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Antigen-specific primary cytotoxic T lymphocyte (CTL) responses in acquired immune deficiency syndrome (AIDS) and AIDS-related complexes (ARC)

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SUMMARY

Alloantigen specific primary cytotoxic T lymphocyte (CTL) responses were examined *in vitro* in 10 patients with AIDS and nine with AIDS-related complex (ARC). The lymphocytes from patients with AIDS and ARC expressed significantly less ($P < 0.01$) CTL activity (mean ± s.d.; 4·7 ± 9% and 10 ± 11% respectively) when compared with CTL activity in normal healthy heterosexual controls (28 ± 9·5%). When data were analysed for individual patients, lymphocytes from nine of 10 patients with AIDS and six of nine with ARC had deficient or no CTL activity. *In vitro* addition of purified human interleukin-2 (IL-2) during the generation of CTL resulted in significant enhancement ($P < 0.05$) of CTL activity in ARC group (mean ± s.d.; 27 ± 18) but not in AIDS group (mean ± s.d.; 8 ± 8%). The presence of IL-2 augmented the induction of CTL activity in three of nine patients in AIDS group and in five of six in ARC group. *In vitro* addition of lectin-free supernatant (SN) obtained from cultures stimulated with PHA as well as with lymphoid cell restored the CTL functions in three of six AIDS patients and in one patient with ARC who did not respond to exogenous IL-2. The CTL activity developed in the presence of SN was higher than that manifested in the presence of IL-2 in both AIDS (SN versus IL-2; mean ± s.d., 18 ± 15·6% versus 8 ± 8%) and in the ARC group (SN versus IL-2; mean ± s.d., 35 ± 13·9% versus 27 ± 18·3%). Lymphocytes from three AIDS patients, however, failed to develop any CTL activity in the presence of either IL-2 or SN. These results demonstrate that: (i) the lymphocytes from majority of patients with AIDS and with ARC have deficient ability to develop into alloantigen specific primary CTL effectors, and (ii) the defective CTL functions are restored by the addition of purified IL-2 or SN in all patients with ARC and only in a subset of patients with AIDS, suggesting heterogeneity of pre-CTL to respond to IL-2 and some differentiation factor in order to differentiate in CTL effectors.

Keywords AIDS, AIDS-related complex specific cytotoxicity interleukin-2

INTRODUCTION

The acquired immune deficiency syndrome (AIDS) is a disorder characterized clinically by the presence of opportunistic infections and/or malignancies especially Kaposi's sarcoma. Recent reports have shown that T-cell lymphotrophic retrovirus belonging to HTLV-III subgroup that preferentially attack helper phenotype T cells to be the aetiological agent of AIDS (Popovic *et al.*, 1984; Sarngadharan *et al.*, 1984).

The antigen specific CTL have been shown to play an important role in defence against viruses

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and neoplasms. Transfer of H-2 restricted CTL with high degree of specificity were recently shown to protect T-deficient mice with influenza pneumonia (Yap, Ada & McKenzie, 1978; Lin & Askonas, 1981; Wells *et al.*, 1983). Similarly, mice and rat with established tumours were cured or their lives were prolonged following transfer of tumour-specific CTL (Eberlein, Rosenstein & Rosenberg, 1982; Cheever *et al.*, 1982; Fernandez-Cruz, Wooda & Feldman, 1980).

The primary antigen specific functional CTL are generated *in vitro* and *in vivo* when mononuclear cells are exposed to alloantigens or to altered autoantigens. These are induced as a result of sequential interactions of macrophages, helper T cells, their biologically active products with CTL precursors (Miller, Schilling & Phillips, 1977; Lutz & Fitch, 1979; Woodward, Fernandez & Daynes, 1979; Schmid, Larsen & Rouse, 1981; Cantor & Boyse, 1975; Wagner & Rollinghoff, 1978; Pilarski, 1979; Okado & Henney, 1980; Larson & Coutinho, 1979; Bonnard, Yasaka & Jacobson, 1979; Farrar, Mizel & Farrar, 1980; Wagner *et al.*, 1982; Schmid *et al.*, 1981). Abnormality in any of these interactions could lead to lack of generation of functionally active effector CTL. The requirement for macrophages and helper T cells can be overcome by interleukin 2 (IL-2) (Schmid *et al.*, 1981). To investigate the possibility of deficient CTL as one of the causes of increased frequency of viral infections and high incidence of tumours in AIDS, we have examined the antigen specific primary CTL function and determined whether *in vitro* addition of IL-2 and lectin-free supernatants (SN) can augment CTL function in patients with AIDS and AIDS-related complex (ARC).

MATERIALS AND METHODS

Patients. Ten male homosexuals with a diagnosis of AIDS and nine with AIDS related complex (ARC) attending the Immunology Clinic of the University of California Irvine Medical Center, California, USA, were studied. Nine age-matched healthy heterosexual males served as normal control. The diagnosis of AIDS was established according to criteria of Center for Disease Control (Selik, Haverkos & Curran, 1984). The diagnosis of ARC was established by the presence of persistent generalized lymphadenopathy, low grade fever, recurrent diarrhoea over 3 months with at least two of the following laboratory abnormalities; depressed Leu-3^+ (helper phenotype) T cells, decreased ratios of Leu-3^+/Leu-2^+ T cells, decreased response to concanavalin A, pokeweed mitogens, cutaneous anergy to recall antigens and T cell lymphopaenia.

Isolation of peripheral blood mononuclear cells (MNC). Fresh heparinized venous blood was diluted 1:2 with Hanks' balanced salt solution (HBSS) MNC were separated on Ficoll-Hypaque density gradient. Cells were washed three times with HBSS and resuspended in RPMI-1640 containing 100 u/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamycin, 2 mM L-glutamine (GIBCO Grand Island, New York, USA), and 10% heat-inactivated pooled AB serum (Irvine Scientific, Irvine, California, USA), hereafter referred as complete medium (CM).

Generation of alloantigen specific CTL in vitro. Effector CTL against alloantigens (HLA) were induced as described previously (Sharma & Terasaki, 1974). In brief, MNC (1×10^6/1·5 ml) from patients with AIDS, ARC or from normal controls were mixed with equal number of irradiated (3,000 R) allostimulator MNC in CM in 15 ml polypropylene tubes (Falcon, Oxnard, California, USA). The *in vitro* influence of IL-2 or SN on the generation of CTL was determined by activation of MNC in the absence or presence of purified IL-2 (150 u/1·5 ml; gift from Dr Steve Gillis, Immunex Corporation, Seattle, Washington, USA) or SN (100 μl/1·5 ml; Association of Biomedical System, Buffalo, New York, USA). The characteristics of IL-2 preparation used have been described (Stern *et al.*, 1984). The cultures were incubated at 37°C in a 5% CO_2 humid atmosphere. After 138 h of incubation, cultures were harvested, cells were resuspended in CM and lymphocytes and lymphoblasts were counted. The cytotoxicity of cultured cells was determined against specific PHA-induced lymphoblasts (cells from donor of stimulators cells) in 5 h ^{51}Cr release assay.

Target cells. Peripheral blood mononuclear cells (1×10^6/ml) from the donor of stimulator cells were cultured in a 25 cm^2 flask (Corning Glass Works, Corning, NY, USA) in a total volume of 5 ml of CM with an optimal concentration of PHA (2 μg/ml). The cultures were incubated for 72 h at

37°C. At the end of incubation, cells were harvested, washed three times, resuspended in 100 μl (100 μCi) of $^{51}Cr_2O_4$, and incubated for 45 min. Cells were then harvested, washed three times and resuspended in CM at a concentration of 1×10^6/ml.

Cytotoxicity of effector CTL. The cytotoxicity of *in vitro* activated effector cells was determined as described previously (Sharma, 1976). Briefly, a constant number of specific target cells (10^4) and effector cells were mixed in 0·4 ml polyethylene microtubes at effector/target ratios of 5:1, 10:1 & 20:1 in a total volume of 0·2 ml CM. The tubes were centrifuged for 20 sec. in microcentrifuge (Beckman Instruments, Fullerton, CA, USA) and then incubated for 5 h at 37°C in 5% CO_2 humid atmosphere. After incubation, tubes were mixed and centrifuged for 2 min. The supernatants (100 μl) were carefully removed and transferred to corresponding new microtubes. The percentage of ^{51}Cr released into supernatant and percentage of ^{51}Cr remaining in the residue plus supernatant is calculated. The per cent specific ^{51}Cr was calculated as follows:

$$\frac{\text{Average per cent test release} - \text{average per cent spontaneous release}}{\text{Average per cent maximum release} - \text{average per cent spontaneous release}} \times 100$$

RESULTS

Generation of antigen specific CTL in AIDS and ARC

Alloantigen activated T lymphocytes from 10 patients with AIDS had a CTL activity (mean ± s.d.) of 4·7 ± 9·2% (Fig. 1). This was significantly lower ($P < 0·01$) than the CTL activity (28 ± 9·5%) from the nine normal donors. Lymphocytes from only one patient developed normal CTL activity, whereas lymphocytes from nine of 10 patients expressed deficient or no cytotoxicity. The CTL activity in the group of patients with ARC (10 ± 11%) was significantly lower ($P < 0·01$) than in the control group. Lymphocytes from six of nine patients failed to express CTL activity. The increase in the number of effector cells did not cause any increase in the CTL activity (Fig. 2).

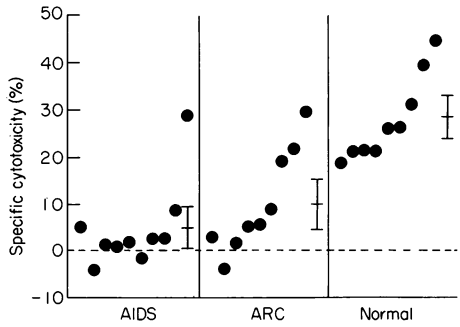

Fig. 1. Alloantigen specific induced-cytotoxicity by *in vitro* activated T lymphocytes from patients with AIDS and ARC and from normal healthy controls.

Effect of IL-2 on the generation of CTL in AIDS and ARC

Tables 1 and 2 show the results of *in vitro* effect of purified IL-2 on the induction of CTL activity in AIDS and ARC patients with deficient CTL responses. The data demonstrate that the addition of IL-2 during activation of lymphocytes augmented the generation of CTL activity in patients with AIDS and with ARC. In AIDS, lymphocytes from three of nine patients developed CTL activity after incubation with IL-2. The mean CTL activity of lymphocytes from AIDS group (nine patients) activated in the presence of IL-2 was 8 ± 8%, which was not significantly different from their baseline CTL activity. Incubation of lymphocytes from ARC group with IL-2 resulted in significant increase ($P < 0·05$) in CTL activity (mean ± s.d.; from 10 ± 11% to 27 ± 18%). Addition of IL-2 restored the CTL responses in four of six non-responder patients with ARC and further augmented the CTL activity of three other ARC patients. Alloactivation of lymphocytes from the normal donors in the presence of exogenous IL-2 showed variable results (Table 3).

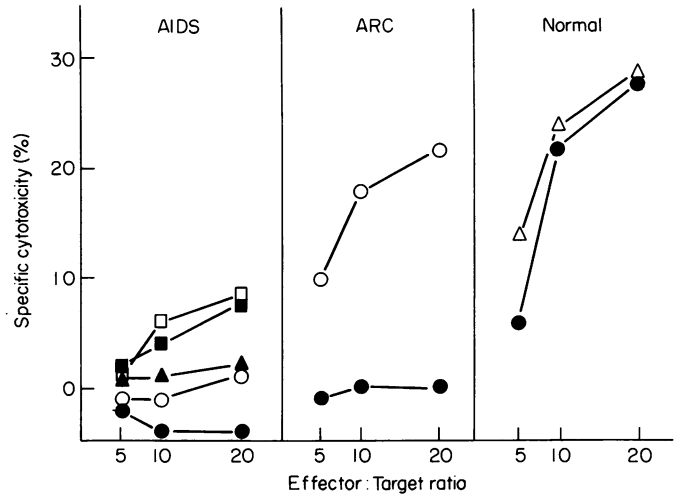

Fig. 2. Activity of alloantigen induced effector CTL determined at various effector:target cell ratios. (●, ■, ▲, ○, □), Cytotoxicity manifested by effector cells from different patients and normal healthy controls.

Table 1. Generation of alloantigen-specific cytotoxic T lymphocytes in patients with AIDS *in vitro*. Effect of interleukin-2 (IL-2) and activated culture supernatant (SN)

Donor of alloantigen-activated lymphocytes*		% Specific cytotoxicity of lymphocytes activated in the presence of:		
		None	IL-2	SN
1-AIDS	PCP,KS,CM†	−4	−2	3
2-AIDS	PCP	1	3	2
3-AIDS	CMV,MAI	9	5	6
4-AIDS	KS	3	26	39
5-AIDS	PCP	−1	13	41
6-AIDS	PCP	3	10	11
7-AIDS	PCP	0	5	12
8-AIDS	KS	2	1	13
9-AIDS	PCP	5	3	11
10-AIDS	KS	29	15	39

* Peripheral blood mononuclear cells (MNC) were cultured with irradiated allogeneic MNC in the absence and presence of purified IL-2 or SN (supernatants of culture stimulated with PHA and lymphoid cells at 37°C in a humidified 5% CO_2 atmosphere for 5 days). On day 6, cytotoxicity of activated cells was determined against specific PHA-induced lymphoblast cells.

† PCP = *Pneumocystic carinii* pneumonia, KS = Kaposi's sarcoma, CM = cryptococcal meningitis, CMV = Disseminated cytomegalovirus infection, MAI = *Mycobacterium avium intracellulare*, disseminated

Table 2. Generation of alloantigen-specific cytotoxic T lymphocytes in patients with AIDS related complex (ARC) *in vitro*. Effect of interleukin-2 (IL-2) and activated culture supernatant (SN)

Donor of alloantigen-activated lymphocytes*	% Specific cytotoxicity of lymphocytes activated in the presence of:		
	None	IL-2	SN
11-ARC	2	20	39
12-ARC	3	16	45
13-ARC	−4	27	21
14-ARC	6	65	61
15-ARC	9	21	20
16-ARC	5	−2	24
17-ARC	19	23	26
18-ARC	22	32	46
19-ARC	30	40	31

* Peripheral blood mononuclear cells were cultured with irradiated allogeneic MNC in absence and presence of IL-2 or SN at 37°C in a humidified 5% CO_2 atmosphere for 5 days. On day 6, cytotoxicity of activated lymphocytes was determined against specific PHA-induced lymphoblast cells.

Table 3. Generation of alloantigen specific cytotoxic T lymphocytes in normal donors *in vitro*. Effect of interleukin-2 (IL-2) and activated culture supernatant (SN)

Donor of alloantigen-activated lymphocytes*	% Specific cytotoxicity of lymphocytes activated in the presence of:		
	None	IL-2	SN
20-Normal	17	34	38
20-Normal	27	54	54
21-Normal	32	40	39
22-Normal	21	41	22
23-Normal	27	27	43
24-Normal	45	36	46
25-Normal	21	14	32
26-Normal	21	11	32
27-Normal	40	17	44

* Peripheral blood mononuclear cells were cultured with irradiated allogeneic MNC in the absence and presence of IL-2 and SN at 37°C in a humidified 5% CO_2 atmosphere for 5 days. On day 6, cytotoxicity of activated lymphocytes was determined against specific PHA-activated lymphoblast target cells.

Effect of SN on the generation of antigen specific CTL in AIDS and ARC

Tables 1 and 2 show the results of experiments designed to determine the effect of SN on the induction of CTL in patients with AIDS and ARC whose lymphocytes failed to develop CTL activity even in the presence of IL-2. The CTL activity induced in the presence of SN in AIDS patients was $18{\cdot}0 \pm 15{\cdot}6\%$ which was significantly higher ($P < 0{\cdot}05$) than CTL activity expressed by lymphocytes that were induced in the absence of SN ($4{\cdot}7 \pm 9\%$). The addition of SN during the activation of lymphocytes restored the CTL functions in three additional patients. The addition of either IL-2 or SN, however, did not restore the ability of lymphocytes from three patients from AIDS to develop CTL activity. The SN was able to reconstitute the CTL activity in a patient with ARC who did not respond to exogenous IL-2.

DISCUSSION

The results of this study demonstrate that majority of patients with AIDS and ARC were unable to manifest alloantigen specific primary CTL responses.No differences were observed between AIDS with Kaposi's sarcoma and AIDS with opportunistic infections.

Patients with AIDS and ARC have decreased helper T cell numbers and functions (Siegel & Fox, 1983; Fahey *et al.*, 1984; Gupta & Safai, 1983; Gupta *et al.*, 1984; Gottlieb *et al.*, 1981). Since helper cells and macrophages, and their biologically active products, IL-2 and IL-1 respectively, are required for the differentiation and clonal expansion of antigen-driven CTL-P into effector CTL (Miller *et al.*, 1977; Lutz & Fitch, 1979; Woodward *et al.*, 1979; Cantor & Boyse, 1975; Wagner & Rollinghoff, 1978; Okado & Henney, 1980; Larson & Coutinho, 1979; Bonnard *et al.*, 1979; Farrar *et al.*, 1980; Wagner *et al.*, 1982; Schmid *et al.*, 1981; Gillis, 1983), the failure to induce CTL in patient groups could be the result of quantitative/qualitative abnormalities in these cells. When added during CTL generation, purified IL-2 was able to restore the CTL function in 30% patients with AIDS and most of the patients in ARC group. Thus dysfunction in helper T cells was responsible for the lack of display of CTL responses in these patients. Similar results of the effect of exogenous IL-2 on the AMLR in AIDS and ARC have been recently reported (Gupta *et al.*, 1984). Interleukin-2 did not, however, reconstitute the CTL activity in several of the patients, although lymphocytes from some of these patients when examined expressed proliferative response or were induced by exogenous IL-2 to proliferate in response to alloantigens (data not shown). This would suggest that IL-2 alone is not sufficient to induce complete differentiation of lymphocytes into effector CTL. Wagner *et al.* (1982) also reported that IL-2 preparations although capable of inducing lymphocytes to proliferate but failed to cause maturation of CTL-precursors (CTL-P) into CTL effectors. Lymphocytes from several patients, who did not respond to IL-2 were, however, induced to differentiate into CTL-effectors in the presence of lectin-free supernatant of PHA and lymphoid cells stimulated cultures (SN). In addition, SN mediated a greater increase in CTL activity as compared to increase mediated by IL-2 (Tables 1 to 3). These findings demonstrate that in addition to IL-2 some factor(s) yet to be characterized, present in SN is required to manifest CTL responses and failure to develop CTL activity in these patients is probably due to a defect in cells that produce this CTL differentiation factor (CTLDF). Several independent groups earlier reported that CTLDF is indeed involved in different allogeneic and syngeneic system during the induction of antigen specific functionally active CTL (Wagner *et al.*, 1982; Raulet & Bevan, 1982; Reddhease *et al.*, 1982). The CTL functions in three patients with AIDS, however, were not restored either by IL-2 or SN. These might represent a true deficiency of CTL-P cells or increase in suppressor cell activity.

In conclusion, the majority of patients with AIDS have profound impairment in their ability to generate antigen specific primary CTL responses. The induction of CTL function activity in AIDS by *in vitro* incubation of lymphocytes with IL-2 and SN showed three patterns: (a) a complete lack of response, (b) response to both IL-2 and SN, and (c) response to SN alone. In contrast, defective CTL functions were augmented in all patients in ARC group by IL-2 or SN. These results could provide a rationale for clinical use of IL-2 in AIDS and ARC.

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