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Upregulation of Human Immunodeficiency Virus (HIV) Replication by CD4 Cross-Linking in Peripheral Blood Mononuclear Cells of HIV-Infected Adults

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This study was conducted with peripheral blood mononuclear cells from 67 human immunodeficiency virus (HIV)-infected adults. It supports the hypothesis that cross-linking of CD4 molecules by HIV gp120 can result in HIV upregulation and spread of infection. Underlying mechanisms include activation of latent infection by factors in addition to, or other than, tumor necrosis factor alpha.

Dynamic virus replication occurs throughout the course of human immunodeficiency virus (HIV) disease, but the nature of the force driving the replicative machinery remains elusive. We have previously reported that ligation of CD4 molecules can induce tumor necrosis factor alpha (TNF-α) secretion (10) and NFκB activation (2) in $CD4^+$ T cells. In HIV-infected patients there is a high probability of the ligation of $CD4^+$ molecules in vivo by HIV envelope protein gp120 expressed on infected cells, by free or complexed gp120 in circulation, or, in lymph nodes, by HIV virions trapped in the processes of follicular dendritic cells (17). To test the hypothesis that CD4 cross-linking (CD4XL) can activate infected cells, we performed CD4XL in vitro in peripheral blood mononuclear cells (PBMC) from HIV-infected individuals and concurrently determined the levels of TNF-α secretion and HIV p24 antigen (Ag) in the culture supernatants. CD4XL in PBMC was performed as described before (10) with anti-CD4 monoclonal antibody (MAb) Leu3a or OKT4 at a concentration of 3 μg of MAb/10^6 cells/ml. A combination of phorbol 12-myristate 13-acetate (PMA) at 0.1 mM plus 15 mg of phytohemagglutinin (PHA) per ml was used as an additional stimulus for PBMC activation. Cells were cultured for 7 days, and supernatants were tested for TNF-α and p24 Ag by enzyme-linked immunosorbent assay with commercial kits.

Based on the responses to CD4XL in PBMC, patients could be divided into three groups (Fig. 1): group 1 (43% of patients), showing upregulation of TNF-α and of HIV; group 2 (36% of patients), showing upregulation of TNF-α without upregulation of HIV; and group 3 (21% of patients), showing a failure to upregulate either TNF-α or HIV. No patient manifested upregulation of HIV without induction of TNF-α. The status of HIV expression was confirmed in randomly selected patients by reverse transcriptase PCR for HIV *gag* in lysed cells (12) and for HIV RNA in supernatants by nucleic acid sequence-based amplification (13). Shown in Fig. 2 are results for a representative patient from group 1 who, in contrast to a patient from group 2, manifested significantly increased intensity of HIV *gag* in CD4XL-PBMC compared to that in medium-treated cells. Similarly, higher numbers of HIV RNA copies were detected in culture supernatants of CD4XL-PBMC than in medium-treated cultures for patients from group 1 but not for patients from group 2 (Table 1). As expected, regardless of their response to CD4XL, stimulation of PBMC with PMA-PHA resulted in increased numbers of HIV RNA copies and levels of p24 Ag, as well as induction of TNF-α in cultures from all patients.

A significant relationship between CD4XL-induced p24 Ag and TNF-α production in the culture supernatants was observed (Spearman's rank correlation coefficient [r] = 0.43, $P < 0.003$), leading to the speculation that in patients from group 1, production of TNF-α by T cells or monocytes augmented viral transcription either directly or via activation of NFκB (9, 14). We examined the role of TNF-α further in the CD4XL system by adding neutralizing anti-TNF-α antibody to the cultures. Unexpectedly, although addition of anti-TNF-α antibody abrogated measurable TNF-α in the cultures, it could not inhibit viral upregulation (Table 2). Similar effects have been reported for CD30 cross-linking of $CD4^+$ T cells from HIV-infected patients (7), which resulted in HIV replication and increased TNF-α secretion, but the HIV replication was not abrogated

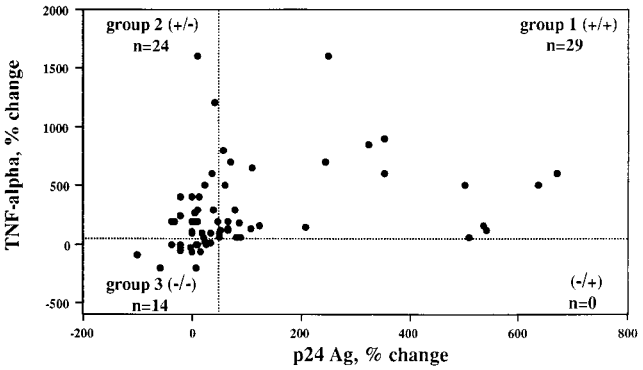

FIG. 1. Effect of CD4XL on TNF-α secretion and p24 Ag levels in PBMC cultures from HIV-infected patients. Data points represent changes in TNF-α and p24 Ag as percentages of values for medium-treated cells. Increases in TNF-α or p24 Ag of greater than 50% were considered indicative of upregulation; the cutoff levels are indicated by dotted lines. + and −, positive and negative, respectively, for TNF-α (left) and p24 Ag (right) upregulation.

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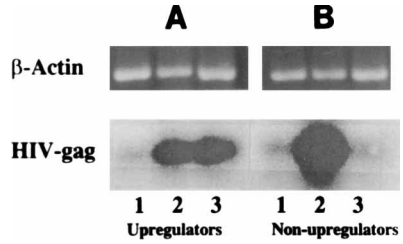

FIG. 2. HIV *gag* RNA expression in PBMC cultures. PBMC were collected after culture, cells were lysed in RNAzol solution, and reverse transcriptase-PCR for HIV *gag* was performed. Results for representative patients from group 1 (A) and group 2 (B) are illustrated. Compared to that in medium-treated cells (lanes 1), HIV *gag* expression was increased in response to CD4XL only in the patient in group 1 (lane 3, panel A) and in response to PMA-PHA in both patients (lanes 2).

by anti-TNF-α neutralizing antibody. These findings do not exclude a role for TNF-α in HIV replication, because effects of induced TNF-α may occur via intracytoplasmic TNF-α–TNF-α receptor interaction. In fact, intracytoplasmic injection of anti-TNF-α antibody, but not exogenously provided antibody, results in blocking of TNF-α activity (16). The observed CD4XL-induced virus upregulation in this group of patients resulted, in all probability, from a combination of TNF-α-dependent and -independent mechanisms; the latter effects could be ascribed to cellular activation, as there is ample evidence that HIV replication resulting from cellular activation can occur independently of TNF-α induction.

The observation that a substantial number of patients fell into the second group, manifesting CD4XL-induced TNF-α secretion without augmentation of viral replication, was unexpected. A possible explanation for the absence of HIV upregulation in this group is that CD4 ligation leads to induction of HIV-specific suppressor factors, either directly or via secretion of TNF-α. The chemokines MIP-1α, MIP-1β, and RANTES have been shown to be produced by many cell types, including $CD8^+$ T cells and B cells, and are known to be potent suppressors of HIV replication (4, 11). Constitutive secretion of the $CD8^+$-T-cell-derived HIV-specific suppressor factor (5) or of the cytokine interleukin-16 (8) in our cultures could also prevent HIV upregulation. Another reason for the failure of virus upregulation may be a dominance of defective (e.g., long terminal repeat-defective) and/or replication-incompetent virus in the PBMC (3, 6). Concurrent antiviral therapy might also prevent viral upregulation in response to CD4XL. Finally, the possibility of CD4 signal-associated direct inhibition of virus replication exists (15), but this is unlikely because HIV-suppressive effects have been ascribed only to signaling via the CDR3 region of the CD4 molecule (1) and not to signaling via the CDR2 region, which is the binding site employed by HIV gp120 and MAb Leu3a.

In patients from group 3, the failure of TNF-α secretion and of viral upregulation upon CD4XL may be attributed to masking of CD4 by endogenous gp120, downmodulation of CD4 molecules in infected cells, or blocking of CD4 by CD4 binding factors, such as interleukin-16. As cells from these patients were responsive to PMA-PHA activation, it is uncertain if they were true nonupregulators.

Patients with the first type of response (group 1), i.e., manifesting CD4XL-induced increases in TNF-α and p24 Ag in PBMC cultures, presented the expected response, in support of the hypothesis that ligation of CD4 molecules activates latently infected cells to promote virus replication. These findings imply the existence of a vicious cycle in HIV infection wherein the virus not only evolves mechanisms for destruction and suppression of the immune system but also can utilize the immune system to its own advantage for facilitating HIV replication and in the propagation of infection. CD4XL elicits cellular activation without proliferation and hence might be involved early in HIV infection and lead to virus spread; this mechanism may also be operative to some extent throughout the disease course. Attempts to interrupt the process of CD4XL in vivo, especially in the early stages of the disease, might be of importance as a strategy to prevent HIV disease progression.

TABLE 1. Effect of CD4XL upon TNF-α secretion, HIV p24 Ag production, and HIV RNA levels in culture supernatants of PBMC from HIV-seropositive donors

Patient	Amt of substance detected under culture condition[a]									Patient group
	Medium			PMA-PHA			CD4XL			
	TNF-α	p24	RNA	TNF-α	p24	RNA	TNF-α	p24	RNA	
1	0.2	2.0	7,800	2.1	3.9	29,000	1.4	15.4	41,000	1
2	0.1	1.5	1,300	1.7	5.9	3,400	0.7	6.8	12,000	1
3	0.1	0.4	1,900	1.9	5.9	37,000	0.6	2.4	29,000	1
4	0	1.1	900	2.0	7.0	35,000	0.6	8.1	6,800	1
5	0	1.9	1,970	2.2	7.3	25,000	2.0	2.3	2,600	2
6	0.3	0.6	<400	2.1	1.9	7,700	0.1	0.7	<400	2

[a] Patients' PBMC were cultured as indicated, and supernatants were collected on day 7. TNF-α levels (expressed as optical density values) and p24 Ag levels (expressed as ratios of actual and cutoff optical density values) were determined by enzyme-linked immunosorbent assay, and the numbers of HIV RNA copies/100 μl of culture supernatant were determined by nucleic acid sequence-based amplification. In all instances, results for p24 Ag concurred with results for HIV RNA. Patients were grouped as described in the text based upon TNF-α and HIV p24 Ag production in response to CD4XL in PBMC.

TABLE 2. Effect of anti-TNF-α neutralizing antibody on CD4XL-induced TNF-α and p24 Ag production

Patient	Amt of substance detected under culture condition(s)[a]									
	Medium		PMA-PHA		CD4XL		CD4XL + anti-TNF-α		CD4XL + rabbit immunoglobulin	
	TNF-α	p24	TNF-α	p24	TNF-α	p24	TNF-α	p24	TNF-α	p24
1	0.18	2.03	2.1	3.92	1.44	15.37	0.12	25.62	1.5	12.4
5	0	1.92	2.16	7.33	2.02	2.33	0.18	4.41	2.0	5.2
7	0.03	1.23	2.3	1.1	1.36	2.51	0.08	3.52	1.2	3.0
8	0	3.33	1.94	3.62	1.33	4.7	0	5.86	1.9	5.6
9	0	2.87	1.93	11.44	1.28	18.59	0	17.71	1.2	17.2

[a] Anti-TNF-α antibody or rabbit immunoglobulin was added to PBMC cultures from patients of group 1 at initiation of CD4XL, and culture supernatants were examined for TNF-α and p24 Ag. In CD4XL-cultures treated with anti-TNF-α antibody, TNF-α was undetectable but p24 Ag production did not decrease. TNF-α levels are expressed as optical density values, and p24 Ag levels are expressed as ratios of the actual and cutoff optical density values.

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