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Anti-Human Immunodeficiency Virus Type 1 Antibodies of Noninfected Subjects Are Not Related to Autoantibodies Occurring in Systemic Diseases

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Indeterminate Western blot (WB) (immunoblot) patterns for anti-human immunodeficiency virus type 1 (HIV-1) antibodies are often observed when testing serum samples from noninfected individuals. We investigated here the possible involvement of some frequently occurring autoantibodies (anti-SmB/B′, U1snRNP [68 kDa, A, and C], Ro/SS-A [60 and 52 kDa], and Jo-1) in the generation of such indeterminate HIV-1 WB. In particular, the role of a reported sequence homology between p24 *gag* and the SmB/B′ autoantigen was investigated. Serum samples were obtained from 50 healthy controls, 51 patients with systemic lupus erythematosus (SLE), 46 with systemic sclerosis, 6 with Sjögren's disease, 3 with mixed connective tissue disease, and 41 healthy subjects with persistent indeterminate HIV-1 WB. Reactivity to HIV-1 p24 *gag* was slightly but not significantly more frequent in patients with SLE than in controls (25.5% versus 14.0%; $P > 0.1$), whereas reactivity to HIV-1 p17 *gag* was significantly more frequent in the former subjects (23.5% versus 8.0%; $P = 0.03$). Simultaneous reactivity to p17 and p24 was observed in patients with SLE (11.8%; $P = 0.014$) or systemic sclerosis (8.7%; $P = 0.049$) but not in controls. There was no association found between the presence of any autoantibody and the occurrence of indeterminate HIV-1 WB nor between the presence of p24-reactive antibodies and anti-SmB/B′; this indicates that most p24-reactive antibodies are directed to epitopes other than the proline-rich sequences shared by p24 *gag* and SmB/B′.

Western blot (WB) (immunoblot) analysis is the test most frequently used for confirming the presence of antibodies to human immunodeficiency virus type 1 (HIV-1) or HIV-2 in serum samples. WB results are considered indeterminate when reactivity is restricted to only a few viral proteins. While indeterminate WBs (IWBs) may be indicative of early seroconversion, they are more often seen in subjects not infected with HIV (7, 14). In most cases, IWBs result from cross-reactions with HIV-1 *gag* proteins, especially p17 and p24 (2, 7, 15, 25). Some investigators presented evidence that IWBs with reaction to p24 *gag* occurred much more often in subjects with systemic lupus erythematosus (SLE), Sjögren's syndrome, or systemic sclerosis than in the general population (5, 22, 23). These results suggested the involvement of endogenous or exogenous retroviruses in the pathogenesis of these diseases. On the other hand, cross-reactivity with p24 could result from molecular mimicry between retroviral capsid protein and some autoantigens (10). In particular, the presence of p24-reactive antibodies found in the serum of certain SLE patients has been explained by a cross-reaction between similar proline-rich sequences present in both HIV-1 p24 and the SmB/B′ autoantigens (6).

Whether IWBs are more frequent in patients with autoimmune disorders than in other noninfected subjects is still a matter of controversy (12). The aims of the present study were therefore (i) to compare the frequency of IWBs generated by serum samples from noninfected patients with manifestations of autoimmunity and from healthy control subjects, (ii) to investigate the possible role of some commonly occurring autoantibodies in the generation of IWBs in noninfected subjects, and (iii) to assess whether anti-SmB/B′ antibodies might be responsible for reactions with p24 in subjects with or without autoimmune disorders.

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MATERIALS AND METHODS

Subjects. Serum samples were obtained from 50 healthy controls negative for HIV-1 and HIV-2 antibodies (Cobas Core anti-HIV-1/HIV-2 enzyme immunoassay [EIA], F. Hoffmann-La Roche Ltd., Basel, Switzerland), 51 patients with SLE, 46 with systemic sclerosis, 6 with Sjögren's disease, 3 with mixed connective tissue disease (MCTD), and 41 healthy subjects with persistent HIV-1 IWB pattern and without any sign of seroconversion during the following 3 months. Of these 41, 12 had p24 alone; 3 had p17 alone; 3 had p55 alone; 2 had p24 and p17; 11 had p24 and p55; 3 had p17 and p55; 3 had p17, p24, and p55; 1 had p55 and p66; 1 had p24, p32, and p55; 1 had p17, p55, and one nonviral band; and 1 had p17, p32, p51, p55, and one nonviral band. HIV-2 infection or seroconversion to HIV-1 was ruled out in the group with persistent IWBs by performing HIV-2 WB and HIV-1 p24 antigen determination and by repeating the complete serological examination 3 months later. None of the subjects of this group or of the other groups of the study were at risk for HIV infection.

Autoantibody assay. All sera were tested for the presence of autoantibodies with a commercially available WB kit (Western Blot Autoantibody Profile Kit; Immunovision Inc., Springdale, Ark.). Only autoantigens that stained brightly with reference sera were considered (SmB/B′, U1snRNP [68 kDa, A, and C], Ro/SS-A [60 and 52 kDa], and Jo-1).

HIV serological assays. All sera were examined by WB for anti-HIV-1 (NEW LAV-BLOT I; Sanofi Diagnostics Pasteur S.A., Marnes-la-Coquette, France). This assay allows the detection, in the serum of HIV-1-infected individuals, of gp160 *env* (a tetrameric form of gp41 [20], gp120 *env* (genuine gp120 plus a trimeric form of gp41 [20]), p66 *pol*, p55 *gag*, p51 *pol*, gp41 *env*, p32 *pol*, p24 *gag*, and p17 *gag*. Results were interpreted according to Centers for Disease Control (CDC) criteria, which state that the minimum band requirement for a Western blot to be considered positive are any two of p24 *gag*, gp41 *env*, or gp120/gp160 *env* (3). WB were considered indeterminate in the case of reaction with one or few viral proteins not fulfilling CDC criteria for positivity (3). Sera were also tested for anti-HIV-2 (NEW LAV-BLOT II). HIVAG-1 monoclonal antibody (Abbott Laboratories, Abbott Park, Ill.) was used for HIV-1 p24 antigen detection.

Statistical analysis. The analysis was done with Fisher's exact test considering one-sided P values of <0.05 significant.

TABLE 1. Prevalence of HIV-1 WB reactivities in serum samples

Sample type (no.)	No. (%) of samples exhibiting reaction; P^a							
	p17 *gag*[b]	p24 *gag*[b]	p17 and p24	p55 *gag* without p17 or p24	p32 *pol*[c]	p66 *pol* isolated	IWB (any pattern)	Nonviral bands
Control (50)	4 (8.0)	7 (14.0)	0 (0)	1 (2.0)	2 (4.0)	0 (0)	13 (26.0)	9 (18.0)
SLE (51)	12 (23.5);**0.030**	13 (25.5);0.115	6 (11.8);**0.014**	1 (2.0)	2 (3.9)	0 (0)	20 (39.2);0.114	31 (60.8);<**0.0001**
MCTD (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (100)
Sjögren's syndrome (6)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	3 (50.0)
Systemic sclerosis (46)	10 (21.7);0.052	7 (15.2);0.547	4 (8.7); **0.049**	2 (4.3)	2 (4.3)	1 (2.2)	17 (37.8);0.156	18 (39.1); **0.019**

[a] Comparison with control group (anti-HIV-1/HIV-2 screening EIA negative) using Fisher's exact test. Significant one-sided *P* values are shown in boldface type.
[b] Isolated or in any combination.
[c] Isolated or with *gag* bands.

RESULTS

An indeterminate HIV-1 WB result was observed in 51 of 156 (32.7%) of the subjects from all groups but that initially selected for having IWB. IWB was found slightly more often in patients having SLE (20 of 51) or systemic sclerosis (17 of 46) than in controls (13 of 50), but the difference was not statistically significant. Similar results were found when considering, in particular, the frequency of reactions to p24 *gag* (13 of 51, 7 of 46, and 7 of 50, respectively). The frequency of reactions to p17 *gag* was significantly higher in SLE patients (12 of 51, $P < 0.05$) than in controls (4 of 50). The association of p17 and p24 was more often seen in subjects with SLE (6 of 51, $P < 0.02$) and systemic sclerosis (4 of 46, $P < 0.05$) than in controls (0 of 50). Reactions with nonviral bands were observed much more regularly in patients with SLE (31 of 51, $P < 0.0001$) or with systemic sclerosis (18 of 46, $p < 0.002$) than in controls (9 of 50). WB reactions with p24 *gag* were, in general, more intense in patients with autoimmune disorders than in healthy subjects. Complete data are shown in Table 1. A positive WB result according to CDC criteria (p24 *gag*, p55 *gag*, and gp160 *env*) was obtained from a 70-year-old man with systemic sclerosis.

The prevalence of autoantibodies in the various groups studied here (Table 2) was found as could be expected (4, 24). There was no significant association (Fisher's exact test) between the occurrence of IWB and the presence of defined autoantibodies (anti-Sm, U1snRNP, Ro, Jo-1), either when each group of subjects was considered separately (patients with SLE or with systemic sclerosis or controls) or when all serum samples were analyzed as a whole ($n = 197$). No significant association was found between reaction to HIV-1 p24 and reaction to SmB/B′ in any group, including the group of healthy subjects with persistent IWB.

DISCUSSION

In the present study, IWB patterns were repeatedly observed in patients with autoimmune disorders as well as in healthy subjects. The occurrence of such a pattern was not significantly different in the various groups of subjects investigated, with the exception of p17 *gag* being more frequent in patients with SLE and of p17 *gag* plus p24 *gag* being more frequent in patients with SLE and those with systemic sclerosis. These results are at variance with those of Talal et al. (23) and Dang et al. (5), who described a more frequent reactivity to p24 *gag* in subjects with SLE, Sjögren's syndrome, or systemic sclerosis than in the general population. The discrepancies between results by Talal, Dang et al. (5, 22, 23), and us might be attributed to technical differences between manufacturers in the preparation of WB strips. In particular, one manufacturer (Diagnostics Pasteur) reports performing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of HIV-1 proteins under reducing conditions, while another (Biotech/Du Pont) does not mention the use of a reducing agent. Such a difference is of importance since both p17 and p24 have the potential to form one intramolecular disulfide bridge which may affect the reac-

TABLE 2. Prevalence of autoantibodies in serum samples

Autoantigen	No. of reactions for sample type (no. of samples)					
	Anti-HIV-1/HIV-2 screening EIA negative (50)	Persistent anti-HIV-1 IWB (41)	SLE (51)	MCTD (3)	Sjögren's syndrome (6)	Systemic sclerosis (46)
Sm						
B′	1	0	11	1	0	2
B	0	1	11	1	0	2
U1snRNP						
68 kDa	0	0	2	0	0	0
A	0	1	4	2	0	3
C	0	0	1	1	0	0
Ro/SS-A						
60 kDa	1	1	1	2	0	3
52 kDa	0	1	3	2	4	2
Jo-1						
53 kDa	0	1	0	1	0	1
Unidentified	11	9	29	2	2	24

tivity of conformation-sensitive epitopes. Indeed, Blomberg et al. (1) have shown that p24-reactive antibodies from certain noninfected persons with no evidence of autoimmune disease recognize a C-terminal peptide in the reduced, but not the oxidized, form. Another possibility is that whereas we recorded all reactivities whatever their intensity, Talal et al. (22) did not take weak reactivities into account. However, this explanation is not sufficient. Indeed, Talal et al. mention that in the group of individuals with weak reactions to p24, they also found a clear-cut difference between normal subjects (4% positive reaction) and patients with Sjögren's syndrome (28% positive reaction).

In none of the groups studied was there a correlation between the occurrence of autoantibodies and that of anti-HIV-1 antibodies, a result in agreement with that of Ranki et al. (21). Moreover, we did not observe any relationship between antibodies to p24 *gag* and antibodies to SmB/B′ in any of the groups investigated.

IWBs may be due to antibodies directed against lymphocytic proteins that are found in viral preparations and comigrate in SDS-PAGE with authentic HIV proteins (18). More often, however, IWBs result from reactions with HIV proteins, especially p17 *gag*, p24 *gag*, and sometimes monomeric or oligomeric gp41 *env* (2, 7, 11, 15, 20, 25). In the case of IWBs with *gag* proteins, we and others have shown that reactions with p17 and p24 in healthy noninfected subjects are due to cross-reactive antibodies elicited by several different immunogens (2, 7, 13, 15–17, 19, 25). Those immunogens have not been identified yet, with the exception of a herpes simplex virus and a streptococcal protein which account for a minority of cases only (16, 17). Our present results in a group of 41 healthy subjects with persistent IWB pattern indicate that the causative antibodies are not cross-reacting autoantibodies recognizing p17 or p24 epitopes as the result of a molecular mimicry between autoantigens and HIV *gag* proteins. Similarly, in patients with autoimmune disorders investigated here, there was no association between reactivity to p17 or p24 and the presence of autoantibodies. In particular, we found no association between the occurrence of reactivity to p24 and the occurrence of anti-SmB/B′ antibodies. Thus, our results do not support those of De Keyser et al. (6), who concluded that autoantibodies to SmB/B′ are responsible for the frequent reactivity to p24 *gag* observed in SLE patients, as the consequence of a cross-reaction between the several proline-rich sequences present in both p24 *gag* and SmB/B′ antigens. Our results suggest that most p24-reactive antibodies are directed to epitopes other than the proline-rich sequences shared by p24 and SmB/B′.

We observed that *pol* bands were rare in HIV IWBs. There was no association between occurrence of the p32 *pol* band and the presence of autoantibodies to U1snRNP A, a 32-kDa polypeptide which could comigrate with p32 *pol* in SDS-PAGE. There was no association either between antibodies to Ro/SS-A (52 kDa) and p51 *pol*.

No sample with antibodies to U1snRNP 68 kDa reacted with HIV *env* proteins, in spite of the extensive homology responsible for cross-reactivity between the two proteins, as described by Douvas and Takehana (8).

The 70-year-old patient with systemic sclerosis reported here, in whom HIV-1 infection was unlikely, had a positive WB by CDC criteria but an IWB according to other criteria. The 160-kDa band seen in WB was indistinguishable from that obtained from sera from HIV-infected patients, thus raising the possibility of a true cross-reaction with the tetrameric form of gp41 (20), as described by Healy and Bolton (11). However, no reaction was observed when a competitive confirmatory EIA (Envacor; Abbott Laboratories) was used, which indicates that possible cross-reacting antibodies were of low affinity or reacted with an epitope not recognized by the anti-HIV-1 conjugate. Nor was a reaction observed when a third-generation anti-HIV-1/HIV-2 sandwich EIA (Abbott) was used in which the recombinant *env* protein contained the 185 N-terminal amino-acids of gp41 (9), raising the possibility that the cross-reaction was directed to an epitope located in the C-terminal moiety of gp41. Since the patient died, further investigations could not be undertaken.

Nonviral bands appeared significantly more often in HIV-1 WBs of patients with autoimmune disorders than in healthy controls. This probably reflects the high prevalence of antibodies to lymphocytic proteins in patients with autoimmune diseases, which are present in the viral preparations used for WBs. Some of these bands were close to HIV-1 proteins on the blots, thus raising the possibility that they had been misinterpreted as authentic viral proteins in previous studies.

In summary, our results show that the frequency of HIV-1 IWB patterns was nearly as high in healthy subjects as in patients with autoimmune disorders; that there was no association between the autoantibodies investigated and IWBs in any group of subjects studied, and that most p24-reactive antibodies of noninfected subjects were directed to epitopes other than the proline-rich sequences common to p24 and SmB/B′.

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