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Cyclosporin A inhibits lymphokine production but not the responses of macrophages to lymphokines

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Summary. Cyclosporin A (Cs A) exerted a dose-related inhibitory effect on antigen (ovalbumin, OVA) and phytohaemagglutinin (PHA)-induced transformation of guinea-pig lymph node cells (LNC). Whereas 0·05 μg/ml was sufficient to depress these responses markedly, it required 100-fold this concentration of Cs A to inhibit the production of lymphocyte activating factor (LAF) by lipopolysaccharide (LPS) stimulated peritoneal macrophages.

Addition of Cs A together with insoluble concanavalin A (iCon A) to LNC cultures resulted in suppressed lymphokine production, as assessed by measurement of migration inhibition factor (MIF), the generation of macrophage procoagulant activity (MPCA) and the release of lymphocyte-derived-macrophage chemotactic factor (LDCF). Cs A also inhibited MIF and procoagulant production by sensitized peritoneal exudate cells in response to antigen, at the same concentrations which blocked lymphocyte transformation. In contrast, Cs A had no direct effect on the migration of peritoneal cells from capillary tubes, or on the responses of macrophages to preformed MIF, the lymphokine inducing MPCA or LDCF. Overnight incubation of macrophages with Cs A did, however, result in mild inhibition of their basal level of procoagulant activity.

Abbreviations: Cs A, cyclosporin A; DTH, delayed-type hypersensitivity; FCS, foetal calf serum; iCon A, insoluble concanavalin A; LAF, lymphocyte activating factor (=interleukin 1); LDCF, lymphocyte-derived macrophage chemotactic factor; LPS, lipopolysaccharide; MAF, macrophage activating factor; MIF, macrophage inhibition factor; MLR, mixed leucocyte reaction; MPCA, macrophage procoagulant activity; NGPS, normal guinea-pig serum; OVA, ovalbumin; PEC, peritoneal exudate cells; PHA, phytohaemagglutinin; TCGF, T-cell growth factor; TG, thioglycollate.

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INTRODUCTION

Cyclosporin A (Cs A) can be distinguished from conventional pharmacological immune suppressants by its selective inhibitory effect on T-cell proliferation (White, Plumb, Pawelec & Brons, 1979) and by its apparent lack of side effects, in particular myelotoxicity, at therapeutic dosage in laboratory animals (Borel, Feurer, Magnée & Stähelin, 1977, Thomson, Whiting & Simpson, 1982). Its ability to impair humoral responses to T-dependent antigens, delayed-type hypersensitivity (DTH), reactions, allograft rejection, experimental graft-versus-host disease and allergic encephalomyelitis has been well documented (reviewed by Green, 1981). These reports have been accompanied by several accounts of its inhibitory effects on T-cell responses *in vitro*, which include depression of antigen and mitogen-induced lymphocyte transformation (Borel & Wiesinger, 1977;

Burckhardt & Guggenheim, 1979; White *et al.*, 1979), primary and secondary mixed leucocyte reactions (MLR; Hess & Tutschka, 1980; Horsburgh, Wood & Brent, 1980; Wang, Heacock, Collins, Hutchinson, Tilney & Mannick, 1981; Hess, Tutschka & Santos, 1982) and the generation of cytotoxic T cells (Hess & Tutschka, 1980).

Whilst evidence is emerging that Cs A may interfere with T-cell replication by blocking production of T-cell growth factor (TCGF = interleukin 2 = IL2) and possibly also by abrogating the response of T cells to interleukins (Larsson, 1980; Bunjes, Hardt, Röllinghoff & Wagner, 1981; Palacios, 1981; Andrus & Lafferty, 1982), there is a conspicuous absence of comprehensive or detailed reports of the influence, if any, of Cs A on the production of (or responses of cells to) lymphokines other than IL2. Alberti, Boraschi, Luini & Tagliabue (1981) reported that production of macrophage activating factor (MAF) in response to antigen was depressed in Cs A-treated mice and that spleen cells from these animals had an impaired capacity to release MAF in response to phytohaemagglutinin. In view of the likelihood that Cs A might inhibit production of other lymphokines influencing macrophage behaviour, we have examined the effects of Cs A on both lymphocyte proliferation and the production of migration inhibition factor (MIF), the lymphokine inducing macrophage procoagulant activity (MPCA) and lymphocyte-derived chemotactic factor (LDCF) by activated guinea-pig T cells. In addition, we have investigated whether Cs A influences the responses of macrophages to these lymphokines.

MATERIALS AND METHODS

Cyclosporin A

Cyclosporin A (Cs A; OL 27-400) was kindly provided by Dr J. F. Borel (Sandoz Ltd, Basel, Switzerland). It was dissolved and serially diluted in anhydrous ethanol before further dilution in RPMI 1640 and addition to cell cultures, in which the final ethanol concentration was always 0·05%.

Lymph node cell suspensions

Lymph nodes were obtained from outbred guinea-pigs of either sex (400–600 g) immunized 14 days previously with five times crystallized ovalbumin (OVA, Sigma Chemical Company). The antigen was dissolved in sterile physiological saline (2 mg/ml) and emulsified with an equal volume of Freund's complete adjuvant (Commonwealth Serum Laboratories, Parkville, Victoria) before injection (0·2 ml) into each footpad. The popliteal, inguinal and axillary lymph nodes were finely chopped with scissors, gently teased and the released cells washed twice in Medium 199 after removal of debris by passage through stainless steel gauze. The cells were finally suspended in RPMI 1640 (Gibco, Grand Island, New York) containing 60 μg/ml penicillin, 100 μg/ml streptomycin, 2 g/l $NaHCO_3$ and 5×10^{-5} M 2-mercaptoethanol. Cell viability was determined by exclusion of trypan blue.

Peritoneal exudate cells

These were obtained 3 days after intraperitoneal (i.p.) injection of 25 ml sterile thioglycollate (TG) (BBL Microbiology Systems, Md) pH 6·1, or 4 days after i.p. injection of 25 ml sterile paraffin oil (Marcol 52, Esso, Australia). The peritoneal cavities were lavaged with 50 ml Hanks's balanced salt solution (HBSS) containing 10 u./ml preservative-free heparin (Commonwealth Serum Laboratories, Melbourne) and the cells washed three times in heparin-free HBSS.

Lymphocyte transformation

Triplicate 0·2 ml cultures of lymph node cells ($3{\cdot}75 \times 10^6$ viable cells/ml) in RPMI 1640 containing 10% foetal calf serum (FCS; Flow Laboratories) and various concentrations of PHA-P (Difco) or OVA together with Cs A were set up in 96 well round-bottomed microculture plates (Nunclon, Denmark). They were maintained for 72 hr at 37° in a humidified atmosphere of 5% CO_2 in air and pulsed 16 hr before harvesting with 0·3 μCi [^{3}H]thymidine (New England Nuclear, Boston, Mass., 6·7 Ci/mmole). The cultures were harvested onto glass fibre discs, using a multiple harvester (Skatron AS, Norway) and counted, in 2 ml toluene-based scintillant, in a Packard liquid scintillation counter. Results are expressed as mean counts per minute (c.p.m.) ± 1 SD.

Lymphocyte activating factor (LAF = IL1)

The method used to study the production of LAF was based on that described by Calderon, Kiely, Lefco & Unanue (1975). Washed TG-induced PEC (5×10^6 macrophages/ml in RPMI 1640 with 10% FCS were allowed to adhere to 35 mm plastic petri dishes (Kayline Plastics, Australia) for 3 hr at 37°. Non-adherent cells were removed by washing three times in HBSS with FCS and 1 ml of RPMI 1640 containing 100 μg *E. coli* LPS W (Difco) and various concentra-

tions of Cs A added. The cells were incubated for a further 24 hr; the supernatants were then clarified by centrifugation and dialysed for 24 hr at 4° (two changes) against RPMI 1640. They were then stored at −70°.

LAF activity was determined using 6-week-old guinea-pig thymocytes. Aliquots of the above supernatants were added to triplicate 0·2 ml cultures in microculture plates containing 10^6 thymocytes and PHA-P (1 μg) in RPMI 1640 with 10% FCS. The cultures were maintained at 37° for 72 hr with addition of [^{3}H]thymidine 16 hr before harvest. The cultures were harvested and the degree of lymphocyte activation determined as described above.

Preparation of lymphokine

Lymphokine supernatants were prepared by stimulating lymph node cells with Sepharose-bound concanavalin A (iCon A; Pharmacia, Uppsala, Sweden) in serum-free medium, according to the method of Friedrich, Lazary, Geczy & de Weck (1975). The cell suspensions (10×10^6 viable cells/ml) containing 50 μg/ml iCon A were gently rocked for 24 hr at 37°. The supernatants were clarified by centrifugation, aliquoted and stored at −70°. Control culture supernatants were prepared by adding iCon A immediately before harvest.

Assay of migration inhibition factor

The technique used was that described by David, Al-Askari, Lawrence & Thomas (1964) as employed by Friedrich *et al.* (1975). Washed oil-induced PEC (20×10^6/ml) suspended in RPMI 1640 with 5% normal guinea-pig serum (NGPS) were drawn into 50 μl capillaries (Corning, N.Y.), which were then sealed with Seal-Ease (Clay Adams, New York, N.Y.). The cells were packed by centrifugation at 100 ***g*** for 5 min, the capillaries cut at the interface, then secured within plastic test chambers (Sterilin Ltd, Hill Rise, Richmond) using silicone grease. The chambers were filled either with various dilutions of antigen in RPMI 1640, or with lymphokine supernatants (diluted 1:2) containing 5% heat-inactivated NGPS. Each test was carried out in quadruplicate. After 18 hr incubation, the areas of migration were projected onto paper, traced out and weighed. Results were expressed as mean areas of migration and as percentage inhibition of migration relative to controls. Migration inhibition of >20% was considered significant.

Assay of macrophage procoagulant activity

The ability of OVA or lymphokine supernatants to promote the procoagulant activity of TG-induced peritoneal exudate cells (PEC) from OVA-immunized or normal guinea-pigs respectively, was determined by the method of Geczy & Hopper (1981), with minor modifications. Washed cells (2×10^6) were incubated in a total volume of 0·5 ml RPMI 1640 containing 5% NGPS in Nunc minisorb tubes (Nunc, Roskilde, Denmark) with various concentrations of OVA ($1–10^{-5}$ μg/ml) or lymphokine (0·2%–5%). After overnight incubation (16–20 hr) at 37°, the cell suspensions were washed twice in HBSS, resuspended in 0·6 ml RPMI 1640 and their ability to shorten the recalcification time of normal citrated platelet-poor guinea-pig plasma (centrifuged at 18,000 ***g*** for 30 min) determined. To 0·2 ml cell suspension was added 25 μl brain phospholipid, 0·1 ml plasma and 0·1 ml 0·03 M $CaCl_2$ in 0·9% saline. The clotting times were measured in duplicate in an automatic coagulometer (Calbiochem, according to Schnitger & Gross, Amelung GmbH, Lemgo, Germany). The results are expressed as percentage reduction in recalcification time, calculated using the formula: [(recalcification time (sec) with control PEC − recalcification time of treated PEC)/(recalcification time with control PEC)] × 100. A decrease in recalcification time of 7% was considered significant.

Production and assay of lymphocyte-derived chemotactic factor

LDCF was prepared from normal guinea-pig spleen cells according to the method of Meltzer, Jones & Boetcher (1975). The cells (5×10^6/ml RPMI 1640 with 2·5% NGPS) were incubated with 50 μg/ml PHA-P in Linbro 24 well tissue culture plates (Flow Laboratories) for 3 days and the supernatants stored at −70°. Cs A was added at the start of the incubation and PHA to control cultures at harvest. Chemotaxis by mononuclear TG-PEC was assayed in a 48-well microchemotaxis chamber (Neuroprobe, Bethesda, Md), as described in detail elsewhere (Falk, Goodwin & Leonard, 1980). The upper wells of the chamber were filled with 0·5 ml cell suspension (10^6 ml RPMI 1640 with 2·5% NGPS) and the lower wells contained the LDCF preparations (diluted 1:3 in the same medium) or control supernatants. The chamber was incubated for 90 min at 37°, then the filter sheets (5 μm polycarbonate, 7·6 × 2·5 cm; Neuroprobe, Bethesda, Md) were removed, stained with Wright's stain and mounted on glass slides. Migrated macrophages were

counted with a ×40 objective and eyepiece grid. Five grid fields were counted per well and the mean of triplicate wells determined. The chemotactic response was expressed as the mean total number of macrophages migrated per 5 grid fields in test wells minus the number of macrophages migrated per 5 grid fields in control wells.

Statistics

The significance of differences between means was estimated using the Student's *t* test.

RESULTS

LAF production

The influence of Cs A on the capacity of TG-induced macrophages to produce LAF in response to LPS is shown in Fig. 1. Only at the highest dose of Cs A used (5 μg/ml) was inhibition of LAF production observed; at this dose Cs A had a mild cytotoxic effect. In 20 hr cultures, it reduced the number of viable TG-PEC by 6% as estimated by dye exclusion. No cytotoxic effect was observed at any other Cs A concentration used in this study.

Lymphocyte transformation

In contrast to the relative insensitivity of LAF production to Cs A, a powerful dose-related inhibition of lymphocyte transformation in response to PHA or OVA was observed in the presence of the drug (Figs 2 and 3). This inhibitory effect was more potent at low mitogen concentrations. At supraoptimal PHA concentrations, Cs A was comparatively ineffective in

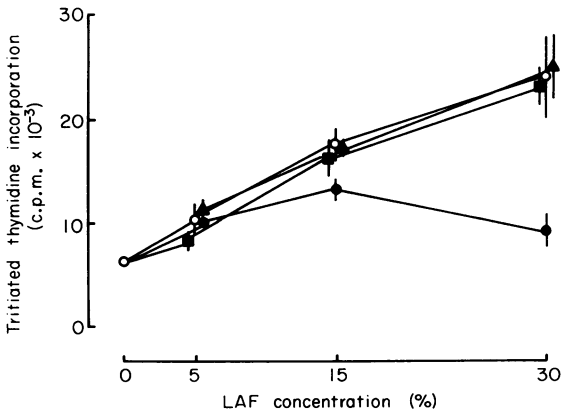

Figure 1. The effect of different concentrations of Cs A on the production of LAF by LPS-stimulated TG-induced macrophages. The macrophages were incubated with LPS and (○) medium, (●) 5 μg/ml Cs A, (■) 0·5 μg/ml Cs A or (▲) 0·05 μg/ml Cs A. Results are means ±1 SD from triplicate cultures.

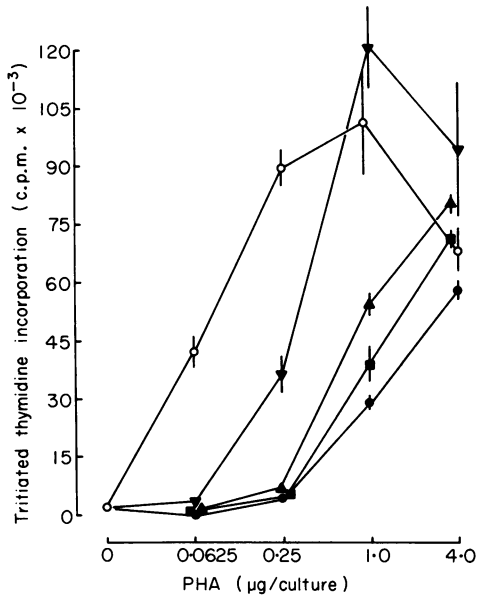

Figure 2. Mitogenic response of normal guinea-pig LNC to PHA in the presence of various concentrations of Cs A. (○) Medium; (●) 5 μg/ml; (■) 0·5 μg/ml; (▲) 0·05 μg/ml; (▼) 0·005 μg/ml. Results are means ±1 SD from triplicate cultures

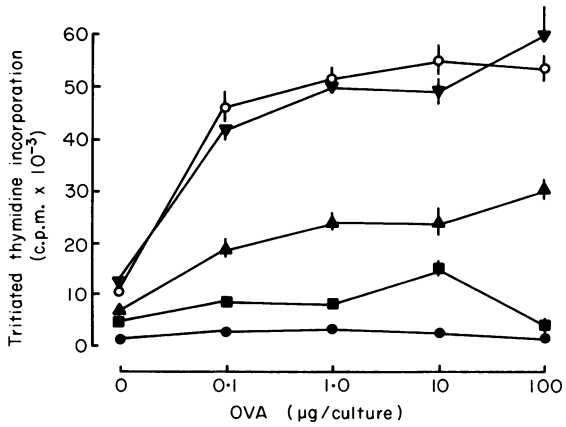

Figure 3. Mitogenic response of OVA-immunized guinea-pig LNC to OVA in the presence of various concentrations of Cs A. (○) Medium; (●) 5 μg/ml; (■) 0·5 μg/ml; (▲) 0·05 μg/ml; (▼) 0·005 μg/ml. Results are means ±1 SD from triplicate cultures.

inhibiting thymidine incorporation. Indeed, in two separate experiments, the lowest dose of Cs A (0·005 μg/ml) actually augmented the response to 4 μg PHA ($P<0·01$).

MIF production and the response of macrophages to MIF

Although the migration of TG-PEC from capillary tubes was unaffected in the presence of Cs A (Table 1), the drug had a potent inhibitory effect on OVA-induced macrophage migration inhibition. Indeed, as little as 0·005 μg/ml Cs A abrogated the response of sensitized cells to low concentrations of antigen. Furthermore, MIF production by iCon A stimulated LNC was either totally (0·5 μg/ml) or minimally (0·05 μg/ml) blocked by addition of Cs A to the cultures (Table 2). In contrast to these observations, Cs A did not affect the response of macrophages to preformed (iCon A-induced) lymphokine (Table 3). Indeed, the degree of migration inhibition was slightly increased in the presence of Cs A, although not in proportion to drug concentration.

Table 1. Effect of Cs A on macrophage migration and OVA-induced macrophage migration inhibition

OVA (μg/ml)	Cs A* (μg/ml)	Area of migration† (mean ±1 SE)	Inhibition (%) of migration
0	—	20·8±2·2	0
	5	19·8±1·5	5
	0·5	23·6±1·6	−13
	0·05	18·2±3·0	12
	0·005	23·2±2·4	−12
100	—	11·4±1·3‡	45
	5	23·4±0·7	−18
	0·5	20·2±1·1	14
	0·05	11·4±1·8§	26
	0·005	10·2±0·6‡	56
10	—	14·2±1·1‡	32
	5	26·0±0·4§	−29
	0·5	20·6±3·5	13
	0·05	16·4±0·6	9
	0·005	12·4±0·8‡	47
1	—	11·2±0·4‡	46
	5	22·2±1·7	−12
	0·5	23·2±2·2	2
	0·05	18·2±0·8	13
	0·005	20·2±2·5	13

* Cs A added at start of incubation.
† Weight (mg) of projected areas of the cells.
Significance of differences from appropriate controls (no antigen); (‡) $P<0·001$; (§) $P<0·01$.

Table 2. Effect of Cs A on iCon A-induced MIF production by LNC

Cs A (μg/ml)	Control	Lymphokine	Inhibition (%) of migration
—	23·8±4·9*	12·3±1·0†	47
0·5	24·2±2·5	23·7±0·2	−3
0·05	27·4±0·7	14·6±4·4†	37
0·005	18·9±1·8	8·6±1·8‡	63

* Results are expressed as the mean weight (mg±1 SE) of the projected areas of the cells.
Significance of difference from control values; (†) $P<0·005$; (‡) $P<0·001$.

Table 3. Effect of Cs A on the response of macrophages to preformed MIF

Cs A (μg/ml)	Control*	Lymphokine	Inhibition (%) of migration
—	23·1±0·5	12·2±0·7†	47
5	20·2±1·3	9·3±1·0	60
0·5	20·4±2·9	8·4±3·4	64
0·05	22·2±0·4	8·1±1·6	65
0·005	21·0±2·0	9·3±2·4	60

* Results are expressed as the mean weight (mg±1 SE) of the projected areas of the cells.
† All values significantly different from corresponding controls, $P<0·005$.

Induction of MPCA and the response of macrophages to MPCA

Cs A also inhibited production of the lymphokine promoting MPCA. During the course of these experiments, it was consistently found that overnight incubation of TG-PEC with Cs A significantly reduced the basal level of procoagulant activity of the cells at 0·5 and 5 μg/ml (Table 4). Consequently, this effect of Cs A has been taken into account in the expression of results from all ensuing experiments using the MPCA assay.

Cs A inhibited the OVA-induced increase in macrophage procoagulant activity observed when TG-PEC from OVA-immunized guinea-pigs were incubated with various concentrations of the antigen (Fig. 4). At

Table 4. Effect of Cs A on macrophage procoagulant activity*

Cs A (μg/ml)	Recalcification time† (sec ± 1SD)	Increase (%) in recalcification time
—	63 ± 3	—
5	72 ± 4‡	14·3
0·5	74 ± 4‡	17·5
0·05	68 ± 5	7·9
0·005	66 ± 2	4·8
0·0005	64 ± 3	1·6

* TG-PEC were incubated overnight in various concentrations of Cs A. The recalcification time of guinea-pig plasma in the presence of the washed cells was then determined.

† Mean recalcification time (sec ± 1 SD) of guinea-pig plasma in these experiments = 118 ± 21.

‡ Significantly different from untreated cell control, $P < 0{\cdot}01$.

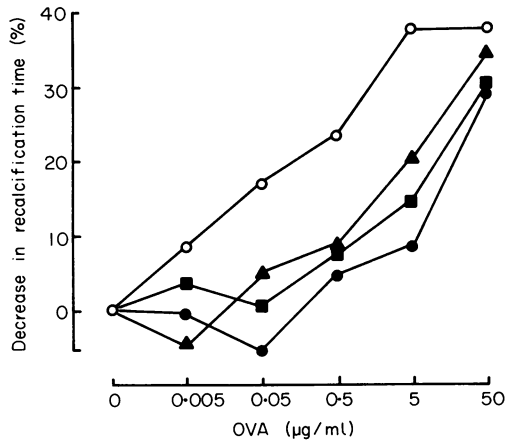

Figure 4. Procoagulant activity of TG-PEC induced by incubating cells from OVA-immunized guinea-pigs with various concentrations of OVA in the presence of (○) medium; (●) 5 μg/ml Cs A; (■) 0·5 μg/ml Cs A or (▲) 0·05 μg/ml Cs A.

low antigen concentrations, as little as 0·05 μg/ml Cs A totally inhibited the response, reflecting the effect of Cs A on OVA-induced macrophage migration inhibition (Table 1). As also seen in the MIF assay, high antigen dose (50 μg/ml) could apparently overcome the inhibitory effect of the drug. The inhibitory effect of Cs A on production of the lymphokine inducing MPCA was also seen when supernatants from lymph node cells stimulated with iCon A in the presence of the drug were tested in the assay (Fig. 5a). At 0·5 μg/ml, Cs A almost totally inhibited production of procoagulant inducing activity. In contrast, the drug did not affect the ability of macrophages to respond to preformed, iCon A-induced lymphokine (Fig. 5b).

LDCF production and the response of macrophages to LDCF

Cs A also exerted a dose-related suppressive effect on the release of LDCF by PHA-stimulated spleen cells. In contrast, the responsiveness of mononuclear TG-PEC to LDCF was unimpaired in the presence of Cs A (Table 5).

DISCUSSION

In the present investigation we have demonstrated using guinea-pig lymph node cells, the inhibitory effect of Cs A on the expression of cell-mediated immunity *in vitro*. The drug was used at concentrations which have previously been found to inhibit certain T-cell responses in other species. These activities include the increased incorporation of tritiated thymidine in response to PHA, Con A or alloantigens (Burckhardt & Guggenheim, 1979; White *et al.*, 1979; Hess & Tutschka, 1980; Horsburgh *et al.*, 1980; Larsson, 1980), T-cell colony formation (Gordon & Singer, 1979) and the generation of cytotoxic T cells in MLR (Hess & Tutschka, 1980; Bunjes *et al.*, 1981; Landegren, Ramstedt, Axberg, Orn & Wigzell, 1981; Wang *et al.*, 1981). We have shown in addition, that Cs A inhibits the production of lymphokines by antigen or mitogen-stimulated T cells. Our observation that the generation of MIF, MPCA and LDCF is impaired in the presence of Cs A is consistent with the reported inhibitory effect of the drug on the production of TCGF by activated murine and human T cells *in vitro* (Bunjes *et al.*, 1981; Palacios, 1981; Andrus & Lafferty, 1982; Hess, Tutschka & Santos, 1982) and also with the marked reduction in serum-borne MAF activity in Cs A-treated mice following antigen challenge (Alberti *et al.*, 1981). Taken together, these observations provide a rational basis for the mode of action of Cs A; they imply that Cs A not only arrests T-cell proliferation but also, by interfering with lymphokine production, particularly at low antigen concentrations, abrogates the recruitment and activation of macrophages including the stimulation of procoagulant activity. Both cells are important media-

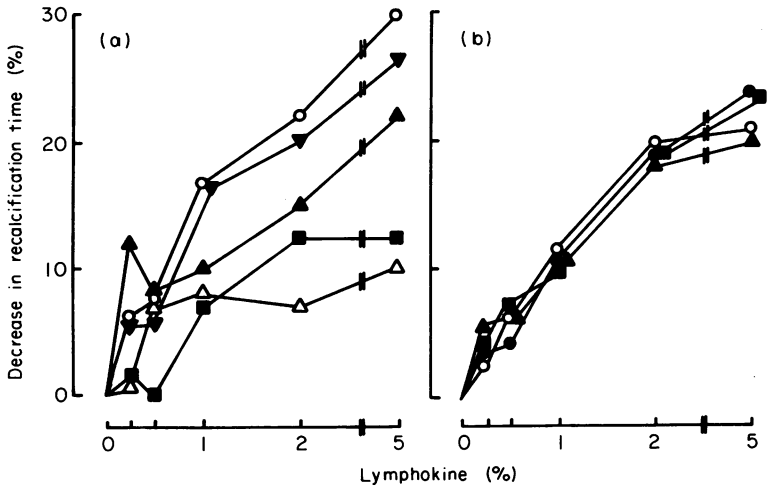

Figure 5. (a) Procoagulant activity of TG-PEC induced by incubating cells with iCon A-induced lymphokine, produced in the presence of different concentrations of Cs A. (○) iCon A supernatant without Cs A; (■) 0·5 μg/ml; (▲) 0·05 μg/ml; (▼) 0·005 μg/ml. The effect of control supernatant to which iCon A was added at the end of the culture period is also shown (△). (b) Procoagulant activity of TG-PEC induced by incubating cells with preformed iCon A-induced lymphokine in the presence of Cs A. (○) iCon A supernatant without Cs A; (■) 0·5 μg/ml; (▲) 0·05 μg/ml; (▼) 0·005 μg/ml.

tors of DTH, which under the appropriate conditions, is severely impaired in Cs A-treated guinea-pigs (Borel *et al.*, 1977). That Cs A acts on the initial T-cell-mediated events in the expression of cellular immunity is further suggested by the failure of the drug to interfere with the activity of established cytotoxic T cells (Landegren *et al.*, 1981; Palacios, 1981; Wang *et al.*, 1981). In addition, the inhibitory effect of Cs A on T-cell proliferation is known to be dependent on its presence at the initiation of the culture period (Borel & Wiesinger, 1977; Keown, Essery, Stiller, Sinclair, Mullen & Ulan, 1981). Furthermore, the drug does not affect the proliferative response of primed T lymphocytes to TCGF (Larsson, 1980; Hess *et al.*, 1982). These studies provide compelling evidence that Cs A inhibits the induction of helper and cytotoxic T-cell populations. On the other hand, no firm conclusions can be drawn, at present, from data relating to the possible influence of Cs A on the generation and expression of suppressor T cells. To date there is evidence that Cs A may permit (Hess & Tutschka, 1980) or inhibit (Palacios, 1981) the generation of suppressor cells in MLR and that it may selectively spare alloantigen-specific suppressor cells in graft bearing animals (Hutchinson, Shadur, Duarte, Strom & Tilney, 1981).

Table 5. Effect of Cs A on LDCF production and the response of macrophages to LDCF

Experiment	Cs A (μg/ml)	Number of macrophages migrating*
LDCF production	0	78±7
	5	20±6†
	0·5	34±5†
	0·05	64±3‡
	0·005	72±13
Response to preformed LDCF§	0	91±10
	5	94±3
	0·5	88±10
	0·05	88±5
	0·005	85±5

* Results are means of triplicates ± 1 SD.
† $P < 0.005$.
‡ $P < 0.01$.
§ Cs A added to LDCF (final concentration 1 in 3) and placed in the lower wells of the chemotaxis chamber.

Several previous reports substantiate our view that the activities of macrophages *per se* are not affected by Cs A. White *et al.* (1979) observed that pig blood leucocytes migrated normally from capillary tubes in the presence of Cs A and McIntosh & Thomson (1980) found that the clearance of intravenous colloidal carbon and sheep erythrocytes was unimpaired in Cs A-treated mice. Whereas in the present study it was

found that Cs A inhibited LAF production only at high dosage (5 μg/ml), and much in excess of that required to suppress lymphocyte transformation, others have observed that the release of LAF from LPS-stimulated mouse macrophages is wholly unaffected by Cs A (Alberti *et al.*, 1981; Bunjes *et al.*, 1981). Conversely, it has been reported that production of LAF by macrophage cell lines in response to LPS or phorbol myristic acetate is impaired by Cs A (Bunjes *et al.*, 1981; Andrus & Lafferty, 1982). The reasons for this apparent discrepancy are at present unclear, but may reflect the use of different cell sources and procedures to solubilize Cs A which, as suggested by Bunjes *et al.* (1981) and Landegren *et al.* (1981), can influence its pharmacological potency *in vitro*.

Recent studies with radiolabelled analogues of Cs A have revealed that the compound binds to both lymphocytes and macrophages (Leoni, Garcia & Allison, 1978; Ryffel, Donatsch, Götz & Tschopp, 1980) and it has been argued that Cs A may interfere with the expression of HLA-DR antigens (Palacios & Möller, 1980) and receptors for TCGF on the surfaces of T cells (Larsson, 1980). It is extremely unlikely that Cs A interferes with receptors for the lymphokines we have studied on the surfaces of macrophages, although by binding to macrophages it may, by some as yet unknown mechanism, have some effect on their basal procoagulant activity, as seen in this study. Further investigation of the interaction between Cs A and these cell populations is clearly merited.

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REFERENCES

ALBERTI S., BORASCHI D., LUINI W. & TAGLIABUE A. (1981) Effects of *in vivo* treatments with cyclosporin-A on mouse cell-mediated immune responses. *Int. J. Immunopharmac.* **3,** 357.

ANDRUS L. & LAFFERTY K.J. (1982) Inhibition of T cell activity by cyclosporin A. *Scand. J. Immunol.* **15,** 449.

BOREL J.F., FEURER C., MAGNÉE C. & STÄHELIN H. (1977) Effects of the new anti-lymphocytic peptide cyclosporin A in animals. *Immunology*, **32,** 1017.

BOREL J.F. & WIESINGER D. (1977) Effect of cyclosporin A on murine lymphoid cells. In: *Regulatory Mechanisms in Lymphocyte Activation. Proceedings of the 11th Leukocyte Culture Conference* (Ed. by D.O. Lucas), p. 716. Academic Press, New York.

BUNJES D., HARDT C., RÖLLINGHOFF M. & WAGNER H. (1981) Cyclosporin A mediates immunosuppression of primary cytotoxic T cell responses by impairing the release of interleukin 1 and interleukin 2. *Europ. J. Immunol.* **11,** 657.

BURCKHARDT J. & GUGGENHEIM B. (1979) Cyclosporin A: *in vivo* and *in vitro* suppression of rat T-lymphocyte function. *Immunology*, **36,** 753.

CALDERON J., KIELY J.-M., LEFKO J.L. & UNANUE E.R. (1975) The modulation of lymphocyte functions by molecules secreted by macrophages. I. Description and partial biochemical analysis. *J. exp. Med.* **142,** 151.

DAVID J.R., AL-ASKARI S., LAWRENCE H.S. & THOMAS L. (1964) Delayed hypersensitivity *in vitro*. I. The specificity of inhibition of cell migration by antigen. *J. Immunol.* **93,** 264.

FALK W., GOODWIN R.H. & LEONARD E.J. (1980) A 48-well microchemotaxis assembly for rapid and accurate measurement of leukocyte migration. *J. immunol. Meths*, **33,** 239.

FRIEDRICH W., LAZARY S., GECZY C. & DE WECK A.L. (1975) Lymphokines. I. Use of insoluble concanavalin A for the production of migration inhibitory factor in guinea-pig lymphocyte cultures. *Int. Archs. Allergy appl. Immunol.* **49,** 504.

GECZY C.L. & HOPPER K.E. (1981) A mechanism of migration inhibition in delayed-type hypersensitivity reactions. II. Lymphokines promote procoagulant activity of macrophages *in vitro*. *J. Immunol.* **126,** 1059.

GORDON M.Y. & SINGER J.W. (1979) Selective effects of cyclosporin A on colony-forming lymphoid and myeloid cells in man. *Nature (Lond.)*, **279,** 422.

GREEN C.J. (1981) Immunosuppression with cyclosporin A: a review. *Diagnost. Histopathol.* **4,** 157.

HESS A.D. & TUTSCHKA P.J. (1980) Effect of cyclosporin A on human lymphocyte responses *in vitro*. 1. CsA allows for the expression of alloantigen-activated suppressor cells while preferentially inhibiting the induction of cytolytic effector lymphocytes in MLR. *J. Immunol.* **124,** 2601.

HESS A.D., TUTSCHKA P.J. & SANTOS G.W. (1982) Effect of cyclosporin A on human lymphocyte responses *in vitro*. III. CsA inhibits the production of T lymphocyte growth factors in secondary mixed lymphocyte responses but does not inhibit the response of primed lymphocytes to TCGF. *J. Immunol.* **128,** 355.

HORSBURGH T., WOOD P. & BRENT L. (1980) Suppression of *in vitro* lymphocyte reactivity by cyclosporin A: existence of a population of drug-resistant cytotoxic lymphocytes. *Nature (Lond.)*, **286,** 609.

HUTCHINSON I.F., SHADUR C.A., DUARTE J.S.A., STROM T.B. & TILNEY N.L. (1981) Cyclosporin A spares selectively lymphocytes with donor-specific suppressor characteristics. *Transplantation*, **32,** 210.

KEOWN P.A., ESSERY G.L., STILLER C.R., SINCLAIR N.R.,

MULLEN R. & ULAN R.A. (1981) Mechanisms of immunosuppression by cyclosporin. *Transplantn. Proc.* **13,** 386.

LANDEGREN U., RAMSTEDT U., AXBERG I., ÖRN A. & WIGZELL H. (1981) Cyclosporin A permits the distinction between specific T and NK activity generated in a human MLC. *Int. J. Cancer*, **28,** 725.

LARSSON E.-L. (1980) Cyclosporin A and dexamethasone suppress T cell responses by selectively acting at distinct sites of the triggering process. *J. Immunol* **124,** 2828.

LEONI P., GARCIA R.C. & ALLISON A.C. (1978) Effects of cyclosporin A on human lymphocytes in culture. *J. Clin. lab. Immunol.* **1,** 67.

MELTZER M.S., JONES E.E. & BOETCHER D.A. (1975) Increased chemotactic responses of macrophages from BCG-infected mice. *Cell. Immunol.* **17,** 268.

MCINTOSH L.C. & THOMSON A.W. (1980) Activity of the mononuclear phagocyte system in cyclosporin A treated mice. *Transplantation*, **30,** 384.

PALACIOS R. (1981) Cyclosporin A inhibits the proliferative response and the generation of helper, suppressor and cytotoxic T-cell functions in the autologous mixed lymphocyte reaction. *Cell. Immunol.* **61,** 453.

PALACIOS R. & MÖLLER G. (1981) Cyclosporin-A blocks receptors for HLA-DR antigens on T cells. *Nature (Lond.)*, **290,** 792.

RYFFEL B., DONATSCH P., GÖTZ U. & TSCHOPP M. (1980) Cyclosporin receptor on mouse lymphocytes. *Immunology*, **41,** 913.

THOMSON A.W., WHITING P.H. & SIMPSON J.G. (1982) Pathobiology of cyclosporin A in experimental animals. In: *Cyclosporin A* (Ed. by D.J.G. White, D.L. Hamilton and D.A.L. Davies). Elsevier, North Holland. (In press.)

WANG B.S., HEACOCK E.H., COLLINS K.H., HUTCHINSON I.F. TILNEY N.L. & MANNICK J.A. (1981) Suppressive effects of cyclosporin A on the induction of alloreactivity *in vitro* and *in vivo*. *J. Immunol.* **127,** 89.

WHITE D.J.G., PLUMB A.M., PAWELEC G. & BRONS G. (1979) Cyclosporin A: an immunosuppressive agent preferentially active against proliferating T cells. *Transplantation*, **27,** 55.