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# Antibodies Reactive with Human Immunodeficiency Virus *gag*-Coded Antigens (*gag* Reactive Only) Are a Major Cause of Enzyme-Linked Immunosorbent Assay Reactivity in a Blood Donor Population

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**Normal blood donors were examined for human immunodeficiency virus (HIV)-reactive antibodies with both virus- and *Escherichia coli*-expressed *env*- and *gag*-coded antigens. The frequency of samples from normal (low-risk) donors that were repeatedly reactive with an HIV enzyme-linked immunosorbent assay blood screening test (Du Pont Co.) was 0.6%. Two classes of HIV serological reactivity were identified: a minor *env*-reactive class (0.03 to 0.06% of donors) and the predominant *env*-nonreactive *gag*-reactive class (*gag* reactive only [GRO]) (0.4 to 0.5% of donors). Assignment of *env* reactivity was made by a synthetic (recombinant) *env* enzyme-linked immunosorbent assay and virus immunoblot. Most GRO sera reacted with p15/p17 bands on HIV immunoblot. Antibody specificity in GRO sera was confirmed by competition-binding studies with viral *gag* and *E. coli*-expressed p55$^{gag}$. This study provides independent verification that *gag*-specific antibodies are present in many *env*-nonreactive sera. More serological and virological studies of individuals with this antibody pattern should be pursued to determine the origin of these *gag*-reactive antibodies.**

Enzyme-linked immunosorbent assays (ELISAs) with disrupted purified virus (3, 27) have proved valuable for screening blood for evidence of exposure to human immunodeficiency virus (HIV), previously known as human T-cell lymphotropic virus type III (HTLV-III)/lymphadenopathy-associated virus or acquired immunodeficiency syndrome-associated retrovirus. It has been suggested that immunoblot reactivity to either antigen p24$^{gag}$ or antigen gp41$^{env}$ be used to validate the HIV ELISA (4–6, 11). Classification of the serum as HIV positive is relatively straightforward if, during such confirmatory testing, evidence of *env*-reactive antibodies is obtained.

Sera will often reproducibly test positive by HIV ELISA but will not show clear evidence of *env*-specific antibodies when reexamined by viral immunoblot. During screening of blood donors, we and others (2, 10, 32, 34) frequently detect reactivity on immunoblots which use HIV-derived antigen at positions of *gag*-coded antigens (p15, p17, p24, or p55) unaccompanied by detectable immunoblot reactivity with envelope bands. The classification of such putative *env*-nonreactive *gag*-reactive sera is difficult. False-negative results for *env*-specific antibodies are possible. There are several reports (11, 19, 20) which demonstrate that the HIV immunoblot technique does not detect *env*-specific antibodies as readily as *gag*-specific antibodies in the early stages of HIV seroconversion. One report (19) shows that reproducibility of detection of gp120$^{env}$ antibodies by immunoblot is affected significantly by variations in the procedures used for preparation of the immunoblot strips. Additionally, we have found in a collaborative study (A. J. Saah, H. Farzadegan, T. H. Lee, S. R. Petteway, C. R. Rinaldo, J. P. Phair, and J. L. Fahey, Abstr. III Int. Conf. AIDS 1987, T.3.1, p. 53) that a standard immunoblot procedure fails to detect *env*-specific antibodies that are present in many serum samples taken during the early stages of infection of HIV-exposed individuals. Related observations have been reported elsewhere (12, 35). In our study, we found that an *env*-specific ELISA (ENV9 ELISA) was more sensitive than HIV immunoblot and similar to radioimmunoprecipitation procedures for detection of *env*-specific antibodies. Because of this demonstrated sensitivity of the ENV9 ELISA, it was used for analysis of donor serum samples in the current study.

In truly *env*-nonreactive sera there are other problems of classification. Contaminating antigens from cells used to culture the virus may affect both HIV ELISA and HIV immunoblot (10, 32). Additionally, the validity of using reactivity to *gag*-coded antigens alone for classifying sera as HIV positive is dubious because of the lack of specificity of this criterion (2, 10, 20, 28, 34). Given the immunological cross-reactivities between *gag*-coded antigens of HIV and those of other viruses, such *gag*-specific immunoreactivity may suggest exposure either to HIV or to other retroviral or retroviruslike antigens (8, 9, 13–15, 20, 21, 26, 27, 34, 36).

Published data on which to base a satisfactory classification of sera showing an *env*-nonreactive *gag*-reactive immunoblot profile are thus not yet available, and our study attempted to address this lack of information. We focused on a subset of blood donor serum samples defined by the following three criteria: (i) they were repeatedly reactive on an HIV ELISA blood screening test, (ii) the presence of HIV *gag*-reactive antibodies in the samples could be confirmed with a source of *gag*-coded antigen that is unequivocally free from human tissue antigenic contamination, and (iii) the subset of sera lacked evidence of *env*-specific antibodies on

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both HIV immunoblot and the recombinant-antigen-based ENV9 ELISA. We refer to this subset of sera as *gag* reactive only (GRO). Additionally, the results of both HIV immunoblots and ENV9 ELISA were used to define the HIV-positive class of sera as the *env*-reactive class.

We used recombinant DNA-derived proteins as antigens with several different techniques for antibody detection to allow a more critical evaluation of the presence of *gag*-reactive antibodies. For a high percentage of the more than 200 HIV ELISA-reactive serum samples that we have examined, there is unequivocal evidence of antibodies that react or cross-react with HIV *gag*-coded antigens unaccompanied by evidence of HIV *env*-specific antibodies.

## MATERIALS AND METHODS

**Serum samples.** Initial questionnaires and counseling, with an emphasis on voluntary self-exclusion, were used to screen out groups known to be at high risk for exposure to HIV. The reactive serum samples were gathered at Blood Bank of Delaware blood donor centers during 1986, mainly from June to October. The HIV ELISA repeatedly reactive rate in these samples was approximately 0.6%, and the HIV-positive rate was 0.03 to 0.06%. Serum was stored at −70°C before testing, and repeated freezing and thawing were avoided.

**Serology.** A preliminary classification of all serum samples was based on repeated tests with an HIV ELISA (HTLV-III ELISA; Du Pont Co.) blood screening kit and on the results of HIV immunoblot testing carried out by a commercial laboratory (Biotech Research Laboratories). Samples were further tested for reactivity to envelope antigens with an *Escherichia coli*-expressed antigen ENV9-based ELISA (16, 22). The 3.5-h assay described in the Du Pont HTLV-III ELISA blood screening kit was the HIV ELISA procedure used to test serum. Additional HIV immunoblot analysis was carried out with commercially available HIV immunoblot nitrocellulose strips (Du Pont).

The recombinant antigens ENV9, GAG55, and GAG55A were derived by molecular cloning from HIV HTLV-IIIB (23). Antigen ENV9 (16, 22) corresponds to an immunodominant region of HIV *env* containing both gp120 and gp41 amino acid sequences coded by nucleotides (23) from coordinates 7196 to 8053. Purification of ENV9 and methods relating to the ENV9 ELISA were carried out as previously described (16).

Antigens GAG55 and GAG55A are very similar to one another and are produced from vectors that were designed to express a peptide corresponding to the complete *gag* open reading frame (D. Tribe and B. Ferguson, unpublished data). Crude preparations of GAG55 or GAG55A (30 ng of bacterial protein per strip) were used to test the *gag*-specific immunoreactivity of serum samples by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot methods (18, 33). For immunoblot analysis, nitrocellulose strips containing antigen were preincubated for 30 min at room temperature in 500 μl of Blotto buffer (5% dried nonfat milk solids, phosphate-buffered saline, 0.05% Tween 20, 0.04% normal goat serum). The strips were then incubated at 4°C overnight with a dilution (generally 1/25) of sample serum. They were then washed exhaustively with phosphate-buffered saline–0.05% Tween 20, and bound antibody was detected with biotinylated anti-human immunoglobulin conjugates and avidin D-horseradish peroxidase. Preabsorption of sera for competition experiments involved incubation of 10 to 20 μl of serum with 1 to 3 volumes of *E. coli* extract (containing 5 mg of bacterial protein per ml) for 2 h at 25°C followed by centrifugation to remove insoluble material.

Purification of GAG55 for ELISA and preparation of ELISA plates were as previously described (16). The specificity of the final GAG55 ELISA plates was checked by competition experiments with panels of human serum and with *E. coli* or GAG55 as the competing antigen. Some evidence of minor reactivity with contaminating *E. coli* proteins was evident in a few samples, and extracts of *E. coli* (500 ng of protein per ml of diluent) were included in diluents to block this reactivity. Other procedures were largely as specified for the commercial HIV ELISA kit, except that the serum was diluted 1/100 for analysis of GAG55 ELISA reactivity.

Anti-*gag* monoclonal antibodies BT2 and BT3 were obtained from Biotech Research Laboratories, and antiimmunoglobulin conjugates were obtained as follows: alkaline phosphatase–anti-human immunoglobulin G (IgG) from Du Pont; peroxidase-conjugated anti-human IgG, IgA, and IgM from Organon Teknika; biotinylated anti-human IgG (heavy and light chain specific); and biotinylated anti-human IgM μ chain and avidin D-horseradish peroxidase from Vector Laboratories.

## RESULTS

**Characterization of recombinant antigens.** The recombinant antigens used in this study were obtained by insertion of regions of HIV HTLV-IIIB into *E. coli* expression vectors. *gag*-coded antigens GAG55 and GAG55A are essentially identical protein sequences covering the complete *gag* open reading frame and thus are similar antigenically to viral protein p55. GAG55A differs from GAG55 in having two amino acids inserted after the N-terminal methionine. Gel electrophoresis has shown that crude extracts of both GAG55 and GAG55A contain a major Coomassie blue band corresponding to the expected 55-kilodalton *gag*-coded antigen.

Both GAG55 and GAG55A react with mouse monoclonal antibodies BT2 and BT3 that are specific for HIV p17 and p24, respectively. They also react with polyclonal rabbit antiserum raised against HIV p24 and with many human HIV-positive sera. Results with both GAG55 and GAG55A demonstrate that antigenicity is associated with an approximately 55-kilodalton protein and some smaller antigenic fragments arising in *E. coli*.

To further characterize our GAG55 and GAG55A antigens, experiments involving competition between recombinant and viral *gag*-coded antigens for reaction with serum antibodies were carried out (27). Six HIV-positive human serum samples were tested by a competition immunoblot technique for reactivity with GAG55A antigen as follows. Serum samples were preabsorbed with GAG55A extract and, in parallel, with a control *E. coli* extract. These absorptions were followed by analysis of the samples with HIV antigen strips. With each HIV-positive sample tested, recombinant *gag*-coded polyprotein was found to substantially if not completely outcompete reactivity on all *gag*-related immunoblot bands (see Fig. 2 for a typical result of such a competition experiment). The specificity of this competition effect was verified by lack of demonstrable competition of *env*- or *pol*-coded bands and by lack of effect of control *E. coli* extracts on any HIV immunoblot band. As a whole, our observations on these HIV-positive samples were taken as evidence that most antibodies in these samples reacting with HIV *gag*-coded immunoblot bands also react with GAG55A antigen.

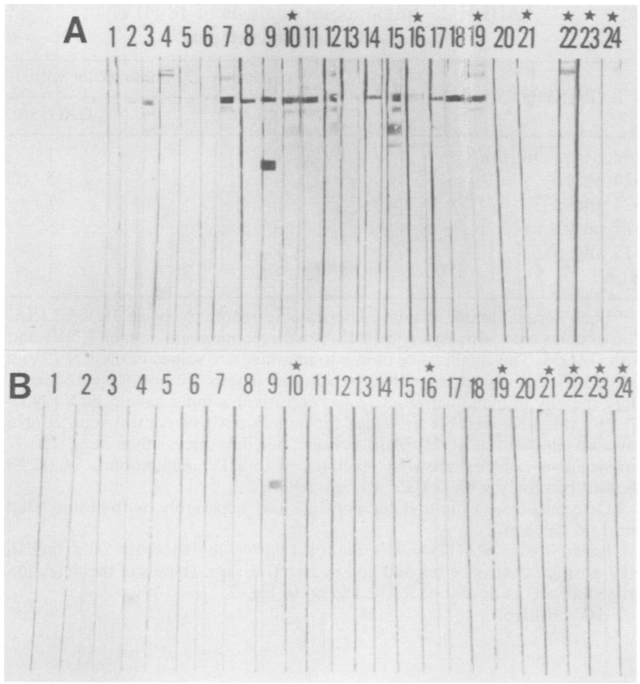


FIG. 1. Immunoblot analysis of HIV ELISA-reactive *env*-nonreactive sera. One GAG55A immunoblot strip (A) and one *E. coli* extract control strip (B) were developed for each serum analyzed. Immunoreactivity with human sera was detected with biotinylated anti-human IgG, IgA, and IgM followed by avidin D-peroxidase-chloronaphthol. Control sera were also tested, and the corresponding strips are starred for clarity and are as follows: strips 10 and 16, monoclonal antibodies BT2 (p17 specific) and BT3 (p24 specific), respectively, detected with anti-mouse IgG; strip 9, HIV-positive human serum; strips 21, 22, and 23, normal human sera; strip 24, HTLV-I-positive human serum. The positive control sera define the expected positions of *gag*-coded bands corresponding to a 55-kilodalton major band plus smaller-molecular-mass bands.

**Analysis of HIV ELISA-reactive sera.** Preliminary tests revealed that many HIV ELISA-reactive serum samples displayed reactivity on HIV immunoblots only at *gag*-coded bands, commonly p15/p17 and less frequently p24 or p15/p17 and p24. Review of donor information did not suggest that HIV ELISA-reactive donors were predominantly from high-risk groups. A follow-up analysis of these serum samples was made with recombinant antigens ENV9 and GAG55 (16, 22) by both immunoblot and ELISA formats. We have used the ENV9 ELISA to test a wide range of HIV-exposed individuals, including those with acquired immunodeficiency syndrome and acquired immunodeficiency syndrome-related complex, asymptomatic HIV-positive individuals, and individuals in the early stages of seroconversion (16, 22; Saah et al., Abstr. III Int. Conf. AIDS 1987) and find it to be a highly sensitive indicator of the presence of *env*-reactive antibodies. Similar synthetic antigens have been reported by others for the detection of HIV-positive sera (7, 28). The follow-up tests with recombinant antigens ENV9 and GAG55 confirmed the occurrence of an *env*-nonreactive *gag*-reactive class of sera and also made unambiguous identification of HIV-positive samples in the population possible.

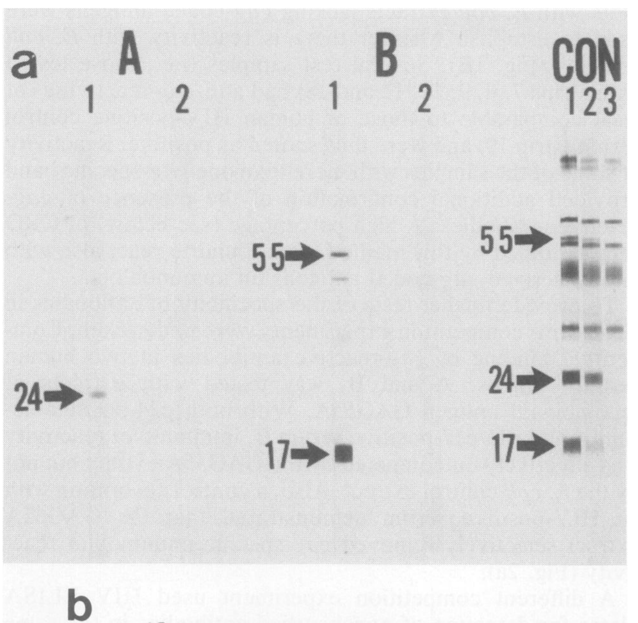
Figure 1 shows a typical result of tests of *env*-nonreactive HIV ELISA-reactive serum samples by immunoblot with recombinant GAG55A antigen. Control reactions with monoclonal antibodies established the position of *gag*-specific bands, including the 55-kilodalton band. Immunoblots with *E. coli* extracts lacking *gag*-coded antigens were used to establish whether there is reactivity with *E. coli* proteins (Fig. 1B). Several test samples (i.e., those tested with strips 7, 8, 9, 11, 12 and 18) had anti-*gag* reactivities at least comparable to those of human HIV-positive control serum (strip 19) and were thus scored as positive. Reactivity of some of the samples with more than one *gag*-specific band provided additional confirmation of the presence of *gag*-reactive antibodies. A high percentage (see below) of GRO sera identified by this method were found to react also with virally derived *gag*-coded antigens on immunoblots.

To provide further tests of the specificity of antibodies in GRO sera, competition experiments were undertaken. Competitive binding of *gag*-reactive antibodies in two human serum samples, A and B, was tested with extracts of recombinant antigen GAG55A. With both p24-positive serum A and p15/p17-positive serum B, immunoblot reactivity was effectively outcompeted by the GAG55A extract but not by the *E. coli* control extract. Also, a control absorption with an HIV-positive serum demonstrated that the GAG55A extract selectively removed *gag*-specific immunoblot reactivity (Fig. 2a).

A different competition experiment used HIV ELISA plates for detection of nonabsorbed antibodies in the same two GRO serum samples. In this case, the antigen extract used for preabsorption was GAG55, but very similar results have been obtained with GAG55A in other experiments. With both serum A and serum B, GAG55 antigen extract substantially decreased the ELISA absorbance compared with the control value (Fig. 2b). Numerous additional experiments showed that there is no interference in the ELISA by control *E. coli* extracts even at dose levels twofold higher than that used in this assay. Similar immunoblot and ELISA competition results have been obtained with many other GRO sera. These competition results, taken with the previously presented GAG55A immunoblot reactivity (Fig. 1), provide strong evidence for the presence of *gag*-reactive antibodies in *env*-nonreactive HIV ELISA-reactive sera.

**Surveys of different subsets of blood donors.** The reaction patterns of a small set of HIV ELISA-reactive serum samples, which were taken from a representative sample of HIV ELISA-reactive blood donors and included at least one HIV-positive donor, were determined. Assays on the samples included HIV and recombinant ELISAs as well as immunoblot analysis with HIV and GAG55A strips (Table 1). Both ENV9 ELISA and immunoblot data were in agreement and indicated that only one serum was HIV *env* reactive. There was considerable agreement between HIV immunoblot and recombinant GAG55A immunoblot; 76% (13 of 17) of the *env*-nonreactive samples were reactive with both HIV *gag*-coded bands and GAG55A-specific bands. One sample in this group was interesting. It gave a stronger IgM-specific HIV immunoblot, and it is possible that it reflected recent exposure to immunogen (1).

Further surveys of *gag* immunoblot reactivity were made with other, larger sets of serum samples and different procedures for detection. For example, a group of 47 HIV ELISA-reactive samples from which (based on HIV immunoblot and ENV9 ELISA testing) known HIV-positive serum was excluded was tested at a 1/25 dilution in an HIV immunoblot using biotinylated second antibody and avidin D-peroxidase detection. Of these samples, 91% (43 of 47) were found to be *gag* reactive, as evidenced by p15/p17 or p24 bands, and 85% (40 of 47) gave bands only at p15/p17. By



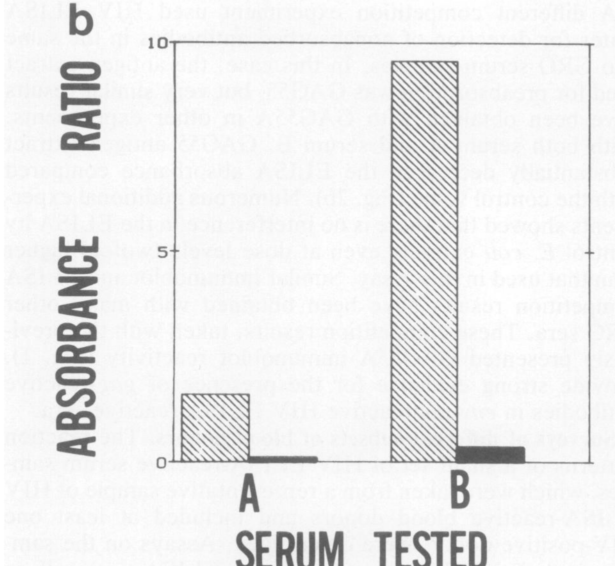




FIG. 2. (a) HIV immunoblot analysis of GRO sera demonstrating competition by recombinant *gag*-coded polyprotein GAG55A. Paired immunoblots (A and B) of representative GRO sera are shown with an immunoblot of an HIV-positive serum (CON). Strip 1 of each pair was preabsorbed with *E. coli*, and strip 2 was preabsorbed with a GAG55A extract (10 μl of serum per 20 μl of extract). For the HIV-positive control serum, two GAG55A absorptions were made (strip 2, 10 μl of serum per 10 μl of extract; strip 3, 10 μl of serum per 20 μl of extract). Arrows indicate the positions of HIV *gag*-coded bands p15/p17, p24, and p55. (b) HIV ELISA analysis of GRO sera demonstrating competition by recombinant *gag*-coded polyprotein GAG55. The GRO sera analyzed were the same as those shown in panel a, and the preabsorption conditions were similar. The values shown are the means of triplicate determinations with each extract. Symbols: ▨, control *E. coli* extract absorption; ■, GAG55 absorption. The absorbance ratio is the ratio of the ELISA value of the sample to that of an HIV-positive control.

TABLE 1. Immunoblot analysis of 18 HIV ELISA-reactive serum samples[a]

| Protein(s) | No. reactive[b] by immunoblot with: | |
|---|---|---|
| | HIV antigen | GAG55A[c] |
| *env*, *gag*, and *pol*[d] | 1 | 1 |
| p24 only | 4 | 3 |
| p24 and p17 | 1 | 1 |
| p17 only | 9 | 8 |
| p17 and p55 | 1 | 1 |
| None | 2 | 1 |

[a] Each serum sample displayed repeatable reactivity in an HIV ELISA. The samples were also tested in ELISAs with recombinant antigen ENV9 and with GAG55. The significance of low to intermediate values in the ENV9 and GAG55 ELISAs is yet to be determined, and details are not presented here. Only the *env* immunoblot-reactive (seropositive) sample gave a high ENV9 ELISA value comparable to values for human positive control sera. It also gave an elevated GAG55 ELISA value, as did three other sera. Of 17 *env*-negative samples showing reactivity with HIV immunoblot, 14 (82%) showed reactivity with GAG55A immunoblot.

[b] Determined with biotinylated anti-IgG and, separately, with biotinylated anti-IgM (μ chain).

[c] Reactivity to GAG55A was ranked relative to reactivity of a human HIV-positive control serum and known negative sera. Details of the detection procedure are as described in the legend to Fig. 2.

[d] HIV positive.

means of a relatively insensitive testing procedure (second antibody-horseradish peroxidase conjugate), clear evidence of GAG55A immunoblot reactivity was found in 18 of 47 of these serum samples, and all 18 were *gag* reactive by HIV immunoblot. In contrast, in similar tests of 100 HIV ELISA-nonreactive serum samples, no definite *gag* reactivity was detected by GAG55A immunoblot.

More evidence demonstrating the occurrence of anti-*gag*-coded antibodies was obtained by using purified GAG55 antigen in an ELISA format. The reactivity of 57 HIV ELISA-reactive *env* (virus immunoblot and ENV9 ELISA)-coded-antibody-negative blood donor serum samples was compared with the reactivity of 69 normal control samples and also with that of 31 HIV-positive samples. The HIV-positive samples were largely from patients with clinically classified acquired immunodeficiency syndrome. In the reactive blood donor group, 44% of the GAG55 ELISA values were greater than any of those in the normal control group and were similar to the greater-than-normal GAG55 ELISA values exhibited by several HIV-positive samples.

It has not yet been possible to do a follow-up analysis on blood samples taken later from many of the HIV-equivocal donors in this study. Second or third blood samples taken as late as November 1987 from 37 HIV ELISA-reactive *env*-nonreactive donors have been investigated, and none were found to be HIV positive. In the HIV ELISA, 26 of 37 were reactive, and 17 of 37 had *gag* reactivity on HIV immunoblot.

## DISCUSSION

The HIV ELISA blood test involves a virus preparation which of necessity is an antigenic mixture. Some components (see Introduction) of this mixture are expected to display immunological cross-reactivity, and it is quite likely that samples in which HIV ELISA reactivity is not caused by HIV exposure could be identified. Key questions in classifying sera that are reactive by the HIV ELISA include

whether all HIV-positive sera have been identified and whether the immunoreactivity measured by the test is relevant to infection with HIV or due to non-HIV-associated cross-reactivities. We have addressed these questions by supplementing conventional HIV immunoblot testing with a range of tests employing *E. coli*-expressed *env*- and *gag*-coded antigens to provide greater specificity in detection of HIV-reactive antibodies. A demonstration that the HIV ELISA signal is caused by antibodies that bind to HIV *gag*-coded antigens may have epidemiological significance, even if it is not possible to confirm that the antibodies are caused by HIV exposure.

Our study has enabled us to identify a subset of HIV ELISA-reactive blood donor serum samples (GRO) that display unambiguous evidence of *gag*-specific antibodies but that as a group are distinct from HIV-positive samples in that they lack detectable *env*-specific antibodies. These GRO sera are negative for *env*-specific antibodies by both recombinant ENV9 ELISA and HIV immunoblot. Under relatively sensitive conditions for detection of antibodies by immunoblot, a high percentage of HIV ELISA-reactive serum samples in this study were found to react with viral *gag*-coded bands (Table 1), and a high percentage (76%) were found to react with both viral immunoblot *gag*-coded bands and with *E. coli*-expressed GAG55A antigen bands. With typical GRO sera, competitive binding of antibodies to HIV antigen and *E. coli*-expressed *gag*-coded polyprotein was demonstrated by both immunoblot and ELISA techniques (Fig. 2). The specificity of competitive binding by the *E. coli*-expressed GAG55A antigen was confirmed in tests with HIV-positive sera (Fig. 2). These tests demonstrated direct binding of antibodies to both recombinant and viral *gag*-coded antigens and competition for binding between *E. coli*-expressed antigen and viral antigen. This provides strong evidence that *gag* reactivity from GRO sera on viral immunoblots is due to reactivity with HIV *gag*-coded antigens and not to human tissue (H9 cell) antigen contamination (17, 32). Confirmation of the presence of *gag*-specific antibodies in HIV ELISA-reactive *env*-nonreactive sera was also obtained when purified GAG55 antigen is used in an ELISA format.

These observations led us to conclude that antibodies which are able to specifically bind to HIV *gag*-coded antigen were present in many of the HIV ELISA-reactive blood donor serum samples that we have examined and that a major portion of HIV ELISA reactivity was caused by these antibodies. HIV ELISA-reactive sera were approximately 0.6% of the total population. With different detection methods and sample sets, various values were obtained for the fraction of these HIV ELISA-reactive serum samples that classified as GRO, but representative results indicate that 77% (14 of 18) HIV ELISA-reactive samples were GRO by HIV immunoblot (Table 1). Results of tests on other, larger sets of HIV ELISA-reactive samples and tests using different testing procedures (including GAG55 antigen; see above) were consistent with this percentage. Using these considerations as well as data for the HIV ELISA-reactive rate and the HIV-positive rate in this population, we estimated the following approximate frequencies of serum classifications as percentages of the total donor population: GRO, 0.4 to 0.5%; HIV ELISA reactive HIV immunoblot negative, 0.1 to 0.2%; HIV positive, 0.03 to 0.06%. It should be noted that these frequencies may not apply to serum samples detected with HIV ELISA from other sources, since there are significant differences in the immunological reactivity of viral antigens and formatting of HIV ELISA tests from different sources (4, 24).

Many of the GRO sera we have examined gave a reaction with the viral p15/p17 immunoblot band (Table 1). We have evidence from antibody competition binding experiments with an *E. coli*-expressed antigen corresponding to the p17 protein (D. Tribe, unpublished data) that most of this reactivity is directed towards the N-terminal *gag* protein p17.

There are several candidates for the possible immunogenic stimulus for the *gag*-reactive antibodies we observe in GRO sera, including endogenous retroviruses, other tissue antigens, and exogenous viruses. Thus, cross-reactivity between core antigens and serum samples from infected subjects has been reported to occur among the human viruses HIV, HIV type 2 (lymphadenopathy-associated virus type II), and HTLV-IV and the monkey viruses simian T-lymphotropic virus type III (African green monkey) and simian T-lymphotropic virus type III (macaque) (8, 9, 13–15, 27). Immunological cross-reactivity of *gag*-coded antigens between HIV and the lentivirus equine infectious anemia virus has also been reported (21). In studies in which we have identified GRO sera in other blood donor populations (N. L. Dock, H. V. Lamberson, T. A. O'Brien, S. R. Petteway, S. Alexander, and B. J. Poiesz, Abstr. III Int. Conf. AIDS, 1987, WP.226, p. 148), viral immunoblot procedures have detected antibodies to HTLV-IV *gag*-coded antigens and also some reactivity to HTLV-I *gag*-coded antigens. Previous work with other retroviral antigens (25, 29–31) document the presence of *gag*-reactive antibodies in human sera and association of these antibodies with disease, and in particular autoimmune disease such as rheumatoid arthritis, in which a retroviral etiology is suspected (25).

This study documents the existence and frequency of antibodies that bind to the *gag*-coded antigens of HIV in samples obtained from a blood donor population from which high-risk individuals were excluded. A number of workers (11, 19), primarily using HIV immunoblot methods, have described the early stages of HIV infection as characterized by exclusive or greater production of *gag*-specific antibody. The possibility that GRO samples in the current study correspond in some cases to the early stages of HIV infection (exposure) should thus be given serious consideration. In our study of a high-risk population (A. Saah et al., Abstr. III Int. Conf. AIDS 1987), 29 of 30 early-HIV seroconversion samples that were GRO by HIV immunoblot analysis were found to be *env* reactive, and thus not GRO, when ENV9 ELISA or radioimmunoprecipitation (gp120/gp160) techniques were used. Thus the GRO samples described here are distinct from both early-HIV seroconversion samples as identified in a high-risk population and from typical HIV-positive samples in patterns of reactivity in the ENV9 ELISA. The GRO sera of the current study were all *env* nonreactive by both ENV9 ELISA and HIV immunoblot, whereas HIV-exposed sera that we have tested, including early seroconversion samples, are almost invariably ENV9 ELISA reactive. On that basis, we consider the antibodies unlikely to be induced by exposure to HIV. We emphasize the needs for continued analysis of individuals of this class and especially for viral isolation studies.

Reactivity to *gag*-coded immunoblot bands in the absence of confirming *env*-specific immunoblot reactivity is sometimes considered a false-positive reaction (2, 10, 20, 34). Our study suggests that most of the sera displaying HIV immunoblot reactivity at the position of band p17 or p24 or both do in fact have antibodies that specifically bind to viral antigens. We suggest that it is prudent to follow the suggestion of Courouce et al. (10) and not use such sera for transfusions until the cause of the (cross-)reactions is known.

## ACKNOWLEDGMENTS

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