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Neutralizing Antibodies against Autologous Human Immunodeficiency Virus Type 1 Isolates in Patients with Increasing CD4 Cell Counts despite Incomplete Virus Suppression during Antiretroviral Treatment

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Antiretroviral-treated human immunodeficiency virus (HIV) type 1-seropositive individuals can remain clinically stable for a long period of time with an increasing CD4 cell count irrespective of incomplete viral suppression. We evaluated the role of neutralizing antibody (NtAb) activity in the etiopathogenesis of this viro-immunological disconnection (defined as an increasing $CD4^+$-cell count despite a persistent, detectable viral load during antiretroviral therapy) in 33 patients failing therapy with two analogue nucleoside reverse transcriptase inhibitors. An HIV NtAb titer of ≥1:25 was detected in specimens from 16 out of 33 (48%) patients. A significant correlation was found between NtAb titers and $CD4^+$-cell counts ($P = 0.001$; $r = 0.546$) but not with HIV RNA levels in plasma. Five patients with a viro-immunological disconnection had an NtAb titer of >1:125, statistically higher than the NtAb titers for the remaining 28 patients with both virologic and immunologic failure ($P < 0.0001$). The HIV-specific humoral immune response could play a role during antiretroviral treatment to improve immunological function despite an incomplete suppression of viral load.

Human immunodeficiency virus type 1 (HIV-1)-seropositive individuals failing antiretroviral treatment can remain clinically stable for a long period of time (7), showing an increasing CD4 cell count irrespective of incomplete viral suppression (6, 8). The clinical implications of such a viro-immunological disconnection (defined as an increasing $CD4^+$-cell count despite a persistent, detectable viral load during antiretroviral treatment) remain uncertain. Different factors, including decreased viral fitness (10), direct effect of protease inhibitors on immune response (1, 13), and effective humoral and cell-mediated immunological activity (2, 15), have been directly related to the lack of HIV disease progression.

Neutralizing antibody (NtAb) activity has been detected in HIV primary infection simultaneously with a decrease of viral load (14) in long-term nonprogressor patients (4) and during antiretroviral therapy in the chronic phase of HIV infection (3, 13), suggesting an active role of this factor in viral clearance. Conversely, the NtAb response is minimal or absent in patients who progress rapidly to AIDS and death (5).

The aim of the present study was to evaluate the NtAb activity against contemporaneous autologous HIV-1 isolates in 33 HIV-infected adult patients failing antiretroviral treatment and to determine the role of NtAb in the etiopathogenesis of the viro-immunological disconnection.

Thirty-three consecutive patients (21 males and 12 females), all failing treatment with a combination of two nucleoside analogues (zidovudine plus lamivudine), were enrolled in this cross-sectional study.

Plasma HIV-1 RNA levels were measured by using an Amplicor HIV Monitor system (Roche Diagnostic Systems, Branchburg, N.J.). To determine if the HIV isolates were syncytium inducing (SI) or non-SI (NSI), an aliquot of viral stock supernatant containing 100 50% tissue culture infective doses was cultured in T25 flasks with 10^6 MT-2 cells. Cultures were maintained up to 4 weeks and were examined for syncytia twice a week.

The titer of NtAb against autologous virus isolated at the time of serum collection was determined by microculture neutralization assay as previously described (13). Sera from two seronegative subjects were used as negative controls. The neutralizing titer was calculated by interpolation (according to the method of Reed and Muench) as the reciprocal of the dilution that reduced the number of infected cultures by 50%.

The levels of RANTES (regulated upon activation, normal T-cell expressed and secreted) and MIP-1α and -1β (macrophage inflammatory protein-1α and 1β) in plasma were measured by a quantitative enzyme-linked immunosorbent assay (Quantikine; R&D Systems, Minneapolis, Minn.).

Data were analyzed by the Mann-Whitney U test, χ^2 test, and linear correlation test.

The correlation between NtAb titer and immunological and virological characteristics of patients is reported in Table 1. An HIV NtAb titer of ≥1:25 was detected in 16 out of 33 (48%) patients; high levels of NtAb (>1:125) were present in 6 (18%) patients. A significant correlation was found between NtAb

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TABLE 1. Characteristics of patients according to NtAb activity against contemporaneous autologous HIV-1 isolate

Parameter	Value for NtAb titer			*P*
	<1:25	1:25–1:125	>1:125	
No. of patients	17	10	6	
Median no. of $CD4^+$ cells/μl (range)	21 (2–220)	40 (3–101)	305 (23–440)	0.001
Median log HIV RNA copies/ml (range)	5.65 (4.52–6.38)	5.28 (4.45–5.9)	5.03 (4.18–5.36)	0.25
No. with SI phenotype/no. with NSI phenotype	11/6	10/0	2/4	
Mean level of MIP-1α in plasma ± SD (pg/ml)	1,826 ± 1,765	2,178 ± 2,345	2,048 ± 1,944	NS[a]
Mean level of MIP-1β in plasma ± SD (pg/ml)	1,267 ± 1,363	1,058 ± 1,063	1,160 ± 1,099	NS
Mean level of RANTES in plasma ± SD (pg/ml)	10,708 ± 10,344	10,309 ± 11,050	11,300 ± 11,844	NS

[a] NS, not significant.

titers and $CD4^+$-cell counts ($P = 0.001$; $r = 0.546$) and the number of days required to isolate HIV strains from plasma ($P = 0.026$; $r = 0.367$) (data not shown). No significant correlation was found between HIV RNA copy number and NtAb titer ($P = -0.25$; $r = 0.17$). However, the patients with the highest NtAb titers (>1:125) had the lowest viral load.

Twenty-one out of 27 patients (77%) with NtAb titers of <1:125 had an SI viral strain, while 4 out of 6 patients (66%) with an NtAb titer of >1:125 showed an NSI isolate. No difference with regard to β-chemokine levels in plasma was found between patients with or without NtAb activity. None of the 33 patients appeared to be homozygous (Δ32/Δ32) or heterozygous (WT/Δ32) for the partial deletion of the CCR-5 allele (data not shown).

Table 2 shows the virological and immunological parameters of antiretroviral-treated patients according to the presence of viro-immunological disconnection, defined as an increase of >100 $CD4^+$ cells with respect to the pretreatment value despite a persistent, detectable viral load (>4 $\log_{10}$). Five patients with viro-immunological disconnection had an NtAb titer of >1:125, statistically higher than the NtAb titers for the remaining 28 patients with both virologic and immunologic failure ($P < 0.0001$). Particularly, in this last group, NtAb titers for 17 individuals were below the limit of detection of the assay, titers for 10 individuals were intermediate (1:25 to 1:125), and only one individual had a high NtAb titer (1:933).

Determinations of β-chemokine levels in plasma showed similar results in two groups, although patients with viro-immunological disconnection showed the highest production of RANTES (data not shown). No statistical difference was detected with regard to the HIV-1 viral loads of the two groups, while a significantly higher $CD4^+$-cell count, with a mean increase of 118 lymphocytes with respect to the pretreatment level, was found in patients with viro-immunological disconnection. Moreover, a retrospective analysis of pretherapy $CD4^+$-cell counts showed a level of >200 $CD4^+$ cell/mm^3 in all but one patient with viro-immunological disconnection and in 3 out of 28 patients with virological and immunological failure.

The presence of NtAb activity against autologous HIV-1 primary isolates was detected in viremic patients treated with a double-nucleoside antiretroviral regimen during the chronic phase of HIV infection. The HIV-specific humoral immune response could play an important role in controlling HIV disease progression. NtAb may provide protection by blocking HIV infection and limiting the spread of cell virus (4), thus moderating the concentration of infectious cells and slowing the rate of $CD4^+$-cell depletion (9). In this study, in patients with virological failure during antiretroviral treatment, a significant correlation between NtAb titers and $CD4^+$-cell counts was found. Particularly, the presence of a significant NtAb titer against autologous virus was detected in patients with a $CD4^+$-cell count of >200 cells/mm^3 before starting therapy and an increase of $CD4^+$-cell count during antiretroviral treatment.

Patients treated at an earlier stage of chronic HIV-1 infection have a higher humoral immune response than individuals with pretreatment CD4 cell counts of <200 cells/mm^3 (2, 3). However, other factors, including decreased viral fitness, adherence to treatment, immunological effects of drugs, resistance to antiretroviral drugs, and HIV-specific cellular immune response, can play a role in viro-immunological disconnection during antiretroviral treatment.

The lack of an evident association between NtAb titer and viral load in this study agrees with previous reports (4, 12) and casts doubts on the ability of NtAb activity to suppress completely the replication of the contemporaneous autologous virus in vivo. On the other hand, the evidence of lower viremia

TABLE 2. Virological and immunological parameters of patients according to viro-immunological disconnection

Parameter	Value for patients with virological failure and:		*P*
	Immunological disconnection	Immunological concordance	
No. of patients	5	28	
Median NtAb titer (range)	1:631 (1:281–1:1,413)	<1:25 (<1:25–1:933)	<0.0001
Median no. of $CD4^+$ cells/μl (range)			
Pretreatment	207 (103–247)	86 (4–245)	0.02
At enrollment	325 (263–440)	22 (2–220)	<0.0001
Median log HIV RNA copies/ml at enrollment (range)	5.04 (4.18–5.36)	5.52 (4.45–6.38)	0.1
No. with SI phenotype/no. with NSI phenotype	1/4	22/6	0.03
Median duration of treatment[a] (range) (days)	369 (150–1,510)	444 (171–1,329)	0.8

[a] Treatment with zidovudine plus lamivudine.

in patients with very advanced HIV infection and high NtAb titers (>1:125) could explain the role of NtAb in partial viral clearance, reduction of virus escape variants, and slower HIV disease progression (11). In this study, the production of β-chemokines does not seem to differ significantly between patients with different levels of NtAb activity.

In conclusion, the presence of significant titers of NtAb against autologous contemporaneous HIV-1 isolates in patients with sustained CD4 cell counts in spite of virologic failure has interesting clinical implications. A longitudinal, randomized clinical trial needs to be done to analyze whether this phenomenon is associated with a sustained clinical benefit and to evaluate the advantage of switching therapy in instances of virological and immunological disconnection.

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