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Prospect for prevention of human immunodeficiency virus infection: Purified 120-kDa envelope glycoprotein induces neutralizing antibody

(acquired immunodeficiency syndrome/subunit vaccine/infected cell membrane/sequence)

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ABSTRACT **This study initiates an effort to develop a safe vaccine against the acquired immunodeficiency syndrome (AIDS) that is caused by infection with a retrovirus designated human immunodeficiency virus (HIV) [formerly human T-cell lymphotropic virus type III (HTLV-III)]. Other retrovirus models have shown that purified external glycoprotein subunits are immunogenic. The external envelope glycoprotein of HIV (gp120) has a molecular size of 120 kDa, is responsible for virus infectivity, and induces strong antibody response in humans. Purified HIV virus preparations contain relatively little gp120 so HIV-infected cells were used as the antigen source. The gp120 was localized on cell membranes and was solubilized with low levels of nonionic detergent. The glycoprotein was further purified by immunoaffinity chromatography over a resin prepared from IgGs isolated from patients. Homogeneity was achieved following extensive dialysis and polyacrylamide gel electrophoresis. The gp120 isolated from infected cells was shown to be structurally identical by peptide maps to virion gp120 and the amino-terminal amino acid sequence confirmed that the molecule was specified by the HIV genome. Goat, horse, and rhesus monkey (*Macaca mulatta*) immune sera to gp120 precipitated the homologous antigen and neutralized the *in vitro* infectivity of HIV. The induction of neutralizing antibody indicates that a gp120 subunit vaccine against HIV is theoretically possible.**

Human immunodeficiency virus (HIV) [formerly human T-cell lymphotropic virus type III (HTLV-III)] is a pathogenic human retrovirus that is structurally and genetically related to the subfamily Lentivirinae (1). Epidemiological studies combined with virus isolation and antibody detection in acquired immunodeficiency syndrome (AIDS) or AIDS-related complex cases, especially in blood donors and recipients, have defined the association of HIV infection to AIDS (2–5). The virus contains an RNA genome capable of coding for at least six gene products (6–9). The envelope (*env*) gene products and the internal structural gene (*gag*) products are the most antigenic in humans because antibodies to these gene products are readily detectable in patients by a variety of tests (4, 10–12). Furthermore, most sera from individuals exposed to the virus contained low levels of antibodies capable of neutralizing the *in vitro* infectivity of HIV (13, 14). We (15) and others (10, 12) have reported some of the characteristics of the HIV envelope gene products. The primary gene product of this gene is a 160-kDa glycosylated protein (gp160) that is processed by proteolysis into a gp120 external glycoprotein and a gp41 transmembrane protein (10, 12, 15).

Initial purification studies using sucrose-banded virions showed that very little gp120 was present. The levels observed were 0.06–0.10 μg/mg of virus protein. However, purification of HIV gp120 to homogeneity was possible using infected cells or cell culture fluids as the source of the glycoprotein. The purified material is immunogenic in goats, horses, and rhesus monkeys in that antibody to gp120 precipitated gp120 and neutralized the infectivity of HIV in cell culture. These results suggest that the establishment of protective humoral immunity to HIV may be theoretically possible in humans.

MATERIALS AND METHODS

Preparation of Cell Extracts. Actively growing HIV-infected H9 cells were centrifuged and suspended in labeling medium consisting of three parts RPMI 1640 containing 10% (vol/vol) fetal calf serum, one part supernatant from above (i.e., conditioned medium), and equal activities of [^{35}S]methionine and [^{35}S]cysteine. Typically, 0.7 ml of infected cells were suspended in 100 ml of labeling medium containing both [^{35}S]methionine and [^{35}S]cysteine at 15 μCi/ml (1 Ci = 37 GBq). Cells were incubated at 37°C for 4 hr, collected by centrifugation, and washed with 0.15 M NaCl/0.02 M sodium phosphate, pH 7.2 (PBS). Cell extraction methods used were comparable to a detergent-extraction method described (16). Briefly, the extraction buffer was PBS that contained 1% Triton X-100, 0.5% sodium deoxycholate (DOC), 0.1% $NaDodSO_4$, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. Cells were extracted at the ratio of 7 ml of buffer to 0.1 ml of cell pellet, and extracts were clarified (20 min at 10,000 × *g*) prior to immunoprecipitation. Generally, 1.0 ml of cell extract was treated with 0.01 ml of test serum, and immune complexes were concentrated and analyzed as described (16). Actively growing cells were collected, labeled, washed with PBS, and then gently suspended in 0.01 M sodium phosphate, pH 7.2, at the ratio of 1 ml to 0.1 ml of cell pellet. This suspension was incubated on ice for 5 min and then vigorously Vortex mixed for 15 sec. Four volumes of PBS containing 1 mM phenylmethylsulfonyl fluoride were then added to the above mixture, and the suspension was vigorously homogenized for 45 sec with a motorized Potter–Elvehjem homogenizer. The resulting homogenate was then clarified (20 min at 5000 × *g*), and the supernatant fraction was discarded. The cell membrane pellet was then resuspended in PBS containing 0.1% Triton X-100 to a final

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Abbreviations: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; DOC, deoxycholate.

volume equivalent to one-half of the final volume of the PBS extraction. That is, 0.1 ml of original cell pellet was extracted with 2.5 ml of PBS containing Triton, homogenized as above, and clarified at 100,000 × *g*. The supernatant fraction (i.e., partially solubilized cell membranes) was collected for further purification by immunoaffinity chromatography.

Immunoaffinity Chromatography. The general methods used have been reported (17). For this purification, a pool of sera was prepared from six selected AIDS patients that contained mainly antibodies highly reactive with the envelope gene products. The characterization used radioimmunoprecipitation of metabolically labeled cell extracts as well as immunoprecipitations of radioiodinated HIV p24 and bacterially expressed antigen-121 (refs. 18 and 19, and unpublished results). Precipitation of p24 measured reactivity with the *gag* gene (18), whereas precipitation of antigen-121 measured reactivity with the gp41 of the envelope gene (19). The IgG fraction of the pool was coupled to Sepharose 4B-CL. Fresh cell extracts, prepared as above, were then adjusted to 1.0 M KCl and 0.01 M Tris, pH 8.5, before mixing with the IgG-Sepharose. HIV antigens were then adsorbed to the resin overnight on a roller mill at room temperature. This resin was poured into a column and washed with three bed volumes of 1.0 M KCl, 0.01 M Tris (pH 8.5), and 0.1% Triton X-100. The column was washed with 1.0 M KCl and 0.01 M Tris (pH 8.5) until the 280-nm absorbance of the effluent was background level that indicated the Triton had been removed. The adsorbed antigens were then eluted from the resin with 4.0 M $MgCl_2$. The elution was monitored by the refractive index and 280-nm absorbance of the eluate. The eluate was then extensively dialyzed against water until a precipitate formed in the dialysis bag. The contents of the dialysis bag was clarified, and the supernatant fraction was lyophilized to dryness. This step was repeated if any turbidity remained when the lyophilized material was resolubilized in water.

Radioiodination and Two-Dimensional Oligopeptide Maps. Immunoaffinity column eluates from cell culture fluids or cell extracts were adjusted to pH 7.2 with 0.2 M sodium phosphate buffer and radioiodinated ($Na^{125}I$) using the chloramine-T method (20). The gp120s were concentrated by immunoprecipitation with human AIDS patient serum and localized on 10% polyacrylamide gels by autoradiography. Radiolabeled bands were excised from the gel and digested with α-chymotrypsin. Digests were analyzed by electrophoresis and ascending chromatography on thin-layer cellulose plates (17). Iodinated peptides were visualized by autoradiography using Kodak SB x-ray film.

Amino-Terminal Sequence Analysis of gp120. The purified gp120 was lyophilized and subjected to automated Edman degradation in an Applied Biosystems (Foster City, CA) gas-phase sequenator (21). Each cycle was derivatized to the phenylthiohydantoin amino acid with 25% (vol/vol) trifluoroacetic acid. The phenylthiohydantoin-amino acid derivatives were identified and quantitated by HPLC as described (22).

$NaDodSO_4$/PAGE Purification of gp120. The water-soluble preparation of gp120 from the immunoaffinity column eluate was purified to homogeneity using preparative $NaDodSO_4$/PAGE. Samples containing approximately 100 μg of the glycoprotein were electrophoresed on 10% polyacrylamide gels, and protein bands were visualized using the 0.25 M KCl method (23). Immediately following electrophoresis, the gel was incubated in 4°C 0.25 M KCl for 15 min. The gel was removed from this solution, wrapped in plastic, and incubated overnight at 4°C. The overnight incubation was necessary for accurate localization of the protein bands. The sensitivity of band detection was equivalent to staining with Coomassie blue G 250. A gel slice containing 100 μg of gp120 was excised, mascerated, and forced through a 200-mesh copper screen. The gel particles were suspended in PBS and used for immunizations.

Immunizations with gp120. Goat, horse, and rhesus monkey (*Macaca mulatta*) immune sera were prepared using either the water-soluble fraction from the dialyzed immunoaffinity column eluate (goat and rhesus) or PAGE-purified gp120 (goat and horse). The primary and secondary intramuscular immunizations consisted of 50 μg (goat) or 100 μg (horse) of soluble protein or protein immobilized in acrylamide gel emulsified with Freund's complete adjuvant. The secondary immunization was 21 days following the primary. All animals were bled 10 days following the last immunization. The rhesus monkeys were immunized with three doses of 20 μg of soluble gp120 emulsified with threonylmuramyl dipeptide (24). The primate immunizations were intramuscular and spaced 21 days apart.

Virus Neutralization Assay. Infectious virus was quantitated by the reverse transcriptase assay described for HIV (25), and neutralizing antibody titers were defined as the serum dilution that inhibited development of enzyme activity by 50% compared to untreated controls (unpublished results). Briefly, serum dilutions were mixed with virus and incubated 30 min at 37°C. The virus/serum mixture was then used to infect H9 cells. Virus spread, as reverse transcriptase, was measured after 7–10 days in culture.

RESULTS

Purification of HIV gp120. The objective of this project was the purification of biochemically significant quantities of the HIV gp120 external glycoprotein. In previous studies gp120 was readily detected in either cell extracts or cell-culture fluids but was not significantly present in sucrose-gradient purified virions (15). Infected cells were initially selected as the source of this protein rather than cell-culture fluids because cells contain more gp120 per unit of volume. Also, biohazard containment requirements per unit of volume for cells were easier to meet than for cell culture fluids.

To detect and locate HIV structural antigens, actively growing infected cells were metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine and extracted with the buffer containing Triton X-100, DOC, and $NaDodSO_4$. The clarified whole-cell extract was then immunoprecipitated with a patient serum that contained antibodies to both *env* and *gag* gene products. The autoradiogram of this precipitate is shown in Fig. 1*A*, lane 1. The envelope gp160 and gp120 were readily detected as well as the p55 *gag* gene precursor. The cellular pool of the p24 core protein was less than that of the precursor. This extraction method effectively solubilized a large percentage of the total cell protein including both *gag* and *env* proteins. The following gp120 extraction procedure was then devised to concentrate *env* proteins while discarding *gag* proteins. Metabolically labeled cell pellets were resuspended in hypotonic buffer (12). Following swelling, cells were disrupted by Vortex mixing, and the suspension was brought to nearly isotonic conditions by the addition of four volumes of PBS. The immunoprecipitate of the supernatant fraction is shown in Fig. 1*A*, lane 2. Appreciable levels of gp120 and gp160 were not detected, suggesting that these molecules were still associated with the cell membrane pellet fraction. Also, the majority of p55 and p24 were detected in the PBS-soluble supernatant fraction. Aliquots of the cell membrane pellet were then suspended in PBS and homogenized in the presence of Triton X-100 concentrations ranging from 0.05%–1.0% because the *env* gene products were known to be soluble in 1% Triton X-100 (15). At all concentrations tested, equivalent amounts of gp120 and gp160 were detected in immunoprecipitates of clarified homogenates. The protein patterns of immunoprecipitated radioactivity were the same as shown in Fig. 1*A*, lane 3, which shows results from the

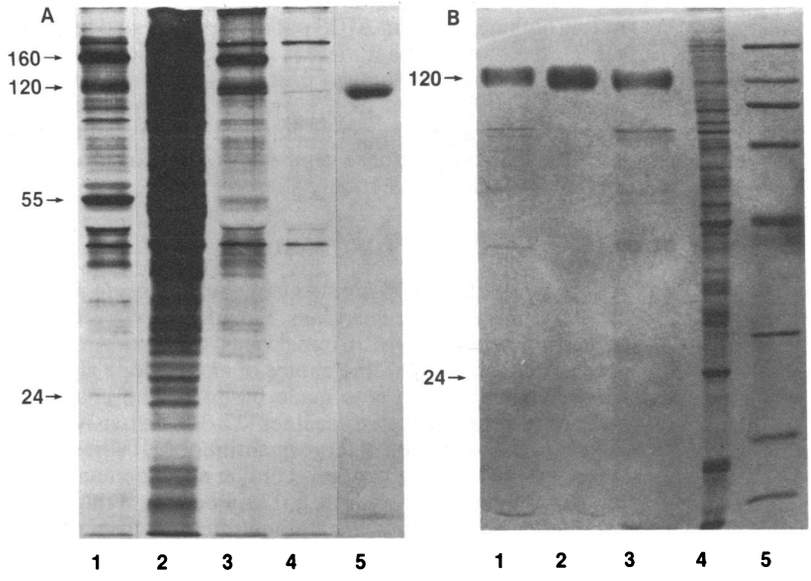

FIG. 1. Autoradiograph of immunoprecipitates of ^{35}S-labeled HIV infected cell fractions (*A*) and Coomassie blue stained gel of HIV gp120 purified from infected cells (*B*). (*A*) Lanes: 1, Triton X-100, DOC, and $NaDodSO_4$ extract of labeled cells precipitated with human serum; 2, PBS extract of labeled cells precipitated with human serum; 3, 0.05% Triton X-100 extract of labeled cells precipitated with human serum; 4, sequential 1.0% Triton X-100 extract of the pellet shown in lane 3, also precipitated with human serum; 5, immunoaffinity-purified gp120 isolated from labeled infected cells. (*B*) Lanes: 1 and 3, soluble fraction of immunoaffinity-purified HIV gp120; 2, soluble fraction of immunoaffinity-purified gp120 following extensive dialysis; 4, sucrose-banded HIV virions; 5, molecular size standards, myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase B (93 kDa), bovine albumin (68 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (21 kDa), and lysozyme (14 kDa). Each standard was present at approximately 1 μg of protein.

0.05% Triton extraction. The extraction of gp120 and gp160 from membranes was quantitative as a second extraction with 1% Triton did not yield additional immunoprecipitable antigen (Fig. 1*A*, lane 4). The unexpected low level of detergent required to solubilize gp120 necessitated an examination of the detergent extract for the presence of intact virions. The 0.05% Triton extract was subjected to centrifugation at 100,000 × *g* for 1 hr. The pellet was then solubilized in cell extraction buffer containing Triton, DOC, and $NaDodSO_4$, clarified, and immunoprecipitated. Neither envelope antigens nor core antigens were detected following conditions known to pellet intact virions. Virus antigens were detected only in the high-speed soluble fraction (data not shown). The detergent extract was then adjusted to 1.0 M KCl and 0.01 M Tris, pH 8.5, and subjected to batch immunoaffinity adsorption using a resin prepared from a serum pool of IgGs from six envelope-reactive AIDS patients. The resin was washed and then eluted with 4.0 M $MgCl_2$. The autoradiogram of the PAGE-analyzed dialyzed eluate is shown in Fig. 1*A*, lane 5. The major soluble radioactive species recovered in the eluate was gp120. Trace levels of other proteins were also detected, and significantly, no gp160 was detected even though it was present in the starting material (Fig. 1*A*, lane 3). The gp160 bound to the resin and was eluted but became insoluble upon dialysis.

The analytical purification of radiological quantities of gp120 was then expanded to isolate preparative quantities of gp120 from large-volume cell pellets. In a typical isolation, 200 ml of packed HIV-infected cells was extracted 1 ml at a time. The total final volume of PBS was 10 liters. The mixture was clarified, and the membrane pellet was then extracted with a total final volume of 5 liters of 0.1% Triton in PBS. After addition of salt and pH adjustment, the clarified soluble fraction was mixed with 300 ml of immunoaffinity resin. Following overnight batch adsorption, the resin was poured into a column, washed, and eluted. The eluate was analyzed by PAGE, and the Coomassie blue stained gel is shown in Fig. 1*B*. Lanes 1 and 3 are representative of gp120 purified as described above, and lane 2 shows the degree of homogeneity achieved following another dialysis of the lyophilized material. The second dialysis effectively removed many of the remaining impurities. The yield of purified gp120 from infected cells was approximately 2 μg/ml of packed cells. As stated earlier, sucrose-gradient purified virions do not contain appreciable Coomassie blue stainable gp120 (Fig. 1*B*, lane 4) although most preparations of purified virus do contain trace quantities detectable by the immunoblot technique (data not shown).

Identification of gp120 by Oligopeptide Maps and Amino Acid Sequence. The gp120 purified from infected cell extracts was then compared to the gp120 isolated from infected cell culture fluids using two-dimensional oligopeptide map techniques. The results shown in Fig. 2 show no differences in the chymotryptic oligopeptide maps of the gp120 from either source. The peptides detected in the gp120 map from cell culture fluids (Fig. 2*A*) were also detected in the map of gp120 from infected cells (Fig. 2*B*). The gp120 maps correspond to those obtained over one year ago, indicating structural stability of the envelope gene on simple passage (15).

To unequivocally demonstrate that the molecule characterized in this report was indeed specified by the *env* region of the HIV genome, an amino-terminal sequence analysis was performed (21). The results obtained by this analysis for the first 18 amino acid residues of the amino terminus of the envelope protein were compared to the predicted sequence of the HIV envelope gene (7). The following sequence was obtained: Thr-Glu-Lys-Leu-Trp-Val-Thr-Val-Tyr-Tyr-Gly-Val-Pro-Val-Trp-Lys-Glu-Ala and was a perfect match with the predicted sequence of the BH10 clone of HIV. The tyrosine at position 10 distinguished this sequence from the

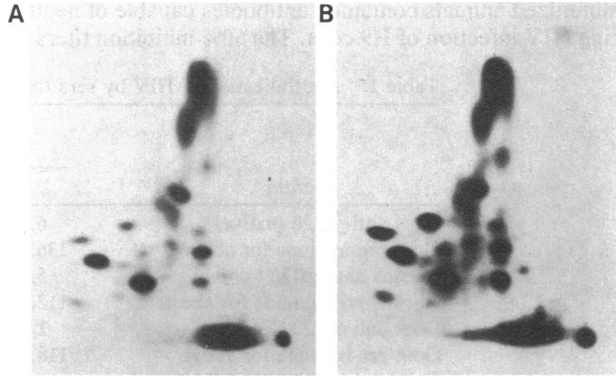

FIG. 2. Two-dimensional α-chymotrypsin oligopeptide maps of HIV gp120 purified from cell culture fluids containing virus (*A*) and HIV gp120 purified from infected cells (*B*). The origin for electrophoresis is in the lower right corner of each map.

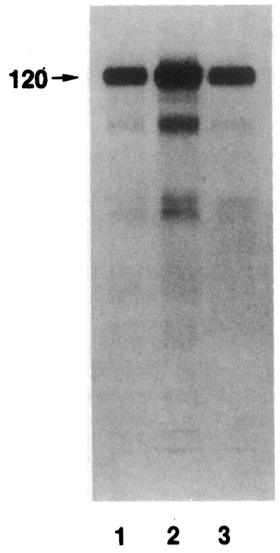

FIG. 3. Autoradiograph of ^{125}I-labeled HIV gp120 immunoprecipitated with goat anti-HIV native gp120 (lane 1), goat anti-HIV PAGE-purified gp120 (lane 2), and human AIDS serum (lane 3).

BH8 sequence (7). The gp120 was thus confirmed as specified by the genome of one HIV variant.

Induction of Precipitating and Neutralizing Antibody. To determine whether the purified gp120 retained antigenicity, a goat was immunized with an antigen preparation equivalent to that shown in Fig. 1*B*, lane 2. This preparation was designated native gp120. A second goat was immunized with PAGE-purified gp120. The goat immune sera, after only two inoculations, were then tested for the ability to precipitate radioiodinated gp120 (20). Goat antiserum to native gp120 (Fig. 3, lane 1) and goat antiserum to PAGE-purified gp120 (Fig. 3, lane 2) both precipitated radiolabeled gp120 as well as or better than a reference serum from an AIDS patient (Fig. 3, lane 3). Identical results were obtained with the horse and rhesus immune sera (data not shown). The major band precipitated by all sera was gp120 but six minor bands were also detected. They were p93, p80, p52, p46, p35, and p32. The latter two were quite faint. This observation suggested that the radioiodinated gp120 preparation contained partially degraded gp120 molecules in agreement with previous observations (15).

Finally, since antiserum directed against gp120 precipitated the homologous protein, the virus neutralization capabilities of the animal immune sera were determined. The results shown in Table 1 demonstrate that early bleed sera from all immunized animals contained antibodies capable of neutralizing HIV infection of H9 cells. The 50% inhibition titers for the animal immune sera ranged from 1:20 to 1:60 compared to 1:40 obtained from an AIDS patient serum tested under the same conditions. The different neutralization titers may reflect differences in antigen presentation or differences in animal responsiveness to the antigen. In any event, the purified external glycoprotein did elicit a virus neutralizing humoral immune response equivalent to the response observed in at least one human patient.

DISCUSSION

A vaccine against AIDS would be one where a safe product, available in significant quantities, would induce a protective immune response against an initial virus infection. Among the options considered (26), the choice of an envelope glycoprotein subunit approach was made based on the analogy to other retrovirus protective vaccines (27–31). Extensive experience in the production of large quantities of HIV has shown that purified virion preparations contain many contaminating cellular proteins and very little gp120. Because of this lack of gp120 in purified virions, membrane fractions from infected cells were used as the starting material for the large-scale isolation of the glycoprotein. The molecule was found to be soluble in water following detergent extraction from membranes. Further, the gp120 was determined to be highly antigenic in the rhesus monkey, goat, and horse. The immune sera generated after only two inoculations precipitated the challenge antigen and neutralized the *in vitro* infectivity of HIV in H9 cells. Data to be published elsewhere show that the goat anti-gp120 sera are also cytolytic to HIV-infected cells in the presence of complement (P. L. Nara, M. A. Gonda, M. Popovic, W.G.R., and P.J.F., unpublished data). This is exactly analogous to antibodies prepared in goats to the envelope gp70 of Friend murine leukemia virus (27). The immune sera prepared against HIV gp120 appear capable of both neutralizing free virus and destroying virus-infected cells, both of which may represent methods of virus transmission in nature.

It will be important to determine whether active immunity to the gp120 of one particular HIV isolate could neutralize and be protective against challenge by one or more of the distantly related HIV isolates (32, 33). With murine retroviruses, immunization of animals with a single species of mouse leukemia virus gp70 first gave rise to type-specific neutralizing antibody. With additional immunization, a broadly neutralizing antibody developed so that a late serum could neutralize a variety of virus types (27). Immune sera will be analyzed for analogous responses. Alternatively, a subunit vaccine could be composed of gp120s of several isolates to overcome the known envelope gene variability of HIV (34). Mutational changes of the lentivirus envelope glycoprotein, compatible with genetic drift, have been described in sheep

Table 1. Neutralization of HIV by sera that contain antibodies to gp120

Serum	Reverse transcriptase levels* at three serum dilutions			Inhibition titers
	1:10	1:40	1:160	
Goat anti-gp120 (native)	6,400	141,000	100,000	1:20
Goat pre-immune for above	136,000	104,000	86,000	
Rhesus anti-gp120 (native)	5,900	83,400	107,600	1:30
Rhesus pre-immune for above	112,700	122,100	99,800	
Goat anti-gp120 (PAGE)	2,100	3,600	168,000	1:60
Goat pre-immune for above	118,000	103,000	92,000	
Horse anti-gp120 (PAGE)	2,300	105,400	69,800	1:30
Horse pre-immune for above	91,300	67,700	82,000	
Human AIDS patient	1,800	86,000	130,000	1:40

*cpm of Mg^{2+}-dependent reverse transcriptase detected in virus pellets (23). The inhibition titers were determined graphically from the above values.

infected with visna virus (35) and horses infected with equine infectious anemia virus (36). The known relatedness of HIV to animal lentiviruses suggest that sequential changes in the envelope gene may be occurring during HIV infection as well. This phenomenon is anticipated and will hopefully be overcome by the induction of a very broad neutralizing and cytolytic response that will encompass all natural HIV types.

Useful vaccination requires that gp120 induce neutralizing antibodies in the rhesus monkey before the critical experiments are performed in the chimpanzee challenge model. The chimpanzee is susceptible to infection by HIV and readily makes detectable antibody following infection (37). Then, chimpanzees demonstrating HIV gp120 neutralizing antibodies would be challenged with homologous cell-free virus or virus-infected cells. If protection were observed following homologous challenge, immune animals would be challenged with variant HIV isolates either as free virus or virus-infected cells. The response of the immunized chimpanzees should be predictive of whether primary HIV infection can be prevented in humans through immunity to HIV gp120.

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1. Gonda, M. A., Wong-Staal, F., Gallo, R. C., Clements, J. T., Narayan, O. & Gilden, R. V. (1985) *Science* **227,** 173–177.
2. Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G. M., Kaplan, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P. & Markham, P. D. (1984) *Science* **224,** 500–503.
3. Shaw, G. M., Hahn, B., Arya, S. K., Groopman, J. E., Gallo, R. C. & Wong-Staal, F. (1984) *Science* **226,** 1165–1171.
4. Sarngadharan, M. G., Popovic, M., Bruch, L., Schüpbach, J. & Gallo, R. C. (1984) *Science* **224,** 506–508.
5. Safai, B., Groopman, J. E., Popovic, M., Schüpbach, J., Sarngadharan, M. G., Arnett, K., Sliski, N. A. & Gallo, R. C. (1984) *Lancet* **i,** 1438–1440.
6. Wain-Hobson, S., Sonigo, P., Danos, O., Cole, S. & Alizon, M. (1985) *Cell* **40,** 9–17.
7. Ratner, L., Haseltine, W., Patarca, R., Livak, K. J., Starcich, B., Josephs, S. F., Doran, E. R., Rafalski, J. A., Whitehorn, E. A., Baumeister, K., Ivanoff, L., Petteway, S. R., Jr., Pearson, M. L., Lautenberger, J. A., Papas, T. S., Ghrayeb, J., Chang, N. T., Gallo, R. C. & Wong-Staal, F. (1985) *Nature (London)* **313,** 277–284.
8. Muesing, M. A., Smith, D. H., Cabradilla, C. D., Benton, C. V., Lasky, L. A. & Capon, D. J. (1985) *Nature (London)* **313,** 450–458.
9. Sodroski, J., Patarca, R., Rosen, C. A., Wong-Staal, F. & Hazeltine, W. A. (1985) *Science* **229,** 74–77.
10. Allan, J. S., Coliga, J. E., Barin, F., McLane, M. F., Sodroski, J. G., Rosen, C. A., Haseltine, W. A., Lee, T. H. & Essex, M. (1985) *Science* **228,** 1091–1093.
11. Barin, F., McLane, M. F., Allan, J. S., Lee, T. H., Groopman, J. E. & Essex, M. (1985) *Science* **228,** 1094–1096.
12. Veronese, F. D., DeVico, A. L., Copeland, T. D., Oroszlan, S., Gallo, R. C. & Sarngadharan, M. G. (1985) *Science* **229,** 1402–1405.
13. Weiss, R. A., Clapham, P. R., Cheingsong-Popov, R., Dalgleish, A. G., Carne, C. A., Weller, I. V. D. & Tedder, R. S. (1985) *Nature (London)* **316,** 69–72.
14. Robert-Guroff, M., Brown, M. & Gallo, R. C. (1985) *Nature (London)* **316,** 72–74.
15. Robey, W. G., Safai, B., Oroszlan, S., Arthur, L. O., Gonda, M. A., Gallo, R. C. & Fischinger, P. J. (1985) *Science* **228,** 593–595.
16. Robey, W. G., Kuenzel, W. J., Vande Woude, G. F. & Fischinger, P. J. (1982) *Cancer Res.* **42,** 2523–2531.
17. Robey, W. G., Dekaban, G. A., Ball, J. K., Poore, C. M. & Fischinger, P. J. (1985) *Virology* **142,** 183–196.
18. Sarngadharan, M. G., Bruch, L., Popovic, M. & Gallo, R. C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3481–3484.
19. Chang, N. T., Huang, J., Ghrayeb, J., McKinney, S., Chanda, P. K., Chang, T. W., Putney, S., Sarngadharan, M. G., Wong-Staal, F. & Gallo, R. C. (1986) *Nature (London)* **315,** 151–154.
20. Elder, J. H., Jensen, F. C., Bryant, M. L. & Lerner, R. A. (1977) *Nature (London)* **267,** 23–28.
21. Hewick, R. M., Hunkapiller, M. M., Hood, L. E. & Dryer, W. J. (1981) *J. Biol. Chem.* **254,** 7990–7997.
22. Henderson, L. E., Copeland, T. D. & Oroszlan, S. (1980) *Anal. Biochem.* **102,** 1–7.
23. Hager, D. E. & Burgess, R. R. (1980) *Anal. Biochem.* **109,** 76–86.
24. Allison, A. C. & Schreiver, A. B. (1985) in *Monoclonal Antibodies and Cancer Therapy*, eds. Reisfeld, R. A. & Sell, S. (Liss, New York), pp. 397–411.
25. Popovic, M., Sarngadharan, M. G., Read, E. & Gallo, R. C. (1984) *Science* **224,** 497–500.
26. Fischinger, P. J., Robey, W. G., Koprowski, H., Gallo, R. C. & Bolognesi, D. P. (1985) *Cancer Res.* **45,** 4694–4699.
27. Schäfer, W. & Bolognesi, D. D. (1977) in *Contemporary Topics in Immunobiology*, eds. Hanna, M. A., Jr., & Rapp, F. (Plenum, New York), Vol. 6, pp. 127–167.
28. Hunsmann, G., Schneider, J. & Schulz, A. (1981) *Virology* **113,** 602–612.
29. Lewis, M. G., Mathes, L. E. & Olsen, R. G. (1981) *Infect. Immun.* **34,** 888–894.
30. Hunsmann, G., Pedersen, N. C. & Theilen, G. H. (1983) *Med. Microbiol. Immunol.* **171,** 233–241.
31. Osterhaus, A., Weijer, K., Uytdehaag, F., Jarrett, O., Sundquist, B. & Morein, B. (1985) *J. Immunol.* **135,** 591–596.
32. Wong-Staal, F., Shaw, G. M., Hahn, B. H., Salahuddin, S. Z., Popovic, M., Markham, P., Redfield, R. & Gallo, R. C. (1985) *Science* **229,** 759–762.
33. Benn, S., Rutledge, R., Folks, T., Gold, J., Baker, L., McCormick, J., Feorino, P., Piot, P., Quinn, T. & Martin, M. (1985) *Science* **230,** 949–951.
34. Hahn, B. H., Gonda, M. A., Shaw, G. M., Popovic, M., Hoxie, J. A., Gallo, R. C. & Wong-Staal, F. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4813–4817.
35. Clements, J. E., Pederson, F. S., Narayan, O. & Haseltine, W. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4454–4458.
36. Montelaro, R. C., Parekh, B., Orrego, A. & Issel, C. J. (1984) *J. Biol. Chem.* **259,** 10539–10544.
37. Gajdusek, D. C., Amyx, H. L., Gibbs, C. J., Jr., Asher, D. H., Rodgers-Johnson, P., Epstein, L. G., Sarin, P. S., Gallo, R. C., Gilden, R. V., Maluish, A., Arthur, L. O., Montagnier, L., Mildva, D. & Mather-Wagh, V. (1985) *Lancet* **i,** 55–56.