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Cyclosporine affects the function of antigen-presenting cells

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SUMMARY

The immunosuppressant drug cyclosporine (CsA) is known to affect T-cell function. We have studied the effect of CsA on the specific proliferative response of T-cell lines to antigen. In addition to blocking IL-2 release by specifically activated T-cell lines, CsA also affected the ability of irradiated spleen cells to present preprocessed antigen to T-cell lines. Irradiated spleen cells pulsed with antigen for 2 hr were able to stimulate a proliferative response in T-cell lines. Following a 2-hr pulse with CsA, antigen presentation by these irradiated spleen cells was reduced significantly, suggesting that CsA not only affects T cells, but also affects the function of antigen-presenting cells.

INTRODUCTION

Cyclosporine (CsA) is a powerful immunosuppressant with some selective effects on different populations of cells in the immune system. It appears to affect T-helper (T_h) and T-cytotoxic (Tc) cells, as well as a subpopulation of B cells (Borel *et al.*, 1977; Hess & Tutschka, 1980; Kunkl & Klaus, 1980; Leapman *et al.*, 1981; Bunjes *et al.*, 1981). It has been suggested that the effect of CsA on the generation of cytotoxic T cells is due to a primary action of CsA on T-helper function (Bunjes *et al.*, 1982), and a recent report has shown that CsA inhibits the production of mRNA for IL-2 (Kronke *et al.*, 1984). Orosz *et al.*, (1982) have demonstrated that CsA affected the ability of cloned T-cell lines (including cytotoxic T cells) to proliferate in response to antigens. Mitogenic responses to lymphokines were affected to a lesser degree. Although it has been reported that CsA does not affect macrophage functions (Gunjes *et al.*, 1982), it seemed worthwhile to reassess the effect of CsA on antigen presentation to T-cell lines.

MATERIALS AND METHODS

Mice

Two- to three-month-old male CBA/Ca mice obtained from the National Institute for Medical Research, Mill Hill, London, were used throughout.

Cell lines

The mouse thyroglobulin (MTg) and purified protein derivative (PPD)-specific T-cell lines were established and maintained as described previously (Champion *et al.*, 1985). Briefly, cell lines were re-stimulated with their specific antigen in the presence of syngeneic irradiated spleen antigen-presenting cells every 7–8 days.

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The IL-2-dependent cytotoxic T-lymphocyte line (CTLL) originally developed by M. Schreier was kindly provided by D. Warren (NIMR, Mill Hill, London).

Culture media

Antigen-specific cell lines were cultured in Dulbecco's modified Eagle medium supplemented with 10% (v/v) fetal calf serum (FCS), 10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, 110 μg/ml sodium pyrurate, 1% (v/v) non-essential amino acids (Flow Laboratories, Irvine, Ayrshire), 100 U/ml penicillin and 100 μg/ml streptomycin (DMEM/FCS). The CTLL were cultured in RPMI-1640 supplemented with 5% FCS, 2 mM L-glutamine, 10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin and 18% T-cell growth factor (TCGF) (30-hr cell-free supernatant of concanavalin A (2·5 μg/ml)-stimulated rat spleen cells with 20 mg/ml α methylmannoside).

Proliferation assays

Assays were set up in flat-bottomed microtitre wells containing 2×10^4 line cells with 5×10^5 irradiated (2000 rads) syngeneic spleen cells with antigen and CsA added as appropriate, or 5×10^5 irradiated spleen cells which had been prepulsed with antigens and/or CsA (see 'Results'). All conditions were tested in triplicate cultures in 200 μl DMEM/FCS. After 48 at 37° in a humidified atmosphere of 5% CO_2 in air, the cultures were pulsed with 0·5 μCi [^{125}I] deoxyuridine ([^{125}I]UdR) in 50 μl DMEM. Eighteen hours later, the cultures were harvested onto glass fibre discs using a Titertek Cell Harvester (Flow Laboratories) and counted in a gamma counter.

IL-2 release assay

Cultures were set up as described for proliferation assays. After 15–20 hr culture, 100 μl of the medium were removed from each well and IL-2 was measured by its ability to maintain the growth of CTLL cells (10^4/well) (assessed in a 2 hr proliferation assays).

Cyclosporine (CsA)

This was a gift from Dr Borel, Sandoz Ltd, Basel, Switzerland. CsA (3 mg) was dissolved in 300 μl ethanol. Tween 80 (60 μl) was then added, followed by the dropwise addition of ice-cold RPMI-1640 to a total volume of 3 ml. This stock solution (1 mg/ml) was then further diluted in culture media as required.

Antigen-pulsed irradiated spleen cells

Irradiated spleen cells (10^7/ml in DMEM/FCS) were incubated at 37 for 2 hr with Tg (100 μg/ul) or PPD (50 μg/ml). After two washes in BSS, the cells were further incubated with PPD (50 μg/ml) or Tg (100 μg/ml), respectively, or without antigen in the presence or absence of CSA (0·1 μg/ml). After three washes in BSS, the cells were used as antigen presenters in proliferation assays as described. Control unpulsed cells (with or without CsA) treated in the same way.

RESULTS

Effect of CsA on antigen-specific proliferation of T-cell lines

CsA significantly suppressed the proliferative responses of both thyroglobulin-specific and PPD-specific T-cell lines in a dose-dependent manner (shown for the response of MTg7, a T-cell line specific for Tg, in Fig. 1a).

When these antigen-specific T-cell lines are stimulated with antigen, IL-2 is released into the supernatant during the first 24 hr (B. R. Champion *et al.*, manuscript in preparation). The response of CTLL cells to IL-2 is not affected by CsA. Therefore, it was possible to assess the effect of CsA on the antigen-dependent IL-2 release by these T-cell lines. From Fig. 1b, it can be seen that CsA not only blocks the proliferative response of the T-cell lines to antigen (Fig. 1a), but also blocks the release of IL-2 from such lines

Effect of CsA on processing and presentation of antigen to T-cell lines

In order to dissect the effect of CsA on antigen-specific responses of T-cell lines, we studied the effect of the drug on antigen processing and presentation. Unseparated spleen cells were used in these studies since isolated macrophage and dendritic cell preparations have previously been shown to be relatively in efficient in presenting antigen to our T-cell lines (Champion *et al.*, 1985). Irradiated spleen cells were first pulsed with one

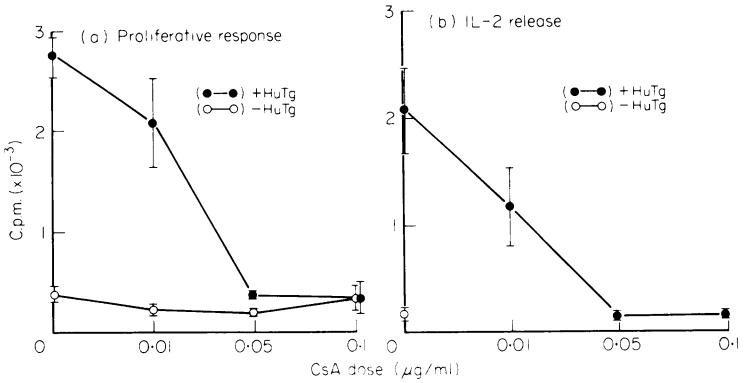

Figure 1. The effect of CsA on the response of thyroglobulin-specific cell line to thyroglobulin. (a) Proliferation: the cell line was cultured with (●——●) or without (○——○) human thyroglobulin (HuTg) in the presence of various concentrations of CsA. Proliferative responses were measured by [^{125}I]UdR uptake measured on Day 3 (3 (c.p.m. × 10 ^{3}). (b) Il-2 release: 24-hr supernatant from linc cells cultured with (●——●) or without (○——○) HuTg in the presence of various concentrations of CsA were assayed for IL-2 content using IL-2-dependent CTLL cells.

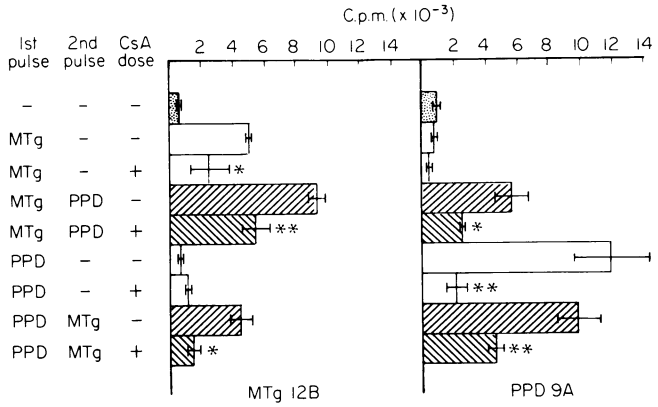

Figure 2. Effect of CsA presentation of antigen to T-cell lines. Irradiated spleen cells were first pulsed for 2 hr with or without mouse thyroglobulin (MTg) or PPD. After washing, these cells were pulsed for a further 2 hr with PPD or MTg in the presence or absence of 0·1 μg/ml CsA. Antigen presentation by such pulsed cells was assayed by their ability to cause a proliferative response following addition to MTg12B and PPD9A cell lines. Proliferative responses were measured at 3 days as described in Fig. 1a. The values indicated by asterisks represent the significance (Student's *t*-test) of these results relative to their respective control without CsA: (*) $P<0·05$; (**) $P<0·01$.

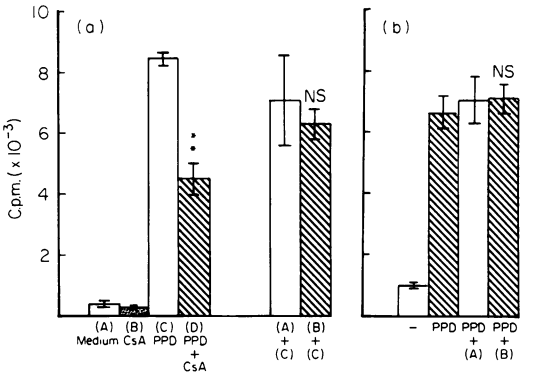

Figure 3. Effect of addition of CsA-pulsed spleen cells on the proliferative responses of a PPD-specific T-cell line. (a) Irradiated spleen cells were pulsed for 2 hr under the following conditions: (A) medium alone; (B) medium containing CsA (0·5 μg/ml); (C) medium containing PPD; (D) medium containing PPD and CsA (0·5 μg/ml). After washing, these cells were assessed for their ability to stimulate a proliferative response in a PPD-specific T-cell line. PPD-pulsed cells were mixed with medium pulsed cells (A + C) or CsA-pulsed cells (B + C) and added to the PPD-specific T-cell line to test for a possible carry over of CsA. (b) (A) or (B) above were added to the T-cell line in the presence of fresh irradiated spleen cells and soluble antigen as a further test of CsA carry-over. Proliferative responses in both (a) and (b) were measured on Day 3. (**) $P < 0{\cdot}01$. NS, not significant.

antigen (Tg orPPD) and then pulsed with the other antigen (PPD or Tg) in the presence or absence of CsA. Following washing, the ability of these presenting cell preparations to stimulate the proliferation of both Tg-specific and PPD-specific cell lines was assessed. In this way, both the effect of CsA on the presentation of preprocessed antigen and the effect on processing and presentation of the second antigen could be studied. The results are shown in Fig. 2. It can be seen that inclusion of CsA during the second pulsing period inhibits the ability of the spleen cells to present already processed antigen as well as antigen being processed in the presence of CsA. It is apparent that T cell IL-2 release is an order of magnitude more sensitive CsA than antigen-presenting cell function, since significant inhibiting effects in the pulsing experiments were not seen with CsA at concentrations lower than 0·1 μg/ml (data not shown), whereas there was significant inhibition of IL-2 release at this concentration (Fig. 1). This result did not appear to be due to an effect on T cells of CsA carried over on spleen cell membranes, since the addition of CsA-treated (and -washed) spleen cells had no effect on either the responses of line cells to antigen-pulsed spleen cells (Fig. 3a) or to soluble antigen in the presence of irradiated spleen cells (Fig. 3b).

DISCUSSION

CsA has been shown to suppress T_h-cell activity in a variety of systems. This inhibition has been attributed by some workers to an inhibition of IL-2 production and IL-2 receptor formation (Larsson, 1980; Palacios, 1981; Bunjes *et al.*, 1981; Lillehoj Malek & Shevach, 1984). Bunjes *et al.* (1981) also suggested that, in addition, CsA could suppress IL-1 production which, in turn, would affect lymphocyte activation. The effects of CsA on lymphokine release appear to be a consistent finding (Thomson *et al.*, 1983). However, some workers do not find any effect on IL-2 recepotor formation (Miyawaki *et al.*, 1983). There have been several reports indicating that CsA does not affect macrophage function, and it is probable that the effect of CsA on prostaglandin E production by macrophages is due to its primary effect on T-cell lymphokine release (Gunn, 1985).

In this report, we present data suggesting that CsA can affect the ability of spleen cells pulsed with antigen to stimulate T-cell lines. This is not due to an effect of CsA on the cells in the irradiated spleen cell preparations, since M. Schreier and D. R. Katz (personal communication) find that T-cell depletion of spleen cells does not affect their antigen-presenting capacity. The effect observed does not appear to be due to CsA carry-over on pulsed spleen cell membranes, since presenting cells pulsed with antigen and then added to presenting cells prepulsed with CsA present antigen normally. We cannot, however, exclude the possibility that antigen-specific clusters arise between antigen-presenting cells and T cells, such that focal release of CsA occurs. This would be difficult to control, for but one approach might be to mix antigen-pulsed presenting cells with presenting cells pulsed with the same antigen and CsA, and to examine their ability to trigger T cells. It is also possible that the observed CsA effect is due to diminished IL-1 production. The ability of glutaraldehyde-fixed cells to present antigen to T cells suggests that IL-1 production may not be a prerequisite for T-cell stimulation, although the presence of membrane-bound IL-1 on such cells cannot be excluded. The recent report by Walden, Nagy & Klein (1985) suggesting that antigen-coupled liposomes containing inserted class II molecules could stimulate T-cell clones implies that not all T cells have a stringent requirement for IL-1. Further experiments to clarify the role of IL-1 in the CsA sensitivity of anigen-presenting cell function are in progress. Unfortunately, since CsA treatment of prepulsed accessory cells inhibited T-cell stimulation, we cannot yet draw any conclusions as to the effect of the drug on antigen processing. This effect on the ability of prepulsed cells to stimulate T-cell lines would seem to be an effect on presentation. Although there is the possibility that additional processing could occur during the period following pulsing, there is some evidence to suggest that there is no difference in the IL-2 release of T lybridomas stimulated with either fixed or non-fixed pulsed presenting cells (Shimonkevitz *et al.*, 1984). This suggests that processing does not play a major role after the 2-hr pulse period. Furthermore, there is some evidence to indicate that presentation of PPD is relatively in sensitive to chloroquine, suggesting that processing is not a major part of the accessory cell function in the response to PPD (Guidos, Wong & Lee, 1984).

Therefore, the inhibitory effect of CsA on T-dependent antibody responses is clearly multifactorial. CsA directly affects T-cell function by inhibiting lymphokine release, and can also interfere with those initial events leading to T-cell activation by inhibiting accessory cell function. The sensitivity of B cells responsive to type 2 T-independent (TI-2) antigens (Kunkl & Klaus, 1980) to CsA may therefore not be an intrinsic property of the B cell itself, but may reflect differences in the requirements for accessory cell function between TI-2 and TI-1 antigens.

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