Potentiation of the Immune Response in HIV-1$^+$ Individuals

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Abstract

T cells from HIV-1$^+$ individuals have a defect in mounting an antigen specific response. HIV-1 Tat has been implicated as the causative agent of this immunosuppression. We have previously shown that HIV-1 Tat inhibits antigen specific proliferation of normal T cells in vitro by binding to the accessory molecule CD26, a dipeptidase expressed on the surface of activated T cells. We now demonstrate that the defective in vitro recall antigen response in HIV-1 infected individuals can be restored by the addition of soluble CD26, probably by serving as a decoy receptor for HIV-1 Tat. The restored response is comparable to that of an HIV-1$^-$ individual, suggesting that early in HIV infection there is a block in the memory cell response, rather than deletion of these cells. (*J. Clin. Invest.* 1996. 97:1545–1549.) Key words: DP IV • CD26 • serine protease • immunotherapy • immunosuppression

Introduction

A general defect in immune responsiveness has been observed in HIV-1$^+$ individuals, even early after infection when CD4$^+$ T cells are still present at relatively normal frequency (1, 2). We have previously reported that Tat, a product of HIV-1, binds with nanomolar affinity to CD26 (3), a cell surface molecule involved in T cell costimulation (4), and we have proposed that this interaction leads to immunosuppression (5). Thus, we set out to test whether dysfunction of CD26 is involved in the immune defect seen in HIV-1$^+$ individuals.

CD26, also known as DP IV, is a dipeptidase which is expressed on the surface of a variety of mammalian tissue (6–8), including T lymphocytes (9). It is a postproline cleaving ectoenzyme with a specificity for removing Xaa-Pro dipeptides from the NH_2 terminus of proteins (10). Using potent and specific boronic acid analog inhibitors of DP IV, XaaboroPro (11), we have established that inhibition of DP IV activity blocks antigen specific T cell proliferation. Neither mitogen nor anti-CD2 mediated proliferation of T lymphocytes, however, is impaired by blocking DP IV with these inhibitors (5).

HIV-1 Tat has a similar functional profile as the DP IV inhibitors; it suppresses antigen specific, but not mitogen induced, activation of peripheral T cells (2). We have confirmed this finding (5), and we have demonstrated that HIV-1 Tat partially inhibits DP IV enzyme activity (3). Thus, it is possible that the immuno-suppressive effects of HIV-1 Tat are mediated through its interaction with the DP IV molecule.

T cells derived from HIV-1 infected individuals early in the disease process act like normal T cells cultured in the presence of HIV-1 Tat or XaaboroPro (1, 2, 5); namely, they are unresponsive to recall antigen, but are activated normally by mitogens. Based on these observations, we tested our hypothesis that the defect in recall antigen response seen in HIV-1$^+$ individuals is mediated through DPIV inhibition, by performing in vitro reconstitution experiments with purified soluble CD26 (sCD26).[1]

Here we report a significant increase in the in vitro recall antigen response of HIV-1 infected individuals in the presence of sCD26. This enhanced response is antigen dependent, because culturing the cells in the presence of sCD26 alone induced minimal to no response.

Methods

Reagents. Serum-free AIM V medium (GIBCO BRL, Grand Island, NY) was used for all the in vitro proliferation assays. sCD26 was purified from porcine and murine kidneys, as previously described (12). PHA was purchased from Sigma Chemical Co. (St. Louis, MO). Tetanus Toxoid (TT) was obtained from Connaught Laboratories Ltd. (Ontario, Canada). *Candida albicans* extract was obtained from Greer Laboratories (Lenoir, NC). [^{3}H]TdR with specific activity of 4 Ci/mmol was purchased from ICN Radiochemicals (Irvine, CA).

Blood donors. HIV-1–infected individuals were recruited by Drs. John Mazzullo and Paul Skolnik (New England Medical Center Hospital, Boston), and Bruce Walker (Massachusetts General Hospital, Boston). All of them were asymptomatic, and their CD4 counts ranged between 420 and 830. HIV-1$^-$ volunteers served as control.

Preparation and stimulation of human PBMC. PBMC were isolated from heparinized blood, diluted 1/1 with PBS, on a Ficoll-Hypaque gradient (Pharmacia Biotechnology, Piscataway, NJ). All experiments with HIV-1$^+$ blood were carried out in a BL3 facility. The recovered live cells were washed several times in PBS, resuspended in serum free AIM V medium, and plated at 2–5 $\times$ 10^5 cells/well with various dilutions of antigen plus or minus sCD26 (1–5 μg/ml). Cultures were incubated for the indicated time periods in 5% CO_2 in air atmosphere, and [^{3}H]TdR (0.5 μCi/well) was added 24 h before harvesting. Radio-activity incorporated into DNA was measured by liquid scintillation counting.

1. *Abbreviation used in this paper:* sCD26, soluble CD26.

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Data analysis. All assays were done in triplicate or quadruplicate. The mean values for [^{3}H]TdR incorporation are reported. The Candida specific response was determined as follows: Candida specific response = (Candida cpm − cells alone cpm). The Candida + sCD26 specific response was determined as follows: Candida + sCD26 specific response = [(Candida + sCD26 cpm) − sCD26 cpm]. The index of enhancement seen in the presence of sCD26 was calculated as follows: Enhancement = (Candida + sCD26 specific response)/(Candida specific response).

Results

sCD26 enhances suboptimal antigen response of HIV-infected individuals. PBMC from thirteen asymptomatic HIV infected individuals (CD4 counts between 400 and 1500) were tested for responsiveness to stimulation with the recall antigen, Candida, or the mitogen, PHA. All the patients responded well to PHA, whereas the response to Candida varied widely, from only slightly above background to optimal. No difference in the level of viral RNA could be detected in the PBMC of these asymptomatic individuals (data not shown). To test whether sCD26 could reconstitute an antigen specific response, PBMC from the same HIV-1$^+$ patients were preincubated with 1–5 μg/ml sCD26 for 30 min and then stimulated with various doses of Candida extract. In the presence of sCD26, three types of antigen specific responses were observed, as shown in Fig. 1, *A–C* and Table I. The first group demonstrated a defective response to Candida which was strongly enhanced in the presence of sCD26 (10- to 117-fold) (Fig. 1 *A*; Table I). The second group responded well to Candida, and was only slightly enhanced in the presence of sCD26 (1.1 to 3.5 fold) (Fig. 1 *B*; Table I). The third group also responded well to Candida; however, the addition of sCD26 inhibited the recall antigen response (0.3–0.8-fold) (Fig. 1 *C*; Table I).

In 9/13 patients the addition of sCD26, in the absence of antigen, did not activate the T cells, while in the remaining 4 patients a modest response to sCD26 alone was observed (3–9-fold over background). Although viral replication was once thought to be minimal during the early stages of infection, current data suggest that the virus replicates at a significant rate throughout the disease. Thus, it is possible that T cells specific for HIV-1 are present and there is sufficient HIV antigen to elicit a response in the presence of sCD26. Furthermore, accumulation of partially activated T cells to other pathogens may be present.

sCD26 only modestly enhances suboptimal responses of HIV-1$^-$ individuals. Analyzing in vitro T cell proliferative responses of normal individuals, Morimoto and his collaborators have shown that sCD26 can modestly enhance a suboptimal response (1.25–11 fold), and actually inhibits optimal responses (13). We have obtained much stronger sCD26 dependent enhancement of the recall antigen response with PBMC from HIV-1$^+$ individuals (see Group 1 in Table I). As demonstrated in Table II, the response pattern of normal subjects in the presence of sCD26 resembles that seen by Morimoto; namely, we observed only a modest enhancement with PBMC which respond minimally to Candida (1.4–2.6-fold) (Table II). In the same individuals, stimulation with TT yielded an extremely vigorous response which was not affected or slightly inhibited by the addition of sCD26 (Table II). With PBMC from other HIV-1$^-$ individuals which were highly responsive to stimulation with Candida, we noticed a slight inhibition in the presence of sCD26 (Table II). The fact that we did not observe as strong an enhancement of the recall antigen response in T cells from uninfected controls as in HIV-1$^+$ T cells implies a fundamental difference in the activation state of the T cells in the two groups.

Discussion

Memory T cells are CD26^{bright}, but the role that CD26 plays in these cells is not yet understood. It is possible that CD26 renders them more responsive to challenge with antigen, probably by acting as a costimulatory molecule and, thus, protecting them from apoptosis or anergy. Inhibition of DP IV enzymatic activity in vitro or in vivo has been shown to cause a block of immune responsiveness (5, 14), suggesting that CD26 plays a regulatory role in the immune system.

A number of diseases are associated with alterations in the level of cell surface CD26 expression. Graves disease (15), multiple sclerosis (16) and rheumatoid arthritis (17), all thought to have an autoimmune etiology, are associated with elevated levels of CD26^{bright} cells. This may facilitate disease processes, because the memory cell population is known to have fewer activation requirements than naive cells. Large

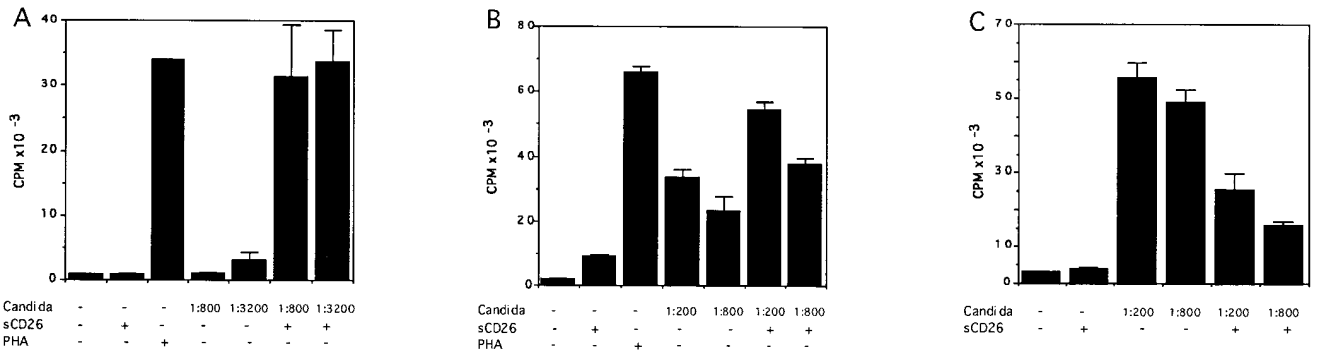

Figure 1. sCD26 enhancement of Candida specific responses in HIV-1$^+$ individuals. In vitro response of HIV-1$^+$ individuals to stimulation with sCD26, PHA, Candida extract or Candida extract in the presence of sCD26. The dilution of Candida extract is specified. The error bars designate the standard error of the mean. (*A*) A minimal antigen specific response that is strongly enhanced in the presence of sCD26 (patient No. 1). (*B*) an antigen specific response that is minimally enhanced in the presence of sCD26 (patient No. 6). (*C*) a strong antigen specific response that is inhibited in the presence of sCD26 (patient No. 12).

Table I. sCD26 Enhancement of an Antigen-specific Response in HIV-1$^+$ Individuals

				[^{3}H] Thymidine incorporation, cpm		
		CD4 Count	Dilution of Candida extract	Candida specific response*	Response to Candida +sCD26‡	Enhancement Index
Group 1	1§	540	1:800	260	30425	117.0
			1:3200	2258	32724	14.5
	2	420	1:200	1163	14294	12.3
			1:800	2038	20406	10.0
			1:3200	4184	1761	0.4
	3	674	1:200	52455	25932	0.5
			1:800	831	39940	48.1
			1:3200	206	19923	96.7
	4	674	1:200	4470	19849	4.4
			1:800	530	14048	26.5
Group 2	5	629	1:200	20098	23671	1.2
			1:800	4908	17124	3.5
	6§	760	1:200	31265	45203	1.4
			1:800	21201	28683	1.4
	7	710	1:800	43376	56367	1.3
			1:3200	44600	55487	1.2
	8		1:800	10816	11778	1.1
	9	493	1:200	9739	18996	2.0
			1:800	10941	13254	1.2
	10	430	1:200	23626	31924	1.4
			1:800	11846	17734	1.5
			1:3200	9283	20758	2.2
Group 3	11	622	1:800	16656	11966	0.7
	12§	576	1:200	52335	21257	0.4
			1:800	45749	11686	0.3
	13	1490	1:800	26072	4639	0.2

*‡See Methods for calculation. §Data for patients 1, 6, and 12 are shown in Fig. 1.

numbers of highly reactive memory cells that are potentially crossreactive to autoantigens may contribute to the pathology of these diseases. HIV-1 infection, on the other hand, is associated with a decrease in the level of CD26^{bright} cells (18, 19). This could signify a specific loss of the memory cell population or, conversely, may be due to a block in the full activation of T cells, resulting in a defective memory response. The latter possibility is more likely, because we are able to reconstitute the immune response of HIV-1 infected PBMC with sCD26, which suggests that T cells specific for recall antigens are present in these patients. Thus, we conclude that the memory cell population has not been lost, but rather the activation process is blocked in HIV infected cells. Our data suggest that HIV-1 Tat mediated inhibition of DP IV indeed plays a role in the defective recall antigen response in HIV-1$^+$ individuals.

Although HIV-1 Tat is a nuclear protein, it has been unequivocally shown that this molecule is secrete from infected live cells, because its transactivating capacity can be detected in bystander cells (20, 21). Furthermore, secreted HIV-1 Tat has been suggested to serve as growth factor for Kaposi's sarcoma cells (21). More recently, it has been proposed that HIV-1 Tat induces functional unresponsiveness in bystander T cells (22) and is responsible for induction of apoptosis in uninfected T cells (23, 24). It is likely that the local concentration of secreted Tat protein is sufficiently high to exert its effect on CD26 on the surface of bystander T cells, although it cannot be detected in the serum at significant level.

It has been shown recently that in vitro activation of HIV-1$^+$ PBMC induces high levels of programmed cell death (AICD) (25). Most reagents that block AICD in HIV-1$^+$ PBMC have no effect on, or only minimally enhance, an antigen specific T cell response (26–28), suggesting that sCD26 does not simply rescue cells from AICD. Ameisen's group has demonstrated that a costimulatory signal through CD28 rescues HIV-1$^+$ cells from AICD and more importantly, restores a defective antigen specific response (25), supporting our theory that the activation process is blocked in HIV infected cells. We have previously shown that CD28 cross-linking overcomes the block in proliferation of HIV-1$^-$ memory T cells, induced by in vitro culture with HIV-1 Tat (5).

Table II. sCD6 Enhancement of an Antigen-specific Response in Uninfected Controls

		[3H] Thymidine incorporation, cpm		
	Stimulus	Candida specific response*	Response to Candida +sCD26‡	Enhancement Index
Control 1	Candida 1:200	12839	10282	0.8
Control 2	Candida 1:300	13305	11568	0.9
Control 3	Candida 1:250	20071	28060	1.4
	Candida 1:1000	4514	12952	2.9
	Tetanus 1:400	46634	41240	0.9
	Tetanus 1:1600	38166	32390	0.8
Control 4	Candida 1:200	7885	20654	2.6
	Candida 1:400	8197	24601	3.0
	Tetanus 1:1000	14254	11747	0.8

*‡See Methods for calculation.

No significant response to recall antigen was seen in most cases when T cells of HIV-1$^+$ individuals with CD4 counts under 300 were tested in vitro, and the addition of sCD26 had no effect. These results are expected, because it is well documented that at the advanced stage of disease the CD26$^+$ CD4 cells are lost. Thus, our approach for bolstering the immune response with sCD26 is only applicable at the early stages of HIV-1 infection. However, at this time point, therapy with sCD26 may raise the general immune responsiveness, resulting in improved CTL activity against HIV-1–infected cells, as well as increased capacity to deal with opportunistic infections.

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