Expression of killer inhibitory receptors on cytotoxic cells from HIV-1-infected individuals

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SUMMARY

Dysfunction of cytotoxic activity of T and natural killer (NK) lymphocytes is a main immunological feature in patients with AIDS, but its basis are not well understood. It has been recently described that T and NK cell-mediated cytotoxicity can be regulated by HLA killer inhibitory receptors (KIR). In this work, we have determined on cytotoxic T cells and NK cells from HIV-1-infected individuals the expression of the following KIR molecules: p58, p70, and ILT2 (immunoglobulin-like family KIR) as well as CD94 and NKG2A (C-lectin-type family KIR). With some exceptions, no significant changes were found on the expression of immunoglobulin-like KIR in either CD8$^+$ or CD56$^+$ cells. Interestingly, the percentages of CD8$^+$ and CD56$^+$ cells expressing CD94 were significantly increased in these individuals. We also show that, *in vitro*, IL-10 up-regulates CD94 expression on CD8$^+$ and CD56$^+$ cells obtained from normal individuals, suggesting that the augmented expression observed in HIV-infected individuals could be related to the high levels of IL-10 previously described in HIV-1-infected individuals.

Keywords NK KIR CD94 cytotoxic T lymphocytes HIV-1 and NKG2

INTRODUCTION

Alterations in the cytotoxicity mediated by T lymphocytes (CD3$^+$CD8$^+$) and natural killer (NK) cells (CD3$^-$CD56$^+$) are a common feature in HIV-1 infection. [1–7]. The underlying causes of these cellular defects have been extensively studied. Abnormalities in the mechanisms of anergy and/or apoptosis of HIV-1-specific T cell clones [1], as well as CD3-mediated stimulation defects [8], have been described in HIV-1-infected individuals. NK cell alterations include a defective triggering of NK cytotoxicity after binding target cells, as well as inability to release NK cytotoxic factors [9]. Nevertheless, these data cannot explain the full extent of alterations in the cytotoxic mechanisms observed in HIV-1 patients.

Recently, HLA-specific killer inhibitory receptors (KIR) have been proposed as regulators of cytotoxic events in HIV-1-infected individuals [10,11]. KIR participate in the regulation of the cytotoxic function of NK and some Tc cells by recognizing specific motifs of HLA molecules [12–15]. These receptors belong to two different structural families. The first group comprises immunoglobulin-superfamily member molecules p58, p70, p70/p140 [16] and the immunoglobulin-like transcript 2 (ILT2) [17], which interact with classical HLA class I antigens expressed on target cells. The second group, which recognizes the non-classical HLA-E class I antigen [18–20], is conformed by heterodimers sharing a common C-type lectin glycoprotein chain, namely CD94, and a second chain encoded for by a member of the NKG2 gene family [21,22]. Thus, CD94/NKG2A heterodimer is considered to be functionally inhibitory, while CD94/NKG2C heterodimer triggers NK cytotoxicity [18,23].

Since cytotoxic cells are involved in protection against viral infections including HIV-1 [24,25], it could be speculated that the cytotoxic dysfunctions reported in HIV-1-infected individuals may be related to the modulation of KIR molecules on Tc and NK cells. Therefore, we have analysed the expression of KIR (p58.1, p58.2, p70 and ILT2) as well as CD94 and NKG2A molecules on peripheral blood T and NK cells of patients with HIV-1 infection.

PATIENTS AND METHODS

Patients and controls

HIV-1$^+$ infected individuals ($n = 44$) were studied for expression of HLA-specific killer inhibitory receptors on CD8$^+$ and CD56$^+$ peripheral blood cells. Blood was obtained during routine

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follow-up visits for the monitoring of disease progression. All subjects gave informed consent under the auspices of the appropriate Research and Ethics Committees. Peripheral blood mononuclear cells (PBMC) were obtained by density gradient centrifugation (Lymphoprep, Nycomed Pharma, Oslo, Norway). Patient classification was according to their total $CD4^+$ cell numbers and clinical stage. Cells were analysed using a FACScount cell counter (Becton Dickinson, Mountain View, CA). At the time of the study all patients were receiving anti-retroviral therapy and were not suffering from other infectious diseases. Healthy HIV-1$^-$ donors ($n = 16$) were included as controls.

Fluorescence analysis

KIR expression was studied by double immunofluorescence on $CD8^+$ and on $CD56^+$ PBMC as previously described [26]. Cells were incubated for 30 min at 4°C in the presence of 1% bovine serum albumin (BSA) with the following MoAbs: GL183 (anti-p58.2), EB6 (anti-p58.1), HP-3B1 (anti-CD94) from Immunotech (Marseille, France), HP-F1 specific for the immunoglobulin-like transcript 2 (ILT2) (a gift from Dr M. Lopez-Botet, Hospital de la Princesa, Madrid, Spain) and MoAb Z199 specific for NKG2A (a gift from Dr A. Moretta, University of Genoa, Italy). After extensive washing, cells were incubated with FITC-conjugated goat anti-mouse IgG antibodies (Becton Dickinson). FITC-conjugated anti-NKB1 (p70), from Becton Dickinson, was also used. Subsequently cells were incubated for 30 min at 4°C with PE-conjugated anti-CD56 (Leu-19) or anti-CD8 (Leu-2A). Fluorescence analysis was performed on a FACSort cytometer (Becton Dickinson) after acquisition of 10^4 events. Viable cells were selected using forward and side scatter characteristics. Resulting profiles were analysed using the Lysis II software.

T cell enrichment and culture

PBMC from healthy individuals were obtained by density gradient centrifugation and adherent cells removed by incubating the PBMC in 100-mm plastic Petri dishes (Costar, Cambridge, MA) at 37°C for 60 min. Non-adherent cells were passed over a nylon-wool column to deplete residual B cells and macrophages [27]. This resulted in a population of T cells (NWT) that was $CD3^+$ >90%, $CD56^+$ <10%, $CD19^+$ <1%, $CD14^+$ <1% cells as determined by flow cytometry. Cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 1% L-glutamine, 1% penicillin/streptomycin (Bio-Whittaker, Verviers, Belgium). Anti-CD3 MoAb (OKT3) was used to coat 24-well culture plates. Cells were cultured at a concentration of 10^6 cells/ml in the presence of IL-10 (10 ng/ml) (Pharmingen, San Diego, CA) and IL-2 (25 U/ml) (Hoffmann-La Roche, Nutley, NJ). OKT3-mediated stimulation was repeated every 5 days.

Statistical analysis

Statistical significance between two means was determined by Student's *t*-test. $P < 0.05$ was considered significant.

RESULTS

Expression of KIR on $CD8^+$ T cells

The expression of CD94, measured by the MoAb HP3B1, on $CD8^+$ cells was significantly increased ($P < 0.0001$) in HIV-1-infected individuals (31.6 ± 25.1) when compared with normal controls (4.6 ± 4.9) (Table 1). A representative flow cytometry analysis from an HIV-1-infected individual is shown in Fig. 1. In order to

analyse further whether this CD94 augmentation was associated with the inhibitory or activatory fractions, the expression of the inhibitory NKG2A molecule was analysed. NKG2A was increased on $CD8^+$ T cells in some patients. On the other hand, no significant changes were found in the expression of immunoglobulin-like KIR (p58, p70 and ILT2) on $CD8^+$ cells (Tables 1 and 2).

Expression of KIR on $CD56^+$ cells

The expression of CD94 on $CD56^+$ cells was significantly increased ($P < 0.001$) in HIV-1-infected individuals (61.2 ± 15.2) compared with normal controls (35.0 ± 19.0) (Table 1 and Fig. 2). No changes in the expression of NKG2A were found on $CD56^+$ cells. No significant changes were found in the expression of immunoglobulin-like KIR in $CD56^+$ cells (Tables 1 and 2). No relationship between the expression of CD94 on T or NK cytotoxic cells and the number of $CD4^+$ cells or the clinical stage was found.

IL-10 mediates CD94 expression on activated T cells

Enriched T cell populations from healthy individuals stimulated by immobilized anti-CD3 MoAb were incubated in the absence or presence of IL-10. The expression of CD94 was determined every 5 days. CD94 was widely expressed on $CD8^+$ T cells (Fig. 3) after 20 days in the presence of IL-10. An increased expression of CD94 on $CD56^+$ cells after IL-10 treatment was also found, although to a lesser extent (data not shown).

DISCUSSION

The cause(s) underlying the dysfunction of the cytotoxic activity of Tc and NK cells described in HIV-1$^+$ individuals are not completely understood despite previous reports which suggest a variety of causes [1–7]. It has been recently proposed that changes in the expression of HLA-specific KIR on cytotoxic T lymphocytes and NK cells would contribute, at least in part, in these deficiencies [10,11]

We have found that the number of $CD8^+$ and $CD56^+$ cells expressing CD94 was significantly increased in HIV-1-infected individuals. The relevance of this observation is the fact that CD94 is the invariant component of the C-lectin-type KIR, broadly expressed on cytotoxic cells [28], although the functional significance of these findings is controversial. Thus, CD94 may be found associated to the NKG2A molecule giving rise to the functionally differentiated HLA-specific inhibitory receptor termed CD94/NKG2A. CD94 can also be associated with NKG2C, a molecule defined in humans by molecular means, constituting the NK
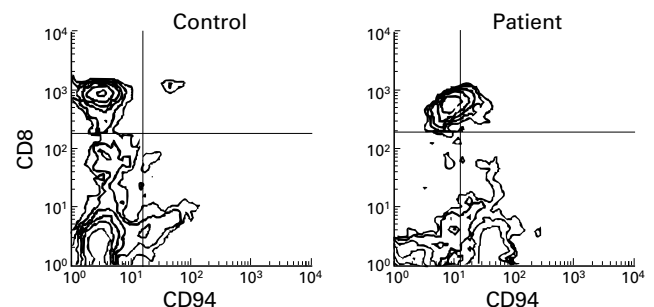

Fig. 1. A representative flow cytometry diagram showing an elevated expression of CD94 on $CD8^+$ cells from HIV-1-infected individuals.

Table 1. Expression of killer inhibitory receptors (KIR) and CD94 on CD8$^+$ and CD56$^+$ subpopulations of lymphocytes from HIV$^+$ individuals

	Ref . no.	CD4 total cells/μl	CD8			CD56			Clinical stage
			(%)*	KIR† (p58.1/p58.2/p70)	CD94†	(%)*	KIR† (p58.1/p58.2/p70)	CD94†	
Controls	1	703	30	1/7/1	2	24	7/15/8	13	–
	2	669	29	4/4/3	3	14	17/13/52	54	–
	3	914	18	0/1/2	3	4	21/28/9	62	–
	4	749	24	0/1/0	2	12	20/33/9	33	–
	5	459	22	1/0/0	7	19	39/35/0	49	–
	6	464	30	2/0/4	3	14	18/6/33	16	–
	7	1296	22	1/1/0	4	9	30/24/13	22	–
	8	823	23	5/1/0	1	12	36/19/8	50	–
	9	556	19	–/–/4	17	40	–/–/6	16	–
HIV$^+$ individuals	1	723	65	–/–/1	44	10	–/–/10	60	A1
	2	677	60	–/–/0	97	7	–/–/1	86	A1
	3	641	63	–/–/5	98	15	–/–/75	63	A1
	4	638	43	–/–/–	9	13	–/–/–	72	A1
	5	628	63	5/1/0	4	5	12/6/0	60	A1
	6	567	46	–/–/10	16	13	–/–/31	62	A1
	7	553	34	–/–/3	16	7	–/–/11	61	A2
	8	541	35	1/7/0	19	17	22/11/1	29	A2
	9	533	27	–/–/0	44	6	–/–/2	67	A2
	10	477	45	–/–/–	33	9	–/–/–	64	C3
	11	458	39	–/–/–	53	6	–/–/–	57	A2
	12	419	54	–/–/2	14	13	–/–/4	36	A2
	13	366	59	17/6/0	12	20	72/22/1	53	B2
	14	353	59	–/–/–	13	7	–/–/–	48	A2
	15	307	49	–/–/0	29	9	–/–/2	56	A2
	16	283	42	–/–/4	52	18	–/–/8	80	B2
	17	261	25	2/2/0	10	16	4/11/1	39	B3
	18	179	42	10/4/–	25	31	12/10/1	83	B3
	19	178	45	–/–/0	13	9	–/–/4	60	A3
	20	172	72	6/5/0	8	8	19/40/7	51	B3
	21	168	64	–/–/3	25	14	–/–/11	79	A3
	22	151	36	–/–/14	8	10	–/–/740	70	C3
	23	143	37	–/–/–	40	22	–/–/–	71	B3
	24	127	56	–/–/2	54	10	–/–/74	38	C3
	25	51	48	–/–/1	35	21	–/–/3	62	B3
	26	45	35	–/–/–	52	6	–/–/–	86	B3

*Referred to the total number of lymphocytes.

†Referred to the percentage of the cellular subpopulation indicated.
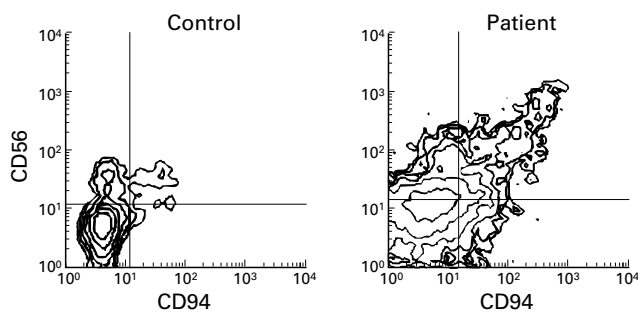
–, Not determined.

Fig. 2. A representative flow cytometry diagram showing an elevated expression of CD94 on CD56$^+$ cells from HIV-1-infected individuals.

cell-triggering receptor designated as CD94/NKG2C heterodimer. In addition, CD94 could bind to other molecules of the NKG2 family whose expression and function on the cell membrane have not yet been described, and the possibility that CD94 may be expressed as a homodimer can not be excluded.

The number of cells expressing KIR belonging to the immunoglobulin-like superfamily is also restricted to a minor subpopulation of CD8$^+$ cells. These results are in agreement with data previously reported [10,11], which have shown that this expression is limited to a subset of specific T cells reactive against HIV-infected target cells.

Understanding the regulation of the expression of these receptors is relevant for both the biology of cytotoxic cells and HIV-1 infection progression. Expression of most leucocyte surface antigens is regulated by cytokines. Whereas very little is known about the regulation of immunoglobulin-like KIR on NK or T cells, it has been recently described that IL-15 up-regulates the expression of

Table 2. Expression of immunoglobulin-like transcript 2 (ILT2), NKG2A and CD94 on CD8^{+} and CD56^{+} subsets from HIV-infected individuals

	Ref. no.	CD4 total cells/μl	CD8				CD56				Clinical stage
			(%)*	ILT2†	NKG2A†	CD94†	(%)*	ILT2†	NKG2A†	CD94†	
Controls	10	628	35	10	4	9	11	36	30	35	–
	11	929	16	3	6	4	23	30	38	35	–
	12	794	19	13	5	12	11	34	25	44	–
	13	913	20	14	6	10	9	31	22	52	–
	14	864	19	37	6	12	18	37	36	38	–
	19	834	20	ND	2	6	11	ND	18	35	–
	16	829	26	ND	5	9	13	ND	15	49	–
		821·56 ± 99·92	21·46 ± 6·41	11·51 ± 12·81	4·59 ± 1·46	8·33 ± 2·75	13·05 ± 4·62	33·48 ± 5·32	24·99 ± 8·76	40·62 ± 7·19	–
HIV[+] individuals	27	458	39	17	33	53	6	50	33	57	A2
	28	477	45	19	2	33	9	28	49	64	C3
	29	638	43	15	6	9	13	7	22	72	A1
	30	353	59	25	6	13	7	22	53	48	A2
	31	143	37	36	2	4	22	39	20	71	B3
	32	45	5	14	15	52	6	27	66	86	B3
	33	615	47	ND	5	13	18	ND	23	58	A2
	34	604	31	ND	3	6	5	ND	11	53	B3
	35	476	18	ND	3	9	6	ND	21	57	A1
	36	538	42	ND	2	6	8	ND	13	42	A1
	37	577	26	ND	4	8	9	ND	17	28	A1
	38	196	8	ND	2	6	18	ND	30	61	C3
	39	256	16	ND	8	13	16	ND	30	56	A2
	40	96	34	ND	5	14	16	ND	11	69	B3
	41	117	32	ND	10	24	6	ND	38	63	B3
	42	185	24	ND	6	15	37	ND	18	76	A3
	43	269	38	ND	8	28	9	ND	25	70	A2
	44	339	40	ND	2	6	7	ND	35	58	A2
		286·12 ± 198	28·10 ± 14·09	19·84 ± 8·31	4·81 ± 7·38	12·87 ± 15·08	10·27 ± 8·10	24·69 ± 14·68	25·24 ± 15·13	58·89 ± 13·24	

*Referred to the number of total lymphocytes.

CD94 on NK cells [29] and cytotoxic T cells [30]. It might be speculated that during HIV-1 infection, where a number of immune alterations occur as the disease progresses, several factors may account for the increased KIR expression observed. Among them, the influence of elevated levels of some cytokines with inhibitory effects on T cell function could be relevant to the
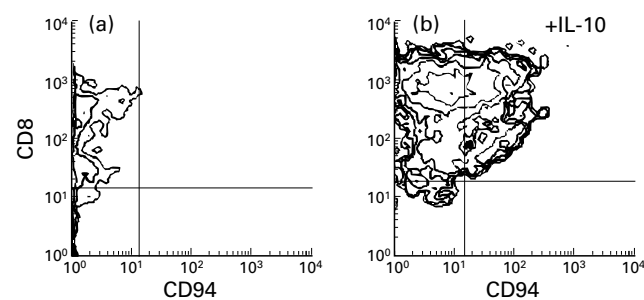

Fig. 3. Induction of CD94 expression on activated T cells treated with IL-10. T cells were stimulated with immobilized anti-CD3 MoAb and cultured during 20 days (a) in the absence or (b) in the presence of exogenous IL-10 (10 ng/ml). A representative experiment is shown.

phenomenon described. Of particular interest in HIV-1 infection is IL-10, which is elevated in HIV-1-infected individuals and is involved in regulating immunity to HIV-1 [31,32]. Indeed, we have shown that prolonged activation of T cells in the presence of a discrete amount of IL-10 induces the expression of CD94 in T cells. It has also been described that IL-10 interferes with HIV-1 replication [33]. Whether or not these two facts are related, requires further investigation.

Taken together, these results suggest that the enhanced expression of CD94 on HIV-1-infected individuals could be involved in the dysfunction of T and NK cytotoxicity demonstrated in HIV-1-infected patients. These results also suggest that IL-10 could have the capacity to induce the expression of CD94 on CD8^{+} and CD56^{+} cells. Whether these changes in the Tc and NK compartment may influence the outcome of individuals with HIV-1 infection is an important issue to a better understanding of the precise role of cytotoxic cells in HIV-1-infected individuals.

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