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Inhibition of the generation of cytotoxic lymphocytes by potassium ion channel blockers

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SUMMARY

Recent studies with patch-clamp technique have shown the presence of voltage-gated K^+ channels in human T lymphocytes and natural killer cells. Blockers of voltage-gated K^+ channel currents (4-Aminopyridine, 4-AP, and tetraethylammonium, TEA), were used here in a pharmacological approach to examine a role of K^+ channels in the differentiation of precursors of cytotoxic cells into functionally active cytotoxic lymphocytes. The data presented here demonstrated that activation of peripheral blood lymphocytes with CCRF-HSB-2, 3163 and other allogeneic lymphoid cells for 5 days in mixed lymphocyte culture (MLC) renders them cytotoxic to the respective target cells. Both 4-AP and TEA (2–4 mM), when added to cultures, inhibited the development of cytotoxic effectors in a dose-dependent manner. Maximum inhibition of the generation of cytotoxic lymphocytes occurred when 4-AP was present at the start of cultures. Little or no inhibition was, however, observed when 4-AP was added 1 day of incubation. The results also demonstrate that the addition of recombinant IL-2 (rIL-2) overcame the 4-AP- or TEA-mediated inhibition of the generation of cytotoxic lymphocytes in a dose-dependent manner. The maximum reversal of 4-AP-induced inhibition occurred when exogenous IL-2 was added at Day 0 or 1. Taken together, these data suggest a role of K^+ channels in the generation of cytotoxic lymphocytes.

INTRODUCTION

Activation of peripheral blood mononuclear cells (PBMC) with alloantigens in mixed lymphocyte culture (MLC) results in the generation of antigen-specific cytotoxic T lymphocytes (CTL). In addition to CTL, effectors with characteristics of activated killer cells (AKC) are also induced during MLR (Sharma & Terasaki, 1974a,b; Sharma, 1976; Sharma *et al.*, 1977; Sharma & Odom, 1979; Strausser *et al.*, 1981; Vanky, Argov & Klein, 1981; Vanky *et al.*, 1982; Vose & Bonnard, 1982). Both antigen-specific CTL and broadly specific AKC are involved in providing defence against viruses and malignant or transformed cells. These cells have also been implicated in the allograft rejection.

The generation of functionally active CTL requires the participation of Ia^+ accessory cells, helper T cells, precursors of CTL (P-CTL) and/or biologically active proliferative and differentiation soluble factors (Lutz & Fitch, 1979; Schmid, Larsen & Rouse, 1981; Wagner & Rollinghoff, 1978; Sharma & Gupta, 1984). The early sequence of events that occur prior to the growth and differentiation of P-CTL into the effector CTL are, however, not completely understood. Recently, several distinct types of ionic channels have been identified in immune cells by the use of gigaohm-seal patch-clamp technique (Chandy *et al.*, 1985a). Voltage-gated potassium (K^+) channels seem to be the predominant ion channels present in human T cells and were shown to play a role in the induction of T-cell proliferation (De Coursey *et al.*, 1984; Matteson & Deutsch, 1984). In this study, we have examined the effect of K^+ channel blockers on the induction of functionally active effector cytotoxic lymphocytes. The results demonstrate that K^+ channel blockers inhibit the generation of cytotoxic effectors *in vitro* and the inhibition can be reversed by exogenous IL-2.

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MATERIALS AND METHODS

Isolation of peripheral blood mononuclear cells

Fresh heparinized venous blood was diluted 1:1 with Hanks' balanced salt solution (HBSS). The PBMC were separated on Ficoll–Hypaque density gradient (Sharma & Gupta, 1985). Cells were washed three times with HBSS and resuspended in RPMI-1640 containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (Gibco, Grand Island, NY) and 10% heat-inactivated pooled AB serum (Irvine Scientific, Irvine, CA), hereafter referred to as complete medium (CM).

Cell lines

The cell lines used in this study were 3163 (provided by Dr G. Grainger, UCI, CA) (human B-cell leukaemia), and CCRF-HSB-2 (T-cell acute lymphoblastic leukaemia; ATCC, Rockville, MD).

Channel blockers

4-Aminopyridine (4-AP) was obtained from Sigma Chemical Co., St. Louis, MO. Tetraethylammonium was obtained from Eastman Kodak Co., Rochester, NY. 4-Ap and TEA were recrystallized before use in the experiments.

Generation of induced cytotoxic lymphocytes

Since MLR results in the induction of both alloantigen-specific CTL and activated killer cells, effectors generated after activation of PBMC with 3163, CCRF-HSB-2 cells or allogeneic PBMC will be referred to as induced cytotoxic lymphocytes or effectors. These were produced by incubation of PBMC ($1{\cdot}2 \times 10^6/1{\cdot}5$ ml) with irradiated cells from 3163 or CCRF-HSB-2 lines at a concentration of $1 \times 10^5/1{\cdot}5$ ml in polypropylene tubes at 37° in a 5% CO_2 humid atmosphere (Sharma & Terasaki, 1974a; Sharma, 1976). The allogeneic MLC contained 1×10^6 responder cells and 1×10^6 stimulator cells in a total volume of 2 ml. After 5 days of incubation, cells were centrifuged, washed with HBSS and were resuspended in 0·5 ml of CM and viable cell numbers were determined. The cytotoxicity of induced effectors was measured against specific target cells in a 4-hr ^{51}Cr-release assay (Sharma & Elias, 1987).

Effect of 4-AP and TEA on induction of cytotoxic lymphocytes

The PBMC ($1{\cdot}2 \times 10^6/1{\cdot}5$ ml) were cultured together with irradiated stimulator cells in the absence and presence of various concentrations of 4-AP or TEA. After 5 days of incubations, the cytotoxicity of cultured cells was then determined against the specific target cells.

Effect of exogenous rIL-2 on 4-AP- or TEA-induced inhibition of induced cytotoxicity

The PBMC were activated with stimulator cells (CCRF-HSB-2 or 3163) without and with 4-AP (2 mM) or TEA (4 mM) in the absence and presence of various concentrations of rIL-2 for 5 days. The cytotoxicity of effector cells generated without 4-AP or TEA, with 4-AP or TEA, and with 4-AP or TEA plus rIL-2 was then determined against the specific target cells.

Target cells

Cultured cells of 3163-, CCRF-HSB2- or PHA-stimulated lymphoblasts were incubated with 100 μCi $Na_2{}^{51}CrO_4$ solution for 45 min. Cells were then washed three times and resuspended in CM at a concentration of 1×10^6/ml.

Cytotoxicity of induced effectors

The cytotoxicity of *in vitro*-acitivated lymphocytes was determined by the Sharma and Terasaki assay as previously described elsewhere (Sharma & Elias, 1987). In brief, a constant number of specific target cells (10^4) and effector cells were mixed in 0·4 ml polyethylene tubes at effector/target ratios of 10:1 in a total volume of 0·2 ml of CM. The tubes were centrifuged for 20 seconds and then incubated for 4 hr at 37° in CO_2 humid atmosphere. After incubation, the tubes were mixed and centrifuged for 2 min. The supernatants were carefully removed and radioactivity was measured in a gamma counter. The percentage of ^{51}Cr release was calculated as: ^{51}Cr in 1/2 supernatant/^{51}Cr release in 1/2 supernatant + ^{51}Cr residue × 100. The percentage specific release was: test release − spontaneous release/maximum release × 100. The spontaneous release is the mean of triplicate c.p.m. released by targets incubated alone, and the maximum release is the mean of triplicate c.p.m. obtained after lysis by 1% Triton X-100.

RESULTS

Effect of 4-AP and TEA on the generation of cytotoxic lymphocytes

The 4-AP and TEA blocker of voltage-gated K^+ channels were used to determine their influence on the generation of cytotoxic lymphocytes against CCRF-HSB-2 or 3163 cells. The results shown in Fig. 1 demonstrate that PBMC activated for 5 days with CCRF/HSB-2 or 3163 displayed a marked cytotoxic activity against both the target cells. The addition of 4-AP at the time of initiation of cultures inhibited the development of cytotoxic effectors in a dose-dependent manner. A complete inhibition of the generation of effectors was observed at 2–4 mM concentrations. Similarly, TEA inhibited the CCRF-HSB-2-

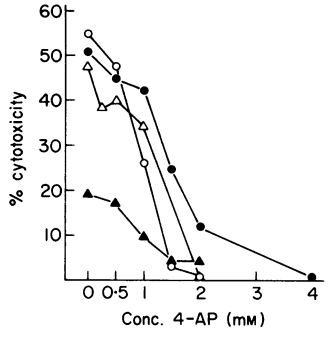

Figure 1. The effects of 4-AP on the generation of cytotoxic lymphocytes against CCRF-HSB-2 and 31363 target cells. PBMC ($1 \times 10^6/1{\cdot}5$ ml) from different donors were cultured with 1×10^5 irradiated CCRF-HSB-2 cells or 3163 cells in the presence or absence of various concentrations of 4-AP. On Day 6, cultured cells were resuspended in the complete medium and then their cytotoxicity was determined against CCRF-HSB-2 or 3163 target cells as decribed in the text. (○), (●) Represent the 4-AP-mediated inhibition of cytotoxicity of donor 1 and 2 against CCRF-HSB-2 target cells: (△), (▲) represent the inhibition of cytotoxicity against 3163 target cells of two donors.

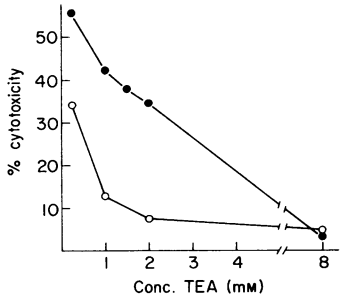

Figure 2. The effect of TEA on the generation of cytotoxic lymphocytes against CCRF-HSB-2 cells. PBMC from donor 1 (○) and donor 2 (○) were cultured with irradiate stimulator (CCRD-HSB-2) cells for 5 days in the presence and absence of various concentrations of TEA. The cytotoxicity of cultured cells was determined against the CCRF-HSB-2 target cells, as described in the text.

Table 1. Effect of K^+ channel blockers on the viability of effector cells generated in mixed cultures*

	No viable cells $\times 10^6$ Conc. (mM) of K^+ channel blockers				
Blocker	0	1	2	4	8
TEA	0·99±0·47	0·83±0·20	0·78±0·16	0·93±0·24	0·99±0·27
4-AP	0·73±0·29	0·85±0·23	0·81±0·28	0·8±0·21†	

* Lymphocytes ($1·2 \times 10^6$/1·5 ml) were cultured with irradiated (3000 rads) stimulator cells in the absence and presence of various concentrations of K^+ channel blockers. At the end of incubation period, cells were harvested, washed and resuspended in 0·5 ml complete medium. Viability was assessed by trypan blue dye exclusion on Day 6. The data are the mean ± SD of five experiments.

† Mean of two experiments.

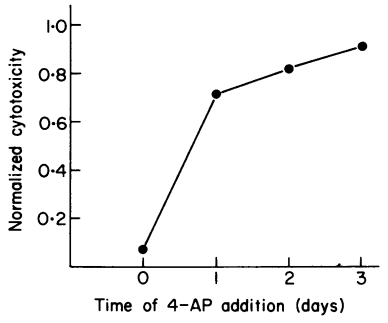

Figure 3. The time-course of 4-AP-induced inhibition of the generation of cytotoxic lymphocytes. The 4-AP (2 mM) was added at different times to the mixed cultures, and after 5 days of incubation the cytotoxicity of cultured cells was determined. The data are normalized by dividing the values obtained in the presence of 4-AP with value obtained in the absence of 4-AP.

induced cytotoxicity (Fig. 2). These blockers did not, however, decrease significantly the viability of cells in the mixed cultures after 5 days of incubation (Table 1).

Time-course of 4-AB-induced inhibition of cytotoxic lymphocytes

4-AP was added at different times to the mixed culture of PBMC and CCRF-HSB-2, and after 5 days of incubation the cytotoxicity of cultured cells was determined. Figure 3 shows the time-course effect of 4-AB-mediated block of cytotoxic lymphocytes generation. The maximum inhibition occurred when 4-AP was added at the time of initiation of cultures (0 time). An inhibition of the induction of cytotoxicity of approximately 30% and 20% was observed when 4-AP was added on Days 1 and 2 of cultures, respectively.

Effect of rIL-2 on 4-AP- or TEA-induced inhibition of cytotoxic lymphocytes

In order to determine whether functional K^+ channels are involved in the events preceding the IL-2 production, we added rIL-2 to the cultures with 4-AP or TEA at various time intervals and then determined the cytotoxic activity. The results (Table 2) demonstrate that the addition of recombinant IL-2 at various concentrations prevented the inhibition of cytotoxicity by 4-AP or TEA. The concentrations ranging from 12·5 to 50 units/1·5 ml were able to reverse the inhibitory effect of 4-AP and TEA. The restoration of cytotoxicity by rIL-2 was dose-dependent. Table 3 shows the time-course for IL-2-mediated recovery of induced cytotoxocity. The reversal of 4-AP-induced inhibition was seen when rIL-2 was added at up to Day 3.

Table 2. Effect of *in vitro* rIL-2 on 4-AP- or TEA-mediated inhibition of induced cytotoxic lymphocytes*

		% specific cytotoxicity				
			+IL-2 (units/1·5 ml added to mixed culture			
Exp. no.	Blocker (4-AP or TEA)	−IL-2	12·5	25	37·5	50
1	—	55				
	+	−0·3	20	49	52	41
2	—	51				
	+	2	28	57	ND	44
3	—	47				
	+	1	20	35	ND	28
4	—	24				
	+	4	13	ND	20	33
5	—	40				
	+	5	50	50	60	ND
6	—	33				
	+	6	19	24	ND	27

* PBMC (1×10^6/1·5 ml) were cultured with cell lines CCRF-HSB-2 (experiment nos 1,2,6); 3163 (experiment nos 3,4); or WIL-2 (experiment no. 5) cells in the absence and presence of 4-AP or TEA and various concentrations of rIL-2. The cytotoxicity of cultured cells was then determined against ^{51}Cr-labelled target cells.

Effect of 4-AP and IL-2 on the generation of alloantigen-specific cytotoxic T lymphocytes

In order to determine the specificity, PBMC were cultured together with irradiated allogeneic stimulator cells and then their cytotoxic activity determined against specific target cells. The results in Table 4 shows that lymphocytes from donor A cultured alone had no cytotoxicity against the allogeneic PHA-stimulated target cells from donor B, whereas, lymphocytes from donor A after activation with B cells displayed a marked cytotoxicity against B target cells. Lymphocytes from donor B after activation with A cells did not express any cytotoxic activity against B-target cells. Lymphocytes activated with alloantigens in the presence of 4-AP failed to develop specific cytotoxicity (Table 4). The addition of IL-2 was, however, able to reverse the effect of 4-AP. Lymphocytes cultured alone with IL-2 showed little cytotoxicity against B-target cells in comparison with specific cytotoxicity displayed by lymphocytes activated with B cells.

DISCUSSION

The activation of lymphocytes with alloantigens results in the proliferation and differentiation of precursors of cytotoxic cells

Table 3. Time-course rIL-2-mediated reversal of 4-AP-induced inhibition of cytotoxic lymphocytes generation*

Exp. no.	Mixed cultures	4-AP	rIL-2	% specific cytotoxicity
1	PBMC+HSB-2	−	−	50
		+	−	5
		+	+ (Day 0)	51
		+	+ (Day 1)	53
		+	+ (Day 2)	43
		+	+ (Day 3)	20
2	PBMC+HSB-2	−	−	51
		+	−	12
		+	+ (Day 0)	45
		+	+ (Day 2)	42
		+	+ (Day 3)	18
3	PBMC+HSB-2	−	−	55
		+	−	1·5
		+	+ (Day 0)	57
		+	+ (Day 1)	80
		+	+ (Day 2)	70
		+	+ (Day 3)	60

* PBMC were activated with CCRF-HSB-2 cells in the presence or absence of 4-AP. rIL-2 (50 units/1·5 ml) was added to the mixed cultures at various time intervals and cytotoxic activity was determined.

Table 4. Generation of alloantigen-specific cytotoxic T lymphocytes *in vitro*: effect of 4-AP and IL-2*

Exp. no.	Culture	% specific cytotoxicity
1	A	0·3
	A+Bx	17
	B†+Ax	1·5
	A+Bx+4-AP (0·95 mM)	8
	A+Bx+4-AP (1·9 mM)	1
	A+Bx+4-AP (1·9 mM) +IL-2	18
2	A	−5
	A+Bx	43
	A+IL-2	8

* PBMC from donor A were cultured alone or with irradiate (R = 2750) allogenic PBMC of donor B (Bx) in the presence and absence of 4-AP and/or IL-2 for 5 days at 37° in a 5% CO_2 humidified atmosphere. On Day 6, cytotoxicity of effector cells was determined against specific PHA induced lymphoblast target cells as described (Sharma & Gupta, 1985).

† PBMC from the donor B were activated with Ax and then their cytotoxicity was determined against B-target cells.

into functionally active cytotoxic lymphocytes. In this study we examined the role of K^+ channels on the generation of cytotoxic lymphocytes. Voltage-gated K^+ channels have been shown to be present in both human T lymphocytes and natural killer cells and also in murine cytotoxic cloned cells (De Coursey *et al.*, 1984; Schlichter, Sidel & Hagiwana, 1986; Chandy *et al.*, 1985b; Fukushima, Hagiwana & Henkart, 1984). These were shown to play a role in T-lymphocyte proliferation, IL-2 receptors expression, IL-2 production and cytolysis of target cells in cell mediated lymphobysis (Chandy *et al.*, 1984, 1985a). Voltage-gated channels are blocked by pharmacological agents 4-AP and TEA (De Coursey *et al.*, 1984). In attempt to examine whether K^+ channels are involved in the generation of functionally active cytotoxic lymphocytes, we found here that 4-AP or TEA, when added at the time of onset of mixed cultures, were able to inhibit the generation of cytotoxic effectors against 3163 (human B-leukaemia cells) and cells from CCRF-HSB-2 (human T-cell acute lymphoblastic leukaemia). 4-AP also inhibited the development of alloantigen-specific cytotoxic effector cells in MLC and against B cells (WIL-2; data not shown here). The concentrations that abrogated the cytotoxic effectors induction were within the comparable range that produced blockage of K^+ currents (De Coursey *et al.*, 1984), suggesting a role of K^+ channels in differentiation of precursors of cytotoxic cells into active cytotoxic lymphocytes. Maximum inhibition of cytotoxicity induction by 4-AP occurred when 4-AP was present in culture during the entire period of mixed culture incubation.

Macrophages or IL-1 and helper T cells are required for the activation of T cells resulting in clonal expansion and differentiation into functionally active cytotoxic effectors (Lutz & Fitch, 1979; Schmid *et al.*, 1981; Wagner & Rollinghoff, 1978; Sharma & Gupta, 1985). Exogenous IL-2 has been shown to substitute the function of IL-1 and helper T cells (Schmid *et al.*, 1981). Since IL-2 is required for the generation of cytotoxic lymphocytes, and 4-AP blocks the IL-2 production, it is likely that the 4-AP-mediated inhibition of cytotoxic activity expression, at least in part, is a result of suppression of IL-2 production or inhibition of steps which lead to the synthesis of IL-2. This is supported by our present data which shows that exogenous IL-2 was able to overcome the blocking effect of these channel blockers. The restoration of cytotoxicity by IL-2 in the presence of 4-AP was in a dose-dependent manner. It is possible that IL-2 reversed the 4-AP-mediated inhibitory effect by simply inducing a different set of effector cells. It was shown earlier that 4-AP can inhibit alloantigen-induced proliferative responses in MLR (Chandy *et al.*, 1984). The alloantigen-specific CTL generated in MLR were also inhibited by 4-AP, and the 4-AP effect was overcome by addition of IL-2 (Table 4). The IL-2 by itself did not generate significant cytotoxicity compared with cytotoxicity displayed by allo-antigen-specific CTL to the specific target cells. These findings suggest, therefore, that it is unlikely that IL-2 reverses the channel blocker's effect by activation of new set of effector cells.

Recently, we reported that $CD4^+$ and $CD8^+$ phenotypes express K channels which can be blocked by 4-AP (Chandy *et al.*, 1985b; Chandy *et al.*, 1988). Both $CD8^+$ and $CD4^+$ cytotoxic effector cell are generated in MLR and their respective cytotoxic activity against specific target cells could be blocked by 4-AP. Together, these findings suggest that $CD4^+$ and $CD8^+$ cells could be the targets for K channel blockers. It is, however, not clear as yet which of the phenotypes is the cellular target during the generation of cytotoxic effector cells. The 4-AP is shown to suppress the production of IL-2, but not the expression of IL-2 receptors (Chandy *et al.*, 1984). Since $CD4^+$ cells are the major cell type that produces IL-2, and $CD4^+$ cells do express K^+ channels, the possibility can not be ruled out that 4-AP mediate inhibition of generation of cytotoxic effector cells

by directly acting on these cells and preventing the release of IL-2, which is required for the proliferation and generation of cytotoxic effector cells. The K^+ channels are also present on cultured macrophages (Ypey & Clapham, 1984). These could also serve as the target for 4-AP, and 4-AP may inhibit the production of IL-1, which is required for the release of IL-2 at optimal levels (Mizel, 1982).

In conclusion, the results of this study demonstrate that K^+ channel blockers inhibit the generation of cytotoxic lymphocytes in MLR without significantly affecting the viability of responding lymphocytes. The finding that IL-2 is able to reverse the K^+ channel blockers'-mediated inhibition of cytotoxic lymphocyte induction suggests that inhibition of the generation of cytotoxicity could be due to the suppression of IL-2 production or blockage of events preceding the IL-2 production.

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REFERENCES

CHANDY, K.G., DE COURSEY T.E., CAHALAN M.D. & GUPTA S. (1985a) Voltage-gated K^+ channels are required for T lymphocyte activation. *J Immunol.* **135,** 787.

CHANDY, K.G., DE COURSEY T.E., CAHALAN M.D., MCLAUGHLIN C. & GUPTA S. (1984) Voltage-gated channels are required for T lymphocyte activation. *J. exp. Med.* **160,** 369.

CHANDY, K.G., DE COURSEY T.E., SHARMA B., CAHALAN M.D. & GUPTA S. (1988) Voltage-gated K^+ channels are required for target cells lysis by human allo-reactive cytotoxic T cells. (*submitted*).

CHANDY K.G., SHARMA B., DE COURSEY T.E., CAHALAN M.D. & GUPTA S. (1985b) K^+ Channel requirement for human T-cell mediated cytotoxicity. *J. Allergy. Clin. Immunol.* **75,** 181.

DE COURSEY T.E., CHANDY K.G., GUPTA S. & CAHALAN M.D. (1984) Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis. *Nature* (*Lond.*), **307,** 465.

FUKUSHIMA Y., HAGIWARA S., HENKART M. (1984) Potassium current in clonal cytotoxic lymphocytes from the mouse. *J. Physiol.* (*Lond.*), **351,** 645.

LUTZ C.T. & FITCH F.W. (1979) Assessory cell requirements for the generation of cytotoxic T lymphocytes. *J. Immunol.* **122,** 2598.

MATTESON D.R. & DEUTSCH C. (1984) K^+ channels in T lymphocytes: a patch clamp study using monoclonal antibody adhesion. *Nature* (*Lond.*), **307,** 468.

MIZEL S.B. (1982) Interleukin 1 and T cell activation. *Immunol. Review.* **63,** 51.

SCHLICHTER L., SIDEL N. & HAGIWANA S. (1986) Potassium mediated killing by human natural killer cells. *Proc. natl. Acad. Sci.* U.S.A. **83,** 451.

SCHMID D.S., LARSEN H.S. & ROUSE B.T. (1981) The role of accessory cells and T-cell growth factor in induction of cytotoxic T lymphocytes against herpes simplex virus antigens. *Immunology*, **44,** 755.

SHARMA B. (1976) *in vitro* immunization of lymphocytes to cultured human tumor cells: parameters of generation of cytotoxic lymphocytes. *J. natl. Cancer Inst.* **57,** 743.

SHARMA B. & ELIAS A.N. (1987) Effect of merthimazole on human lymphocyte proliferation and natural killer cell activity. *Gen. Pharmac.* **18,** 449.

SHARMA B. & GUPTA S. (1985) Antigen-specific primary cytotoxic T lymphocytes (CTL) response in acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC). *Clin. exp. Immunol.* **62,** 296.

SHARMA B. & ODOM L.F. (1979) Generation of Killer lymphocytes *in vitro* against human autologous leukemia cells using leukemic blasts and BCG extract. *Cancer Immunol. Immunother.* **7,** 99.

SHARMA B. & TERASAKI P. (1974a) Immunization of lymphocytes from cancer patients. *J. natl. Cancer Inst.* **52,** 1925.

SHARMA B. & TERASAKI P.I. (1974b) *In vitro* immunization to cultured human tumor cells. *Cancer Res.* **34,** 115.

SHARMA B., TUBERGEN D. G., MINDEN P. & BRUNDA M. (1977) *In vitro* immunization against human tumor cells with bacterial extracts. *Nature* (*Lond.*), **845.**

STRAUSSER J.L., MAZUMDER A., GRIM E.A., LOTZ M.T. & ROSENBERG S.A. (1981) Lysis of solid tumors by autologous cells sensitized *in vitro* to alloantigens. *J. Immunol.* **127,** 266.

VANKY F.S., ARGOV, S. & KLEIN E. (1981) Tumor biopsy cells participating in systems in which cytotoxicity of lymphocytes is generated. Autologous and allogeneic studies. *Int. J. Cancer*, **27,** 273.

VANKY F.S., GORSKY T., GORSKY Y., MASUCCI G. & KLEIN E. (1982) Lysis of tumor biopsy cells by autologous T lymphocytes activated in mixed cultures and propagated with T-cell growth factor. *J. exp. Med.* **155,** 83.

VOSE B. & BONNARD G.D. (1982) Human tumor antigens defined by cytotoxicity and proliferative responses of cultured lymphoid cells. *Nature* (*Lond.*), **296,** 359.

WAGNER H. & ROLLINGHOFF M. (1978) T.T. Cell interactions during *in vitro* cytotoxic allograft responses. I. Soluble products from activated Ly 1+ T cells trigger autonomously antigen primed Ly 23+ T cells to cell proliferation and cytotlytic activity. *J. exp. Med.* **148,** 1523.

YPEY D.L. & CLAPHAM D.E. (1984) Development of a delayed outward rectifying K conductance in cultured mouse peritoneal macrophages. *Proc. natl. Acad. Sci.* U.S.A. **81,** 3083.