Antibodies to HIV-1 in Sera from Patients with Mycobacterial Infections[1,2]

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The human immunodeficiency virus (HIV) ([6]), a human retrovirus (lentivirus) earlier referred to as lymphadenopathy-associated virus (LAV) ([2]) or human T-lymphotropic virus III (HTLV-III) ([16]), has been identified as the etiological agent of the acquired immunodeficiency syndrome (AIDS). The selective tropism of HIV for T-4 lymphocytes leads to T-cell depletion and the consequent lowering of T-cell defenses ([10]), giving rise to a variety of opportunistic infections. Organisms such as the *Mycobacterium avium*-complex and *M. tuberculosis* are reported to be present in as many as 50% of the AIDS patients examined for acid-fast bacilli ([7]). It has also been reported that the immunological changes in lentivirus infections resemble those of lepromatous leprosy ([1]).

Both direct and indirect methods have been developed for identifying HIV-infected persons ([12]). Almost all HIV-infected persons are seropositive for specific antibodies, and the virus can be isolated from 50% to 80% of them ([14, 18]). A number of serological tests, including the enzyme-linked immunosorbent assay (ELISA) ([5, 19]) and the Western blot (WB) ([5, 20]) and immunofluorescence (IF) ([4, 11]) assays, are available for the detection of anti-HIV antibodies. The ELISA and WB assay are being used in most laboratories for the screening and confirmation of HIV infection. However, both false-positivity and crossreactivity by WB assay have been documented ([3, 8, 17, 21]).

The present study was undertaken to investigate patients (of north India origin) with mycobacterial infections for the presence of anti-HIV-1 antibodies and the possible antigenic crossreactivity between these two pathogens.

MATERIALS AND METHODS

Subjects. Sera were collected from 348 patients with different types of leprosy (classified according to Ridley-Jopling criteria), 33 patients with pulmonary tuberculosis, and 29 healthy contacts of leprosy patients. There were two control groups. The first control group consisted of 30 normal healthy European subjects, unlikely to have had significant exposure to environmental mycobacteria; they were included to demonstrate that the assay conditions were adequate to prevent false-positive reactions. The second control group consisted of 38 normal healthy Indians, not closely exposed to tuberculosis or leprosy. This group would have had greater exposure to environmental mycobacteria than the European controls.

ELISA. All of the 478 sera were tested for antibodies to HIV-1 IgG by an ELISA using the Wellcozyme HTLV-III kit (U.K.).

Western blot assay. Sixty-nine randomly chosen sera from among those tested by ELISA were also tested for anti-HIV-1 IgG antibodies to the different protein antigens on nitrocellulose strips using the Biotech/DuPont kit (U.S.A.). These included 43 samples from leprosy patients (14 LL, 13 BB, 12 BT, 3 TT, 1N), 21 from tuberculosis patients, and 5 from healthy contacts of leprosy patients. Thirty sera from the Euro-

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TABLE 1. *Western blot (WB) reaction patterns in different types of leprosy.*

Leprosy type[a]	No. tested by WB	No. showing reactions (%)	No. showing reactions to HIV antigens						Total bands
			p55	p24	>p66	p66	p17	gp41	
LL	14	11 (78.6)	6	4	2				12
BB	13	12 (92.3)	9	7	3	2	2	1	24
BT	12	7 (58.3)	5	1		2	1		9
TT	3	2 —	1	1					2
N	1	1	1	1		1			3
Total	43	33 (76.7)	22	14	5	5	3	1	50

[a] LL = lepromatous leprosy, BB = borderline borderline; BT = borderline tuberculoid; TT = tuberculoid; N = neuritic leprosy.

pean control group and 33 sera from the Indian control group were also tested by this method.

RESULTS

None of the 478 sera were positive for anti-HIV-1 antibodies by the ELISA. Thirty-three of 43 leprosy patients (Table 1), 7 of the 21 tuberculosis patients, and 4 of the 5 healthy contacts of leprosy patients showed one, two, or multiple bands to the different HIV-1 proteins by the Western blot assay (Table 2). A reaction to p55 was observed in 31 of 69 sera tested, while no reaction was observed to gp120, gp160, or p31 antigens (Fig. 1).

Reactions to the p24 protein antigen, one of the specific markers for HIV-1 infection, were seen in 17 samples: 14 leprosy patients, 2 healthy contacts, and 1 tuberculosis patient. A reaction to the gp41 antigen, another specific marker of HIV-1, was seen in 1 BB leprosy patient in combination with p24, p55, and a band above the p66 region (Table 3). One of the 33 normal healthy Indians showed a p17 band (Fig. 2), while none of the 30 European control sera showed any reaction (Fig. 3).

DISCUSSION

An ELISA is used as a screening test for the diagnosis of HIV-1 infection. In this study of 478 sera from northern India subjects, none was positive for HIV-1 by ELISA. Miller, *et al.* ([15]) have also reported similar findings on 311 leprosy sera screened by them for anti-HIV-1 antibodies by ELISA. However, they had not tested any of their sera by the WB method. Earlier studies ([3, 8]) have shown that although sera may be nonreactive by competitive ELISA (Wellcome) for HIV-1 antibodies, they may still show "false-positive" reactions by the WB assay. Hence, 69 randomly chosen samples from persons infected with or exposed to mycobacteria along with 63 suitable controls, although all were negative by ELISA, were tested by us by WB assay. It is interesting that 44 of the patients (tuberculosis and leprosy) and healthy contacts of leprosy patients showed crossreactivity to various HIV-1 proteins, while the remaining 25 were totally nonreactive. In contrast, only 1 out of 63 controls showed a single band at the p17 region. This subject had earlier suffered from high proteinuria and albuminuria and is now normal.

TABLE 2. *Frequency of HIV bands observed in patients with mycobacterial infections and healthy contacts.*

Reaction to HIV antigen	Leprosy patients (N = 43)	Healthy contacts (N = 5)	TB patients (N = 21)	Total (N = 69)	
				No.	%
>p66	5 (3BB, 2LL)	2	0	7	10
p66	5 (2BB, 2BT, 1N)	1	0	6	9
p55	22 (9BB, 6LL, 5BT, 1TT, 1N)	3	6	31	45
p51	0	1	0	1	1
gp41	1 (BB)	0	0	1	1
p24	14 (7BB, 4LL, 1BT, 1TT, 1N)	2	1	17	25
p17	3 (2BB, 1BT)	0	1	4	6

TABLE 3. *WB reaction patterns of sera from patients with mycobacteria infections and healthy contacts.*

Reaction to HIV antigens	Leprosy patients (N = 43)	Healthy contacts (N = 5)	TB patients (N = 21)	Total (N = 69)
p24 alone	5 (3LL, 1BB, 1TT)	0	1	6
p24 + p55	2 (2BB)	0	0	2
p24 + p17	1 (BT)	0	0	1
p24 + p66	1 (LL)	1	0	2
p24 + p55 + p17	1 (BB)	0	0	1
p24 + p55 + p66	2 (1BB, 1N)	0	0	2
p24 + p55 + >p66	1 (BB)	1	0	2
p24 + p55 + gp41 + >p66	1 (BB)	0	0	1
p55 alone	14 (6LL, 3BB, 4BT, 1TT)	1	5	20
p66 alone	1 (BT)	0	0	1
>p66 alone	1 (LL)	0	0	1
p17 alone	1 (BB)	0	0	1
p55 + p66	1 (BT)	0	0	1
p55 + p17	0	0	1	1
p66 + >p66	1 (BB)	0	0	1
p55 + p51 + p66	0	1	0	1
Total	33 (12BB, 11LL, 7BT, 2TT, 1N)	4	7	44

False-positive WB reactions to the various HIV-1 antigens have been reported in blood donors from Sweden ([3]), France ([8]), and The Netherlands ([21]) but the rate has been quite low, less than 0.7%. There has been no report so far on crossreactivity by WB assay in mycobacterial infections. It is therefore interesting to find such a high rate of crossreactivity (44/69, 64%) in this sample of patients.

An isolated antibody reactivity to the p24 protein of HIV-1 in WB may represent false-positivity, especially in low-risk populations, and can persist for about 2 years without signs of HIV-1 infection ([21]); or it may represent early HIV-1 infection ([9, 13, 17]). A high rate of crossreactivity to the core proteins by Western blot has been observed in patients with multiple sclerosis (42.2%), cutaneous T-cell lymphoma (24%), and in patients with dermatological disorders (12.7%) ([17]) which may be due to either an infection with a virus immunologically related to the known human retroviruses or an early infection with HIV-1. In the present study, sera from 14 leprosy patients, 2 healthy contacts of leprosy patients, and 1 tuberculosis patient have shown antibodies to the p24 antigen alone or in combination with other bands. One BB leprosy patient showed a thin gp41 band in addition to the p24, p55, and a band above p66. This patient has been followed up for 18 months and his condition has improved. His bacterial index (BI) which was 2 has become negative, but he continues to receive multiple drug therapy for leprosy. He has not shown any signs of HIV infection so far. In an earlier report ([21]), among 150 healthy Finnish persons tested by WB one had antibodies to p24 and p55 of HIV-1, but in the present study none of the 63 normal persons showed antibodies to these two core HIV-1 proteins.

There may be certain antigens in HIV-1 and mycobacteria which may be similar, accounting for the crossreactivity observed in this study. Conceivably this could be related to a similar immunologic impairment in HIV-1 and mycobacterial infections, especially lepromatous leprosy ([1]). Therefore, it would be worthwhile to conduct both immunologic and genetic studies to look for common antigenic loci and similar gene sequences between these two unrelated pathogens.

Our study indicates that a significant proportion of leprosy sera react with several HIV-1 antigens. In a country such as India, where leprosy and tuberculosis are highly prevalent, the results of the Western blot assay should be interpreted with great caution using stringent criteria for the diagnosis

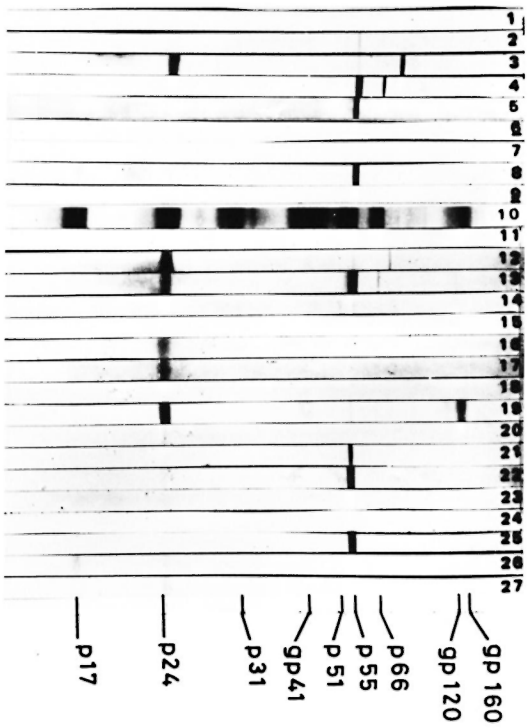

FIG. 1. Reactivity of representative samples tested for anti-HIV-1 by WB. Lane 1 = negative control; lane 10 = high positive control; lane 19 = weak positive control; lanes 2, 9, 12, 16, 17, 18, 20, 22 = LL sera; lanes 3, 4, 5, 6, 8, 21, 25, 27 = BB sera; lanes 7, 11, 14, 15, 23, 24, 26 = BT sera; lane 13 = N serum.

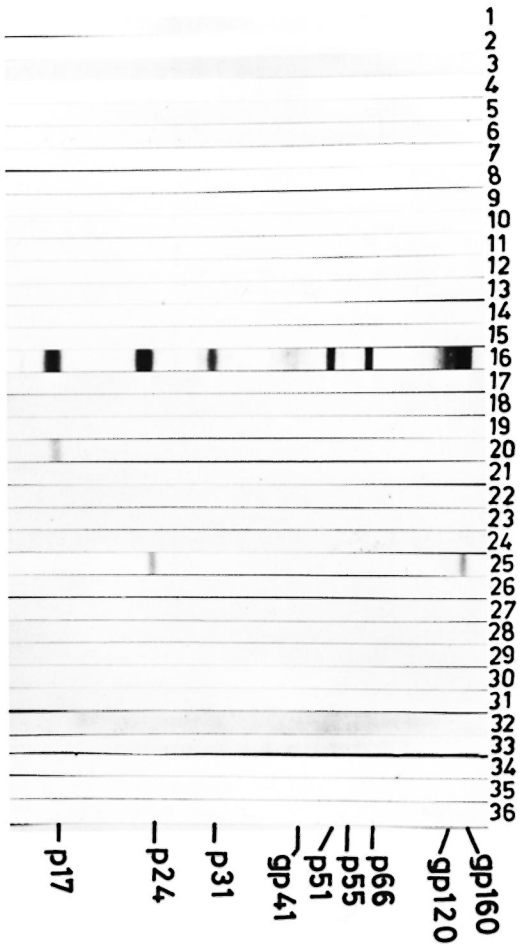

FIG. 2. Reactivity of normal human sera from Indians to HIV-1 antigens by WB. Lane 7 = negative control; lane 16 = high positive control; lane 25 = weak positive control; remaining lanes = normal healthy Indian sera (33); lane 20 = normal healthy subject who had suffered from proteinuria and albuminuria showing p17 band.

of HIV-1 infection. Additional confirmatory tests for HIV-1 already in practice may be useful in such situations.

SUMMARY

Sera from 478 persons (348 leprosy patients, 33 tuberculosis patients, 29 healthy contacts of leprosy patients, 38 normal healthy Indians, and 30 normal healthy Europeans) were screened for anti-HIV-1 IgG antibodies by ELISA. None was positive. In addition, 132 samples (from 43 leprosy patients, 21 tuberculosis patients, 5 healthy contacts of leprosy patients, 33 normal healthy Indians, and 30 normal healthy Europeans) were also tested by Western blot assay for anti-HIV-1 IgG antibodies. Only 1 of the 63 healthy subjects expressed a prominent p17 band. One or more bands were found in 44 (leprosy patients 33/43, tuberculosis patients 7/21, and leprosy contacts 4/5) of the remaining 69 sera. Antibody to the HIV-1-specific antigen p24 was expressed by 17 of these subjects (14/43 leprosy patients, 1/21 tuberculosis patients, and 2/5 leprosy contacts), either as a single band or in combination with other bands. This raises the possibility of a common antigenic pattern between HIV-1 and mycobacteria, especially *Mycobacterium leprae.*

RESUMEN

Usando un ensayo inmunoenzimático se buscaron anticuerpos IgG anti-HIV-1 en los sueros de 478 personas (348 pacientes con lepra, 33 pacientes con tuberculosis, 29 contactos sanos de pacientes con lepra, 38 individuos sanos de la India, y 30 europeos sanos).

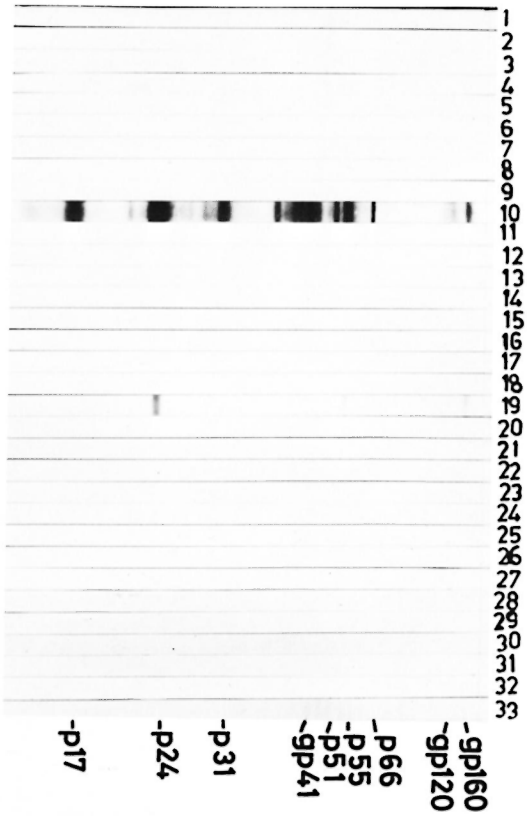

FIG. 3. Reactivity of normal healthy European sera to HIV-1 antigens by WB. Lane 1 = negative control; lane 10 = high positive control; lane 19 = weak positive control; remaining lanes = normal healthy European sera from 30 subjects negative for anti-HIV-1 IgG antibodies.

Ninguno resultó positivo. Además, 132 muestras (de 43 pacientes con lepra, 21 pacientes con tuberculosis, 5 contactos sanos de pacientes con lepra, 33 indúes y 30 europeos sanos) también se probaron por inmunoelectrotransferencia ("Western blot assay") para buscar anticuerpos IgG anti-HIV-1. Sólo 1 de 63 sujetos sanos expresó una banda p17 prominente. Una o más bandas fueron encontradas en 44 de los 69 sueros restantes (33/43 pacientes con lepra, 7/21 pacientes con tuberculosis, 4/5 contactos de pacientes con lepra). Diez y siete de estos individuos (14/43 pacientes con lepra, 1/12 pacientes con tuberculosis, y 2/5 contactos de pacientes con lepra) tuvieron anticuerpos contra el antígeno p24 específico de HIV-1 de manera aislada o en combinación con otras bandas. Esto introduce la posibilidad de que pueda existir algún patrón antigénico común entre HIV-1 y las microbacterias, especialmente *Mycobacterium leprae.*

RÉSUMÉ

Des échantillons de sérum provenant de 478 individus (348 malades de la lèpre, 33 patients atteints de tuberculose, 29 contacts sains de malades de la lèpre, 38 indiens en bonne santé et 30 européens également en bonne santé) ont été passés en revue afin de mettre en évidence les anticorps IgG anti HIV 1, au moyen de méthodes ELISA. Aucun sérum n'a été trouvé positif. En outre, on a également étudié 132 échantillons, provenant de 43 malades de la lèpre, 21 sujets tuberculeux, de 5 contacts sains de malades de la lèpre, de 33 indiens normaux en bonne santé, et de 30 européens également en bonne santé, en utilisant une épreuve de Western blot pour les anticorps IgG anti HIV 1. Un seul seulement des 63 individus en bonne santé a révélé une bande p17. Dans 44 des échantillons de sérum, parmi les 69 autres, on a observé une ou plusieurs bandes : chez 33 malades de la lèpre sur 43, chez 7 sujets tuberculeux sur 21, et chez 4 contacts sur 5. Chez 17 de ces individus, un anticorps contre l'antigène spécifique P24 de HIV-1, était exprimé : chez 14 malades de la lèpre sur 43, chez 1 malade de tuberculose sur 21, et chez 2 contacts lépreux sur 5 parmi les contacts de malades de la lèpre. Ces anticorps étaient exprimés soit sous forme d'une bande unique, soit en combinaison avec d'autres bandes. Ces résultats font entrevoir la possibilité d'un profil antigénique commun entre HIV-1 et les mycobactéries, en particulier *Mycobactérium leprae.*

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