INTERLEUKIN-9 RECEPTOR α CHAIN mRNA FORMATION IN CD8+ T CELLS PRODUCING ANTI-HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 SUBSTANCE(S)

M.M. HOSSAIN[1], H. TSUCHIE[1*], M.A. DETORIO[1,2], H. SHIRONO[2], C. HARA[2], A. NISHIMOTO[2], A. SAJI[2], J. KOGA[2], N. TAKATA[3], J.K. MANIAR[4], D.G. SAPLE[4], K. TANIGUCHI[5], S. KAGEYAMA[6], H. ICHIMURA[7], T. KURIMURA[7]

[1]Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, 565 Osaka, Japan; [2]JCR Pharmaceutical Co. Ltd., Kobe, Japan; [3]Hiroshima University Medical Hospital, Hiroshima, Japan; [4]Grant Medical College, Mumbai, India; [5]Eiken Chemical Co. Ltd., Tokyo, Japan; [6]Toyama Medical and Pharmaceutical University, Toyama, Japan; [7]Kyoto Prefectural University of Medicine, Kyoto, Japan.

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Summary. – A search for gene(s) associated with anti-human immunodeficiency virus type 1 (HIV-1) activity of CD8+ T cells was attempted using molecular cloning and the relation between the anti-HIV activity of CD8+ T cells and the interleukin-9 receptor α chain (IL-9R-α) mRNA expression from the cDNA clones obtained was examined. The anti-HIV-1 activity of CD8+ T cell culture supernatants was assessed by measuring the level of HIV-1 replication in a CD4+ T cell line transfected with an infectious HIV-1 DNA clone. IL-9R-α mRNA was assayed by reverse transcriptase-polymerase chain reaction (RT-PCR). Of 5 cases showing high level of anti-HIV-1 activity (more than 80% suppression of HIV-1 replication), the mRNA was detected in 4 cases. Of 10 cases showing low level of anti-HIV-1 activity (less than 80% suppression of HIV-1 replication), the mRNA was detected in one case. Soluble recombinant human IL-9 receptor (rhIL-9sR) did not suppress HIV-1 replication at a concentration of 1 μg/ml. These data suggest that the IL-9R-α mRNA formation in CD8+ T cells may correlate with and play some role in the anti-HIV-1 activity of CD8+ T cells from HIV-1-infected individuals.

Key words: CD8+ T cells; anti-HIV-1 activity; cytokines; interleukin-9 receptor

Introduction

CD8+ T cells are involved in the control of HIV-1 replication *in vivo*, especially during the phase of clinical latency and in long-term non-progressors (LTNPs). The suppressive effect of CD8+ T cells may be mediated by a soluble factor(s) (Walker *et al.*, 1986; Walker and Levy, 1989; Brinchmann *et al.*, 1990; Mackewicz and Levy, 1992; Cao *et al.*, 1995; Tsuchie *et al.*, 1997). CC-chemokines RANTES, MIP-1 α and MIP-1 β have been reported to suppress HIV replication (Cocchi *et al.*, 1995; Gao *et al.*, 1997). Recent studies have identified CC-chemokine receptors (CCRs), especially CCR5, a receptor for RANTES, MIP-1 α and MIP-1 β, as co-receptors for macrophage-tropic HIV-1 strains, indicating that the CCRs inhibit HIV-1 infection by interfering with viral entry (Dragic *et al.*, 1996; Huang *et al.*, 1996). Several groups using an HIV-1 long terminal repeat (LTR)-driven chloramphenicol acetyl transferase assay have reported that CD8+ T cell-derived soluble factors suppressed HIV-1 transcription in transiently transfected CD4+ T cells (Chen *et al.*, 1993; Mackewicz *et al.*, 1995;

*Corresponding author.

Abbreviations: AIDS = acquired immunodeficiency syndrome; CAF = CD8+ T cell anti-viral factor; CCR = CC-chemokine receptor; ELISA = enzyme-linked immunosorbent assay; FCS = foetal calf serum; G3PDH = glyceraldehyde-3-phosphate dehydrogenase; HIV-1 = human immunodeficiency virus type 1; HPLC = high performance liquid chromatography; IFN = interferon; IL-2 = interleukin-2; IL-9R-α = interleukin-9 receptor α chain; LTNP = long-term non-progressor; LTR = long terminal repeat; PBS = phosphate-buffered saline; PBMC = peripheral blood mononuclear cell; PCR = polymerase chain reaction; PHA = phytohaemagglutinin; rhIL-9sR = soluble recombinant human IL-9 receptor; RT-PCR = reverse transcription/polymerase chain reaction; SDS = sodium dodecyl sulfate

Copeland *et al.*, 1995). Levy *et al.* (1996) characterised biochemically the factor and designated it as CD8$^+$ T cell antiviral factor (CAF). In our previous studies (M.M. Hossain, unpublished results), we have also found that CD8$^+$ T cells from HIV-1-infected individuals produced a soluble anti-HIV factor(s) which suppressed both HIV-1 replication and transcription in CD4$^+$ T cells. Unlike CAF, this factor was inactivated at 100°C in 10 mins and at 65°C in 20 mins.

In attempting molecular cloning of the anti-HIV substance from mRNA of CD8$^+$ T cells producing high level of anti-HIV activity by use of subtractive hybridisation probes, a cDNA clone corresponding to IL-9R-α was obtained. Unlike the other 16 clones, this was also found to be more related to the anti-HIV activity of CD8$^+$ T cells. The relationship between the production of an anti-HIV-1 substance(s) by CD8$^+$ T cells and the formation of mRNA encoding IL-9R-α in CD8$^+$ T cells was examined in HIV-infected and HIV-seronegative individuals.

Materials and Methods

Subjects and CD8$^+$ T cell preparation. Blood samples were obtained from 6 Indian, 8 African and 1 Japanese HIV-infected individuals (P-1 to P-15). The Japanese HIV-1-infected individual (P-1) was a LTNP whose CD8$^+$ T cells were producing high level of an anti-HIV substance(s) and was used for molecular cloning of cDNA as described below. Blood samples were also collected from 4 Japanese and 1 African HIV-seronegative individuals (H-1 to H-5). The African HIV-seronegative individual (H-5) was the spouse of an African HIV-1-infected man. CD8$^+$ T cells were separated from peripheral blood mononuclear cells (PBMCs) using Dynabeads M-450 (Dynal, Oslo, Norway) and cultured at a density of 5 x 10^6 cells/ml in a growth medium [RPMI 1640 medium with 10% foetal calf serum (FCS), 50 U/ml anti-human interferon (IFN)-α serum (JCR Pharmaceutical Co., Kobe, Japan) and 0.5 U/ml recombinant interleukin-2 (IL-2, Takeda Chemical Industries, Osaka, Japan)] containing 1% phytohaemagglutinin P (PHA, Difco Laboratories, Detroit, MI). Supernatants of the CD8$^+$ T cell cultures were collected, filtered (0.22 μm pore size) and kept at -70°C until used.

Construction of cDNA library. Poly(A)$^+$ RNA prepared from the CD8$^+$ T cells of a Japanese HIV-1-infected LTNP (P-1) by use of Quick Prep mRNA Purification Kit (Pharmacia Biotech Tokyo, Japan) was employed for molecular cloning of cDNA. By use of Great Lengths cDNA Synthesis Kit (Clontech Laboratories Inc., Palo Alto, CA), double-stranded cDNA was synthesised, ligated to *Eco*RI-*Not*I-*Sal*I adaptors, and phosphorylated. cDNA fragments bigger than 500 bp were obtained by using Sephadex G50 columns, ligated to *Eco*RI-digested pUC118 vector, and employed for transformation of *E. coli* strain DH5-α for preparation of a cDNA library.

Subtracted cDNA probe. To improve the chances of identifying cDNA clones related to anti-HIV activity, a subtracted cDNA was generated with a Subtractor Kit (Invitrogen Corp., San Diego, CA) and used as a probe for screening of the cDNA library. Single-stranded cDNA was prepared from P-1 and hybridised to photobiotinylated mRNA from CD8$^+$ T cells from HIV-seronegative donors without any HIV suppression activity. As a result, cDNA sequences common to both pools hybridised, leaving differentially expressed genes unhybridised. The photobiotinylated mRNA-cDNA hybrids were complexed with free streptavidin and removed from the hybridisation mixture by phenol-chloroform extraction (Sive and John, 1988), leaving unhybridised (substracted) cDNA in the water phase. The subtracted cDNA was radiolabelled and used as a probe for screening of the cDNA library.

Colony hybridisation. For screening, 20,000 transformants from selected plates were transferred to nylon membranes (Hybond-N$^+$, Amersham, Cambridge, UK) and processed as previously described (Grunstein and Hogness, 1975). Hybridisation was carried out at 65°C in 6 x SSC, 1% sodium dodecyl sulfate (SDS), and 0.5% non-fat dry milk pH 7.0 for 12 – 16 hrs. A radioactive probe was prepared from the subtracted cDNA by use of Random Primer Kit (Invitrogen Corp., San Diego, CA). The radioactivity of hybridisation mixture was adjusted to approximately 1 x 10^6 cpm/ml. Colonies hybridising strongly with the probe were selected, plated again and a secondary hybridisation was performed for the identification and isolation of individual positive colonies.

DNA sequencing. Double-stranded plasmid DNA templates were prepared in a plasmid DNA purification mini-system (BIO 101, Vista, CA) and sequenced from both ends using universal forward and fluorescent reverse M13 primers. The reaction was carried out using PRISM Sequencing Kit (Applied Biosystems Inc., Foster City, CA) and the reaction products were analysed in an automated ABI 373 DNA sequencer. Nucleic acid and protein databases search was performed at the National Center for Biotechnology Information Server using the BLAST algorithm (Altschul *et al.*, 1990) .

Assay for anti-HIV-1 activity of CD8$^+$ T cells. PMBCs (1 x 10^6) from 4 HIV-1-infected individuals and CD8$^+$ T cell-depleted PBMCs (1 x 10^6) from HIV-seronegative donors per well were mixed and co-cultured in 24-well microplates in 1 ml per well of the growth medium containing 1% PHA. Alternatively, to examine the anti-HIV-1 activity of CD8$^+$ T cells, CD8$^+$ T cell-depleted PBMCs from HIV-1-infected individuals were co-cultured with CD8$^+$ T cell-depleted PBMCs from HIV-seronegative donors. One-half volumes of the culture supernatants were collected and replaced with the same amount of fresh growth medium without PHA every 2–3 days. The culture supernatants were assayed for HIV-1 p24 antigen by a commercially available HIV-1 p24 Kit (HIVAG-1, Abbott GmbH Diagnostika, Wiesbaden-Delkenheim, Germany).

To examine the anti-HIV-1 activity in the culture supernatants of CD8$^+$ T cells, we utilized a CD4$^+$ T cell line, TALL-1, transfected with pNL432 plasmid DNA carrying a full-length of HIV-1 genome (Adachi *et al.*, 1986). Briefly, 1 x 10^6 cells were transfected with 1 μg of pNL432 and incubated in RPMI-1640 medium containing 10% FCS for 5 hrs at 37°C. Then, the transfected cells were cultured in the presence or absence of 50% dilutions of the CD8$^+$ T cell culture supernatants. One-half volumes of the culture supernatants were replaced with fresh 50% dilutions of CD8$^+$ T cell culture supernatants every 3 days and the supernatants were assayed for HIV-1 p17 by an enzyme-linked immunosorbent assay (ELISA). Additionally, the transfected cells were cultured in the presence or absence of rhIL-9sR (R & D Systems, Minneapo-

lis, MN) in the growth medium at concentrations of 1, 10, 100 and 1,000 ng/ml to examine its effect on HIV-1 replication. One-half volumes of the culture supernatants were replaced with the fresh medium containing the same concentration of rhIL-9sR every 3 days and the culture supernatants were tested for HIV-1 p17.

Detection of IL-9R-α mRNA by RT-PCR. Poly(A)⁺ RNA preparation from 2 x 10^6 CD8⁺ T cells was used for the first-strand cDNA synthesis with Ready-To-Go T-primed First Strand Kit (Pharmacia Biotech, Tokyo, Japan). One μl of the first strand cDNA sample was subjected to a 25-cycle amplification (denaturation at 94 °C for 1 min, annealing at 65°C for 1 min, and elongation at 72°C for 2 mins) by PCR in 100 μl of PCR buffer containing 10 mmol/l Tris.HCl pH 8.3, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 0.001% gelatin, 0.25 mmol/l each of dNTPs, 100 pmoles of either IL-9R-α-specific or glyceraldehyde-3-phosphate dehydrogenase (G3PDH)-specific primers, and 2.5 U of Taq DNA polymerase (Takara Biochemicals Co., Tokyo, Japan). The primers used for the amplification of IL-9R-α cDNA encoding transmembrane and cytoplasmic domain of IL-9R-α were IL-9R-A (5'-GTGGCCAGGCAACACCCTTGTTGC-3') and IL-9R-B (5'-GTCAGGGACCTAGAATGTCCAGGA-3'). The primers for the amplification of G3PDH cDNA were G3PDH-A (5'-TGAAGGTCGGAGTCAACGGATTTGGT-3') and G3PDH-B (5'-CATGTGGGCCATGAGGTCCACCAC-3'). Five μl of the PCR product was electrophoresed in a 2.5% NuSieve GTG agarose gel and the presence of IL-9R-α gene product was examined under UV light after ethidium bromide staining.

Quantitative determination of IL-9-α cDNA by high performance liquid chromatography (HPLC). To analyse the difference in levels of mRNAs encoding IL-9R-α and G3PDH in CD8⁺ T cells, a first strand cDNA sample containing 10 amoles of G3PDH cDNA was used in PCR for amplification of IL-9R-α cDNA. The amount of IL-9R-α cDNA was determined by HPLC and expressed as the square of the IL-9R-α-specific peak. For determination of the copy number of G3PDH cDNA (target), a constant volume of each first strand cDNA was co-amplified with competitor DNA (G3PDH PCR Mimic Clontech Laboratories Inc., Palo Alto, CA) using serial ten-fold dilutions ranging from 0.001 to 100 amoles. After reaction, the products derived from the target and competitor cDNA were resolved by HPLC, and the amount of target cDNA was determined from the amount of competitor cDNA in which the amounts of competitor and target products appeared equal.

Results

Molecular cloning of cDNA

A cDNA library was generated to search for mRNA formation in CD8⁺ T cells producing high level of anti-HIV activity. Twenty-one cDNA clones were isolated by subtractive hybridisation (Table 1). Of these clones, 10 were similar to known cellular sequences (# 1, 2, 3, 4, 11, 16, 17, 18, 19 and 21), 6 had nucleotide sequences which have not been reported yet (# 5, 6, 7, 12, 14 and 20), and 5 could not be sequenced successfully (# 8, 9, 10, 13 and 15). IL-9R mRNA was detected only in CD8⁺ T cells of P-1 but not in the HIV-seronegative donor (H-1). mRNAs corresponding to other DNA clones (# 1, 4, 5, 6, 7, 12, 14, 16, 17, 18, 20 and 21) were found in CD8⁺ T cells of both P-1 and H-1.

Anti-HIV-1 activity of CD8⁺ T cells and IL-9R-α mRNA formation

The results of experiments on HIV-1 replication in naturally infected PBMCs and CD8⁺ T cell-depleted PBMCs from 4 HIV-1-infected individuals and on IL-9R-α mRNA formation in CD8⁺ T cells are shown in Table 2. A complete suppression of HIV-1 replication in a PBMC culture containing CD8⁺ T cells and of IL-9R-α mRNA formation was detected in one patient (P-2). However, high level of HIV-1 replication in a CD8⁺ T cell-depleted PBMC culture was observed. A relative delay in HIV-1 replication in the non-depleted PBMC culture in comparison with that in the CD8⁺ T cell-depleted PBMC culture was seen in one patient (P3) in which IL-9R-α mRNA was not detected. IL-9R-α mRNA but not HIV-1 production was detected in both the non-depleted and CD8⁺ T cell-depleted PBMC cultures of two patients (P-4 and P-5).

The results of utilising the supernatants from the CD8⁺ T cell cultures of HIV-infected individuals for determination of suppression of HIV-1 replication in transfected CD4⁺ T cells are shown in Table 3 and Fig. 1. In 5 out of the 15 cases tested,

Table 1. Characterization of cDNA clones from CD8⁺ T cells

Clone No.	Description	mRNA expression in CD8⁺ T cells	
		P-1	H-1
11	IL-9 receptor	+	–
1	Nucleic acid binding protein	+	+
2	*Alu* repeat	NT	NT
3	*Alu* repeat	NT	NT
4	Type I 5'-iodothyronine deiodinase	+	+
16	NRF1 protein	+	+
17	Type I 5'-iodothyronine deiodinase	+	+
18	NKG-5	+	+
19	*Alu* repeat	NT	NT
21	MIP-1 α	+	+
5		+	+
6		+	+
7	Novel sequence	+	+
12		+	+
14		+	+
20		+	+
8			
9			
10	Sequencing unsuccessful		
13			
15			

NT = not tested.

Table 2. Anti-HIV activity of CD8⁺ T cells in naturally infected PBMCs and formation of IL-9R-α mRNA in CD8⁺ T cells

Patient	Clinical stage	HIV infection	Cell preparation	HIV-1 p24 (pg/ml) on day 15	18	21	24	27	IL-9R-α mRNA
P-2	2	HIV-1	PBMCs	<30	<30	>30	<30	<30	+
			CD8⁺-depleted PBMCs	31	137	2,166	10,694	35,884	
P-3	4	HIV-1	PBMCs	<30	36	220	1,628	12,034	–
			CD8⁺-depleted PBMCs	<30	40	1,163	10,483	88,902	
P-4	2	HIV-1	PBMCs	<30	<30	<30	<30	<30	+
			CD8⁺-depleted PBMCs	<30	<30	<30	<30	<30	
P-5	2	HIV-1	PBMCs	<30	<30	<30	<30	<30	+
			CD8⁺-depleted PBMCs	<30	<30	<30	<30	<30	

Table 3. Relationship between levels of the anti-HIV activity and the IL-9R-α mRNA in CD8⁺ T cells from HIV-infected patients and HIV-seronegative donors

Patient code	Clinical stage	HIV infection	p17 pg/ml (%)[a]	IL-9R-α mRNA
Exp. 1				
Medium			720(100)	
P-1	2	HIV-1	55(7)	+ (8604416)[b]
H-2		negative	1550(215)	–
H-3		negative	765(106)	–
H-4		negative	1275(177)	–
Exp. 2				
Medium			722(100)	
P-1	2	HIV-1	<10(<5)	+ (8604416)
P-6	2	HIV-2	244(33)	– (319942)
P-7	2	HIV-2	363(50)	– (265931)
P-8	2	HIV-1	650(90)	– (22668)
Exp. 3				
Medium			2100(100)	
P-1	2	HIV-1	<10(<5)	+ (8604416)
P-9	2	HIV-1	310(15)	– (99317)
P-10	2	HIV-1	362(17)	+ (3059192)
P-11	2	HIV-1	400(19)	+ (5122696)
P-12	2	HIV-1	1240(59)	– (328776)
P-13	2	HIV-1	1830(87)	+ (3799348)
P-14	2	HIV-1	1990(94)	– (464651)
P-15	2	HIV-1	2300(109)	–
H-5		negative	65(3)	+ (2067405)

[a]Values on day 9. [b]Values in parentheses are squares of IL-9R-α mRNA-specific peaks in HPLC.
(+) = present; (–) = absent.

a high level of anti-HIV-1 activity (more than 80% suppression of HIV-1 replication) was observed (P-1, P-9, P-10, P-11, and H-5). In 4 out of these 5 cases (P-1, P-10, P-11, and H-5), IL-9R-α mRNA was detected. In one case (P-9), a high level of anti-HIV-1 activity (85% suppression of HIV-1 replication) was observed but IL-9R-α mRNA was not detected. Among the 3 Japanese HIV-seronegative individuals (H-2, H-3 and H-4), neither anti-HIV-1 activity nor IL-9R-α mRNA was detected. However, both the anti-HIV-1 activity and IL-9R-α mRNA were detected in the African HIV-seronegative woman (H-5) whose spouse was HIV-1-positive. Among the 10 cases which showed a low level of anti-HIV-1 activity (less than 80% suppression of HIV-1 replication), IL-9R-α mRNA was detected in one case (P13).

The data on the level of IL-9R-α mRNA detected by gel electrophoresis were confirmed by PCR and HPLC using the first-strand cDNA sample containing the same amount of G3PDH cDNA (Table 3).

Table 4. Effect of rhIL-9sR on HIV-1 replication

	HIV-1 p17 (pg/ml)	
	Day 9	Day 12
Medium	872	5,076
rhIL-9sR		
1 μg/ml	765	4,391
100 ng/ml	775	8,614
10 ng/ml	946	9,535
1 ng/ml	1,000	11,355
CD8⁺ T cell culture supernatant		
50%	<10	<10
5%	413	574
0.5%	575	908
0.05%	984	2,384

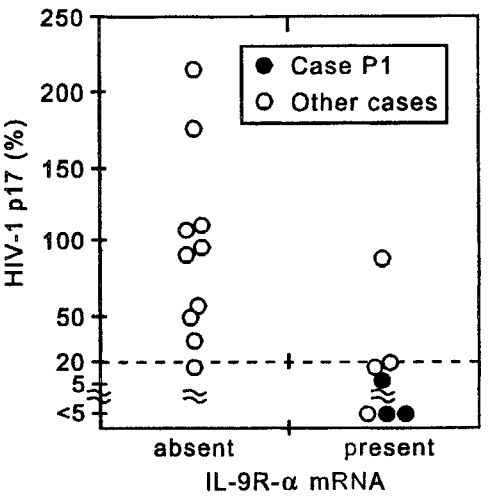

Fig. 1
Anti-HIV-1 activity and IL-9R-α mRNA formation – properties of 21 cDNA clones from CD8⁺ T cells
Anti-HIV-1 activity in culture supernatants expressed as reciprocal of HIV-1 p17 in % (ordinate). The experiment with the cDNA clone from CD8⁺ T cells from case P1 repeated independently 3 times.

rhIL-9sR did not suppress HIV-1 replication even at a concentration of 1μg/ml in the assay, whereas the culture supernatant of CD8⁺ T cells from P-1 did in a dose-dependent manner (Table 4).

Discussion

In our experiments on molecular cloning of cDNA using subtractive hybridisation, a cDNA clone corresponding to IL-9R-α was obtained. In addition, the correlation between levels of the anti-HIV-1 activity and IL-9R-α mRNA in CD8⁺ T cells was observed in 13 out of 15 cases. We used two different assay procedures for the anti-HIV activity of CD8⁺ T cells (the assay using naturally HIV-infected PBMCs (the co-culture assay) and another assay using the T cell line transfected with infectious HIV-1 DNA). In the co-culture assay, a contact between the CD4⁺ and CD8⁺ T cells was necessary for the optimal inhibition of HIV replication in some cases (Tsuchie *et al.*, 1997). In addition, in some cases too, HIV production in supernatants of PBMC cultures was undetectable even after depletion of CD8⁺ T cells. It is possible that, in such cases, virus load in PBMCs was very low and HIV production was not detected during the observation period. It is also possible that a small number of CD8⁺ T cells with a very high level of anti-HIV activity remained in the CD8⁺ T cell-depleted PBMC cultures.

To exclude the possibility that all kinds of mRNA might be intensively formed in CD8⁺ T cells in the cases positive for IL-9R-α mRNA, the first strand cDNA sample containing the same amount of G3PDH cDNA was used for PCR and the relationship between of the two cDNAs was analysed semiquantatively by HPLC. These data showed that large amounts of IL-9R-α mRNA were present in CD8⁺ T cells in those cases in which G3PDH mRNA was found in small amounts.

IL-9 stimulates the proliferation of a variety of hematopoietic lineages through its interaction with IL-9R (Uyttenhove *et al.*, 1988; Van Snick *et al.*, 1989; Yang *et al.*, 1989). IL-9R consists of two chains, α chain specific for its ligand and chain specific for signal transduction (Renauld *et al.*, 1992). A sequence analysis has revealed that IL-9R can be expressed in membrane-bound and soluble forms (Yang *et al.*, 1989). Soluble cytokine receptors can be generated through proteolytic cleavage of the membrane-bound receptors or by direct translation of spliced mRNAs specifically encoding the soluble forms, possibly by mRNA splicing in case of the soluble form of IL-9R (Heaney and Golde, 1996). IL-9 has been reported to enhance HIV replication in naturally and experimentally infected CD4⁺ T cells (Mackewicz *et al.*, 1994).

The relationship between the levels of IL-9R-α mRNA and anti-HIV activity in CD8⁺ T cells observed in our study raises the following possibilities: (1) A soluble form of the extracellular domain of IL-9R-α chain may exert inhibitory activity on HIV-1 replication and represent an anti-HIV substance itself. (2) A soluble form of IL-9R may induce formation of mRNA encoding an anti-HIV substance(s) in CD8⁺ T cells through binding to some receptor on the cells. (3) Correlation between an anti-HIV substance production and IL-9R-α mRNA formation may be accidental. Soluble receptors competing with a membrane-bound receptor for ligand may decrease receptor-mediated signal generation (Renauld *et al.*, 1995). Several cytokines including tumour necrosis factor, IFN-γ, IL-2 and IL-4 are inhibited by soluble

receptors (Larrick and Wright, 1992). In our assay, rhIL-9sR produced by the baculovirus expression system did not show any anti-HIV-1 activity even at a concentration of 1 μg/ml. The mature rhIL-9sR has a predicted M_r of approximately 26 K, and our preliminary data suggested the size of the active substance between 15 and 40 K.

In our assay using the T cell line transfected with infectious HIV-1 DNA, CC-chemokines RANTES, MIP-1α and MIP-1β did not suppress HIV-1 replication at a concentration of 200 ng/ml (unpublished results). In addition, the anti-HIV activity was not inactivated by neutralising antibodies against CC-chemokines but was inactivated at 100°C in 10 mins or at 65°C in 20 mins unlike CAF as reported by Levy *et al.* (1996). These data suggest the presence of some anti-HIV factor(s) different from CC-chemokines and CAF. It is well known that a strong non-cytotoxic anti-HIV response of CD8⁺ T cells and the production of a soluble anti-HIV factor(s) correlate with high numbers of circulating CD4⁺ T cells and a healthy clinical state (Levy *et al.*, 1996; Gomez *et al.*, 1994). The present study delineates a new aspect of the suppression of HIV replication by CD8⁺ T cells and may be useful for the potential therapeutic strategy for long-term survival of HIV-infected individuals, although the mechanism of the phenomenon involved is so far unclear.

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References

Adachi A, Gendelman HE, Koenig S, Folks T, Willey R, Rabson A, Martin MA (1986): Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J. Virol.* **59**, 284–291.

Altschul SF, Gish W, Miller W (1990): Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410.

Brinchmann JE, Gaudernack G, Vartdal F (1990): CD8⁺ T cells inhibit HIV replication in naturally infected CD4⁺ T cells: Evidence for a soluble inhibitor. *J. Immunol.* **144**, 2961–2966.

Cao Y, Qin L, Zhang L, Safrit J, Ho DD (1995): Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. *N. Engl. J. Med.* **332**, 201–208.

Chen C-H, Weinhold KJ, Bartlett JA, Bolognesi DP, Greenberg ML (1993): CD8⁺ T lymphocyte-mediated inhibition of HIV-1 long terminal repeat transcription: a novel antiviral mechanism. *AIDS Res. Hum. Retroviruses.* **9**, 1079–1086.

Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P (1995): Identification of RANTES, MIP-1α, and MIP-1β as the major HIV-suppressive factors produced by CD8⁺ T cells. *Science* **270**, 1811–1815.

Copeland KFT, McKay PJ, Rosenthal KL (1995): Suppression of activation of the human immunodeficiency virus long terminal repeat by CD8⁺ T cells is not lentivirus specific. *AIDS Res. Hum. Retroviruses.* **11**, 1321–1326.

Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, Cayanan C, Maddon PJ, Koup RA, Moore JP, Paxton WA (1996): HIV-1 entry into CD4⁺ cells is mediated by the chemokine receptor C-C CKR-5. *Nature* **381**, 667–673.

Gao M, Tsuchie H, Detorio MA, Hossain MM, Owatari S, Zhang J, Taniguchi K, Nishimoto A, Shirono H, Koga J, Takata N, Matsumoto T, Yoshizaki K, Kurimura T (1997): Interleukin-16 does not suppress HIV-1 replication in naturally infected peripheral blood mononuclear cells. *AIDS* **11**, 538–539.

Gomez AM, Smaill FM, Rosenthal KL (1994): Inhibition of HIV replication by CD8⁺ T cells correlates with CD4 counts and clinical stage of disease. *Clin. Exp. Immunol.* **97**, 68–75.

Grunstein M, Hogness DS (1975): Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.

Heaney ML, Golde DW (1996): Soluble cytokine receptors. *Blood* **87**, 847–857.

Huang Y, Paxton WA, Wolinsky SM, Neumann AU, Zhang L, He T, Kang S, Ceradini D, Jin Z, Yazdanbakhsh, Kunstman K, Erickson D, Dragon E, Landau NR, Phair J, Ho DD, Koup RA (1996): The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nature Medicine* **2**, 1240–1243.

Larrick JW, Wright SC (1992): Native cytokine antagonists. *Bailliere's Clin. Haematol.* **5**, 681–702.

Levy JA, Mackewicz CE, Barker E (1996): Controlling HIV pathogenesis: the role of the noncytotoxic anti-HIV response of CD8⁺ T cells. *Immunol. Today* **17**, 217–224.

Mackewicz CE, Levy JA (1992): CD8⁺ cell anti-HIV activity: nonlytic suppression of virus replication. *AIDS Res. Hum. Retroviruses* **8**, 1039–1050.

Mackewicz CE, Ortega H, Levy JA(1994): Effect of cytokines on HIV replication in CD4⁺ lymphocytes: lack of identity with the CD8⁺ cell antiviral factor. *Cell. Immunol.* **153**, 329–343

Mackewicz CE, Blackbourn DJ, Levy JA (1995): CD8⁺ T cells suppress human immunodeficiency virus replication by inhibiting viral transcription. *Proc. Natl. Acad. Sci. USA* **92**, 2308–2312.

Renauld J-C, Druez C, Kermouni A, Houssiau F, Uyttenhove C, Van Roost E, Van Snick J (1992): Expression cloning of the murine and human interleukin 9 receptor cDNAs. *Proc. Natl. Acad. Sci. USA* **89**, 5690–5694.

Renauld J-C, Kermouni A, Vink A, Louahed J, Van Snick J (1995): Interleukin-9 and its receptor: involvement in mast cell

differentiation and T cell oncogenesis. *J. Leukocyte Biol.* **57**, 353–360.

Sive HL, John TS (1988): A simple subtractive hybridization technique employing photoactivatable biotin and phenol extraction. *Nucleic Acid Res.* **16**, 10937.

Tsuchie H, Detorio MA, Hossain MM, Tesfamariam N, Trickett A, Lam-Po-Tang PR, Yamada O, Ichimura H, Dwyer JM, Kurimura T(1997): Suppression of HIV replication in vitro by CD8$^+$ T-cells from HIV-infected and HIV-seronegative individuals. *Int. J. STD. AIDS* **8**, 307–310.

Uyttenhove C, Sympson RJ, Van Snick J (1988): Functional and structural characterization of P40, a mouse glycoprotein with T-cell growth factor activity. *Proc. Natl. Acad. Sci. USA* **85**, 6934–6938.

Van Snick J, Goethals A, Renauld J-C, Van Roost E, Uyttenhove C, Rubira MR, Moritz RL, Simpson RJ (1989): Cloning and characterization of a cDNA for a new mouse T cell growth factor (P40). *J. Exp. Med.* **169**, 363–368.

Walker CM, Moody DJ, Stites DP, Levy JA (1986): CD8$^+$ lymphocytes can control HIV infection in vitro by suppressing virus replication. *Science* **234**,1563–1566.

Walker CM, Levy JA (1989): A diffusible lymphokine produced by CD8$^+$ T lymphocytes suppresses HIV replication. *Immunology* **66**, 628–630.

Yang Y-C, Ricciardi S, Ciarletta A, Calvetti J, Kelleher K, Clark SC (1989): Expression cloning of a cDNA encoding a novel human hematopoietic growth factor: human homologue of murine T-cell growth factor P40. *Blood* **74**, 1880–1884.