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Human immunodeficiency virus-1 (HIV-1) gp120 superantigen-binding serum antibodies. A host factor in homosexual HIV-1 transmission.

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Research Article

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Human Immunodeficiency Virus-1 (HIV-1) gp120 Superantigen-binding Serum Antibodies

A Host Factor in Homosexual HIV-1 Transmission

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Abstract

HIV-1 gp120 is an immunoglobulin superantigen which can bind to preimmune serum Ig. We hypothesize that levels of such preimmune antibodies vary in the population and might affect host resistance or susceptibility to viral transmission. This study tests two predictions: (*a*) levels of preimmune anti-gp120 Igs are a polymorphic trait; and, (*b*) these levels are correlated with resistance or susceptibility to HIV-1 transmission. The first prediction was confirmed in a longitudinal study of a low-risk seronegative population. In this group, levels of both endogenous anti-gp120 IgM and IgG varied widely, but were characteristic and stable for each individual. The second prediction was addressed in a study of participants of the Multicenter AIDS Cohort Study, in which men "susceptible" and "resistant" to HIV infection were identified based on numbers of sexual partners and eventual seroconversion. Specimens consisted of archival sera obtained > 2 yr before seroconversion. Men in the susceptible population (low-risk seroconverters) were distinguished by low levels of anti-gp120 IgG. We conclude that the level of preimmune anti-gp120 IgG is a polymorphic population trait, and low levels are a potentially specific and significant factor in homosexual transmission of HIV infection. (*J. Clin. Invest.* 1996. 98:1794–1801.) Key words: immunoglobulin • AIDS • superantigen • VH3 • disease transmission

Introduction

The mechanism of initial HIV-1 transmission and infection is an area of intense investigation. The usual modes of viral transmission are through mucosal or direct blood contact. However, both modes are inefficient. For example, in the case of receptive anal intercourse, the probability of infection per encounter has been estimated at 1% or less (1). A number of parameters are known to influence the efficiency of viral infection (2, 3). Some factors such as macrophage tropism and viral load, are primarily due to the donor's strain and viral burden. Epidemiological studies have also identified factors which are intrinsic to the host including, pre-existing conditions which disturb the integrity of the mucosal epithelium and impaired immune status which may compromise the acute antiviral immune response (4–6). Other than this, there is little information concerning the existence of innate traits conferring host susceptibility.

One potential host trait is the recently identified binding activity of preimmune serum antibodies to the HIV-1 envelope glycoprotein gp120 (7–10). gp120 can be categorized as a member of the newly defined class of B cell superantigens (IgSAg)[1] (7, 11–14) by the following criteria: (*a*) The frequency of preimmune B cells that bind gp120 (4–6%) is much greater than would be expected for B cells that bind an antigen in a primary immune response (0.01%) (7, 8). (*b*) Antibodies from these B cells are enriched in the VH3 gene family of human Igs. (*c*) Biologically, this reactivity is reflected by the proliferation and activation of preimmune B cells in vitro, and is correlated with the activation and eventual loss of gp120-binding VH3 B cells during clinical HIV infection (8–10).

Analytically, SAg-binding anti-gp120 antibodies (SAg_{gp120}-binding Ig) are distinguished from conventional postinfection anti-gp120 antibodies in several respects. (*a*) IgM is the predominant isotype, and IgG is below the limit of detection by direct ELISA. Conversely, IgG predominates in postinfection antibodies (8). SAg_{gp120}-binding Ig activity is thus poorly detected using IgG-specific reagents often used in serodetection. (*b*) SAg_{gp120}-binding IgM titer is much lower than postinfection anti-gp120 IgM, requiring detection with high antibody concentration (1% serum, vs $\leq$ 0.01% for postinfection serum) by direct ELISA. In fact, ELISA absorbances at 0.1% serum, a typical serodiagnostic concentration, is at the limit of detection ($<$ 0.1 OD). (*c*) Some commonly used blocking agents (e.g., albumin and milk at $>$ 100 μg/ml) impair measurement of these antibodies. (*d*) These antibodies are not detectable in serum by Western analysis. This in part reflects the fundamentally lower sensitivity of Western vs ELISA assay. In addition, while certain linear peptides compete with native gp120 for binding to VH3 antibodies, their avidity is low (100- to 1,000-fold molar excess of peptide:Ig) compared to fluid-phase binding to native gp120 (1- to 10-fold molar ratio of gp120:Ig) (8, 13). These observations indicate that the native epitope is not efficiently represented as a linear epitope, suggesting that gp120 denaturation during Western analysis may compromise this assay of gp120–VH3 interaction.

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1. *Abbreviations used in this paper:* ARI, anal receptive intercourse; mod-SpA, iodine monochloride-modified *Staphylococcus aureas* protein A; SAg, superantigen.

What role might such preexisting SAg_{gp120}-binding Ig play in HIV infection? These preimmune antibodies may affect viral infection, either negatively (through the usual mechanisms of immune complex removal [2, 15]), or positively (by enhancement mechanisms demonstrated for antibodies and complement proteins in vitro [16–22]). To establish if either of these effects are significant in vivo, we have developed an epidemiologic study of viral transmission.

We first investigated whether SAg_{gp120}-binding Ig levels differ in the population. If so, we could test the prediction that levels of these antibodies correlate with resistance and/or susceptibility to HIV-1 infection. As will be shown, the first hypothesis was confirmed, and such antibodies were correlated with susceptibility to homosexual viral transmission.

Methods

Subjects. Five groups of individuals were selected for this study. A low-risk seronegative group from a study of patients with acne vulgaris was used to study the stability of SAg_{gp120}-binding Ig levels in individuals over time. From this group (n = 13), we obtained serum drawn biweekly over a 4-mo period (23).

The other four groups were participants of the Multicenter AIDS Cohort Study (MACS), an ongoing prospective cohort study of 4,954 homosexual and bisexual men studied at four centers (Chicago, Baltimore, Los Angeles, and Pittsburgh) starting in 1984 (24). Subjects used in the study were approved by the UCLA Institutional Review Board. A total of 252 serum samples were obtained from frozen specimens collected at the baseline MACS visit (4/84–9/84) equally divided (n = 63) among four groups of individuals that were seronegative at the baseline visit. The categorization was done using a combination of their serostatus as of the end of 1993 and the estimated number of anal receptive intercourse (ARI) partners in the 2.5 yr before the second MACS visit (10/84-3/85) The criteria were described by Detels et al. (25) as: (*a*) "resistant" individuals were in the top 10th percentile of ARI partners (median 195, range 108–500) that remained seronegative through the end of 1993, (*b*) "susceptible" individuals were in the lowest 30th percentile of ARI partners (median 1, range 0–12) who had a first HIV-1 seropositive visit at MACS visit 5 (1/86-9/86) or later, (*c*) "low risk" individuals were in the lowest 30th percentile of ARI partners (median 0, range 0–0) who remained seronegative through the end of 1993; and (*d*) "high risk" individuals were in the highest 10th percentile of ARI partners (median 75, range 5–400) who subsequently seroconverted at MACS visit 5 or later (Table I). We also obtained serial serum samples from eight individuals from the MACS population. Samples were drawn at 6-mo intervals over the course of 2 yr.

SAg_{gp120}-binding Ig and other antibodies. SAg_{gp120}-binding Ig activity was detected by a direct ELISA as described previously (8). In some cases, IgG was detected with a more sensitive indirect ELISA, consisting of biotinylated $F(ab')_2$ goat anti–human IgG and peroxidase-streptavidin (Southern Biotechnology Associates, Birmingham, AL). Briefly, plates were coated overnight with 1 μg/ml HIV-1 $gp120_{SF2}$ (gift of Dr. K. Steimer, Chiron Corporation, Emeryville, CA). Purified human polyclonal IgM (Sigma Chemical Co., St. Louis, MO) and IgG (Sigma Chemical Co.) were used as SAg_{gp120}-binding Ig standard reagents. Absorbances were determined for serial dilutions of each standard. Test sera were analyzed in relation to the standard curves, using serum dilutions achieving absorbances within the linear dose response of the standard curves. We arbitrarily define the standard immunoglobulin reagents (1 mg/ml stock) as 1 U/ml of SAg_{gp120}-binding activity. Units of SAg_{gp120} Ig were calculated using Microplate Manager (Bio-Rad, Hercules, CA) software. For values of 0, half of the lowest value from that analysis was added for log transformation.

Binding activity of antibodies were also quantitated under the same experimental conditions using wells coated with 0.5 μg/ml soluble modified protein A (mod-SpA, a VH3 superantigen; see below) or 1 μg/ml tetanus toxoid (an irrelevant antigen). Total IgM, IgG, and IgA were quantitated by capture ELISA as previously described (8).

Fractionation of VH3 (modified protein A-binding) IgG. *Staphylococcus aureus* protein A (SpA) expresses binding sites for both VH3 family Ig and the constant region domain of IgG. Iodine monochloride modification of SpA (mod-SpA) selectively inactivates IgG-binding activity of protein A, without affecting its Ig-SAg binding activity (26, 27). To produce mod-SpA, we followed the protocol of Sasso et al. (26). Quantitation indicated that 17% of the original IgG sample was recovered in the eluate. This closely matched the 15% yield previously reported for IgG $F(ab')_2$ (26). The modified SpA-bound and unbound IgG pools were tested for effectiveness of the fractionation by direct ELISA. Wells (3690; Costar Corp., Cambridge, MA) were coated with modified SpA at 1 μg/ml in coating buffer, blocked with 1% BSA/0.05% Tween-20/PBS, and washed with 0.5% Tween-20/PBS. The two IgG pools were each titrated twofold serially into blocking buffer and incubated 1 h at room temperature. IgG was detected with biotinylated $F(ab')_2$ goat anti–human IgG and peroxidase-streptavidin. Binding activity of the fractions for gp120 was tested as described above.

Peptide competition was performed by preincubation of gp120 peptides (20 amino acids in length) from both the C2 domain (No. 1960) and the C3 domain (No. 1990) overnight at 4°C in 0.5% Tween-20/PBS, as previously described (13) with 2.5 ug/ml mod-SpA+ IgG, or 2.5 and 12.5 μg/ml mod-SpA− IgG. These peptides were selected because of the presence or absence (in 1960 and 1990, respectively) of a decamer sequence defining a competitive antagonist SAg_{gp120} ligand for gp120–VH3 interaction.

Statistical analysis. A log (base 10) transformation of all experimental outcomes was made before the analysis, resulting in measurements that could be more accurately described using normal distribution methods. To determine whether there was a temporal trend in the data from the first group of 13 biweekly SAg_{gp120}-binding Ig measurements, two separate linear regression models with time as the independent variable were fit, and we determined if the average slope over time for either variable was significantly different from zero. To compare the consistency of the responses from the same individual over time, we incorporated the within individual correlation into the model using a random effects approach.

To investigate differences among the four MACS populations for each of the measured IgG and IgM variables, analysis of covariance methods were used. Therefore, for each log-transformed variable we could determine differences among group means while controlling for any potential differences in the mean level across the four MACS centers. To ensure that differences among groups were not affected by the use of these normal distribution models, we confirmed the results using nonparametric Wilcoxon tests on the group medians. Spearman correlation coefficients among the variables were calculated and statistically compared to zero. All computations were done using SAS 6.09 software (SAS Institute, Cary, NC).

Table I. Multicenter AIDS Cohort Study Populations

	Median sexual partners	Range	HIV status
Susceptible	1	0–5	positive
Low risk SN	0	0–0	negative
High risk SC	75	5–400	positive
Resistant	195	108–500	negative

MACS participants were categorized into four groups based on median number of sexual partners and eventual serostatus. SN, seronegative; SC, seroconverter.

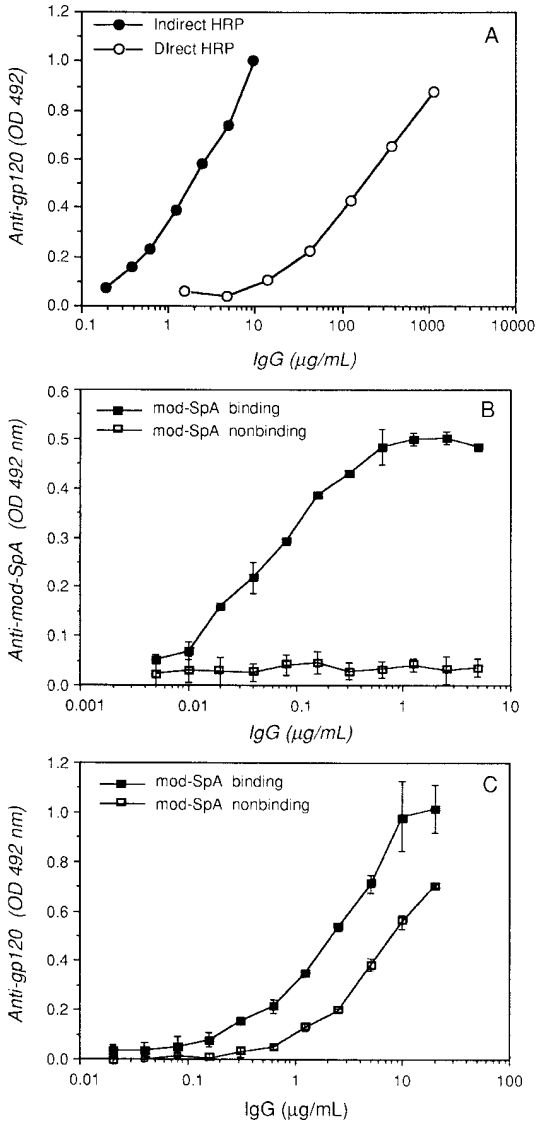

Figure 1. Association of SAg_{gp120}-binding Ig activity with VH3-enriched IgG. (*A*) Detection of IgG anti-gp120 binding activity was compared using direct and indirect ELISA. (*B* and *C*) Purified IgG from pooled seronegative sera was fractionated using mod-SpA affinity chromatography to obtain VH3-enriched (mod-SpA binding) and VH3-depleted (modSpA nonbinding) IgG. Indirect ELISA was used to measure binding activity for modSpA (*A*) and gp120 (*B*). Values are means±SEM of absorbance (subtracted background was 0.2 absorbance unit). Note the quantitative fractionation of IgG for anti-modSpA, and enrichment (about fivefold) for anti-gp120, in VH3-enriched IgG.

Results

Isotype characterization of SAg-binding anti-gp120 serum antibodies. We have previously characterized naturally occurring SAg_{gp120}-binding Ig as a VH3 SAg-binding activity by two criteria: enrichment of activity in the VH3 subset (by SpA fractionation and VH3 anti-idiotope analysis), and specific competition by peptides from the C2 domain of gp210 (241–250) (8, 9, 13). As a prelude to the present study, SAg_{gp120}-binding Ig activity was characterized for the other major isotypes using immunoglobulins purified from pooled, seronegative donor sera. Naturally occurring anti-gp120 IgG activity is at the limit of detection by direct ELISA (8). Therefore, we first compared detection of this activity in purified IgG by direct versus indirect ELISA. Anti-gp120 activity in purified IgG was detectable by direct ELISA at concentrations above ~ 50 μg/ml; detection by indirect ELISA was 50-fold more sensitive (Fig. 1 *A*).

Detection of antibody levels was about fivefold less efficient in serum. Thus, mean SAg_{gp120}-binding IgG was 2 U/ml (equivalent to 2 mg/ml purified IgG), whereas total IgG was 11 mg/ml (Table II). This presumably reflects the interfering effect of serum components (see Introduction). We were interested to understand how this level of anti-gp120 activity compared to that seen in postinfection subjects. A set of 20 postinfection MACS subjects (4–9 yr HIV seropositive by p24 assay) were tested in this assay; 685±43 U/ml (mean±SEM) was observed. Thus, mean SAg_{gp120}-binding IgG is a very low activity compared to post-infection anti-gp120 IgG.

The SAg-binding specificity of this IgG activity was validated by the two criteria noted above. First, purified serum IgG was fractionated by mod-SpA affinity chromatography column into binding and nonbinding pools, corresponding to VH3-enriched and depleted IgG (Fig. 1 *B*). SAg_{gp120}-binding Ig activity was enriched fivefold in mod-SpA binding IgG (Fig. 1 *C*), comparable to that observed with SpA fractionation of IgM (8). Second, anti-gp120 activity in the mod-SpA binding IgG was efficiently competed by peptide 1960, bearing the

Table II. Antigen-binding IgG in MACS Populations

	Mean±SD	Range	*P* value
Total IgG			
Susceptible	9.24±3.95	3.95–21.81	
Low risk SN	11.34±5.75	4.76–39.00	0.066
High risk SC	11.77±6.62	0.00–35.39	0.026
Resistant	11.83±6.17	1.17–29.16	0.019
SAg_{gp120}-binding IgG			
Susceptible	1.44±1.30	0.04–6.02	
Low risk SN	2.01±1.88	0.00–10.28	0.021
High risk SC	2.10±2.07	0.29–10.00	0.015
Resistant	2.42±2.23	0.38–10.43	0.0004
Anti-modSpA IgA			
Susceptible	11.79±11.42	1.43–60.00	
Low risk SN	14.70±13.81	0.00–60.00	
High risk SC	13.28±10.81	3.23–60.00	
Resistant	12.96±11.29	2.83–60.00	
Antitetanus toxoid IgG			
Susceptible	22.80±20.92	0.00–127.80	
Low risk SN	13.79±13.37	0.00–69.93	
High risk SC	23.35±29.11	0.00–125.00	
Resistant	23.43±26.83	0.00–130.00	

Serum samples from MACS participants categorized as susceptible, resistant, low-risk seronegative (*low-risk SN*), and high-risk seroconverter (*high-risk SC*) were drawn at least 2 yr before seroconversion. Samples were analyzed for titers of antibodies to a variety of antigens. Note that significant differences between susceptible men and other groups were limited to levels of SAg_{gp120}-binding IgG and total IgG.

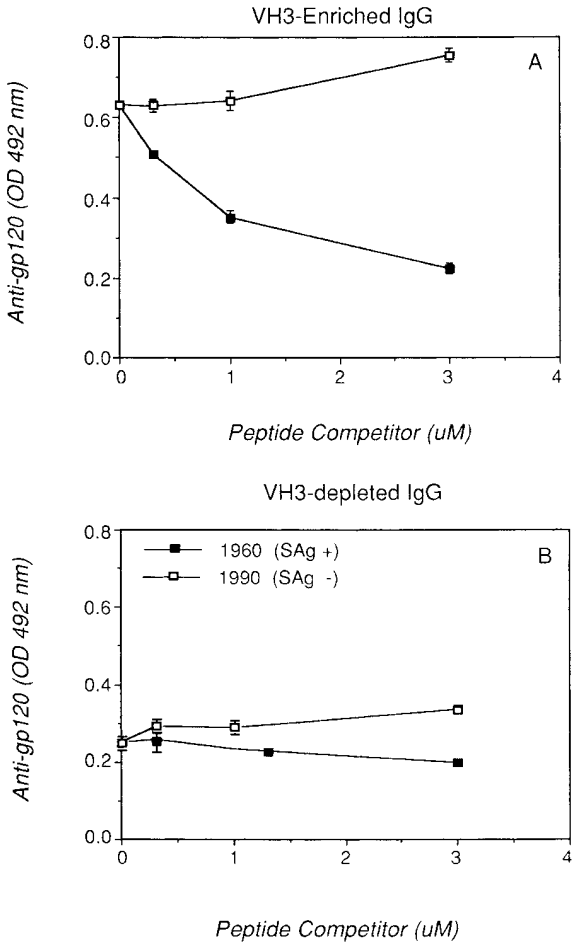

Figure 2. Characterization of SAg_{gp120}-binding IgG by SAg peptide competition. VH3-enriched (*A*) and depleted IgG (*B*) (1 μg/ml) were preincubated with SAg+ and SAg- gp120 peptide competitors (*1960* and *1990*, respectively) at the indicated concentrations. The IgG/peptide solutions were then tested for binding to native gp120 by direct ELISA. Note the competition of gp120-binding by SAg+ but not SAg− peptide.

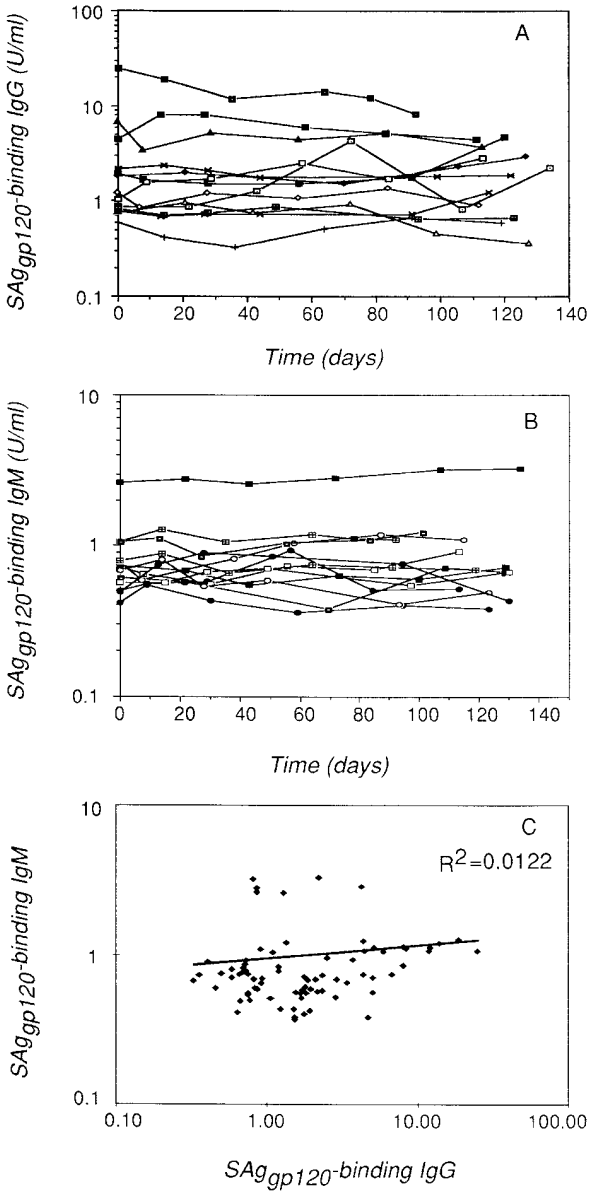

Figure 3. SAg_{gp120}-binding Ig levels in low-risk seronegative individuals over time. Serum samples were collected biweekly from 13 individuals over a 4-mo period, and quantitated for units of SAg_{gp120}-binding Ig activity as described in Methods. (*A*) SAg_{gp120}-binding IgG; (*B*) SAg_{gp120}-binding IgM; (*C*) Correlation of SAg_{gp120}-binding IgM and IgG. Different standards are used to calculate IgM and IgG SAg_{gp120}-binding units, so the relative gp120-binding activity between isotypes cannot be directly compared. Note that for both isotypes, levels of anti-gp120 Ig vary widely among individuals, but that levels in each individual remain constant over time. Also, levels of SAg_{gp120}-binding IgG versus IgM did not correlate, indicating these isotype activities are independently controlled.

VH3 SAg sequence, but not by the irrelevant C3 peptide 1990 (Fig. 2). Modest but specific peptide competition was also observed for anti-gp120 activity of mod-SpA nonbinding IgG, suggesting that a component of this anti-gp120 activity is SAg binding by the peptide criterion. Since 17% of IgG is mod-SpA binding, and has fivefold increased anti-gp120 activity, at least 50% of SAg_{gp120}-binding IgG reflects VH3 SAg-binding activity.

SAg_{gp120}-binding Ig levels in the population are independent and heterogeneous. The first goal of this study was to determine whether the serum levels of endogenous anti-gp120 Ig vary among HIV-1 seronegative individuals. SAg_{gp120}-binding Ig concentrations were measured in a set of serum samples obtained biweekly from a previously described group of individuals at low risk for HIV-1 infection (23). As shown in Fig. 3 *A*, SAg_{gp120}-binding IgG levels varied dramatically among these individuals (0.3–25 U/ml). SAg_{gp120}-binding IgM levels also varied in this population (0.4 – 4 U/ml, Fig. 3 *B*). Values of anti-gp120 IgG or IgM for a given individual (P = 0.602 and 0.532, respectively) were remarkably consistent over the 4-mo period. The intraindividual correlations were 0.89 for IgG and 0.86 for IgM, indicating that the titers remained almost constant over this time. Thus, SAg_{gp120}-binding Ig levels do not reflect sporadic changes in single individuals over time (as might occur due to infectious disease or other acute immune responses). Instead, these data indicate that SAg_{gp120}-binding Ig levels vary considerably within the seronegative population,

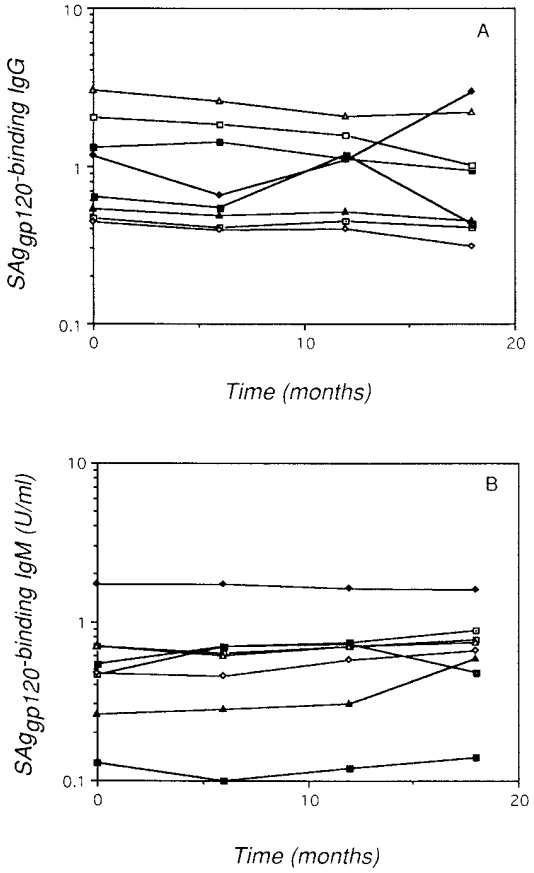

Figure 4. SAg_{gp120}-binding Ig levels in MACS participants over time. Serum samples were collected every 6 mo from eight individuals over a 2-yr period, and quantitated for units of SAg_{gp120}-binding Ig activity as described in Methods. (*A*) SAg_{gp120}-binding IgG; (*B*) SAg_{gp120}-binding IgM. Different standards are used to calculate IgM and IgG SAg_{gp120}-binding units, so the relative gp120-binding activity between isotypes cannot be directly compared. Note that for both isotypes, levels of anti-gp120 Ig vary widely among individuals, but that levels in each individual remain constant over time.

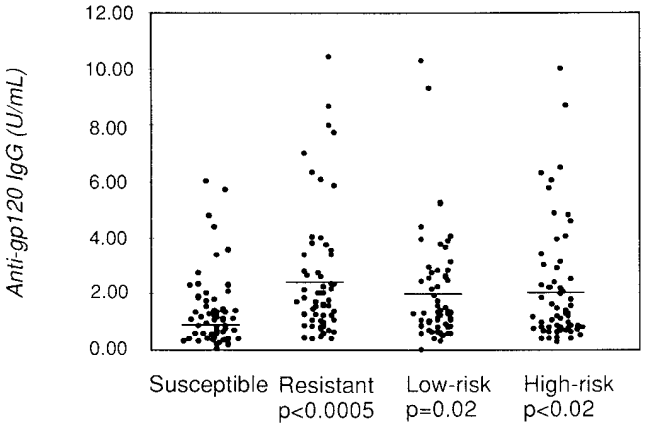

Figure 5. Decreased SAg_{gp120}-binding IgG among susceptible men. Serum samples were collected from MACS participants classified as low-risk seroconverter (*susceptible*), high-risk seronegative (*resistant*), low-risk seronegative (*low-risk SN*), and high-risk seroconverter (*high-risk SC*). These samples were collected at least 2 yr before seroconversion, and so are not reflective of infection-related antigen exposure. Note that susceptible men have significantly lower levels of SAg_{gp120}-binding IgG as compared to all other groups. Bars represent group means.

and that the observed levels within an individual are relatively stable over time.

The nature of the MACS archive allowed us to confirm whether these SAg_{gp120}-binding Ig levels were a stable trait in the MACS population. Eight MACS subjects were selected, based on their divergent SAg_{gp120}-binding Ig levels at the initially tested time point. Archival specimens were then obtained for each subject at 6-mo intervals over a 2-yr period. As in the non-MACS individuals, levels of SAg_{gp120}-binding IgG and IgM were remarkably stable. Thus, none of eight subjects ranged more than twofold in IgM levels, and only one of eight in IgG levels, over the 2-yr observation period (Fig. 4). These results also indicate that the stability of these antibody levels persist for an even more extended times than was testable with the non-MACS archive (2 yr vs 4 mo).

SAg_{gp120}-binding IgG and total IgG is reduced in "susceptible" men. A significant decrease was observed in the mean concentration of SAg_{gp120}-binding IgG in susceptible men compared to all the other groups ($P < 0.0005$ compared with resistant, $P < 0.02$ compared with low-risk seronegatives and high risk seroconverters) (Fig. 5, Table II). By splitting the SAg_{gp120}-binding IgG levels for resistants and susceptibles into quartiles (< 0.80; 0.80–1.314; 1.315–2.289, ≥ 2.29) we compared the odds of anti-gp120 IgG ≥ 2.29 (highest quartile) vs < 0.80 (lowest quartile), and found the odds 5.49 times higher in the resistant group than the susceptible group. Analysis of total IgG levels revealed that the susceptible men also had modestly reduced levels compared to all other groups ($P < 0.02$ for resistant, $P = 0.065$ for low-risk seronegatives, and $P < 0.03$ for high-risk seroconverters) (Table II). There was no difference between any of the groups in IgG binding to other antigens.

A third significant difference was also found between susceptible men and all other groups; the concentration of total IgM was lower in this group compared with resistant men ($P = 0.04$), low-risk seronegatives ($P = 0.02$), and high-risk seroconverters ($P < 0.01$). Again, there was no difference between any of the groups in IgM binding to other antigens (Table III).

Concordance of antigen binding with total IgG and total IgM levels. The foregoing observations indicated that levels of pre-immune SAg_{gp120}-binding IgG distinguished susceptible men from other groups. We further tested the correlation between SAg_{gp120}-binding Ig levels and the other antigen-binding activities, to confirm that the observed difference in SAg_{gp120}-binding IgG titer for the susceptible group was specific, and not merely reflective of the slight overall decrease in antibody levels. We therefore examined the correlation of SAg_{gp120}-binding IgG levels with the levels of anti-modSpA and antitetanus toxoid as well as total IgG and IgM (see Tables IV and VI). Concordance was assessed by a Spearman correlation analysis of the different antibody activities in the entire MACS population used in this study ($n = 252$). While all the IgM antibody activities were highly correlated, the IgG activities showed much lower concordance (Table IV and V). Furthermore, SAg_{gp120}-binding IgG activity only weakly correlated with SAg_{gp120}-binding IgM activity (Table V) and showed no correlation to total IgM (Table VI). Thus, SAg_{gp120}-binding IgG is an antigen-specific humoral immune trait, distinct from

Table III. Levels of Antigen-binding IgM in MACS Populations

	Mean±SD	Range	*P* value
Total IgM			
Susceptible	1.32±0.91	0.36–6.95	
Low risk SN	1.71±1.20	0.41–6.25	0.02
High risk SC	1.64±0.86	0.44–4.59	0.008
Resistant	1.52±0.65	0.49–3.97	0.04
$SAg_{gp\ 120}$-binding IgM			
Susceptible	1.39±1.52	0.06–9.57	
Low risk SN	1.63±2.61	0.17–15.69	
High risk SC	1.89±2.36	0.27–35.69	
Resistant	1.42±1.18	0.13–5.27	
Anti-modSpA IgM			
Susceptible	0.96±0.56	0.11–3.17	
Low risk SN	1.12±0.92	0.0–5.23	
High risk SC	1.09±0.83	0.28–6.3	
Resistant	1.01±0.45	0.33–2.37	
Antitetanus toxoid IgM			
Susceptible	1.30±0.96	0.13–4.03	
Low risk SN	1.26±1.43	0.17–9.64	
High risk SC	1.82±1.63	0.27–7.65	
Resistant	1.58±1.55	0.15–8.88	

Subjects and samples described in Table II were analyzed for antigen-binding IgM activity. As with IgG, the susceptible group was distinguished by low levels of total IgM levels.

the modest population differences seen with total IgG and total IgM.

Discussion

This study observed two population-based features of SAg_{gp120}-binding IgG expression in HIV-1 seronegative individuals. First, SAg_{gp120}-binding IgG levels are a temporally stable trait in each individual. However, within the total population, levels are highly heterogeneous. Second, SAg_{gp120}-binding IgG and total IgG levels are significantly lowered in men susceptible to HIV infection, consistent with the hypothesis that preexisting serum antibodies to HIV-1 may be a host protective factor.

Population polymorphism in SAg_{gp120}-binding Ig levels. The first prediction tested in this study was that the level of pre-immune SAg_{gp120}-binding Ig is stable for a given individual yet polymorphic in the HIV-seronegative (low risk) population. Confirming this prediction, we observed that the levels of both SAg_{gp120}-binding IgM and IgG varied significantly in this group yet remained constant over time. These findings are confirmed by further analysis of serum samples from the MACS that had heterogeneous yet strikingly static levels of SAg_{gp120}-binding Ig over a 2-yr period. This finding is not surprising since stable predominance of selected B cell clones is a common outcome in both the mouse (28–30) and human (31, 32). This biology has been related to both genetic traits (33–35) and prior encounters with cross-reactive antigens (e.g., "antigenic sin" [29]). Thus, both literature precedents and our direct experimental findings validate the prediction that the level of SAg_{gp120}-binding Ig is a natural polymorphic trait in the general population.

Table IV. Correlation of IgG Antigen-binding Activities

	Total IgG	SAg_{gp120}-binding Ig	Anti-ModSpA	Anti-TT
Total IgG	1.00			
SAg_{gp120}-binding Ig	0.21	1.00		
Anti-ModSpA	0.38	0.27	1.00	
Anti-TT	0.23	0.13	0.26	1.00

Spearman correlation coefficients were determined for comparisons of all IgM binding activities tested. Note that all variables correlated weakly, if at all.

Table V. Correlation of IgM Antigen-binding Activities

	Total IgM	SAg_{gp120}-binding Ig	Anti-ModSpA	Anti-TT
Total IgG	1.00			
SAg_{gp120}-binding Ig	0.53	1.00		
Anti-ModSpA	0.77	0.68	1.00	
Anti-TT	0.46	0.70	0.45	1.00

Spearman correlation coefficients were determined for comparisons of all IgM binding activities tested. Note that all variables correlated strongly.

Correlation of low SAg_{gp120}-binding Ig antibody phenotype and susceptibility to HIV infection. The second goal of this study was to test whether SAg_{gp120}-binding Ig levels correlated with susceptibility or resistance to HIV infection. We examined archival preseroconversion sera from MACS participants whose epidemiologically susceptibility to HIV-1 infection was subsequently identified. Notably, we observed that preimmune SAg_{gp120}-binding IgG was decreased in susceptible men versus resistant and control men (by 40 and 30%, respectively). This finding suggests that these antibodies may be protective against HIV-1 infection.

We note, however, that levels of these antibodies did not predict future HIV-1 status among the groups with high-risk behavior. This observation indicates that naturally occurring SAg_{gp120}-binding antibodies are only one factor in resistance to infection. Other factors have been associated with resistance to HIV infection, such as blood levels of CD8+ cells and polymorphonuclear leukocytes (25). A recent report has also associated resistance with the prevalence of certain TAP gene variants and $CD25^+$, $CD8^+$ cells. In the high risk populations,

Table VI. $SAg_{gp\ 120}$-binding IgG Levels Are an Independent Trait

SAg_{gp120}-binding IgG vs SAg_{gp120}-binding IgM	0.27
SAg_{gp120}-binding IgG vs total IgG	0.21
SAg_{gp120}-binding IgG vs total IgM	0.11

Spearman correlation coefficients were calculated for SAg_{gp120}-binding IgG compared with SAg_{gp120}-binding IgM, total IgG, and total IgM. Data were analyzed from either the entire MACS population ($n = 252$) or the susceptible MACS population alone ($n = 63$). Note that SAg_{gp120}-binding IgG did not correlate with total IgM and only weakly correlated with total IgG and SAg_{gp120}-binding IgM.

other known transmission factors (viral factors, other immunological traits, rectal trauma, genital infection, and immunosuppression) are more prevalent, and may predominate (compared with SAg_{gp120}-binding IgG) in the success of viral transmission (3–6, 36–42).

Independence of anti-gp120 IgG antibody levels. The correlation analysis showed that SAg_{gp120}-binding IgG poorly correlated with antibody activities to irrelevant antigen (tetanus toxoid), another VH3 SAg (mod-SpA), and total IgG titers (Table IV). These findings confirmed that reduced SAg_{gp120}-binding IgG is a specific and independent immune trait associated with susceptibility to HIV infection.

Total IgG and IgM titers were also reduced in the susceptible men. However, neither of these results were significantly correlated with SAg_{gp120}-binding IgG levels. There may be two reasons for this discordance. First, the difference in total Ig levels between susceptible and other groups was small, reducing the statistical significance of the correlation with SAg_{gp120}-binding IgG. Second, other subsets of naturally occurring antibodies, such as polyreactive antibodies (43–45) may also bind gp120 or other envelope structures. If so, they would be expected to confer a similar biologic effect.

Relationship of SAg_{gp120}-binding Ig activity among the different isotypes. Concordance was also examined for the levels of SAg_{gp120}-binding IgM and SAg_{gp120}-binding IgG. In all populations tested, Spearman correlation coefficients revealed that these antigen affinities were discordant. These findings indicate that the abundance and/or secretory activity of anti-gp120 B cell clones contributing to the serum repertoire is independently controlled according to isotype. There are good precedents for this observation. Such clones in the IgM repertoire are frequently polyreactive (43, 44), and there is direct evidence for negative selection of such clones in the more mature repertoire (45).

Proposed mechanism of SAg_{gp120}-binding IgG on viral transmission. The most striking observation in this study is the decrease in endogenous SAg_{gp120}-binding IgG levels in the susceptible men versus the resistant men. The lower level of difference observed with low-risk seronegative and high-risk seropositive men probably reflects the unavoidable inclusion of both resistant and susceptible men in those two groups. We envision that the virus is opsonized by such antibodies and cleared, decreasing the efficiency of initial viral transmission. In individuals with low-risk behavior, this mechanism has a significant impact on the small viral inoculum reaching the tissue sites of initial cellular infection. Accordingly, individuals with low levels of these antibodies are at greater risk for successful viral transmission. In contrast, individuals with high-risk behavior are likely to encounter larger viral inocula due to reduced mucosal integrity (due to mucosal trauma, bacterial or parasitic inflammation, etc.) or generalized immunosuppression (due to drug use or other infections). In this setting, the normal range of SAg_{gp120}-binding IgG levels are inadequate to significantly bind and clear the viral inoculum.

The present study is the first report of a potential physiologic role of VH3 SAg_{gp120}-binding antibodies in host resistance. This hypothesis has direct consequences in the area of preventing HIV-1 transmission through vaccination. Antibodies are known to play a role in protection against HIV-1 infection. Unfortunately, their utility as a target of vaccine design has been limited by a number of factors, particularly the strain restriction of neutralizing epitopes (49–53). The present study indicates that targeting the VH3 SAg site of gp120 may be a new alternative for eliciting protective antibody immunity to HIV-1. We are currently studying the mechanism of SAg_{gp120}-binding Igs on viral infection, and immunization strategies to raise the levels of this antibody subset. If validated, such information could be useful as part of an immunization strategy for protection of individuals at risk for HIV-1 infection.

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