tor and other proteases in vitro (*24*) support this possibility. In addition, the recent structural identification of the hst/KS oncogene from a human stomach tumor (*25*) and Kaposi sarcoma (*26*), and the *int*-2 oncogene from a human epithelioma (*27*) may be relevant to the issue of angiogenic helper genes because both oncogenes have significant sequence similarities to HBGF-I and HBGF-II (*2*).

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Autologous Red Cell Agglutination Assay for HIV-1 Antibodies: Simplified Test with Whole Blood

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An antibody detection procedure based on agglutination of autologous red cells has been developed for samples of whole blood. A nonagglutinating monoclonal antibody to human red blood cells conjugated to a synthetic peptide antigen (in this case residues 579 to 601 of the HIV-1 envelope precursor, Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys) permitted the detection of antibodies to the human immunodeficiency virus type 1 (HIV-1) in 10 microliters of whole blood within 2 minutes. Agglutination was specifically inhibited by addition of synthetic peptide antigen but not by unrelated peptides. The frequency of false positive results was 0.1% with HIV-1 seronegative blood donors (n = 874). The false negative results were approximately 1% (n = 81). The autologous red cell agglutination test is potentially suitable for simple, rapid, qualitative screening for antibodies to a variety of antigens of medical and veterinary diagnostic significance.

METHODS FOR DETECTION OF individuals infected with HIV-1 provide a vital key in attempts to curtail the spread of the virus, particularly by way of blood product contamination. In addition, the need to protect health care personnel has highlighted the demand for simple, rapid, inexpensive, and specific tests for the presence of HIV-1 antibodies.

The diagnostic tests already developed to detect HIV-1 antibodies in infected patients include a variety of enzyme-linked immunoassays (EIA), protein immunoblot (Western blot) procedures, and radioimmune precipitation assays (RIA) (*1*). Such procedures are relatively slow, requiring at least 3 hours to complete. Gelatin particle and latex agglutination tests have also been reported (*2*), and these are more rapid but cannot be used on whole blood. We sought to develop a simple qualitative procedure for use with small samples of whole blood that would take 2 min or less to complete. Historically, red cell agglutination has been a widely used technique in serology and immunology (*3*), but these procedures require the use of heterologous red cells coated with a variety of antibodies or antigens. In the test we describe here, the patient's own red cells are used to provide a detection system for antibodies. A nonagglutinating monoclonal antibody (mAb) to human red blood cells is chemically cross-linked to a synthetic peptide antigen. When this conjugate (30 μl) is added to 10 μl of the patient's blood, specific agglutination of the patient's red cells occurs in the presence of antibodies to this antigen. The conjugate reagent consisted of 0.2 mg/ml of conjugate and 2.5 mg/ml of blocking mAb in phosphate-buffered saline containing 10 m*M* sodium azide. The agglutination reaction was done on a plastic plate and scored visually after 2 min on a scale of 4 to 0, where 0 represents no agglutination.

The mAb was prepared by immunizing mice with washed human red blood cells (*4*) and selecting only those antibodies that bound to red blood cells but did not cause agglutination without the addition of antibody to mouse immunoglobulin G (IgG). In the presence of the rabbit antibody to mouse IgG, the mAb to red blood cells caused agglutination of all human red blood cells tested, including those from patients with red cell disorders, and was independent of blood group (*4*). It did not recognize red cells from other species. The synthetic pep-

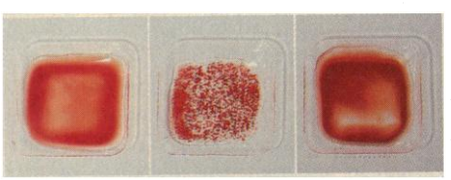

Fig. 1. Agglutination of a HIV-1 seropositive patient's red cells. The synthetic peptide–conjugated mAb to red blood cells causes agglutination (middle well). There is no agglutination in the presence of either unconjugated mAb (left) or with conjugated mAb in the presence of excess (100 μg/ml) free synthetic peptide antigen (right).

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tide antigen derived from gp41 of HIV-1 (residues 579 to 601) was chosen on the basis of the Welling procedure (*5*) and corresponds with the region identified as a major epitope recognized by antibodies from approximately 98% of AIDS patients (*6*). This epitope region has also been studied extensively by Gnann *et al.* (*7*). The synthetic peptide (*8*) was conjugated with the mAb to red blood cells by means of the heterobifunctional reagent *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) (*9*). The degree of substitution of the mAb influenced the solubility of the conjugate; 20 mol of peptide per mole of conjugate became insoluble. The range of 5 to 7 mol of peptide per mole of antibody was optimal. The capacity of the conjugate to bind red blood cells was monitored by using the agglutination test with rabbit antibody to mouse IgG and with HIV-1–positive whole blood collected in heparin. The response was not diminished by storage of the HIV-1–positive whole blood for 24 hours at room temperature.

The addition of the peptide-mAb conjugate to whole blood causes the red cells to become coated with synthetic peptide antigen. For HIV-1 seropositive patients, the presence of antibody to the gp41 peptide causes agglutination of the patients' red cells on addition of mAb conjugate as shown in Fig. 1. No agglutination occurs if unconjugated mAb is used or conjugated mAb in the presence of an excess of free synthetic peptide antigen (Fig. 1). The results of testing of seropositive and seronegative patients as well as healthy blood donors are given in Table 1. In an initial survey, 39 seropositive patients were all detected, but in the follow-up study, one seropositive patient out of 43 failed to give detectable agglutination. In this latter study, agglutination was specifically inhibited by saturating concentrations of free peptide antigen, confirming that agglutination was specific for the peptide with blood from seropositive patients. A false negative level of approximately 1 to 2% has been reported for a similar peptide antigen used in an enzyme immunoassay (*6*). Thus the level of false negative results reflects the properties of the peptide antigen used. In the present study, a blocking mAb antibody was used to prevent false positive agglutination due to antibody to mouse IgG. The false positive rate was 0.1% (n = 874) with healthy blood donors compared to 0.2% for the same samples when tested with the commercial test (Genetic Systems) used by the local blood transfusion service. Apparent false positive reactions with the autologous agglutination test were observed more frequently in hospitalized patients than in healthy blood donors (Table 1). In the hospitalized patients agglutination was inhibited with added free peptide. The cross reactivity to the synthetic peptide observed in false positive cases is not entirely unexpected since antibodies cross-reacting with an HTLV-1–specific peptide have been observed in patients with leukemia and rheumatoid arthritis, and in patients who have received multiple transfusions (*10*).

To evaluate the antigenic specificity of the agglutination we tested a series of synthetic peptides corresponding to other regions of the HIV-1 envelope proteins for their capacity to inhibit the agglutination reaction (Table 2). No unrelated peptide competed, and the synthetic gp41(572–591), which is missing the essential COOH-terminal epitope region (*6*), did not inhibit agglutination. The comparison of agglutination in the presence and absence of added synthetic antigen was useful in aiding in the detection of the occasional weak positive samples. Conversely, failure of added synthetic peptide to inhibit agglutination would suggest the presence of cross-reactive antibodies against the antibody to red blood cells.

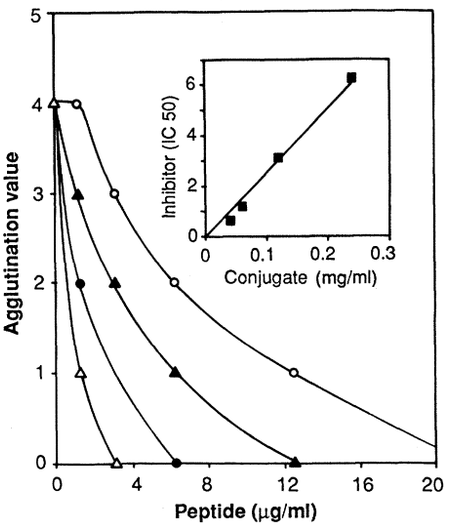

Fig. 2. Inhibition of agglutination with synthetic antigen. Synthetic peptide antigen gp41(579–601) was added to the conjugated mAb to red blood cells as indicated. The final concentration of the conjugated mAb was 0.24 (○), 0.12 (▲), 0.06 (●), and 0.03 (△) mg/ml, respectively. The agglutination assay was performed as described in the text. Agglutination values are given on the ordinate and are scored visually from 4 to 0 for intensity of the agglutination reaction where 0 represents no agglutination.

The lower limits of sensitivity of antibody detection tests can normally be estimated by titration. In the autologous red cell agglutination test, the use of red cells in whole blood precludes direct measurement of antibody titer. A simple alternative estimate of the level of antibody can be obtained by determining dose responses for peptide antigen inhibition of agglutination (Fig. 2). The concentration of peptide antigen required to achieve 50% of the maximum inhibition of agglutination was approximately linear and dependent on the concentration of peptide-mAb conjugate. The dose dependence of the mAb conjugate concentration also affords a means of assessing the apparent titer for the agglutination response.

Table 1. Autologous red cell agglutination test. The agglutination test was performed as described in the text. Blood samples from patients previously shown to be HIV-1 positive were retested by protein immunoblot assay by the Victorian State Reference Laboratory. Hospitalized patients were negative either by EIA (Abbott Laboratories, recombinant indirect ELISA) or protein immunoblot and had been admitted to hospital with a variety of medical conditions. Blood donors were tested by EIA (Genetic Systems) (*12*). False positive or negative values are given in parentheses and were verified by protein immunoblot analysis except for the single blood donor (*1*) who was negative by EIA.

Subjects	Agglutination test		EIA test	
	+	−	+	−
HIV-1–positive patients	42	(1)	43	0
Hospitalized patients	(3)	63	0	66
Healthy blood donors	(1)	873	(2)	872

Table 2. Specificity of peptide inhibition of agglutination. Synthetic peptide (0.125 mg/ml) was added to the conjugated mAb before the whole blood. The agglutination test was performed as described in the text. Common sequences are underlined.

Peptide	Added synthetic peptide (sequence)	Inhibition of agglutination (%)
	None	0
gp41(579–601)	RILAVERYLKDQQLLGIWGCSGK	100%
gp41(572–591)	GIKQLQARILAVERYLKDQQ	0
gp120(193–200)	ASTTTNYT	0
gp120(105–117)	HEDIISLWDQSLK	0
gp120(101–118)	VEQMHEDIISLWDQSLKP	0
gp120(105–129)Y^{129}	HEDIISLWDQSLKPAVKLTPLCVSY	0

These results indicate that an autologous red cell agglutination test can be used as a sensitive and specific test for the presence of circulating antibodies. The procedure is flexible and could be tailored to detect a variety of antibodies for which there was an appropriate antigen for conjugation to the red blood cell antibody. In using the autologous red cell agglutination test for antibodies to HIV-1 it will be essential to use a conjugated antigen with a very low false negative rate. This may require the use of a mixture of peptide epitopes or soluble recombinant protein antigens. It is also possible that the test may be automated, just as automation has been achieved for the gelatin bead agglutination test (*2*) and many turbidimetric or nephelometric assays (*11*). Preliminary results indicate that the conjugated antibody is stable to freeze-drying and can be air-dried onto plastic plates. For field testing, a positive control mAb to the synthetic peptide antigen would be included as a means of quality control for the reagent.

At present the major advantages of the autologous red cell agglutination test are its speed and simplicity. For these reasons it is likely to have significant applications as a "front line" test. In this role it will be an important adjunct to conventional test procedures and may also be useful for assessing vaccination programs. It is hoped that the autologous agglutination test will contribute to the protection of health care workers and aid in the control of the spread of AIDS.

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8. Synthetic peptides were synthesized by the Merrifield procedure [R. S. Hodges and R. B. Merrifield, *Anal. Biochem.* **65**, 241 (1975)] with the aid of an Applied Biosystems Model 430 synthesizer using double coupling cycles supplied by the manufacturer. The *N-t*-butyloxycarbonyl amino acid derivatives were obtained from the Protein Research Foundation (Osaka, Japan). Side chain protection was the same as supplied by Applied Biosystems with the exception of arginine for which the ω-NO_2 derivative was used. Chain assembly was monitored with ninhydrin [V. Sarin *et al.*, *Anal. Biochem.* **117**, 147 (1981)]. The assembled peptides were simultaneously cleaved and deprotected by using anhydrous HF containing 10% anisol (v/v) [J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis* (Freeman, San Francisco, 1966), pp. 44 and 66]. The crude peptide was precipitated with diethylether and washed with ethyl acetate before being extracted with 60% acetonitrile in 0.1% trifluoroacetic acid (v/v). Synthetic peptides were purified by preparative reversed phase chromatography (Amicon C_{18} resin, 250 Å pore size 25 × 400 mm) with a gradient of 1000 ml, 0 to 60% acetonitrile in 0.1% trifluoroacetic acid. The synthetic peptide was approximately 95% pure as judged by analytical reversed-phase high-performance liquid chromatography and by quantitative amino acid analysis after acid hydrolysis.
9. Purified monoclonal antibody (10 mg) was treated with SPDP (110 μg) in phosphate-buffered saline (PBS), *p*H 7, for 60 min. Untreated SPDP was removed by chromatography on Sephadex-G25 using the same buffer. The derivatized antibody contained approximately 6 mol of propyldithiopyridine per mole of antibody as indicated by reduction in the presence of dithiothreitol. The synthetic peptide (3 mg) was reduced in buffer containing 0.1*M* tris-HCl, 1 m*M* EDTA, 4*M* guanidine hydrochloride, 160 m*M* 2-mercaptoethanol, *p*H 8, for 90 min at room temperature. The reduced peptide was recovered by batch chromatography on a Sep-pak (Waters Associates) and rotary evaporation from 60% acetonitrile in 0.1% trifluoroacetic acid. The reduced peptide was dissolved in 0.6 ml of 4*M* guanidine hydrochloride and immediately added to 6 mg of the derivatized antibody in 3.0 ml of 0.1*M* PBS, *p*H 7, containing 100 m*M* NaCl. After overnight incubation the degree of peptide substitution was measured spectrophotometrically at 343 nm. The conjugated antibody was separated from unreacted peptide by chromatography on Superose-12 using a Pharmacia FPLC system equilibrated with PBS. The fractions across the protein peak were assayed by the red cell agglutination assay, bulked, and concentrated in dialysis tubing with powdered Ficoll. Complete separation of the peptide-mAb conjugate from unreacted peptide and aggregated conjugate was essential. This procedure is reproducible, and nine independent peptide-mAb conjugates were prepared and found to be active in agglutinating seropositive patients' red blood cells. Active conjugate was also prepared by using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester as the cross-linking reagent.
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Selection of Variable-Joining Region Combinations in the α Chain of the T Cell Receptor

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Most T lymphocytes express an antigen-specific receptor composed of two subunits, α and β, each of which can exhibit structural variability. A complex selection process operates on T cells during development in the thymus such that cells expressing only particular αβ-receptors migrate to the periphery. The α-chain repertoire was dissected at different stages of the selection process by using the polymerase chain reaction (PCR) technique to amplify only those transcripts of a particular variable region gene (V_{58}). Sequences from these V_{58} cDNAs reveal the predominant expression of four joining (J) segments by T cells in the adult thymus, suggesting that molecular or cellular processes select particular $V_\alpha J_\alpha$ combinations during development. T cells expressing one of these $V_{58}J_\alpha$ chains appear to have been negatively selected at a later stage, since these transcripts were present in the spleen at approximately one-tenth the level in the thymus. Results also indicate that residues present at the $V_\alpha J_\alpha$ junction may be important in an early selection process.

T LYMPHOCYTES RECOGNIZE A FOREIGN antigen only when it is presented with a cell surface product of the major histocompatibility complex (MHC). The T cell receptor (TCR) responsible for this dual recognition is composed of two subunits, α and β, each generated by somatic rearrangement of multiple gene segments (*1, 2*). However, before a T lymphocyte becomes a functional antigen-specific cell in the peripheral lymphoid system, it undergoes selection in the thymus. The selection process, which operates on the αβ-heterodimer, guarantees that a T cell will react with a product of the MHC (a phenomenon resulting in MHC "restriction") and that it will not react with "self" antigens (a phenomenon resulting in self tolerance). The observation that >95% of cells in the thymus die (*3*) and never reach the periphery has long been suggested to be, at least in part, the result of this process.

Despite the progress that has been made in identifying the TCR, the structural basis of MHC restriction remains unknown. It has now been established that the αβ-heterodimer on mature T cells (helper and cytotoxic) is responsible for binding both antigen and MHC product on the presenting cell (*4*). In most cases, attempts to correlate

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