences encoding an amino acid sequence of at least 22 residues but not more than about 146 residues, where the sequence displaying at least one epitope for specifically binding an antibody that recognizes an antigen of HIV-2. This polypeptide is further characterized by the inability to bind to an antibody that recognizes an antigen of HIV-1. Further, the present invention relates to recombinant DNA molecules comprising a DNA segment of this invention and a vector, as well as to a recombinant cell transformed by such a recombinant DNA molecule. Advantageously, the cell may be bacterial, as in the Examples below, or it may be eukaryotic, including mammalian. These aspects of the invention have been referred to above in relation to identification of polypeptides of this invention, and these DNA segments are further described in Example 1.

The present invention also relates to an immunodiagnostic kit, comprising a polypeptide of the invention, as described above. As noted in the Background, such kits, which contain all the reagents needed for serodiagnosis of an infection based on detection of antibodies in human specimens, particularly blood, are one of the main forms of commercial distribution of diagnostic antigens, for example, to detect HIV-1 infection.

The present invention also relates to an antibody that is raised against an antigen of the present invention, and assays for detection of HIV-2 antigens, for instance, in blood or other tissues of HIV-2-infected individuals. A variety of standard means for raising such antibodies, including monoclonal antibodies, by using the polypeptides of the present invention as immunogens in

various animals are well known in the art of peptide immunology and thus require no further explanation here (see for instance, R. Jemmerson, et al., 1986, Bio Techniques 4:18-29 and various references cited therein). As noted above (Background) such antibodies, again most likely in the context of an immunodiagnostic kit, comprising the antibody and reagents required for its use in detecting HIV-2 antigens, are useful in situations where it is desirable to monitor the progress of an HIV-2 infection in terms of production of viral antigens.

The present invention may be understood more readily by reference to the following detailed description of specific embodiments and the Examples and Figures included therein.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the genetic organization of the HIV-2 genome and illustrates the *env* gene fragments inserted into the expression plasmids according to the present invention.

Fig. 2 shows immunologic (Western blot) analysis of recombinant *env* polypeptides expressed by plasmid pMZ921 with human test sera. Panel (A): Lanes 1-4 and 7-10 were coded HIV-2-positive West African sera, and lanes 5 and 6 were coded HIV-2-negative sera. Panel (B): Lanes 1-10 were known HIV-1-positive sera from AIDS/ARC patients. Panel (C): Lanes 1-6 are known HIV-1-and HIV-2-negative human control sera. Locations and sizes (kDa) of recombinant *env* polypeptides are indicated by arrows.

Fig. 3 shows Western blot analysis of recombinant *env* polypeptides expressed by plasmids pMZ997 and pMZ996 with human test sera. Panel (A): pMZ997: lanes 1-5, known HIV-1-positive sera from AIDS/ARC patients; lanes 6-10, HIV-2-positive sear from West African patients; and lanes 11-13, normal (HIV-1- and HIV-2-negative) human sera. Panel (B): pMZ996: Same test sera and serum sequence used in Fig. 3, panel A. Arrows show immunoreacting bands and size of recombinant *env* polypeptides.

DESCRIPTION OF SPECIFIC EMBODIMENTS

In a principal aspect, the present invention relates to a polypeptide comprising at least 22 amino acids and displaying at least one epitope that specifically binds to an HIV-2-specific antibody, which antibody recognizes an antigen HIV-2. This polypeptide is unable to bind to an antibody which recognizes an antigen of HIV-1.

The polypeptides of the present invention were developed during the course of a study involving the development of HIV-2 specific antigens via expression in *E. coli* of HIV-2 *env* polypeptides. Initial attempts to express the entire HIV-2_{NIH-Z} envelope and larger segments of the gp120 and gp35 envelope proteins were not successful. Therefore, due to similar difficulty with expressing the HIV-1 envelope (Samuel et al., 1988, Gene 64:121-134), individual gene fragments coding for specific regions of the HIV-2 envelope were selected and inserted into the cloning site in the pWS50 expression vector (Fig. 1). This work identified an antigenic region of the N-terminal portion of the HIV-2_{NIH-Z} (Zagury, JF, et al., 1988, Proc Natl Acad Sci USA 85:5941-5945) gp35 *env* protein. The recombinant polypeptides from this region are highly sensitive and specific in immunoblot assays for identifying antibodies in the sera of HIV-2-infected, but not HIV-1-infected individuals.

Several recombinant plasmid clones, expressing different regions of genes for the HIV-2_{NIH-Z} gp120 and gp35 proteins, were isolated by the method of this invention, as described below in Example 1. Each of these recombinant plasmids is shown in Fig. 1, and Table 1 (immediately following) summarizes the characteristics of the representative recombinant plasmids expressing specific HIV-2 *env* polypeptides.

Table 1. Summary and characteristics of the HIV-2_{NIH-Z} *env* expression clones

HIV-2 *env* clone	Restriction/Amino Acid coordinates	DNA insert size (bp)	*env* region	Fusion Protein size (kDa)
pMZ933	AvaI-HpaII (nc-220)[1]	746	[2]N-gp120	22
pMZ945	FokI-FokI (214-344)	391	[3]M-gp120	14
pMZ922	HaeIII-HaeIII(333-536)	608	[4]C-gp120	21
pMZ921	HpaII-HpaII(537-707)	510	N-gp35	20 and 16
pMZ997/996	HpaII-HpaII(537-707)	510	N-gp35	20
pMZ1003	StuI-EcoRV(699-856)	485	C-gp35	17

[1]nc = non-coding *env* sequences
[2]N = amino terminus
[3]M = middle
[4]C = carboxyl terminus

Each of the recombinant plasmids shown in Fig. 1 and summarized in Table 1, were introduced into the *E. coli* strain TAP56 and their fusion products were expressed and analyzed for immunoreactivity by Western blotting with human sera, as detailed in Example 2, below. Filter strips containing the resolved proteins were incubated with a 1:400 dilution of HIV-2 or HIV-1 antibody positive human AIDS/ARC sera, or with normal control human sera. The immune complexes thus formed were scored as being either weakly positive (+), strongly positive (++), or negative (-) in the immunoblots.

Only the recombinant fusion proteins expressed by clones pMZ921 (Fig. 2A), pMZ997 (Fig. 3A), and pMZ996 (Fig. 3B) that are encoded by the region encompassing amino acid residues 537 to 707 of the *env* gene, reacted strongly with anti-*env* antibodies present in sera obtained from HIV-2 infected individuals. Moreover, none of these fusion proteins reacted with HIV-1 antibody positive sera or with normal (HIV-1 and HIV-2 antibody negative) human control sera (Figs. 2B and 2C, and Figs. 3A and 3B, lanes 1-5 and 11-13, respectively).

The HIV-1 positive sera tested against these HIV-2 specific immunoreactive *env* polypeptides were previously shown to react strongly in Western blot assay and ELISA with a bacterial recombinant HIV-1 N-terminal gp41 clone 566 and with authentic gp41. Although there appears to be weak antigenic cross-reactivity between the 20 kDa but not the 16 kDa polypeptide species of the HIV-2 *env* clone pMZ921 with antibodies in HIV-1 positive sera (Fig. 2B, lanes 1-10), there is however, a several-fold higher immunoreactivity with HIV-2 positive sera, as shown by the intensely-stained bands (Fig. 2A), suggesting that both the 20 kDa and 16 kDa *env* polypeptides can specifically differentiate between HIV-2 and HIV-1 infections. The 20 kDa polypeptide species expressed by clones pMZ997 (Fig. 3A) and pMZ996 (Fig. 3B) also show high specificity for antibodies to HIV-2 *env* proteins in HIV-2 positive sera, and can also differentiate HIV-2 from HIV-1 infections. A summary of the Western blot results on the reactivity of these $HIV\text{-}2_{NIH\text{-}2}$ recombinant *env* polypeptides is presented in Table 2 (see below).

Table 2. Summary of immunoreactivity of the $HIV\text{-}2_{NIS\text{-}Z}$ *env* polypeptide by Western blot assay.

SERUM CATEGORY	SERUM NUMBER	IMMUNOREACTIVITY OF RECOMBINANT ANTIGENS 933	945	922	921*/997/996	1003
HIV-2 Pos.	1	-	-	-	++	-
	2	-	+	-	++	-
	3	-	+	-	++	-
	4	-	-	-	++	-
	7	-	-	-	++	-
HIV-1 Pos.	1	-	-	-	-	-
	2	-	-	-	-	-
	3	-	-	-	-	-
	4	-	-	-	-	-
	5	-	-	-	-	-
Normal (HIV-1 and HIV-2 Neg.)	1	-	-	-	-	-
	2	-	-	-	-	-
	3	-	-	-	-	-

*Clone pMZ921 was initially characterized against a panel of 10 coded serum samples from Dr. Phyllis Kanki (8 were HIV-2 positive and 2 HIV-2 negative), 10 known HIV-1 positive sera and 10 HIV-1 and HIV-2 negative serum samples, respectively (see Fig. 2A, 2B, and 2C).
++ = intensely immunostained band.
+ = weak immunostained band.

It is of interest to note that Schulz et al. (Schulz, TF, et al., 1989, 3:165-172) have also identified an HIV-2 specific immunoreactive domain (residues 555-761) within the same N-terminal region using bacterially expressed $HIV\text{-}2_{ROD}$ λCro-β-galactosidase fusion proteins. However, extensive cross-reactivity was observed between their immunoreactive fusion proteins and antibodies in HIV-1 positive sera. The *cII-env* fusion proteins produced by the recombinant plasmids of this invention, pMZ921, pMZ997, and pMZ996, respectively, specifically react with antibodies in human sera to HIV-2 *env* glycoproteins.

Other details and advantages of the present invention are presented in the following Examples.

Example 1. Construction and testing expression of vectors for HIV-2 envelope protein fragments.

The following materials and methods were used in this and the subsequent examples, as needed.

HIV-2 subclone. A *Sac*I restriction fragment of the HIV-2_{NIH-Z} provirus (Zagury et al., 1988, supra) was obtained as a subclone in a plasmid which was provided by Dr. Robert Gallo (Laboratory of Tumor Cell Biology, NCI, NIH, Bethesda, MD), and contains the entire *env-nef*-3' LTR sequences of the virus.

Bacterial strains and plasmids. *E. coli* strain TAP56 used in this study was obtained from Drs. T. Patterson and D. Court (Bionetics Research, Inc., NCI-FCRF, Frederick, MD). The genotype of TAP 56 is str^R *r-m+ sup*E *leu*$^-$ *thr*$^-$ *lac*Δ*4169* *gal*K_{amber} *bio*A N^+ *cI*857. The temperature-sensitive mutation (*cI*857) (Lieb, M., 196, J Mol Biol 16:149-163) in the gene coding for the λ repressor allows for the regulated expression of foreign proteins.

The bacterial expression vector pWS50 (Sisk, WP, et al., 1986, Gene 48:183-193) is a derivative of the λP_L promoter vector pJL6 (Lautenberger JA, 1983, Gene 23:75-84) containing the *E. coli LacZ* gene fused distally to and out of translational phase with the *c*II, were obtained from Dr. D. Court, Bionetics Research, Inc., NCI-FCRF), and have been described in detail elsewhere (Sisk, WP, et al., 1986, Gene 48:183-193).

Recombinant DNA techniques. Mini and large-scale plasmid DNA preparations; restriction enzyme analysis of plasmid DNA; purification of restriction DNA fragments from agarose gel slices; limited Bal-31 treatment of purified DNA fragments; Klenow Polymerase and DNA ligation reactions; and transformations of competent *E. coli* cells with ligated material, were all standard procedures following the recommendations of Maniatis et al.

(Maniatis, T, et al., 1982, Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and the product sheets supplied by the manufactures.

Construction of expression plasmids. The HIV-2_{NIH-Z} *Sac*I proviral DNA was digested separately with *Ava*I and *Hpa*II, *Fok*I, *Hae*III, *Hpa*II, *Stu*I, and *Eco*RV to obtain the specific 746bp, 391bp, 608bp, 510bp, and 485bp long *env* gene fragments, respectively. Following treatment of the DNA fragments with the slow form of the exonuclease Bal-31 (International Biotechnology), under conditions for limited digestion to generate blunt ends, the blunt-ended *env* gene fragments were ligated to the *Nru*I blunt ends of the vector pWS50, which was previously linearized with *Nru*I. This allows the open reading frame of each of the *env* gene fragments to be directly fused with the orf of the *c*II gene segment at their 5'-ends and to the *Lac*Z gene at their 3'-ends.

Isolation and characterization of recombinant plasmids. Competent *E. coli* strain TAP56 was transformed with an aliquot of each ligation mixture. Phenotypically, colonies containing recombinant plasmids with the DNA fragments inserted in the correct orf were screened for at 32°C. These appear positive blue (*Lac*Z^+) on ampicillin-containing lactose indicator (X-gal) agar plates, since the pWS50 vector harbors the *Lac*Z gene, and the *E. coli* TAP56 cells carry the lac deletion and *c*I8575+s mutation. Positive blue colonies (*Lac*Z^+) were picked and grown in 1 ml of LB broth containing 50 μg/ml Ampicillin (Amp) at 32°C and induced at 42°C. Hybrid recombinant proteins that were greater than 116 kDa, i.e., larger than the wild-type *E. coli* β-galactosidase protein to which the *env* sequences are fused, were screened for by SDS-PAGE analysis. The β-galactosidase expressing *Lac*Z^+ recombinant plasmids were further confirmed by diagnostic restriction analysis for the presence of the correct HIV-2_{NIH-Z} *env* gene fragments. Thus, the following recombinant plasmids pMZ933, pMZ945, pMZ922, pMZ921, pMZ996, pMZ997, and

pMZ1003, which carry the β-galactosidase deletion, were screened for as white colonies (*LacZ*⁻) on X-gal plates, isolated, and characterized by this approach.

Protein induction and extraction. *E. coli* TAP56 lysogens harboring the recombinant expression plasmids, were initially grown at 32°C in LB broth containing 50 μg/ml Amp in an air shaker to an optical density at 600 of ~0.5. The bacterial cultures were then shifted to 42°C, and protein induction proceeded for another 60 min. in a shaking water bath. Recombinant *env* polypeptides in the induced cell pellets were enriched for by partial purification according to the detergent extraction protocol of Krippl et al., 1984 (Proc Natl Acad Sci USA 81: 6988-6992) as previously applied (Samuel, KP, et al., 1988. Gene 64:121-134). The crude recombinant *env* polypeptides in the potassium thiocyanate pellet fraction were finally solubilized in 8M urea.

Sources of human sera. The HIV-2 positive serum samples used in this study were provided by Dr. Phyllis Kanki (Dept. of Cancer Biology, Harvard School of Public Health, Boston, MA), and were received as coded samples by K.P.S. Some of the HIV-1 positive sera from AIDS or ARC patients were provided by Dr. Larry Arthur (Program Resources, Inc., NCI-FCRF, Frederick, MD) and also from the Centers for Disease Control (Atlanta), and have previously been analyzed against recombinant HIV-1 gp41 clone 566 (Samuel, KP, et al., 1988 supra) or authentic gp41 proteins (Zweig M, et al., 1988, AIDS Res and Human Retroviruses 4:487-492). Human control (normal) serum samples were obtained from the Biological Products Lab. (NCI-FCRF, Frederick).

SDS-PAGE and Western blot analyses. The recombinant HIV-2_{NIH-Z} env gene encoded polypeptides were solubilized in 8M urea, resolved by electrophoresis on SDS-15% polyacrylamide gels (Laemmli, UK, 1970, Nature 227:680-685), and the resolved polypeptides were then electroblotted onto nitrocellulose paper (Towbin H, et al., 1979, Proc Natl Acad Sci USA 76:4350-4354). Strips of the

blotted nitrocellulose paper were cut and incubated in BLOTTO (Johnson DA, et al., 1984, Gene Anal Tech 1:3-8) for 1 hr at room temperature to block nonspecific antigen-binding sites, and then reacted with a 1:400 dilution of the human test sera in TBS (0.9% NaCl, 10mM Tris-HCl, pH7.5) containing 0.5% Tween-20 and 0.5% BSA (Zweig M, et al., 1988, AIDS Res and Human Retroviruses 4:487-492). After incubation at room temperature for 15 hr, and 3 washes with TBS, the filters were incubated with a 1:1000 dilution of peroxidase-conjugated goat anti-human IgG (Boehringer Mannheim). The immune complexes formed were then visualized by staining with a 1:1 solution of 4-chloro-1-naphthol and hydrogen peroxide (Kirkegaard and Perry Labs) as previously described (Zweig M, et al., 1988, AIDS Res and Human Retroviruses 4:487-492).

For construction and expression of vectors for various HIV-2 envelope protein fragments, the bacterial expression vector pWS50 was used in this study because it was designed to identify orf's in DNA of interest, since *c*II hybrid proteins are fused at their carboxyl terminus to the amino terminus of the *E. coli* β-galactosidase protein which can readily be screened for phenotypically as *LacZ*$^+$ blue colonies on X-gal Amp plates, and the tripartite fusion product identified on Coomassie stained SDS-PA gels by their high-level production and larger size (>116 kD) than wild type (116 kD) Several such recombinant plasmid clones, expressing different regions of HIV-2_{NIH-Z} gp120 and gp35 sequences, were isolated by this approach.

To remove the unwanted β-galactosidase sequences from the *c*II-*env* portion of the fusion products, advantage was taken of a unique *Bam*HI restriction site present in the *LacZ* gene, located 6 codons from the *Env*-β-galactosidase fusion point, to create an in-frame or frame-shift termination codon following repair and religation of the *Bam*HI ends with *E. coli* Klenow enzyme and T4 DNA ligase, respectively. The resultant recombinant plasmids, pMZ933, pMZ945, pMZ922, pMZ921, pMZ997, pMZ996, and pMZ1003, and

the origins of the *env* gene sequences they harbor, are summarized in Fig. 1. These expression plasmids were reintroduced into competent *E. coli* TAP56 cells and shown to express the predicted size *cII-env* fusion products (>116 kD) by analysis of total crude induced (42°C) cell extracts by Coomassie staining of SDS-PA gels, and by Western blot analysis against a known HIV-2 positive human serum.

A culture of TAP56 transformed with clone pMZ996 has been placed on deposit with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20852, and has been issued the ATCC deposit number 68091.

The 112 kDa β-galactosidase sequences in the recombinant fusion product expressed by the pMZ921 clone was truncated by utilizing a unique *Stu*I restriction site located at the 3'-end of the 510 bp *Hpa*II-*Hpa*II *env* gene insert of HIV-2_{NIH-Z}, which was directly ligated to the *Eco*RV blunt end of the 485 bp *Stu*I-*Eco*RV insert fragment of pMZ1003, so as to introduce an in-frame translation stop codon.

The pMZ921 clone expresses two *env* polypeptides of 20 kDa and 16 kDa, respectively. The 16 kDa species does not appear to be generated as a result of proteolytic degradation. These polypeptides most likely result from translation initiation occurring simultaneously at the *cII* gene ATG of the vector pWS50 to generate the 20 kDa species, and at an internal ATG translation initiation codon located at position 555 of gp35 to give the 16 kDa species. Moreover, a potential consensus ribosome binding site (AGAG) is also located 10 nucleotides upstream from this internal ATG. Because the 510bp *Hpa*II *env* gene fragment was modified by Bal-31 exonuclease treatment prior to construction of the pMZ997 and pMZ996 clones, it is conceivable that the inserts in these clones lack this potential ribosome binding site, since both clones express a single 20 kDa polypeptide species. Similar observations were previously reported describing reinitiated or internally initiated bacterially expressed HIV-1 *env* fusion

proteins (Crowl R, et al., 1985, Cell 41:979-986). Table 1 (above) summarizes the characteristics of the representative recombinant plasmids expressing specific HIV-2 *env* polypeptides.

Example 2: Reactivity of bacterially expressed HIV-2 *env* polypeptides with human sera.

Each of the recombinant plasmids shown in Fig. 1 and summarized in Table 1, were introduced into the *E. coli* strain TAP56 and grown at 32°C in LB broth containing 50 μg/ml Amp and induced at 42°C as described (Zweig M, et al., 1988, AIDS Res and Human Retroviruses 4:487-492). Aliquots of the urea solubilized polypeptide extracts were fractionated by SDS-PAGE on 15% gels, and the resolved proteins were electroblotted onto nitrocellulose paper. Filter strips containing the resolved proteins were incubated with a 1:400 dilution of HIV-2 or HIV-1 antibody positive human AIDS/ARC sera, or with normal control human sera, as described previously (Zweig M, et al., 1988, AIDS Res and Human Retroviruses 4:487-492). The immune complexes thus formed were detected as described, and their reactivity was scored as being either weakly positive (+), strongly positive (++), or negative (-) in the immunoblots.

The results of the immunoblot assays shown in Fig. 2 and Fig. 3, respectively, show that a HIV-2 specific antigenic region was identified, and is located at the N-terminus of the HIV-2_{NIH-Z} gp35 transmembrane protein (Fig. 2A, 3A, and 3B). Only the recombinant fusion proteins expressed by clones pMZ921 (Fig. 2A), pMZ997 (Fig. 3A), and pMZ996 (Fig. 3B) that are encoded by this region (amino acid residues 537 to 707) of the *env* gene, reacted strongly with anti-*env* antibodies present in sera obtained from HIV-2 infected individuals, recognizing 8 of 8 (pMZ921) and 5 of 5 (pMZ997 and pMZ996) of the HIV-2 positive sera tested. Moreover, neither of the fusion proteins reacted with HIV-1 antibody positive sera or with normal (HIV-1 and HIV-2 antibody negative) human control sera (Figs. 2B and 2C, and Figs. 3A and 3B, lanes 1-5 and

11-13, respectively).

The HIV-1 positive sera tested against these HIV-2 specific immunoreactive *env* polypeptides (see Fig. 2B lanes 1-10, and lanes 1-5 of Fig. 3A and 3B) were previously shown to react strongly in Western blot assay and ELISA with a bacterial recombinant HIV-1 N-terminal gp41 clone 566 and with authentic gp41. Although there appears to be weak antigenic cross-reactivity between the 20 kDa but not the 16 kDa polypeptide species of the HIV-2 env clone pMZ921 and antibodies in HIV-1 positive sera (Fig. 2B, lanes 1-10), there is however, a several-fold higher immunoreactivity with HIV-2 positive sera, as shown by the intensely-stained bands (Fig. 2A), suggesting that both the 20 kDa and 16 kDa *env* polypeptides can specifically differentiate between HIv-2 and HIV-1 infections. The 20 kDA polypeptide species expressed by clones pMZ997 (Fig. 3A) and pMZ996 (Fig. 3B) also show high specificity for antibodies to HIV-2 *env* proteins in HIV-2 positive sera, and can also differentiate HIV-2 from HIV-1 infections. A summary of the Western blot results on the reactivity of these crude HIV-2_{NIH-Z} recombinant *env* polypeptides is presented in Table 2 (see above).

A weak (+) immunogenic determinant is located on the 14 kDa recombinant *env* polypeptide expressed by clone pMZ945, which is encoded by an antigenic region located in the middle (amino acid residues 214-344) of gp120. This polypeptide recognized 2 of 5 HIV-2 positive sera tested, and did not cross-react with any of the 5 HIV-1 positive sera nor with the 3 normal human sera (Table 2). However, the immunoreaction was weak (+) when compared with the strong reactivity (++) obtained with the N-terminal region of gp35 (amino acid residues 537 to 707), which is encoded in the polypeptides expressed by clones pMZ921 (20 kDa and 16 kDa), pMZ997 (20 kDa), and pMZ996 (20 kDa), respectively.

For purposes of completing the background description and present disclosure, each of the published articles, patents and patent applications heretofore identi-

fied in this specification are hereby incorporated by reference into the specification.

The foregoing invention has been described in some detail for purposes of clarity and understanding. It will also be obvious to one of ordinary skill in the arts of genetic engineering and immunodiagnostics that various combinations in form and detail can be made without departing from the scope of the invention.

WHAT IS CLAIMED IS:

1. A polypeptide comprising at least 22 amino acids, said polypeptide displaying at least one epitope that specifically binds to an HIV-2-specific antibody, which antibody recognizes an antigen of human immunodeficiency virus type 2 (HIV-2), said polypeptide being unable to bind to an antibody which recognizes an antigen of human immunodeficiency virus type 1 (HIV-1).

2. The polypeptide according to claim 1, comprising about 146 amino acids that display said epitope.

3. The polypeptide according to claim 2, which includes at least one amino acid sequence found in residues 555 through 700, inclusive, of the envelope glycoprotein gp35 of human immunodeficiency virus type 2.

4. The polypeptide according to claim 3, which further includes at least one amino acid sequence found in residues 537 through 554, inclusive, or in residues 701 through 707, inclusive, of the envelope glycoprotein gp35 of human immunodeficiency virus type 2.

5. A process for producing an antigenic protein fragment of the viral envelope proteins of human immunodeficiency virus type 2 (HIV-2), comprising the steps of:

a. isolating a DNA segment from the 5' region of the transmembrane gp35 coding sequence of the HIV-2 *env* gene;

b. inserting said segment into an open reading frame vector whereby a recombinant plasmid is produced;

c. introducing said recombinant plasmid into a bacterial strain;

d. culturing said bacterial strain under conditions that allow expression of said DNA segment; and

e. isolating said antigenic protein fragment from said bacterial strain.

6. A DNA segment comprising nucleotide sequences encoding a polypeptide comprising at least 22 amino acids, said polypeptide displaying at least one epitope that specifically binds to an HIV-2-specific antibody, which

antibody recognizes an antigen of human immunodeficiency virus type 2 (HIV-2), said polypeptide being unable to bind to an antibody which recognizes an antigen of human immunodeficiency virus type 1 (HIV-1).

7. The DNA segment according to claim 6, wherein said polypeptide includes at least one amino acid sequence found in residues 555 through 700, inclusive, of the envelope glycoprotein gp35 of human immunodeficiency virus type 2.

8. The DNA segment according to claim 7, wherein said nucleotide sequence is included in a 438 bp segment of the *env* gene of human immunodeficiency virus type 2 bounded on the 5' end by an ATG and on the 3' end by a cleavage site for the restriction enzyme *StuI*.

9. A recombinant DNA molecule comprising the DNA segment according to claim 8 and a vector.

10. The recombinant DNA molecule according to claim 9 that is designated as clone pMZ921.

11. A cell transformed by the recombinant DNA molecule according to claim 10.

12. The DNA segment according to claim 6, wherein said polypeptide includes at least one amino acid sequence found in residues 537 through 554, inclusive, or in residues 701 through 707, inclusive, of the envelope glycoprotein gp35 of human immunodeficiency virus type 2.

13. The DNA segment according to claim 12, wherein said nucleotide sequence is included in a 510 bp segment of the *env* gene of human immunodeficiency virus type 2 bounded on both the 5' end and on the 3' end by a cleavage site for the restriction enzyme *HpaII*.

14. A recombinant DNA molecule comprising the DNA segment according to claim 13 and a vector.

15. The recombinant DNA molecule according to claim 14 that is designated as pMZ996 or as pMZ997.

16. A cell transformed by the recombinant DNA molecule according to claim 15.

17. The cell according to claim 16 wherein said cell is a bacterial cell.

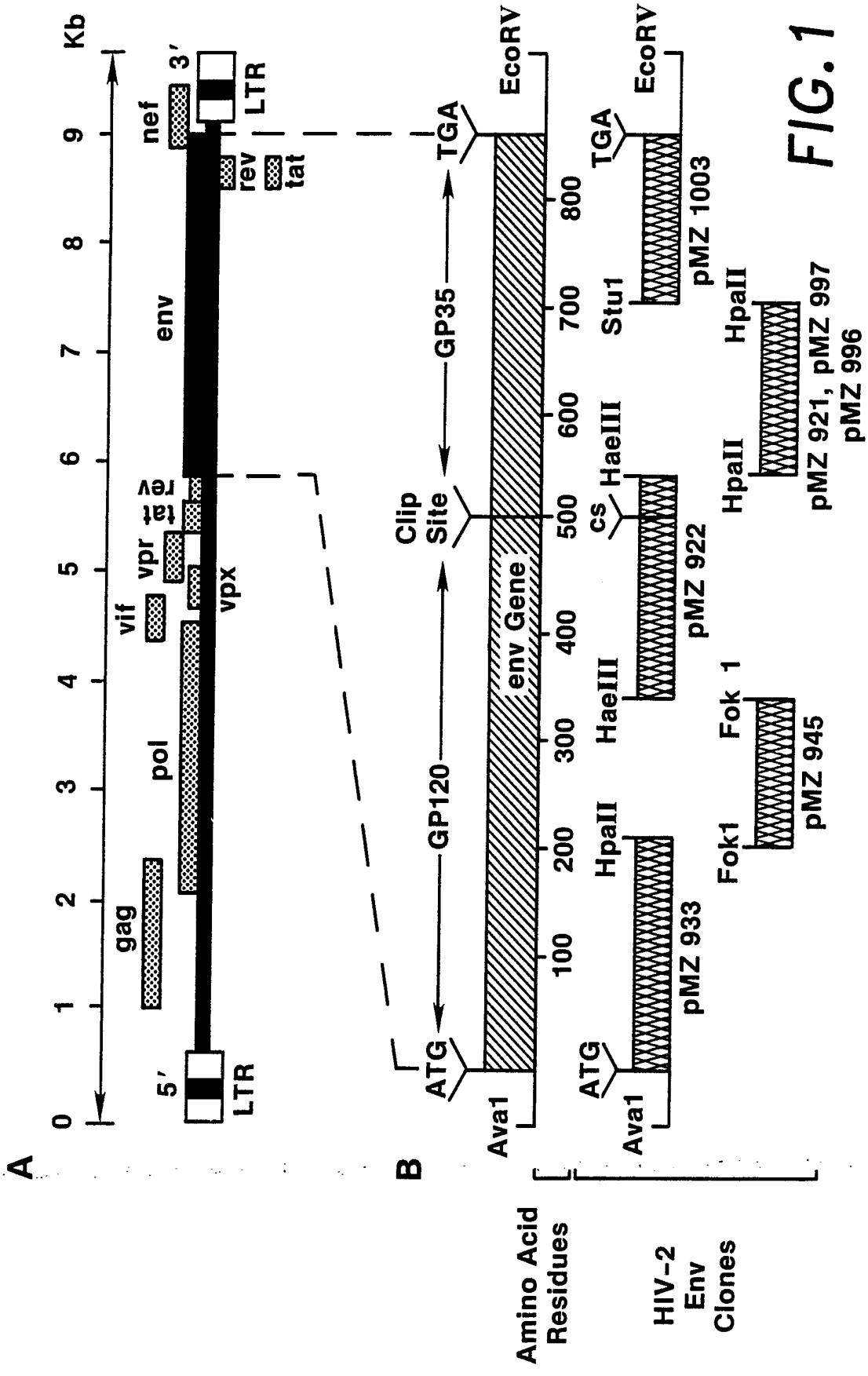
18. The cell according to claim 17 wherein said cell is *Escherichia coli* strain TAP56.

19. The cell according to claim 18, wherein said cell is transformed by clone pMZ996 and has the American Type Culture Collection deposit number of 68091.

20. An immunodiagnostic kit, comprising the polypeptide of claim 1.

21. An antibody prepared by using the polypeptide of claim 1 as an immunogen.

22. An immunodiagnostic kit, comprising the antibody of claim 22.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GP120
Clip Site
GP35
TGA
EcoRV
env Gene
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400
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600
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HpaII
HaeIII
cs
Stu1
Fok 1
pMZ 933
pMZ 922
pMZ 1003
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pMZ 996
pMZ 945
Amino Acid Residues
HIV-2 Env Clones

FIG. 1

FIG. 2A

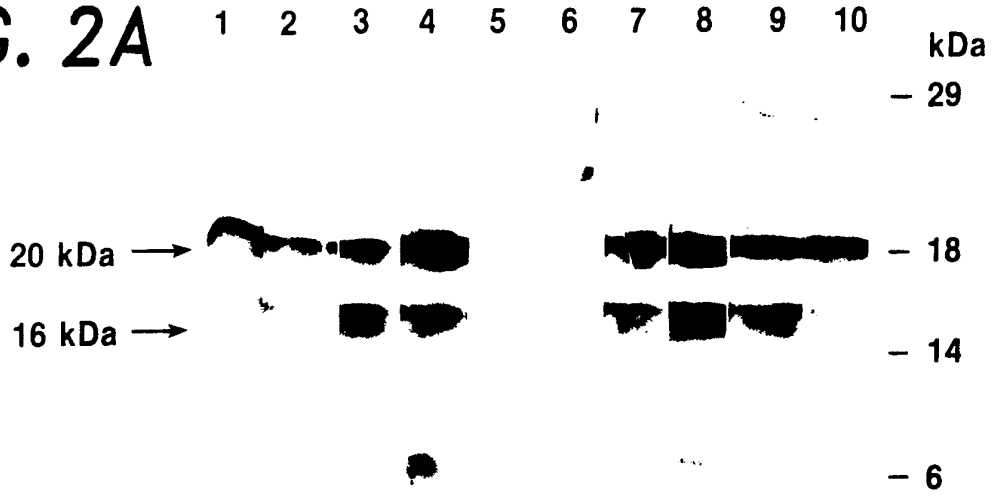


FIG. 2B

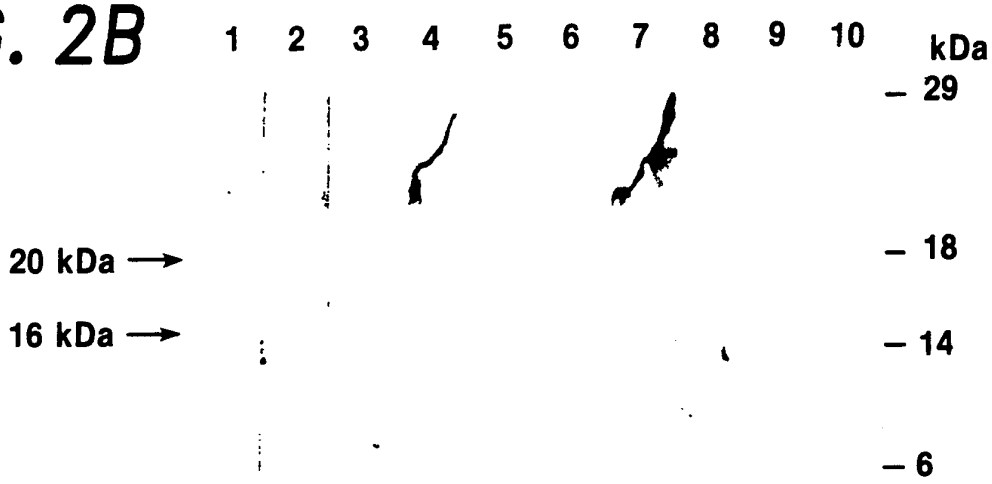
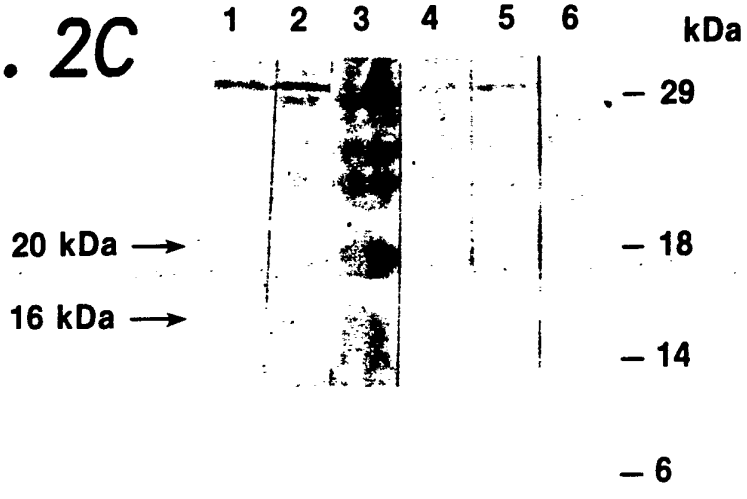


FIG. 2C



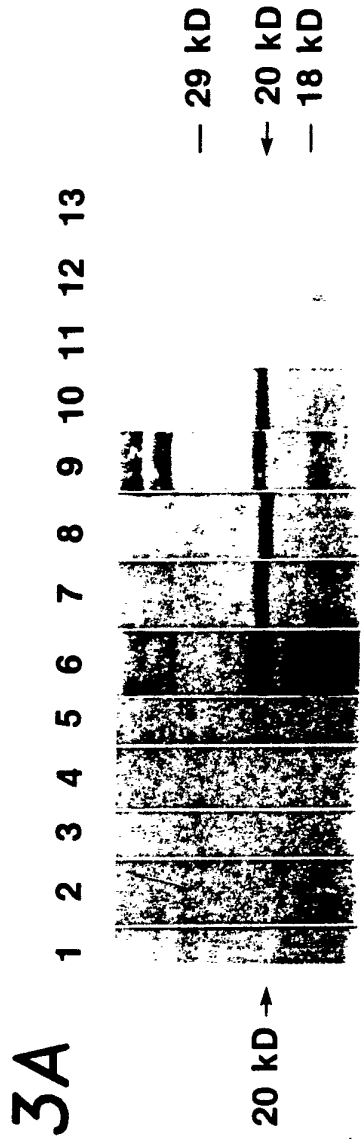

29 kD
20 kD
18 kD
1 2 3 4 5 6 7 8 9 10 11 12 13
20 kD

FIG. 3A

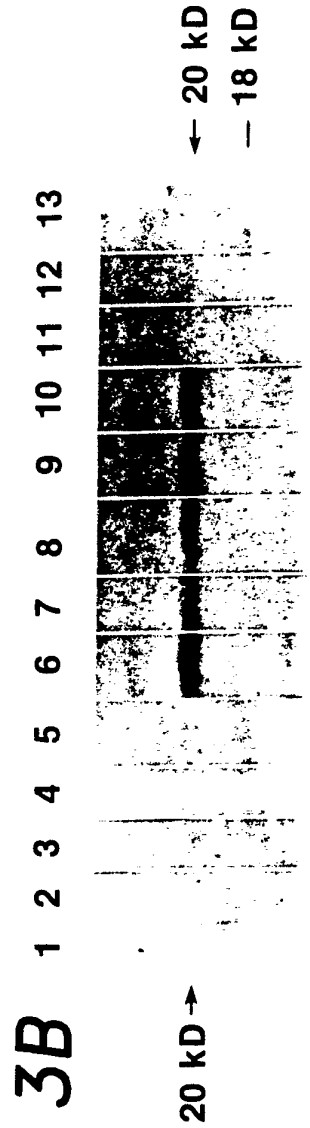

20 kD
18 kD
1 2 3 4 5 6 7 8 9 10 11 12 13
20 kD

FIG. 3B

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05149

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

Accerding to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/02; C07K 5/00; C07D 499/04; C07 H 15/12; C12 N 1/20
US. CL: 530/300, 530/328, 536/320, 536/27, 435/69.1, 252.8

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols		
	530/300	435/69.1	536/320
	530/328	536/27	435/252.8
U.S.			

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

Data bases: Dialog (files 357, 155, 399, 5, 154, 157 and 172)
Automated Patent System (File USPAT 1971-1990)

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	Proceedings National Academy of Sciences, Volume 85, August 1988, "Genetic Variability between isolates of human immunodeficiency virus (HIV) type 2 is comparable to the variability among HIV type 1" pages 5941-5945. (see entire document).	1-4
Y	Gene, Volume 48, 1986 "A plasmid vector for cloning and expression of gene segments: expression of an HTLV-1 envelope gene segment, pages 183-193, see entire document.	1-22

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document 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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]

20 November 1990

Date of Mailing of this International Search Report [2]

23 JAN 1991

International Searching Authority [1]

ISA/US

Signature of Authorized Officer [20]

Lynette F. Smith

Lynette F. Smith

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	EMBO Journal, Vol. 5, No. 11, 1986, "Subregions of a conserved part of the HIV gp 41 transmembrane protein are differently recognized by antibodies of infected individuals", pages 3051-3056, see pages 3052-3054 .	1-22
Y	Gene, Volume 64, January 1988, "Bacterial expression and characterization of nine polypeptides encoded by segments of the envelope gene of human immunodeficiency virus", pages 121-134, see entire document.	1-22

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ **OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]**

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

1. ☐ Claim numbers . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , 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 [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ **OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]**

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

See Attachment VI

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

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

3. ☐ 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 claim numbers:

4. ☒ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.