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$\gamma\delta$ T lymphocyte responses to HIV

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SUMMARY

Natural immunity may be involved in controlling viral spread in hosts infected with HIV. A panel of $\gamma\delta$ T cell receptor-positive lymphocyte clones was isolated from the peripheral blood of healthy HIV$^-$ donors and tested for anti-HIV cytotoxic responses. Twelve of 30 (40%) V$\gamma9^+$/V$\delta2^+$ T cell clones, but none of seven V$\delta1^+$ T cell clones, displayed lytic activity against HIV-infected cells. The V$\gamma9^+$/V$\delta2^+$ clones cytotoxic for HIV-infected cells also lysed Daudi cells. However, not all V$\gamma9^+$/V$\delta2^+$ clones which lysed Daudi targets had the capacity to lyse HIV-infected cells. Some of the $\gamma\delta$ T cell clones were also investigated for potential proliferative responses to HIV-infected cells. One V$\gamma9^+$/V$\delta2^+$ T cell clone (ME8-7) and one V$\delta1^+$ T cell clone (ME18-2) demonstrated proliferative responses toward HIV-infected cells. Another V$\gamma9^+$/V$\delta2^+$ clone (VM39) proliferated in response to cell-free HIV. Taken together, these results provide direct evidence of anti-HIV $\gamma\delta$ T cell responses in healthy, HIV$^-$ persons.

Keywords HIV $\gamma\delta$ T lymphocyte cytotoxicity proliferation

INTRODUCTION

Infection with HIV initiates a progressive degeneration of the immune and central nervous systems in susceptible hosts [1]. The terminal manifestation of HIV infections, which occurs after a median latent period of 8–10 years [2,3], is characterized by clinical symptoms caused by opportunistic infections and certain neoplasms [4]. At this stage of disease progression, many functions of the immune system are severely altered [5]. Nevertheless, on the initial stages of HIV infection, the host's immunosurveillance pathways usually curtail virus replication [6,7], but in most cases probably do not eliminate the virus from the body. It has been speculated that $\alpha\beta$ T cell-mediated immunity plays an important role in these mechanisms [8,9]. However, to our knowledge, the possibility that $\gamma\delta$ T cell clones present in healthy persons are involved in the anti-HIV immunosurveillance has not been analysed.

Approximately 3–10% of human peripheral blood lymphocytes express the $\gamma\delta$ T cell receptor (TCR) [10]. The large majority of these cells express V$\gamma9^+$/V$\delta2^+$ TCR, while V$\delta1^+$ T cells constitute a second major subset of $\gamma\delta$ T lymphocytes in the periphery [11,12]. Increased numbers of $\gamma\delta$ T lymphocytes in persons infected with pathogenic agents such as *Mycobacterium leprae*, *Brucella meliterisis*, *Plasmodium falciparum*, *Leishmania amayonerisis*, and *Toxoplasma gondii* [13–17] indicate that $\gamma\delta$ T cells may contribute to the immune response against certain intracellular pathogens. A few studies (reviewed in [18]) have addressed the role that $\gamma\delta$ T cells may play in human immune responses against viral infection. For example, it has been reported that the number and percentage of $\gamma\delta$ T cells present in the peripheral blood of patients with acute Epstein–Barr virus (EBV) infections are increased in comparison with healthy controls, and remain elevated during the convalescent phase of infection [19]. $\gamma\delta$ T cells from some herpes simplex virus (HSV)-immune donors have been shown to lyse HSV-infected cells [20,21]. Increases in relative and absolute numbers of peripheral blood of $\gamma\delta$ T cells in HIV-1$^+$ persons have been described [22–24], but no functional analyses of these lymphocytes have been reported. Here we report that within a panel of $\gamma\delta$ T cell clones from HIV-1$^-$ persons, some clones display potent anti-HIV activities *in vitro*.

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MATERIALS AND METHODS

Antibodies

The TCRδ1 pan-human $\gamma\delta$ T cell MoAb [25] was generously provided by Dr M. Brenner (Harvard University, Boston, MA). Vδ2-specific MoAb 15D was kindly provided to Dr S. Carding (University of Pennsylvania, Philadelphia, PA). MoAb A13 (Vδ1 specificity) [26] and MoAb BB3 (Vδ2 specificity) [27] were kindly provided by Drs L. Moretta and E. Ciccone (Instituto Nazionale per la Ricerca sul Canro, Genova, Italy). FITC-conjugated TCRδ1, pan-TCR$\alpha\beta$, Vδ1, Vδ2, and Vγ9 T cell subset antibodies were purchased from T cell Diagnostics (Cambridge, MA). FITC-conjugated isotype control IgG1 antibody was purchased from Sigma (St Louis, MO). Mouse anti-human CD4-FITC/CD8-PE,

FITC-conjugated CD8 antibody, and isotype control mouse IgG2a-FITC/IgG1-PE were purchased from Olympus (Lake Success, NY). FITC-conjugated goat anti-mouse antibody and anti-CD4-biotin were purchased from Becton Dickinson (Mountain Ciew, CA). Streptavidin-PE was purchased from Southern Biotechnology Associates (Birmingham, AL). Mouse hybridoma 31-90-25, which produces a MoAb specific for an epitope of the HIV-1 core protein p24, and mouse hybridoma MK-D6, producing a MoAb reactive to murine I-A^d molecules, were obtained from ATTC (Rockville, MD). MoAb ML30 raised against *Myco. leprae* hsp65 and cross-reactive with human hsp58, was generously provided by Dr J. Ivanyi (Tuberculosis and Related Infections Unit, MRC, London, UK) [28].

T cell clones

Peripheral blood mononuclear cells (PBMC) were isolated from blood of healthy HIV^- human donors by density centrifugation over Histopaque-1077 (Sigma). PBMC were washed twice in PBS and samples were stained with either FITC-conjugated TCRδ1 or Vδ1 MoAb, or with anti-CD4-FITC/CD8-PE MoAb. Specific T cell populations were positively selected using a fluorescence-activated cell sorter (FACStar^{plus}; Becton Dickinson) as described [29]. The sorted cells were cloned by limiting dilution at 0·7 cell/well in 96-well round-bottomed microtitre plates (Costar, Cambridge, MA) in RPMI 1640 medium (Biowhittaker, Walkersville, MD) supplemented with 10% human serum (Pel-Freeze, Brown Deer, WI), 100 U/ml penicillin and 100 μg/ml streptomycin (Mediatech, Herndon, VA), and 2 mM L-glutamine (Mediatech) (complete RPMI medium), which was further supplemented with 200 U/ml IL-2 (Biological Modifiers Response Program, NCI, Frederick, MD), 0·25 μg/ml phytohaemagglutinin (PHA; Murex Diagnostics Inc., Dartford, UK), 10^5 irradiated (50 Gy) autologous or allogeneic PBMC/ml medium and 5×10^4 irradiated (110 Gy) LCL721 cells/ml (an EBV-transformed lymphoblastoid B cell line kindly provided by Dr R. DeMars, University of Wisconsin, Madison, WI). Cell cultures were grown in a humidified atmosphere of 5% CO_2 in air at 37°C. Wells were scored for growth 14–21 days after plating. Individual clones were passaged weekly, and after expansion they were phenotyped by flow cytometry using MoAbs reacting with different TCR and CD molecules.

Target cells

Mycoplasma-negative CEM [30], CEMx174 [31], Daudi [32], and Raji [33] cells were passaged twice weekly in RPMI 1640 medium containing 10% fetal bovine serum (FBS; Intergen, Purchase, NY), 100 U/ml penicillin, 100 μg/ml streptomycin (Mediatech), 10 μg/ml ciprofloxacin (Miles Inc., Kankakee, IL) and 2 mM L-glutamine (Mediatech). As a preventive measure against infection with adventitious microorganisms, the cell lines were routinely screened for mycoplasma. CEM or CEMx174 cells were infected with cell-associated HIV_{LAI} [34] 3 days before cytotoxicity assay set up as described [35]. All assays with infected cells were performed in our HIV biocontainment facility. Before HIV-infected cells were used as targets in cytotoxicity assays, they were examined for viability by eosin dye exclusion and for HIV p24 expression by flow cytometry. In brief, to assess p24 expression in HIV-infected cells, the cells were washed with PBS, fixed with 1·5% paraformaldehyde in PBS, washed and permeabilized with 0·3% Tween 20 in PBS [36] and incubated with either a supernate from mouse hybridoma 31-90-25 (reactive with HIV p24) or MK-D6 hybridoma supernate (utilized as a negative control for human cells) for 1 h at 4°C. The cells were washed twice, incubated with FITC-conjugated goat anti-mouse antibody for 30 min at 4°C, washed twice, and the percentage of HIV p24-positive cells (based on the analysis of 5000–10 000 cells) was determined by flow cytometry.

Cytotoxicity assays

One million cells of each target cell type were labelled with 100 μCi of Na_2-^{51}Cr-O_4 (Du Pont, Boston, MA) for 1 h at 37°C. Cells were washed with RPMI 1640 medium, and resuspended in 5 ml of medium and incubated for 0·5 h at 37°C. Effector cells were washed and specific cell numbers were plated in triplicate to yield particular E : T ratios in 96-well round-bottomed microtitre, plates in 100 μl/well complete RPMI medium. Target cells were washed twice more and were then added at 5×10^3 cells/well in 100 μl volume of complete RPMI medium to wells containing plated effector cells. In some assays, as noted, ML30 antibody was added at 25 μg/ml during the assay period. The plates were incubated at 37°C for 5 h. Supernatants were harvested using the Skatron harvesting press and harvesting frames (Skatron Instruments As, Lier, Norway). The maximum (target cells plus detergent), minimum (target cells alone) and experimental (targets plus effector cells) chromium release ct/min were determined using a gamma counter, and the per cent specific lysis was calculated as described [37].

Primary T cell cocultures

Primary $\alpha\beta$ $CD4^+$ T cell clones or lines were infected with cell-free HIV_{LAI} or mock infected [35]. Confirmation of productive infection in primary cells was determined by HIV-1 p24 antigen assay of their supernate (Coulter Corp., Hialeah, FL). The HIV-infected or mock-infected $\alpha\beta$ $CD4^+$ T cells were subsequently cocultured with $V\gamma9^+/V\delta2^+$ T cells or control $\alpha\beta$ $CD8^+$ T cells at a one-to-two cell ratio in complete RPMI medium supplemented with 200 U/ml IL-2 and 0·25 μg/ml PHA. After a 3 day coculture period, the cells were harvested, counted and stained with anti-CD4-biotin, followed by streptavidin-PE, and then either FITC-conjugated pan-$\gamma\delta$ TCR antibody (TCRδ1) or FITC-conjugated CD8 antibody. The absolute number of $CD4^+$ T cells remaining in culture was calculated from the percentage of $CD4^+$ T cells and the cell count.

Preparation of antigens

CEM cells were infected with HIV_{LAI} as described [35]. The supernate of HIV_{LAI}-infected CEM cells was harvested and cell-free HIV was isolated by size exclusion over Sepharose 4B columns [38]. Virus preparations eluted from Sepharose columns were centrifuged 1 h at 8000 ***g*** in a microcentrifuge. The pelleted virus was resuspended in PBS and stored at −20°C. Protein concentration was determined by the BioRad (Hercules, CA) protein assay following the instructions of the manufacturer. The candida antigen [39] was kindly supplied by Dr E. Balish (University of Wisconsin Madison, WI).

Proliferation assays

$\gamma\delta$ T cells were harvested, washed, and plated in triplicate in 96-well flat-bottomed microtitre plates in complete RPMI medium (5×10^4 or $2{\cdot}5 \times 10^4$ cells/well). Irradiated accessory cells (5×10^4/well) were added to appropriate wells. These accessory cells served as stimulator cells or antigen-presenting cells (APC). The dose of irradiation was either 110 Gy (Daudi, Raji, CEM,

HIV_{LAI}-infected CEM, and LCL721 cells) or 50 Gy (PBMC). The following mitogens or antigens were added to the appropriate wells in the presence of absence of APC: 0·25 μg/ml PHA (Murex Diagnostics Inc.), 10 μg/ml candida, and 10 μg/ml HIV. HIV was added directly after thawing frozen stock or was heat-treated for 1 h at 60°C before addition to the appropriate wells. Plates were incubated at 37°C and were pulsed (1 μCi/well) with tritiated thymidine (Du Pont) on day 2 or day 4 (as indicated), and harvested 24 h later. Tritiated thymidine incorporation was measured by liquid scintillation counting [40]. The mean ct/min of triplicate samples was used to calculate the SI as follows: SI = (responder with stimulator cells ct/min – stimulator cells alone ct/min)/ responder cells alone ct/min.

RESULTS

$\gamma\delta$ T cell-mediated lysis of HIV-infected cells

$V\gamma9^+/V\delta2^+$ T cell clones, $V\delta1^+$ cell clones, and $\alpha\beta^+$ T cell clones isolated from the peripheral blood of healthy HIV^- individuals were tested for their ability to lyse uninfected CEM and HIV-infected CEM cells. Typically, 72 h after infection, at least 90% of CEM cells were infected with HIV (Fig. 1). We observed that some $V\gamma9^+/V\delta2^+$ T cell clones isolated from healthy HIV^- donors, such as VM3, were cytotoxic for HIV-infected CEM cells (Table 1). At a 5 : 1 E : T ratio, the VM3 clone was 2·7 times more lytic for HIV-infected CEM cells (70% specific lysis) than for uninfected CEM cells (26% specific lysis). At a 1 : 1 E : T ratio the per cent specific lysis of infected cells was 25%, with no specific lysis of uninfected CEM cells (Table 1). Levels of cytotoxicity for HIV-infected cells mediated by different $V\gamma9^+/V\delta2^+$ T cell clones are summarized in Tables 1 and 2. Table 1 presents clones that displayed at least two-fold higher (i.e. 100% increased) cytotoxicity for infected *versus* uninfected cells for at least one E : T ratio tested. Table 2 presents clones that exhibited less than a two-fold increase in cytotoxicity. The ability of $V\gamma9^+/V\delta2^+$ T cell clones to lyse Daudi, but not Raji cells [41] was also analysed. Most $V\gamma9^+/V\delta2^+$ T cell clones lysed Daudi cells regardless of their capacity to lyse HIV-infected cells (Tables 1 and 2). Further evidence of cytotoxicity was provided by a set of clones derived from donor RS (Fig. 2). $V\gamma9^+/V\delta2^+$ T cell clones RS3-3, RS2-11 and RS5-1 were highly cytolytic for Daudi cells (>90% specific lysis), but consistent with [41], the control $\alpha\beta^+$ T cell clone RS13-6 was not cytotoxic in this assay (Fig. 2a). RS2-11 and RS5-1 clones exhibited substantially greater lysis for HIV-infected CEMx174 cells than uninfected CEMx174 cells, whereas the RS3-3 and RS13-6 clones did not (Fig. 2b). Overall, 40% of $V\gamma9^+/V\delta2^+$ T cell clones tested exhibited selective cytotoxicity for HIV-infected cells.

To determine whether $\gamma\delta$ T cells other than those with $V\gamma9^+/V\delta2^+$ TCR are cytotoxic for HIV-infected cells, seven $V\delta1^+$ T cell clones were isolated from four of the $V\gamma9^+/V\delta2^+$ T cell clone donors. The $V\delta1^+$ T cell clones lysed neither HIV-infected nor uninfected CEM cells, irrespective of $V\gamma9$ coexpression, nor did they lyse Daudi or Raji cells (Table 3). As expected, control of $\alpha\beta$ $CD8^+$ and $\alpha\beta$ $CD4^+$T cell clones derived from the same donors also did not exhibit cytotoxicity for any of the target cell types (data not shown).

To determine whether or not $V\gamma9^+/V\delta2^+$ T cells might affect HIV-infected primary cells *in vitro*, peripheral blood-derived $\alpha\beta$ $CD4^+$ T cells were HIV-infected or mock infected and were subsequently cocultured (1 : 2) with $V\gamma9^+/V\delta2^+$ T cells or control $\alpha\beta$ $CD8^+$ T cells. After a 3 day coculture period, the cells were harvested, counted and stained with MoAbs. The absolute number of $CD4^+$ T cells remaining in the HIV-infected culture was $5{\cdot}6 \times 10^5$ (exp. 1) and $5{\cdot}9 \times 10^5$ (exp. 2). Coculture with

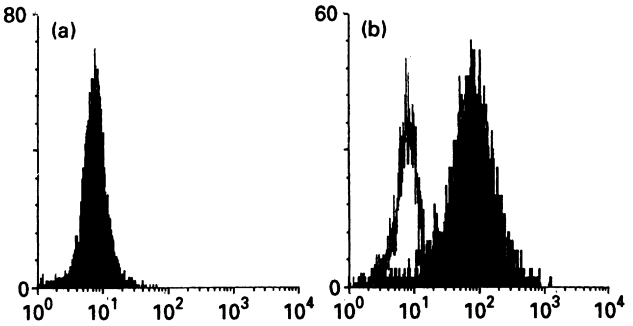

Fig. 1. HIV infection level in CEM target cells. Uninfected CEM cells (a) and HIV-infected CEM cells (b) were incubated with the monoclonal antibody 31-90-25 specific for HIV p24 (solid black) or with negative control MoAb MK-D6 (black outline), and in a second step were stained with goat anti-mouse FITC-conjugated antibody. The abscissa indicates the relative fluorescence intensity. The ordinate indicates the number of cells.

Table 1. $V\gamma9^+/V\delta2^+$ T cell clones with selective cytotoxicity for infected *versus* uninfected cells

		Per cent specific lysis			
Clone	E : T ratio	HIV-CEM	CEM	Daudi	Raji
HH2	10 : 1	34	19	49	8
	5 : 1	30	14	NT	NT
	2·5 : 1	17	9	NT	NT
HH3	10 : 1	40	28	24	2
	5 : 1	32	18	NT	NT
	2·5 : 1	22	11	NT	NT
HH4	10 : 1	11	5	15	1
	5 : 1	7	3	NT	NT
	2·5 : 1	3	2	NT	NT
VM3	10 : 1	77	41	65	29
	5 : 1	70	26	65	23
	1 : 1	25	0	40	12
VM1-1	10 : 1	71	41	68	21
	1 : 1	36	16	33	0
VM4-4	10 : 1	45	17	42	0
	1 : 1	13	0	26	0
VM112	10 : 1	20	5	6	2
	5 : 1	11	7	4	1
	1 : 1	4	4	1	0
VM113	10 : 1	26	20	18	0
	5 : 1	16	9	12	1
	1 : 1	10	2	7	1
ME14-1	10 : 1	13	2	27	0
	2 : 1	0	0	17	0
ME21-1	10 : 1	17	5	56	3
	2 : 1	5	0	44	0

NT, Not tested. $V\gamma9^+/V\delta2^+$ T cell clones from healthy HIV^- donors HH, VM and ME were assayed for cytotoxicity against indicated target cells.

Table 2. $V\gamma9^+/V\delta2^+$ T cell clones with minimal or no selective cytotoxicity for HIV-infected cells

		Per cent specific lysis			
Clone	E : T ratio	HIV-CEM	CEM	Daudi	Raji
HH1	10 : 1	57	55	65	13
	5 : 1	55	43	NT	NT
	2·5 : 1	47	28	NT	NT
VM25	10 : 1	4	6	2	0
VM6-4	10 : 1	20	24	76	8
	1 : 1	0	0	40	0
VM9-3	10 : 1	23	23	72	12
	1 : 1	5	3	35	0
ME5-2	10 : 1	63	51	48	7
	2 : 1	46	40	35	2
ME5-4	10 : 1	42	30	25	2
	2 : 1	19	16	17	1
ME8-6	10 : 1	47	33	18	3
	2 : 1	27	20	15	1
ME8-7	10 : 1	66	49	34	2
	2 : 1	42	39	26	3
ME10-5	10 : 1	60	56	44	14
	2 : 1	41	40	41	6
ME6-1	10 : 1	12	7	12	0
	2 : 1	3	0	9	0
ME9-1	10 : 1	17	16	5	1
	2 : 1	4	9	5	2
ME12-4	10 : 1	8	5	2	0
	2 : 1	1	0	2	0
JS2E12	10 : 1	30	23	48	18
	5 : 1	26	17	49	10
	1 : 1	13	12	17	8
JS1E5	10 : 1	11	10	17	1
	5 : 1	5	4	12	1
	1 : 1	2	2	5	1
KT12	10 : 1	23	23	28	0
KT15	10 : 1	31	23	24	5

NT, Not tested. $V\gamma9^+/V\delta2^+$ T cell clones from healthy HIV^- donors HH, VM, ME, JS, and KT were assayed for cytotoxicity against indicated target cells.

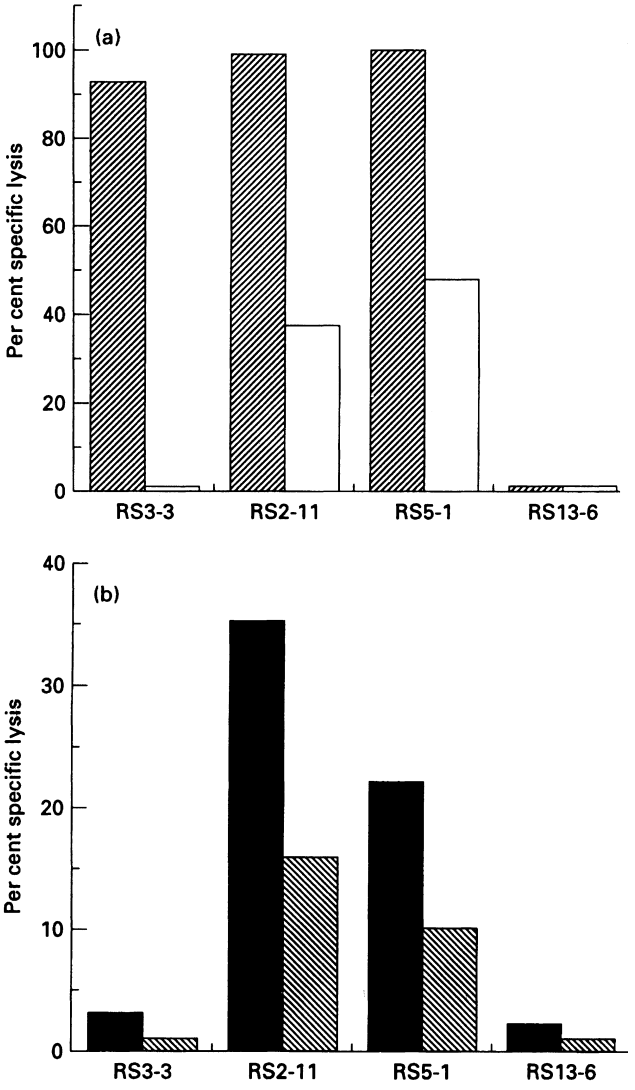

Fig. 2. Cytotoxicity mediated by different T cell clones. (a) Cytotoxicity of the $V\gamma9^+/V\delta2^+$ T cell clones RS3-3, RS2-11, RS5-1, and $\alpha\beta$ $CD4^+$ T cell clone RS13-6 against Daudi cells (▨) and Raji cells (□), (b) Cytotoxicity of the $V\gamma9^+/V\delta2^+$ T cell clones RS3-3, RS2-11, RS5-1, and $\alpha\beta$ $CD4^+$ T cell clone RS13-6 against HIV-infected CEMx174 cells (■) and uninfected CEMx174 cells (▨). The given values correspond to an E : T ratio of 10 : 1.

autologous $V\gamma9/V\delta2$ T cells led to a substantial decrease of $CD4^+$ T cells remaining in these HIV-infected cultures, i.e. $1{\cdot}2 \times 10^5$ (exp. 1) and $2{\cdot}1 \times 10^5$ (exp. 2), respectively. Allogeneic $V\gamma9/V\delta2$ T cells, but not autologous $CD8^+$ T cells, had a similar effect. $V\delta1^+$ T cell clones tested did not influence numbers of $\alpha\beta$ $CD4^+$ T cells from either the HIV-infected or mock infected populations, and numbers of control (mock infected) $\alpha\beta$ $CD4^+$ T cells were unaffected by coculture with $V\gamma9^+/V\delta2^+$ T cells. In certain experiments, an increase in $V\gamma9^+/V\delta2^+$ T cell number was observed when these cells were cocultured with HIV-infected cells compared with uninfected cells (data not shown), which suggested that $V\gamma9^+/V\delta2^+$ T cells might proliferate in response to HIV-infected cells.

γδ cell proliferation in response to HIV-infected cells or cell-free HIV

In subsequent experiments, we examined whether or not $V\gamma9^+/V\delta2^+$ T cells would proliferate in response to HIV-infected cells. We tested four $\gamma\delta$ T cells clones derived from donor ME. One of three $V\gamma9^+/V\delta2^+$ T cell clones, ME8-7, responded to HIV-1 infected, but not to uninfected CEM cells (Table 4). All $V\gamma9^+/V\delta2^+$ T cell clones proliferated in response to Daudi cells. The $V\gamma9^-/V\delta2^+$ T cell clone ME18-2 proliferated in response to HIV-infected CEM cells, but not in response to uninfected CEM cells (Table 4). ME18-2 cells did not respond to Daudi cells, which is in agreement with previous studies of $V\delta1^+$ T cells [29].

$V\gamma9^+/V\delta2^+$ T cell clone VM39 did not lyse HIV-infected CEMx174 cells, but it did proliferate in response to these cells (data not shown). Furthermore, this clone displayed a DNA synthetic response to cell-free HIV-1 (Fig. 3). The VM39 cells also proliferated in response to IL-2, irradiated Daudi cells or PHA, but did not proliferate in response to irradiated Raji cells or candida

Table 3. Per cent specific lysis of target cells by V$\delta1^+$ T cell clones

Clone	E : T ratio	Per cent specific lysis			
		HIV-CEM	CEM	Daudi	Raji
JS2C12	10 : 1	7	11	6	2
V$\gamma9^+$/V$\delta1^+$	5 : 1	4	9	4	5
	1 : 1	3	5	1	3
JS4A12	10 : 1	8	5	7	3
V$\gamma9^+$/V$\delta1^+$	5 : 1	5	3	3	5
	1 : 1	1	6	6	1
VM3-3	10 : 1	0	0	0	0
V$\gamma9^-$/V$\delta1^+$	1 : 1	0	0	0	0
VM17					
V$\gamma9^-$/V$\delta1^+$	10 : 1	11	11	1	0
KT1					
V$\gamma9^-$/V$\delta1^+$	10 : 1	5	7	7	2
ME18-2	10 : 1	11	14	2	1
V$\gamma9^-$/V$\delta1^+$	2 : 1	4	8	1	2
ME18-6	10 : 1	7	9	1	1
V$\gamma9^-$/V$\delta1^+$	2 : 1	4	3	1	0

V$\delta1^+$ T cell clones from healthy HIV$^-$ donors JS, VM, KT and ME were assayed for cytotoxicity against indicated target cells.

antigen (Fig. 3a). The VM39 clone responded to HIV in the presence or absence of additional APC, but it did not respond to irradiated uninfected CEM cells, which were used to propagate virus (Fig. 3b). Thus the possibility that MHC molecules, which are known to be incorporated into HIV particles [42], would account for the $\gamma\delta$ T cell stimulatory principle seems unlikely. Treating the virus preparation of 60°C for 1 h decreased the capacity of the purified virions to induce DNA synthesis in the

Table 4. DNA synthetic response of $\gamma\delta$ T cell clones

Clone	Pulsed on day	Stimulation index*		
		Daudi	CEM	HIV-1-CEM
ME6-1	2	6·2	0†	0
	4	0·6	0·6	0
ME8-7	2	31·3	0	7·1
	4	3·3	0·6	4·1
ME14-1	2	15·2	0	1·7
	4	1·2	0·4	0
ME18-2	2	0	0	5·8
	4	0·3	0	11·3

V$\gamma9^+$/V$\delta2^+$ T cell clones ME6-1, ME8-7, ME14-1, and V$\gamma9^-$/V$\delta1^+$ T cell clone ME18-2 ($2{\cdot}5 \times 10^4$/well) were tested for their proliferative responses to irradiated Daudi cells, CEM cells, or HIV_{LAI}-1 infected CEM cells as stimulator cells in complete RPMI 1640 medium. Plates were pulsed with tritiated thymidine on day 2 and on day 4 and harvested 24 h later.

*Stimulation index was calculated as described in Materials and Methods. The mean DNA synthesis of unstimulated $\gamma\delta$ cells ranged between 40 and 75 ct/min per well.

†The value of 0 indicates that after subtracting mean 'stimulator cells alone' ct/min from mean 'T cell clone plus irradiated stimulator cells' ct/min the resulting number was $\leqslant 0$.

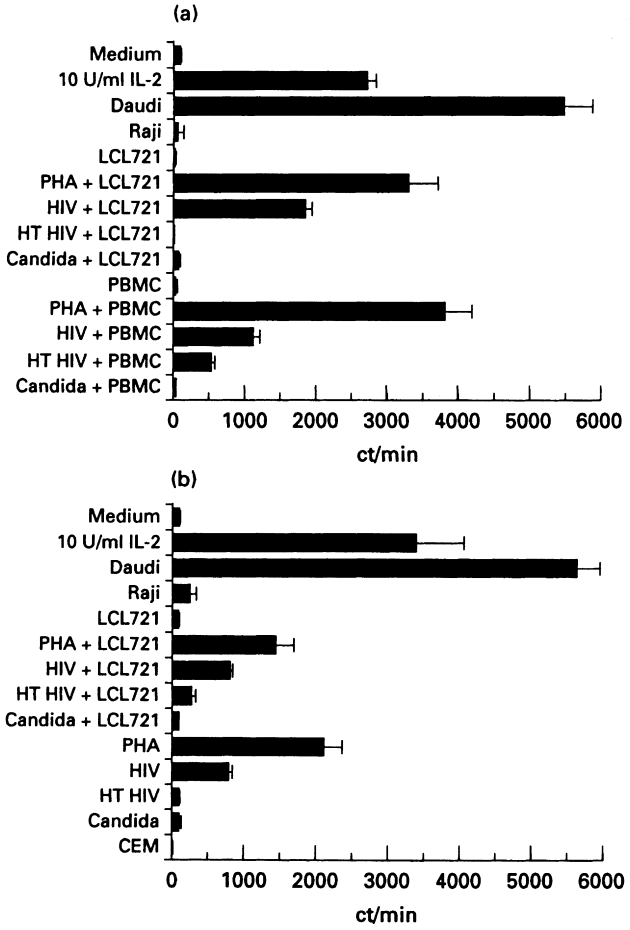

Fig. 3. The DNA synthetic response of the V$\gamma9^+$/V$\delta2^+$ T cell clone VM39 to purified HIV. VM39 cells (5×10^4 cells/well) were incubated in complete RPMI medium with or without HIV, heat-treated (HT) HIV, candida antigen, or phytohaemagglutinin (PHA) as described in Materials and Methods. (a) Either irradiated LCL721 cells or allogenic peripheral blood mononuclear cells (PBMC) served as antigen-presenting cells (APC). (b) Irradiated LCL721 cells or no accessory cells were used as APC. Wells were pulsed with tritiated thymidine on day 4 and were harvested 24 h later. The thick and thin bars correspond to means and s.d., respectively. When appropriate (e.g. from in all but the top two samples in experiment a), mean ct/min for added accessory (stimulator) cells incubated alone were subtracted from mean ct/min for the experimental sample, i.e. from T cell clone plus accessory cells.

VM39 clone (Fig. 3). PBMC and an $\alpha\beta$ CD4$^+$ T cell clone which were tested as control responders, did not proliferate in response to purified virions (data not shown).

Effect of ML30 MoAb on V$\gamma9^+$/V$\delta2^+$ T cell lysis of HIV-infected cells

All of the $\gamma\delta$ T cell clones in this study were isolated from HIV$^-$ donors, and may be considered immunologically naive for HIV encoded proteins. It is possible that the recognized entity may be a virus-induced, cell-derived component capable of triggering $\gamma\delta$ T cells. One such candidate for the recognized molecule is hsp58 [29]. HIV preparations react with the human hsp58-reactive MoAb ML30 on Western blots [38], and this antibody specifically stains the cell surface of some HIV-infected T cells [43]. To investigate the possible involvement of this molecule in recogni-

tion of HIV-infected cells by $\gamma\delta$ T cells, we atempted to block lysis of the target cells with ML30. However, the addition of this antibody did not affect the lysis of HIV-infected CEM cells mediated by $V\gamma9^+/V\delta2^+$ T cells (37% specific lysis in the absence and 39% in the presence of ML30 at E : T ratio of 10 : 1).

DISCUSSION

We have previously reported that primate $\gamma\delta$ T cells can lyse a variety of T cell lines infected with either HIV or simian immunodeficiency virus (SIV) [43–45]. Here we analysed anti-HIV activities of a panel of human T cell clones. We found that HIV infection renders cells susceptible to lysis by a substantial number of $V\gamma9^+/V\delta2^+$ T cell clones from HIV^- donors. It seems unlikely that this activity would be selectively induced by the culture conditions *in vitro* because 60% of $V\gamma9^+/V\delta2^+$ T cell clones and all other T cell clones propagated in the same manner consistently failed to lyse HIV-infected CEM cells. The dominance of the $V\gamma9^+/V\delta2^+$ phenotype among the cytotoxic clones suggests the involvement of the TCR in the lytic activity. The fact that $V\gamma9^+/V\delta2^+$ T cell clones from multiple donors lyse HIV-infected CEM cells suggests that this activity is MHC-unrestricted. Clonal heterogeneity between different $V\gamma9^+/V\delta2^+$ T cell clones may account for differences in the ability of various clones to recognize and lyse HIV-infected cells.

Increased numbers of $\gamma\delta$ T cells in HIV-1 infected individuals are partly due to specific elevations in the $V\delta1^+$ T cell subset [22–24]. In some of these individuals, $V\delta1^+$ T cells expressed surface HLA-DR, indicating possible activation [46]. Kozbor *et al*. [47] have suggested (based on their observation of increases in the absolute numbers of $V\delta1^+$ $CD8^+$ T cells in HIV-infected children) that the $V\delta1$ subset responds to HIV infection and may be cytotoxic for HIV-infected cells. Although we did not observe any cytotoxicity of $V\delta1^+$ T cell clones from HIV^- donors against HIV-infected targets, we did detect a proliferative response towards HIV-infected cells in one $V\delta1^+$ T cell clone. This finding and the apparent direct response of the $V\gamma9^+/V\delta2^+$ VM39 clone to purified HIV particles are compatible with the idea that the increased numbers and activation status of $\gamma\delta$ T cells in HIV-1-infected individuals may be a result of direct HIV stimulation *in vivo*.

It is noteworthy that $V\gamma9^+/V\delta2^+$ T lymphocytes have been shown to lyse cells infected with HSV [21], SIV [43,44] and HHV-6 [48] without previous exposure to corresponding viral antigens. These observations are consistent with the hypotheses that (i) $\gamma\delta$ T cells may function early in viral diseases as front line defenders to remove infected or otherwise stressed cells [49]; and (ii) the antigen(s) recognized may be of cellular origin [21]. These hypotheses are also compatible with the fact that abnormal proteins [50] and many viral infections (reviewed in [51]) can act as stress signals in eukaryotic cells. In particular, this has been well documented in various HSV systems [52–59]. In addition, in some experiments, we used SIV as a control virus. The observation that some $\gamma\delta$ T cell clones cytotoxic for HIV-infected cells also lysed SIV-infected (data not shown), but not uninfected, cells further supports the proposed concepts [21,49].

Since many $\gamma\delta$ T cells (in contrast to $\alpha\beta$ T cells) can respond to non-peptide antigens [60–64], it is possible that $\gamma\delta$ T lymphocytes may have an $\alpha\beta$ T cell-complementing function in immunosurveillance against various infectious agents, including HIV. Our data provide direct evidence for anti-HIV responses in a large proportion of $V\gamma9^+/V\delta2^+$ T cell clones isolated from putatively healthy individuals. These responses may be important for the phenomenon of HIV exposure without infection [65]. Further analysis of $\gamma\delta$ T cell antiviral activities may facilitate the evaluation and development of immunotherapeutic strategies aimed at virus–$\gamma\delta$ T lymphocyte interactions.

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