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Lysis of CD4$^+$ lymphocytes by non-HLA-restricted cytotoxic T lymphocytes from HIV-infected individuals

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SUMMARY

Individuals infected with HIV have elevated numbers of total and activated CD8$^+$ lymphocytes in peripheral blood. CD8$^+$ lymphocytes from HIV-infected individuals have been shown to mediate non-human histocompatibility-linked antigen (HLA)-restricted suppression of viral replication, HLA-restricted killing of cells expressing HIV antigens, and killing of uninfected lymphocytes. We studied CD8$^+$ T lymphocytes that lysed autologous CD4$^+$ lymphocytes, heterologous CD4$^+$ lymphocytes from HIV-infected individuals and uninfected CD4$^+$ lymphocytes. Killing in all cases required T cell receptor (TCR)-mediated recognition or triggering. However, these CD8$^+$ cytotoxic T lymphocytes (CTL) killed HLA class I mismatched CD4$^+$ lymphocytes and CD4$^+$ lymphocytes treated with a MoAb against HLA-A, B and C antigens (PA2.6) which blocks HLA class I-restricted killing. HLA class II-negative CD4$^+$ T lymphoma cells (CEM.NK^R) were also killed by anti-CD3 inhibited CTL. Stimulation of peripheral blood lymphocytes (PBL) from HIV-infected individuals, but not uninfected controls, with concanavalin A (Con A) and IL-2, induced non-HLA-restricted TCR $\alpha\beta^+$, CD8$^+$ CTL which lysed CD4$^+$ lymphocytes. Activation of CD4$^+$ lymphocytes increased their susceptibility to CD8$^+$ CTL-mediated lysis. In HIV infection, a population of non-HLA-restricted CTL which lyse activated CD4$^+$ lymphocytes is expanded. The expansion of CTL with unusual characteristics is interesting, because the stimulus for this expansion is unknown. CTL which recognize activated CD4$^+$ cells could play a role in immune regulation and the pathogenesis of AIDS.

Keywords CD4 cells CD8 cells cytotoxic T lymphocytes HLA restriction HIV AIDS

INTRODUCTION

The expansion of CD8$^+$ lymphocyte subsets that accompanies many viral infections involves a cytotoxic T lymphocyte (CTL) response against viral antigens made and processed within host cells and presented at the cell surface by host MHC class I molecules [1–5]. Generally, CD8$^+$ lymphocyte numbers normalize after acute infection, but following HIV infection, CD8$^+$ lymphocytosis persists for years, suggesting chronic acute infection [6]. A high proportion of the circulating CD8$^+$ lymphocyte population in HIV-infected individuals is phenotypically and functionally activated [7–9]. Distinct CD8$^+$ subsets within the expanded population include HLA-restricted CTL [10], non-HLA-restricted killer cells [11], and non-HLA-restricted suppressor cells [12]. By killing HIV-infected cells [10] and suppressing HIV replication [12], CD8$^+$ lymphocytes may play a protective role in HIV infection. However, by killing uninfected lymphocytes [9,13,14] and invading local tissues, CD8$^+$ cells from HIV-infected individuals can also have negative effects [15–18]. The relative risk of progression to AIDS increases for HIV-infected individuals as the CD8$^+$ lymphocyte count rises [19,20]. This association may represent an indirect effect of increasing HIV replication, or may suggest a direct role for CD8$^+$ lymphocytes in disease progression.

We found that CD8$^+$ lymphocytes that lyse uninfected activated CD4$^+$ lymphocytes could be generated *in vitro* from peripheral blood lymphocytes (PBL) of HIV-infected individuals, but not uninfected controls, by polyclonal activation with concanavalin A (Con A) and IL-2. These CTL were T cell receptor (TCR) $\alpha\beta^+$ and triggered through the TCR, but not restricted by classical HLA class I antigens. Such non-HLA-restricted killing is usually associated with natural killer (NK) and lymphokine-activated killer (LAK) cells, but unlike LAK or NK cells, killing by these CTL involved CD3 and the $\alpha\beta$ TCR. This suggests that target recognition and effector cell activation were mediated by antigen-specific receptors. A subset of CTL with unusual specificity and an unknown role appears to specifically expand following HIV infection.

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SUBJECTS AND METHODS

Subjects

HIV-1-infected individuals were recruited through the Chedoke-McMaster Special Immunology Services Clinic. Infection with HIV-1 was tested by ELISA and confirmed by Western blot. Uninfected HIV$^-$ volunteers were recruited from hospital and laboratory personnel. HLA typing for some subjects was determined by microcytotoxicity assay [21].

Lymphocyte separations

Blood was collected in heparinized vacutainers, diluted with an equal volume of PBS pH 7·2, layered over Ficoll–Paque gradient separation medium (Pharmacia Chemicals, Dorval, Quebec) and centrifuged at 400 ***g*** for 30 min. Interface cells were collected, washed three times in PBS containing 1% fetal calf serum (FCS; Bocknek, Burlington, Ontario), and counted. Cells were resuspended in PBS containing 0·1% bovine serum albumin (BSA) at 3×10^6/ml with sufficient OKT4-coated magnetic beads (Dynal Inc., Great Neck, NY) to ensure a 5:1 bead-to-target cell ratio, and rotated together for 1 h in a 15-ml conical tube at 4°C. After magnetic displacement of beads, unbound cells were removed by pipetting. The beads bearing the positively selected cells were washed twice with PBS containing 0·1% BSA before culture.

Lymphocyte stimulation

Positively selected CD4$^+$ lymphocytes were resuspended at approximately 10^6/ml in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% FCS, 10 mM HEPES, 2 mM L-glutamine, 1% penicillin and streptomycin (all from GIBCO) and 2×10^{-5} M 2-mercaptoethanol (Sigma Chemical Co., St Louis, MO) and stimulated with 5 μg/ml purified phytohaemagglutinin (PHA-P) (Wellcome Diagnostics, Dartford, UK). After 3 days of PHA stimulation, CD4$^+$ cultures were further supplemented with 5 U/ml recombinant IL-2 (Genzyme, Cambridge, MA). Following an additional day in IL-2, CD4$^+$ cells were removed from the beads by vigorous pipetting in a 15-ml conical tube, magnetic displacement of beads, removal of unbound cells, resuspension of beads in PBS containing 0·1% BSA, and repetition of this procedure for a total of three times. Cells were not used for at least 48 h after removal from beads to allow re-expression of surface antigens. Cultures so treated contained a mean of 95% CD4$^+$ lymphocytes determined by flow cytometry (data not shown).

CD4$^+$ lymphocyte-depleted PBL were resuspended at approximately 10^6/ml in lymphocyte medium supplemented with 10 μg/ml Con A (Difco, Toronto, Ontario) for 3 days, after which 5 U/ml IL-2 was added. After a total of 7 days these cultures contained a mean of 90% CD8$^+$ T cells by flow cytometry (data not shown).

Cytotoxicity assays

CD4$^+$ lymphocytes cultured as above were pelleted and incubated in 1 ml medium for 90 min with 200 μCi $Na_2{}^{51}CrO_4$ (New England Nuclear, Boston, MA), washed four times in PBS containing 1% FCS, and resuspended at 10^5/ml in media. In some assays CEM.NK^R cells, a CD4$^+$ T cell line that does not express HLA-class II antigens [22], were used as targets. Target cell suspension (50 μl) was added to duplicate wells of 96-well round-bottomed microtitre plates (Nunclon, Roskilde, Denmark) for each effector : target (E:T) ratio and for minimum and maximum controls. Effector cells were resuspended in medium at 5×10^6/ml and 50, 25 and 12·5 μl added to duplicate wells of the microtitre plates for E:T ratios of 50:1, 25:1 and 12·5:1. A constant volume of 300 μl in each well was obtained by adding either medium or hybridoma supernatants to test wells, medium alone to minimum wells, and by adding 1 N HCl to maximum wells. Following addition of effector and target cells, microtitre plates were incubated at 37°C in a humidified 5% CO_2 incubator for 5 h and 100 μl of supernatant were removed from each well for γ counting. Per cent specific lysis was calculated by the following formula: (experimental ^{51}Cr release − spontaneous release) ÷ (maximum ^{51}Cr release − spontaneous release) × 100. In all assays, spontaneous ^{51}Cr release was < 30% of total ^{51}Cr release. Ten per cent specific lysis was considered positive, since

Table 1. CD3$^+$ CD8$^+$ cytotoxic T lymphocytes (CTL) mediating lysis of autologous CD4$^+$ lymphocytes are present in HIV-infected individuals but not controls

	Per cent specific lysis of autologous CD4$^+$ lymphocytes					
CD8$^+$ effectors	E:T	50	25	12·5	50+OKT3*	50+OKT8
HIV$^+$						
119		26	25	21	0	
119+OKT3†		0	0	0		
139		17	11	10		1
139+OKT3		0	0	0		
140		15	16	13	6	
152		43	33	20	1	
167		35	25	19	8	
188		41	23	14		0
188+OKT3		2	0	0		
203		15	4	5	0	
203+OKT3		0	0	0		
HIV$^-$						
KR		3	0	2		
CP		0	0	0		
DS		0	0	0		
SP		0	0	0		
MW		0	0	0		
AA		0	0	0		
AG		0	0	0		
SG		0	0	0		

CD4-depleted cells from HIV-infected individuals and HIV$^-$ controls were stimulated with concanavalin A (Con A) and IL-2 and tested for cytotoxicity against purified phytohaemagglutinin (PHA)-activated autologous CD4$^+$ lymphocytes.

* Lysis in the presence of OKT3 or OKT8 was measured at an E:T ratio of 50:1 by adding 200 μl hybridoma supernatant to test wells in place of medium to attain a final volume of 300 μl.

† In some cases, effector cells alone were incubated with 1 ml OKT3 hybridoma supernatant for 30 min immediately before the assay. Unbound OKT3 was removed by two washes in PBS containing 1% fetal calf serum (FCS).

Table 2. Lysis of heterologous CD4+ lymphocytes from HIV-infected and uninfected individuals is mediated by CD3+ CD8+ cytotoxic T lymphocytes (CTL)

CD8+ effectors	CD4+ target source	Per cent specific lysis E:T	50	25	12·5	50+OKT3*	50+OKT8
HIV+	HIV+						
112	140		23	20	13	8	
182	140		14	14	11	0	
119	136		15	8	7	1	
136	72		45	27	19	4	6
136	139		40	14	10	1	18
142	140		14	6	0	0	
HIV+	HIV−						
119	KR		45	30	12	2	
119+OKT3†	KR		1	3	1		
119	DS		27	11	2	9	0
119+OKT3	DS		7	3	0		
139	KR		31	27	19	0	
155	KR		29	9	0		5
152	DS		48	26	30	11	
123	DS		21	10	7	6	
203	DS		44	24	18	7	

CD4-depleted cells from HIV-infected individuals were stimulated with concanavalin A (Con A) and IL-2 and tested for cytotoxicity against purified phytohaemagglutinin (PHA)-activated CD4+ lymphocytes from HIV-infected individuals and from HIV− individuals.

* Lysis in the presence of OKT3 or OKT8 was measured at an E:T ratio of 50:1 by adding 200 μl hybridoma supernatant to test wells in place of media to attain a final volume of 300 μl.

† In some cases, effector cells alone were incubated with 1 ml OKT3 hybridoma supernatant for 30 min immediately before the assay. Unbound OKT3 was removed by two washes in PBS containing 1% fetal calf serum (FCS).

this was always greater than three times the s.d. of the mean spontaneous release.

Characterization of effector cells

Inhibition studies with various MoAbs were carried out to determine the phenotype of the cytotoxic cells. Hybridoma supernatants from OKT3 (ATCC CRL8001) anti-CD3, OKT8 (ATCC CRL8014) anti-CD8 or S6F1 (ATCC HB9579) anti-LFA-1 were added to the microtitre plates in some cases, while in other cases, effector cells were incubated for 30 min with 1 ml OKT3 hybridoma supernatant and washed twice with PBS containing 1% FCS before inclusion in cytotoxicity assays. In further tests, effector cells were incubated with 1 μg of anti-αβ TCR MoAb BMA-031 (Behring Diagnostics, Ontario) or 1 ml OKT3 hybridoma supernatant for 30 min and washed as above before inclusion in assays. In other assays, target cells were incubated with hybridoma supernatants from PA2.6 (ATCC HB118) anti-HLA class I A, B and C, OKT4 (ATCC CRL8002) anti-CD4, 7G7B6 (ATCC HB8784) anti-IL-2 receptor, L243 (ATCC HB55) anti-HLA-DR, 4F2C13 (ATCC HB22), or 2 μg anti-CD54 ICAM (84H10; Amacine, Westbrook, ME) for 30 min and washed as above before use as targets. These studies were performed to evaluate the functional relevance of cell surface molecules to target cell recognition and effector cell function.

RESULTS

CD8+ CTL from HIV-1-infected individuals, but not controls, lyse autologous CD4+ lymphocytes

Using Con A-stimulated CD8+ lymphocytes from HIV+ individuals as effector cells, we demonstrated killing of autologous CD4+ lymphocytes. Results in Table 1 show effective lysis of purified autologous CD4+ lymphocytes by CD4-depleted Con A-stimulated effector cells from seven HIV-1-infected individuals. In contrast, following the same *in vitro* treatment used to stimulate effector cells from HIV-infected individuals, none of eight HIV− individuals tested had similar cytotoxic activity against CD4+ target cells (Table 1). Lysis of autologous CD4+ cells by effector cells from HIV-infected individuals was inhibited by antibodies against CD3 or CD8, when either was present throughout the assay or if effector cells alone were treated with anti-CD3 and washed (Table 1).

CD8+ CTL from HIV-1-infected individuals lyse heterologous CD4+ lymphocytes from HIV-1-infected individuals or uninfected controls

CD8+ effector cells from HIV-1-infected individuals effectively lysed purified CD4+ cells from heterologous HIV+ individuals and uninfected CD4+ target cells from HIV− individuals (Table 2). Lysis of heterologous CD4+ cells from HIV+ individuals and heterologous CD4+ lymphocytes from uninfected individuals

Table 3. Anti-HLA class I A, B and C MoAb does not inhibit lysis of autologous or heterologous CD4+ lymphocytes by CD8+ cytotoxic T lymphocytes (CTL) from HIV-infected individuals

CD8+ effector cells	CD4+ targets	Per cent specific lysis E:T 50	25	12·5	50+PA2·6*
188	188	60	54	43	
188	188+PA2.6†	73	62	48	
139	139	17	11	10	
139	139+PA2.6	17	16	17	
119	119	26	25	21	
119	119+PA2.6	34	36	30	
119	KR	50	38	19	
119	KR+PA2.6	41	33	13	
155	KR	29	9	0	25
185	KR	70	55	50	71
214	KR	78	63	56	75
221	KR	51	37	28	40
252	KR	69	51	40	67
Anti-viral and anti-allogeneic HLA controls					
119 anti-HIV CTL	Vacc-env	60	45	28	14
123 anti-HIV CTL	Vacc-pol	54	27	13	28
152 anti-EBV CTL	152 BLCL	70	54	26	8
KR anti-SP CTL	SP BLCL		27		6
SP anti-KR CTL	KR BLCL	59	38	22	19

The ability of PA2.6, a MoAb against HLA class I A, B and C, to inhibit lysis of CD4+ lymphocytes by CTL from HIV-infected individuals was tested. Recombinant vaccinia viruses expressing the pol (Vacc-pol Vcf40) [23] and env (Vacc-env VSc25) [24] genes of HIV were used in anti-HIV CTL studies. These viruses were obtained from Dr B. Moss (NIH) and used to infect autologous B lymphoblastoid cell lines (BLCL) at a multiplicity of infection of 15 for 16 h before their use as target cells. A vaccinia recombinant containing the nucleoprotein of Pichinde virus (VVNP) was constructed in our laboratory [25] and used as a control for these studies. Anti-HIV CTL and Epstein–Barr virus (EBV)-transformed BLCL were generated as previously described [26]. Anti-allogeneic HLA CTL were generated in a 6-day mixed lymphocyte reaction.

* In some cases, 200 μl PA2.6 hybridoma supernatant were added to microtitre plates in place of medium to attain a final volume of 300 μl at an E:T ratio of 50.

† In other cases, target cells alone were incubated for 30 min with 1 ml PA2.6 hybridoma supernatant and unbound antibody removed by two washes in PBS containing 1% fetal calf serum (FCS).

was also inhibited when antibodies against CD3 or CD8 were present in the assay, or if effector cells alone were treated with anti-CD3 and washed (Table 2).

Lack of HLA restriction of CD8+ CTL and increased lysis of activated CD4+ lymphocytes

Lysis of autologous CD4+ lymphocytes or heterologous CD4+ lymphocytes from HIV-infected or HIV− individuals was not inhibited by the anti-HLA-A, B and C MoAb (PA2.6), although this same antibody inhibited alloantigen-specific CTL and class I-restricted anti-viral CTL (Table 3). Anti-CD3 inhibitable lysis of heterologous CD4+ T cells from HIV-infected and uninfected individuals was independent of HLA-A or B identity, and also not inhibitable by antibodies against HLA-class II DR antigens (data not shown). Furthermore, the anti-CD3 inhibi-

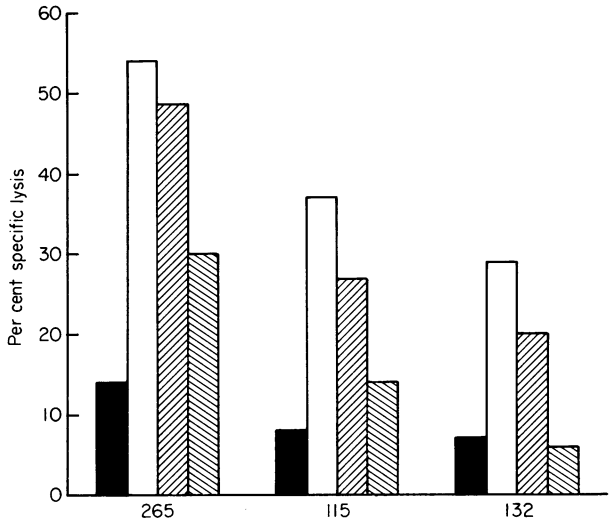

Fig. 1. HLA-class II negative CEM.NK^R cells are lysed by CD8+ cytotoxic T lymphocytes (CTL) from HIV-infected individuals. Effector cells from three HIV-infected subjects (265, 115, and 132) were tested at E:T ratios of 50, 25 and 12·5 and at an E:T ratio of 50 in the presence of OKT3 for specific lysis of CEM.NK^R cells. ■, 50:1+OKT3; □, 50:1; ■, 25:1; ▨, 12·5:1.

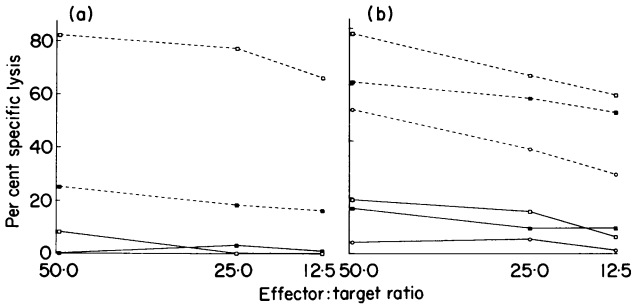

Fig. 2 (a) Activation of autologous CD4+ lymphocytes increases their susceptibility to lysis by CD8+ cytotoxic T lymphocytes (CTL) from HIV-infected individuals. Effector cells from two HIV-infected individuals were tested at three E:T ratios for specific lysis of non-activated (solid lines) and phytohaemagglutinin (PHA)-activated (dashed lines) autologous CD4+ lymphocytes. CD4+ lymphocytes were positively selected from freshly isolated peripheral blood mononuclear cells (PBMC) by magnetic bead separation, removed from beads by vigorous pipetting, and used as targets after 48 h of culture in unsupplemented lymphocyte medium. ■, 119; □, 152. (b) Activation of CD4+ lymphocytes from a non-HIV-infected individual increases their susceptibility to lysis by CD8+ CTL from HIV-infected individuals. Effector cells from three HIV-infected individuals were tested at three E:T ratios for specific lysis of non-activated (solid lines) and PHA-activated (dashed lines) CD4+ lymphocytes from a non-HIV-infected individual. Lysis of non-activated CD4+ lymphocytes was measured against CD4 cells enriched from freshly isolated PBMC by depletion of CD8+ T cells with OKT8-coated magnetic beads and depletion of monocytes by adherence to plastic. ■, 185; □, 214; ○, 221.

Table 4. Cytotoxic T lymphocytes (CTL) mediating non-HLA-restricted lysis of CD4+ lymphocytes are T cell receptor (TCR)$\alpha\beta^+$

CD8+ effectors	CD4+ targets	Per cent specific lysis E:T	50	25	12·5
119	KR		28	19	8
119+OKT3	KR		0	0	0
119+BMA-031	KR		0	0	0
119	DS		24	14	9
119+OKT3	DS		7	3	0
119+BMA-031	DS		2	0	1
139	KR		32	18	8
139+OKT3	KR		15	11	8
139+BMA-031	KR		14	14	4
140	DS		28	20	9
140+OKT3	DS		7	5	1
140+BMA-031	DS		5	1	2
203	KR		30	25	18
203+OKT3	KR		8	2	4
203+BMA-031	KR		8	4	3
203	203		15	4	5
203+OKT3	203		0	1	0
203+BMA-031	203		0	0	0
214	KR		53	44	39
214+OKT3	KR		5	5	0
214+BMA-031	KR		10	6	3
221	KR		18	17	14
221+OKT3	KR		0	0	0
221+BMA-031	KR		0	0	0
252	KR		39	24	11
252+OKT3	KR		0	0	0
252+BMA-031	KR		12	2	4

Immediately before assay, effector cells were incubated with either 1 ml of OKT3 hybridoma supernatant, 1 μg of anti-TCR $\alpha\beta$ framework MoAb BMA-031 in 1 ml medium, or 1 ml of medium alone for 30 min. Effector cells were then washed twice in PBS containing 1% fetal calf serum (FCS) and tested for cytotoxicity.

table CTL lysed HLA-class II negative CEM.NK^R cells (Fig. 1). Results shown in Fig. 2 demonstrate that activation of autologous CD4+ cells (Fig. 2a) or heterologous CD4+ cells from uninfected individuals (Fig. 2b) increased their susceptibility to killing by the CTL under study.

T cell receptor phenotype of CTL recognizing CD4+ lymphocytes

Since the recognition of activated CD4+ lymphocytes by CD3+ CD8+ CTL from HIV-infected individuals was neither HLA-restricted nor HIV-specific, we wished to test the TCR phenotype of the effector CTL by inhibition with MoAb. OKT3 binds to the CD3 ε subunit [27] and inhibits both $\alpha\beta$ and $\gamma\delta$ T cell-mediated cytolysis, while BMA-031 binds the $\alpha\beta$ TCR [28] and inhibits only $\alpha\beta$ T cell-mediated cytolysis. Effector CTL from seven different HIV-infected individuals were preincubated with either OKT3 or BMA-031 and tested against autologous CD4+ lymphocytes and heterologous uninfected CD4+ lymphocytes. In all cases, lysis of CD4+ lymphocyte targets was markedly inhibited (Table 4).

DISCUSSION

We confirmed that CD8+ TCR $\alpha\beta^+$ lymphocytes from HIV-infected individuals kill uninfected CD4+ lymphocytes, and showed that these CTL are not classically HLA-restricted. CD8+ CTL killing autologous CD4+ lymphocytes and heterologous CD4+ lymphocytes from HIV-infected individuals were also not classically HLA-restricted. CD4+ lymphocytes from the NK-resistant T lymphoma-derived cell line CEM.NK^R were inherently susceptible to the CTL, but the sensitivity of peripheral blood CD4+ lymphocytes to killing increased following lectin-mediated activation. This suggests that activated CD4+ lymphocytes selectively express target antigen(s) or accessory molecules at levels necessary for effective CTL recognition, binding and activation. Effector cells were functionally inhibited by anti-CD8, anti-CD3 or anti-TCR $\alpha\beta$ antibodies, but antibodies selected to block target cell molecules had no effect. Since no role for the HLA antigens expected to enable specific recognition by CD8+ CTL was observed, the TCR of these CTL are either HLA-independent or dependent on non-classical HLA molecules.

Non-HLA-restricted antigen-specific TCR-mediated triggering of $\alpha\beta^+$ CTL has been documented in several studies. Non-HLA-restricted CTL with identical TCR rearrangements recognized, via the TCR, a cell surface antigen selectively expressed on activated lymphocytes and some transformed cell lines [29,30]. These CTL were unusually frequent in peripheral blood mononuclear cells (PBMC) (0·15%) [31] and recognized a 140-kD cell surface antigen defined by MoAbs anti-TNK$_{TAR}$ [29] and 4F2C13 [32]. 4F2C13 did not inhibit the lysis of target cells by CTL examined in this study (data not shown). Non-HLA-restricted antigen-specific CTL specifically recognizing tumour-associated mucins [33], bacteria-infected targets [34], idiotypic determinants on B lymphocytes [35] and herpes simplex virus (HSV)-infected lymphocytes [36] have also been described. Apparently a variety of antigens may engage TCR independently of MHC presentation.

Non-MHC-restricted TCR-dependent activation of T cells could be less exceptional than generally believed. The ability of superantigens, anti-TCR antibodies and allogeneic cells to stimulate T cells illustrates that no absolute functional requirement of self-MHC molecules for T cell stimulation exists. *In vivo*, TCR $\gamma\delta^+$ and CD4$^-$CD8$^-$ $\alpha\beta^+$T cells without clear MHC dependence form part of the mature repertoire and selectively expand under certain conditions. Such conditions include immunodeficiency following bone marrow transplantation [37], infection with HIV [38,39] or Epstein–Barr virus (EBV) [40] and autoimmune disease [41]. Factors promoting expansion of non-MHC restricted $\gamma\delta^+$ and CD4$^-$CD8$^-$ cells under these conditions may be related to the factors promoting expansion of non-MHC-restricted CD8+ CTL in HIV infection.

The association of non-HLA restricted CTL with HIV infection raises interesting questions about the origin and role of these CTL. Since CD8+ lymphocyte numbers and CTL activity in general rise in HIV infection, this association might result simply from generalized expansion of CD8+ cells. However, when CD8+ cells are purified from peripheral blood of non-HIV-infected controls and stimulated in the same way as those from HIV-infected individuals, non-HLA-restricted CTL that kill CD4+ lymphocytes are not detectable. E:T ratios are normalized before cytotoxicity assays, so the killing of unin-

fected CD4+ lymphocytes by CD8+ CTL from HIV-infected individuals reflects a specific qualitative and quantitative change in the CD8+ lymphocyte repertoire of HIV-infected individuals. It may be informative to look for CTL activity against CD4+ T cells in HIV- subjects from groups at risk for HIV infection or with other viral infections. Exposure to blood products, semen and other viruses can produce immunological changes similar to HIV infection, including inverted CD4/CD8 T lymphocyte ratio and increased absolute numbers of CD8+ lymphocytes [42,43]. HIV infection appears uniquely to produce the chronic and progressive changes resulting in AIDS, but it may be that other factors cause expansion of CTL that kill CD4+ lymphocytes. What drives this expansion is not known, but since killing by these CTL is mediated by antigen-specific TCR, a specific immune response against antigens present on activated CD4+ T cells seems to occur.

Of the many effects HIV has on the immune system, activation and expansion of CD8+ lymphocytes is probably the most immediate [44,45], chronic and consistent [6–9, 46–49]. Expansion of distinct subsets of CD8+ lymphocytes is well recognized, but it is unclear which, if any, subset is more important or immunologically dominant. Furthermore, it is not clear whether expansion of CD8+ lymphocytes in HIV infection has a positive or negative effect. CD4 lymphocyte counts are a good indicator of disease stage, and changing CD4 counts an important prognostic measure in HIV infection. What high CD8+ lymphocyte counts, rising CD8+ lymphocyte counts, falling CD8+ lymphocyte counts or low CD8+ lymphocyte counts and different CD8+ subsets signify in HIV infection is relatively unknown. Knowledge in this area may open a new window on the pathogenesis of HIV infection and refine current strategies for immunotherapy.

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