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INHIBITION OF A T-DEPENDANT *IN VITRO* ANTIBODY RESPONSE BY CYCLOSPORINE A

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ABSTRACT: Cyclosporine A (CsA) is a widely used immunosuppressive drug which has novel, but poorly understood, effects on the immune system. Substantial evidence exists that CsA preferentially acts by impairing T cell lymphokine production, but evidence also exists that cyclosporine may affect B cells and other antigen-presenting cells as well. In this study, the effect CsA has on the primary *in vitro* antibody response of different populations of mouse lymphocytes toward SRBC, a T-dependent antigen, was examined. At physiologically achievable levels, CsA inhibited antibody plaque formation of naive splenocytes or naive T and B cell mixtures, when added to cultures on day 0, 1, and 2 following antigenic stimulation, but not on day 3. Lymphocytes from animals prestimulated with sub-immunogenic doses of SRBC could be inhibited completely by CsA only on day 0 of culture. Mixed cell cultures of enriched B and T cells stimulated to give a higher PFC response by the addition of T cell lymphokines can still be completely inhibited by CsA. B cells stimulated to produce anti-SRBC antibodies by T cell replacing factors also appear to be highly sensitive to CsA induced inhibition through day 2 of culture. These findings suggest that cyclosporine has direct inhibitory effects on lymphocytes, including B cells, aside from, or in addition to, a reduction of lymphokine production.

INTRODUCTION

Cyclosporine A (CsA) is a relatively new immunosuppressive cyclic polypeptide, which has been used successfully to prolong allograft survival and which has shown remarkable promise in the treatment of autoimmune-related diseases, such as diabetes and uveitis (Stiller, *et al.,* 1984; Nussenblatt, *et al.,* 1982). Experimentally, when CsA is given to a mouse at the time of antigen challenge, a significant inhibition of the antibody response occurs *in vivo* to antigens such as sheep red blood cells (SRBC) or the induction of cytolytic T cell activity in response to alloantigens (Shevach, 1985). Although many investigations have led to a better understanding of the mechanism of action of CsA, significant questions remain concerning the drug's immunosuppressive or immunomodulatory effects. CsA appears to affect an early step in T lymphocyte activation by blocking the transcription of IL-2 mRNA, by blocking expression of IL-2 receptors on certain T_{CTL}, and by inhibiting production of IFN-γ (Kronke, *et al.,* 1984; Lillenhoj, *et al.,* 1984; Thomson, 1983). Although there is some controversy about the roles and relative importance of the multiple, pleiotropic interleukins, including IL-2, in the antibody response (O'Garra, *et al.,* 1988; Tigges, *et al.,* 1989), interference with IL-2 production and possibly other lymphokines is probably the primary reason for suppressed antibody production following CsA treatment (Borel and Gunn, 1986). Although helper T cells seem to be an important target of CsA action, some experimental evidence exists which suggests that other cells, including antigen presenting cells (APC), such as macrophages and B cells, may also be directly inhibited by CsA (Manca, *et al.,* 1985; Paavonen and Hayry, 1980; Furue and Katz, 1988). Some investigators have suggested that different subsets of B cells exist, which are differentially sensitive to CsA, or that two distinct mechanisms for triggering B cells are operating, one which is CsA sensitive and another which is CsA resistant (Klaus and Kunkl, 1983; Muraguchi, *et al.,* 1983; Gorelick, *et al.,* 1987). Originally, work with cyclosporine *in vivo*

seemed to suggest that thymus dependent B cells as well as those B cells responding to T-independent-1 antigens (TI-1) were CsA resistant (Klaus and Kunkl, 1983). These investigators also reported that CsA preferentially blocked the activation of B cells responding to T-independent-2 antigens (TI-2). Recently, B cells that respond to anti-μ antibodies or calcium ionophores were also shown to be exquisitely sensitive to CsA. Their response to phorbol esters was not affected (Shevach, 1985; Klaus, *et al.,* 1985). Although both murine and human B cells, which respond to several polyclonal activators including anti-μ, appear to be cyclosporine sensitive, it has not yet been shown directly that B cells participating in a T-dependent response to a specific antigen *in vitro* are affected by cyclosporine inhibition.

In the present study, a primary *in vitro* plaque-forming response to SRBC was used as a model to establish the sensitivity of a T-dependent antibody response to CsA and to establish whether or not the CsA-induced inhibition of an antibody response to a foreign antigen may be due, at least in part, to the direct effect of the drug on B cells.

MATERIALS AND METHODS

Animals. BALB/c mice were bred and maintained in the Ball State University Biology Department's animal facilities. Mice of either sex from 3 to 6 months of age were used for the experiments.

Antigen. Sheep red blood cells (SRBC) from a single animal were obtained from the Colorado Serum Company (Denver, CO).

Reagents. CsA, obtained from Sandoz Corporation (East Hanover, NJ), was dissolved in 95% ethanol (13%), Tween 80 (7%), and saline at a concentration of 5 mg/ml. This stock solution was further diluted in RPMI 1640 medium to 25 μg/ml and filter sterilized before addition to spleen cell cultures. Rabbit Low-Tox complement (C) was obtained from Cedarlane Laboratories (Westbury, NJ), and Concanavalin A (Con A) type IV was obtained from Sigma Chemical Company (St. Louis, MO). Monoclonal anti-Thy 1.2, anti-Ly 1.2, and anti-Ly 2.2 were generously provided by Dr. F.W. Shen (Memorial Sloan Kettering Cancer Center, New York, NY), and goat anti-mouse immunoglobulin was kindly provided by Dr. Jan Cerny (University of Texas Medical Branch, Galveston, TX). DTAF (fluoroscein)-conjugated affinity purified $F(ab^1)_2$ fragment rabbit anti-mouse Ig was obtained from Pel-freez (Rogers, AR). A nutritive cocktail made of 1% Eagle's essential amino acids, 5% Eagle's non-essential amino acids, 5% L-glutamine, and 1% dextrose in HBSS (pH 7.2) mixed at a 2:1 ratio with fetal calf serum (FCS) was added daily to the lymphocyte cultures (see Mishell and Dutton, 1967).

Preparation of spleen cell and lymphocyte subpopulations. Spleens were removed either from naive animals or from those which had been injected three days earlier with 0.2 ml of a 0.1% SRBC suspension in saline. This dose of SRBC was shown to be subimmunogenic; i.e., not capable of inducing PFC in the spleens of animals 5 days post-injection. The spleens from at least three animals were disrupted between two ground-glass slides and were pooled for all studies. The cell suspension was washed twice in Hanks balanced salt solution (HBSS) plus 1% FCS. Before *in vitro* culture, the cells were diluted to the appropriate concentration in RPMI 1640 medium (Sigma Chemical Company, St. Louis, MO) supplemented with 10% FCS, 100 μg/ml streptomycin, 100 units/ml penicillin, 2 mM glutamine, 25 mM Hepes buffer, and 5×10^{-5}M 2-mercapto ethanol. All other cultures were performed in the same complete medium.

A T-cell-enriched population was prepared by adding spleen cells in Dulbecco's PBS

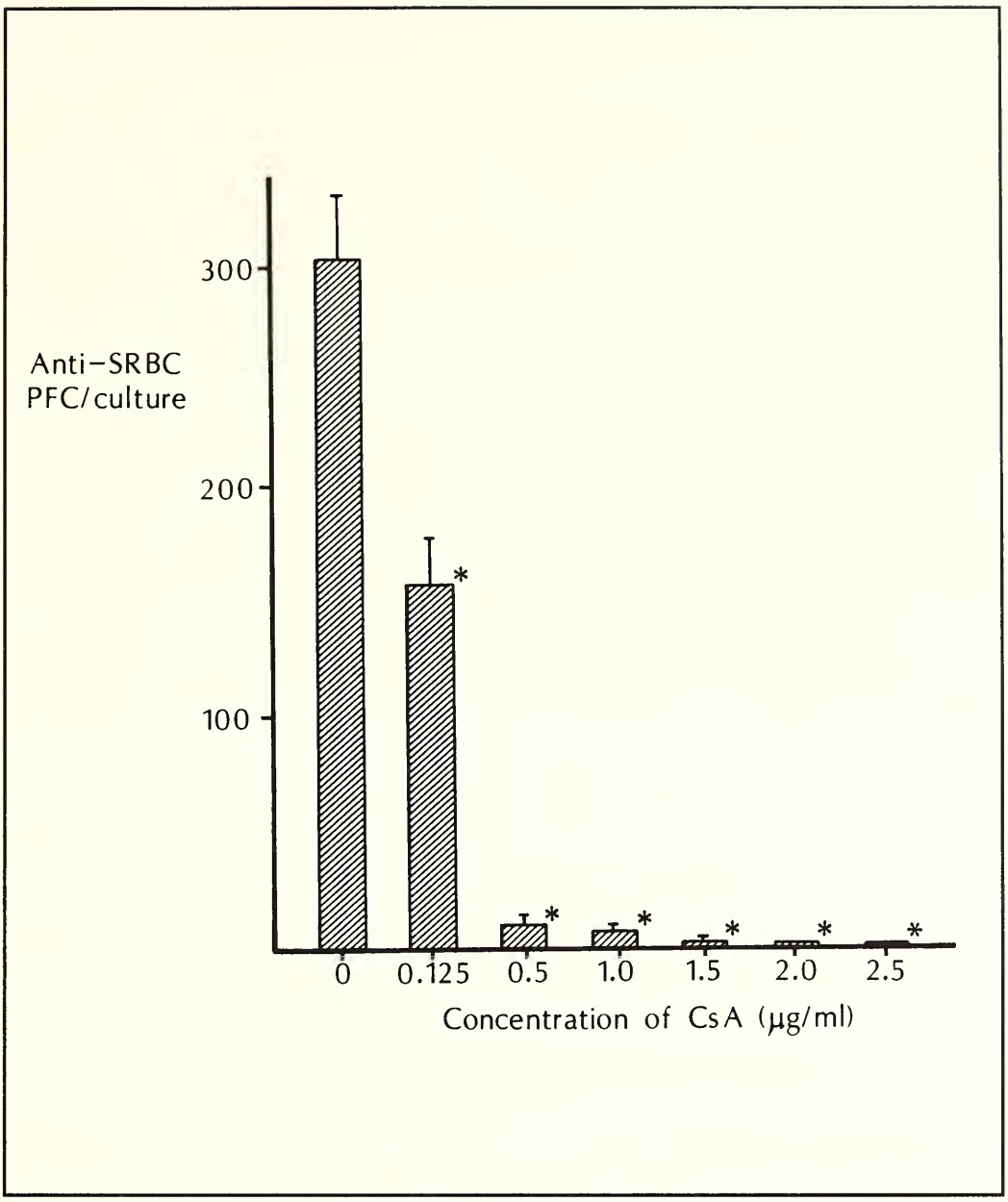

Figure 1. Splenocytes are inhibited by CsA in a dose-dependent manner in an *in vitro* antibody response. Spleen cells from naive mice were cultured at a concentration of 1 x 10^6 cells/well in the presence of SRBC and increasing amounts of CsA or corresponding amounts of medium to which the drug diluent had been added. On day 5, triplicate wells were pooled and assayed in triplicate. The results are shown as the average PFC per well for each experimental culture condition ±SEM. Any data bar shown without a standard error bar represents an average PFC per culture < 5 with a standard error < 5.
* $p < 0.001$ compared with culture without CsA.

(DPBS) plus 5% FCS to plastic petri dishes coated with goat anti-mouse immunoglobulin and harvesting the nonadherent fraction after 70 minutes at 4° C (Wysocki and Sato, 1978). Enriched populations of B cells were made by treating normal spleen cells with anti-Thy 1.2 plus C (Eardley, *et al.,* 1978). This B cell enriched population was found to contain > 94% Ig positive cells by direct immunofluorescent staining with fluoroscein conjugated anti-mouse Ig. To prepare an even more highly purified, positively selected B cell-population, normal spleen cells were added to plastic petri dishes coated with goat anti-mouse

Table 1. Temporal sensitivity of naive or primed splenocytes to CsA inhibition in the PFC response.[a]

Responders	Hour of Addition (Percent Inhibition Induced by Added CsA[b])			
	1	24	48	72
Naive splenocytes				
1 μg/ml CsA	> 99*	> 99*	45*	0
2 μg/ml CsA	> 99*	> 99*	69*	15
Primed splenocytes				
1 μg/ml CsA	> 99*	0	0	0
2 μg/ml CsA	> 99*	27	0	0

[a] Spleen cells from naive or primed animals were cultured at 1.5 X 10^6 per well in the presence of SRBC. CsA at 1 μg/ml or 2 μg/ml was added within 1 hour of culture or 24, 48, or 72 hours later to duplicate sets of triplicate wells. On day 5, the triplicate wells were pooled and assayed for PFC in triplicate windows of Cunningham slides. The average PFC/culture well for each culture condition was then determined. The mean levels of PFC ± SEM in control cultures without CsA were 250 ±30 for naive spleen cells and 400 ±25 for primed spleen cells.

[b] Percent inhibition of PFC was calculated according to the following formula:

$$\frac{\text{PFC in control cultures - PFC in experimental cultures}}{\text{PFC in control cultures}} \times 100$$

* Significant suppression ($p < 0.001$).

Fab. After removing the nonadherent fraction, 5 ml of DPBS + 1% FCS were added to each plate, and the plates were allowed to rock gently at 37° C for 30 minutes. Any remaining adherent cells were then gently removed from the plates by pipetting with a Pasteur pipet. These cells were pelleted by centrifugation and treated with an anti-T cell cocktail, consisting of anti-Ly 1.2, anti-Ly 2.2, and anti-Thy 1.2 plus complement. The resulting cells were washed thoroughly in HBSS and resuspended in complete medium before culture. This purified B cell population was found to be > 98% Ig positive.

Assay Cultures. The ability of whole spleen or selected subpopulations to produce antibody-forming cells *in vitro* was assessed by culturing the cells in complete culture medium with or without additives for five days in the presence of washed SRBC. The cells were cultured in a total volume of ca. 0.2 ml in Falcon 3040 flat-bottom microtiter trays in a humidified 5% $C0_2$ incubator at 37° C. Before incubation, 20 μl of a 1% suspension of SRBC and 40 μl of the nutritive cocktail were added to each well. For three days following culture, nutritive cocktail in the amounts of 20 μl, 10 μl, and 10 μl, respectively, was added to each well. A diluted solution of CsA was added to some of the wells either on

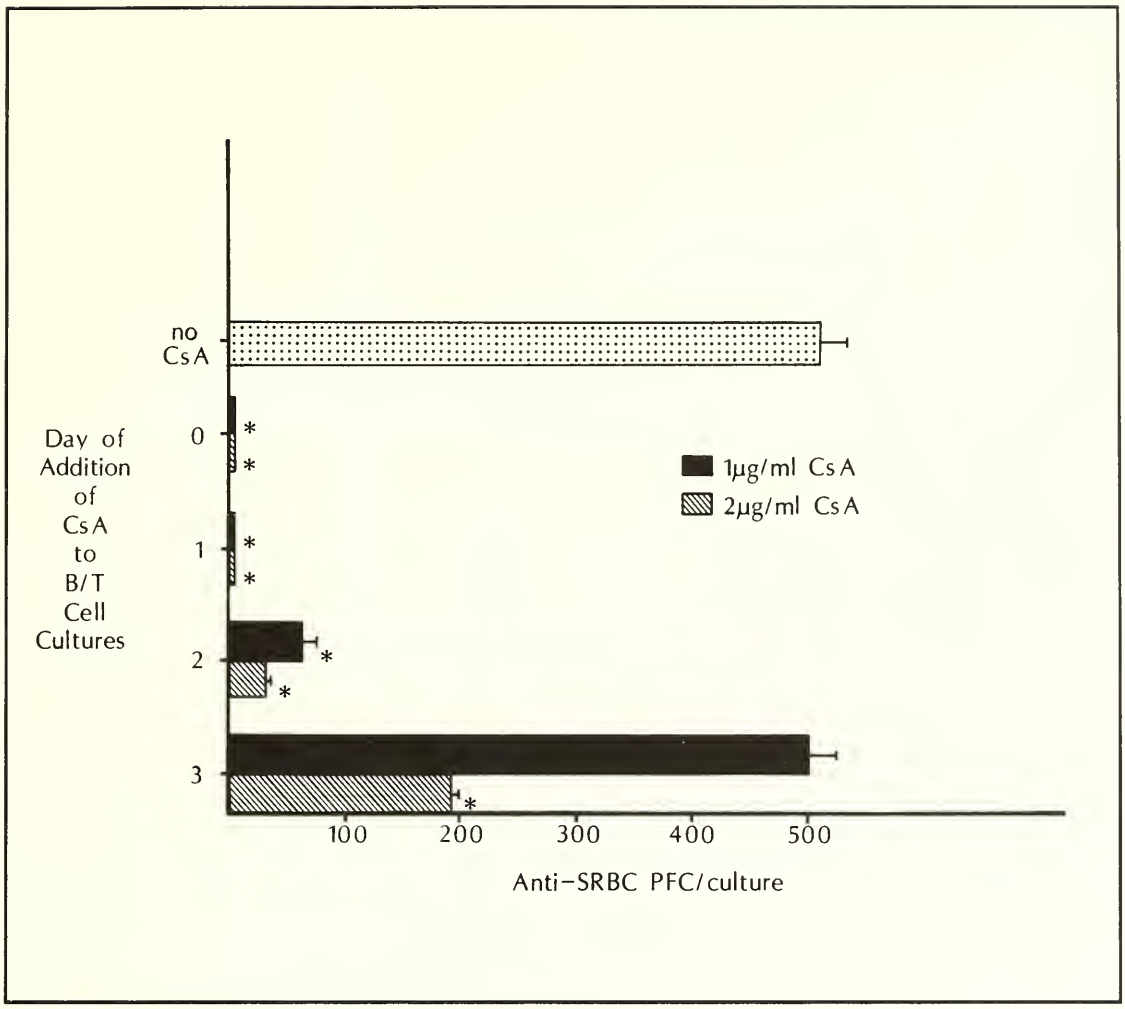

Figure 2. Inhibition of the *in vitro* plaque-forming responses by CsA in B/T cell cultures occurs during the first 48 hours of incubation. Enriched B cells were cultured at a concentration of 1.5 x 10^6 cells/well along with 5 x 10^5 enriched T cells and SRBC. CsA at concentrations of either 1μg/ml or 2 μg/ml was added to three sets of triplicate wells on day 0, 1, 2, and 3 of culture. On day 5, the average number of PFC per culture well for each experimental condition ±SEM was determined and represented as described in Figure 1. The enriched B cells did not produce and PFC when cultured alone with SRBC for 5 days. The negtively selected, enriched T cells produced only 16 ± 19 PFC/culture, when alone at 1.5 x 10^6/well.
*$p < 0.001$ compared with control cultures without CsA.

the day of culture (day 0) or on subsequent days before harvest. As appropriate, a lymphokine-rich supernatant was also added to some culture wells on day 0. Normally, at least six or more culture wells were established for each culture condition. On day 5, the number of IgM producing plaque-forming cells (PFC) to SRBC per culture was determined (see Cunningham and Szenberg, 1968).

Lymphokine Rich Medium. Spleen cells from Sprague-Dawley CD rats were collected, washed, and suspended in culture medium without mercaptoethanol at a concentration of 5 X 10^6/ml in the presence of 5 μg/ml of Con A. These cells were incubated in 250 ml cultures in a 5% $C0_2$ incubator at 37° C. After 48 hours, the suspension was centrifuged, and the supernatant was collected. Alpha methyl-mannoside (Sigma) was added to the conditioned medium at a final concentration of 0.05 M before filter sterilization to re-

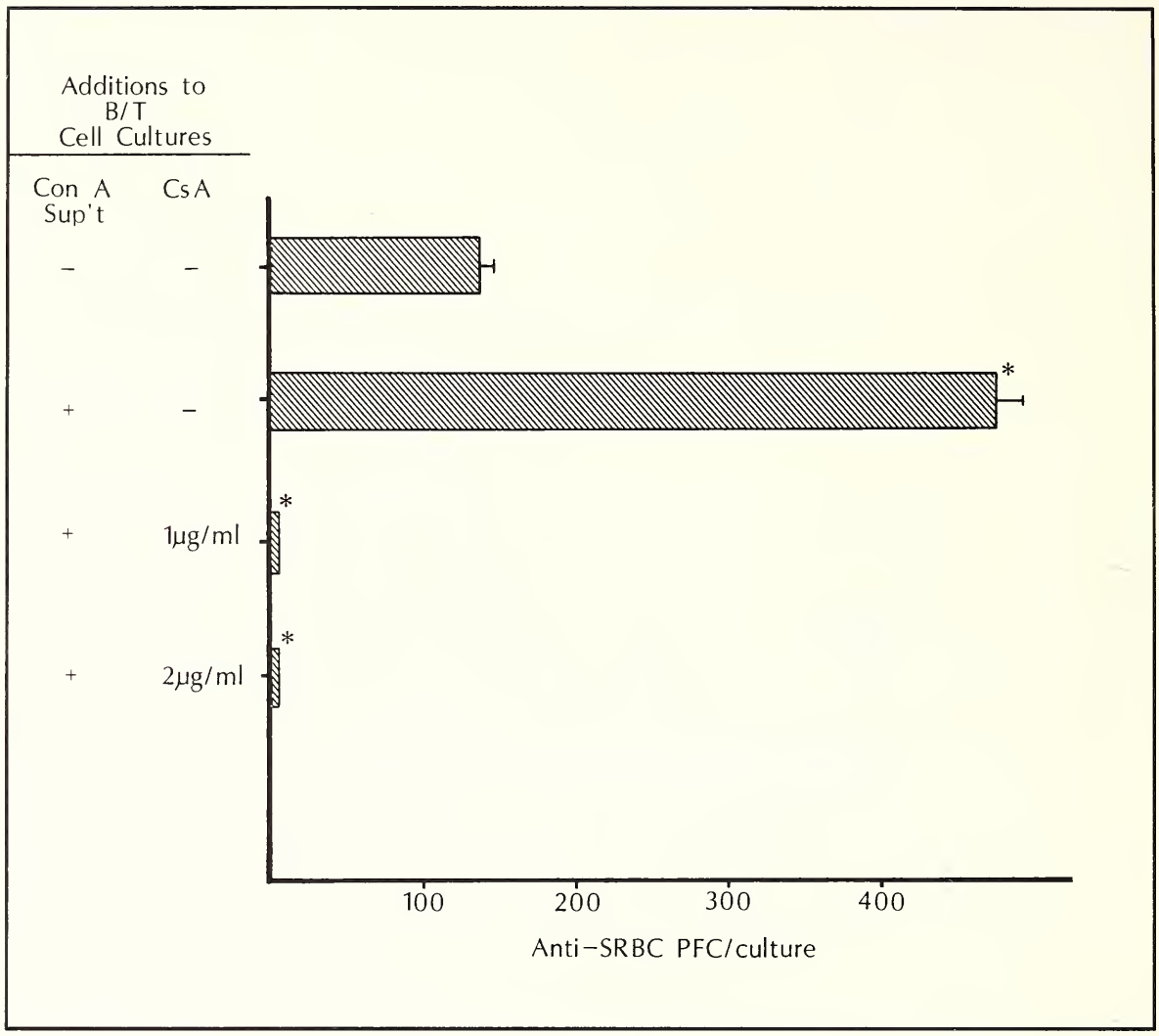

Figure 3. CsA completely inhibits the PFC response of B/T cell cultures even in the presence of enhancing lymphokines. Enriched B cells were cultured at a concentration of 1.5 x 10^6 cells/well along with 5 x 10^5 enriched T cells and SRBC. To some culture wells either 12% CM-1 (Con A supernatants) or 12% CM-1 supernatants plus CsA were added to duplicate sets of triplicate wells before incubation of the cultures. On day 5, the average numbers of PFC ± SEM per culture were determined and represented as described in Figure 1. Neither the enriched B cells nor T cells produced PFC, when cultured alone for 5 days.

* $p < 0.001$ compared with B/T cultures without CsA and Con A supernatant.

move residual Con A. Aliquots were frozen at -20° C until use. The Con A stimulated supernatant (conditioned medium, CM-1) prepared as above was found to contain substantial amounts of IL-2 as determined by its ability to support the proliferation of the IL-2 dependent cytotoxic line CTLL-2 (Gillis, *et al.*, 1978). These supernatants presumably contained several other T cell lymphokines, but no determination was made for any other T cell products affecting B cells.

Statistical analysis. The statistical significance of differences in PFC responses between experimental and control cultures was determined by the student's t-test. A p value of less than 0.001 was considered to be significant. All experiments were repeated two to three times with similar results, and only representative data are presented.

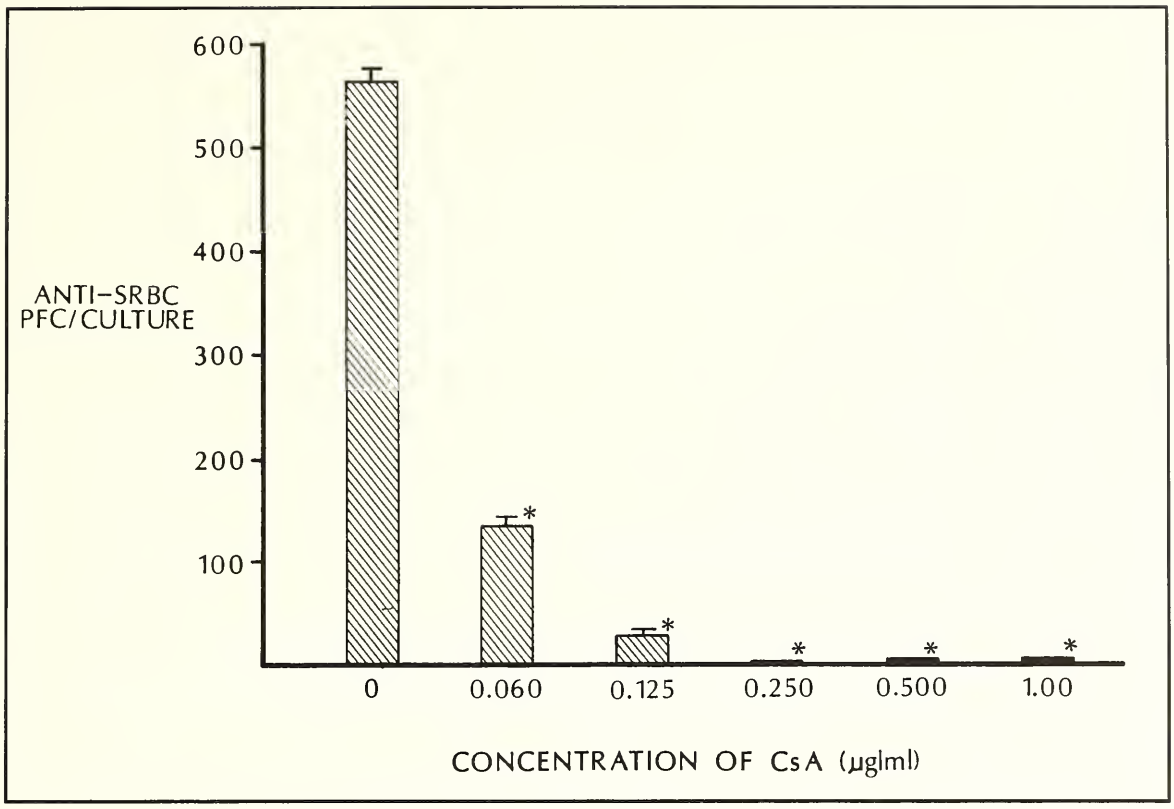

Figure 4. B cells are inhibited by CsA in a dose-dependent manner in an *in vitro* antibody response. Purified B cells were cultured at a concentration of 1.2 x 10^6 cells/well along with 25% CM-1 and SRBC. Increasing amounts of CsA were added to some culture wells, while others received appropriate amounts of diluent alone. Triplicate triplicates of each culture condition were established and cultured for five days. After incubation, triplicate wells were pooled and assayed in triplicate. The results are shown as the average PFC per well for each experimental condition ± SEM. B cells cultured without addition of conditioned medium produced no PFC.
* $p < 0.001$ compared to culture without CsA.

RESULTS

Dose-dependent inhibition of the *in vitro* anti-SRBC plaque-forming response by CsA. To determine whether CsA affected the ability of lymphocytes to respond to an antigen in an *in vitro* antibody response, splenocytes from naive mice were cultured in the presence of SRBC and increasing amounts of CsA or diluent from the beginning of the culture period. Following a five day incubation, the number of anti-SRBC PFC formed was found to be inhibited in a dose-dependent manner with greater than ninety-five percent inhibition at all concentrations of CsA at or over 0.5 µg/ml (Fig. 1). Because serum levels over 1 µg/ml are found to be effectively immunosuppressive in mice (Attridge, *et al.,* 1982; Borel, 1988), the *in vitro* dose of 1 µg/ml or 2 µg/ml CsA was used in most subsequent experiments.

Sensitivity of naive or primed splenocytes to CsA inhibition over time in the PFC response. In order to address the question of whether lymphocytes are sensitive to CsA throughout the sensitization and differentiation period of the PFC culture, naive Balb/c splenocytes were exposed to SRBC on day 0 of culture and CsA (1 µg/ml) was added to

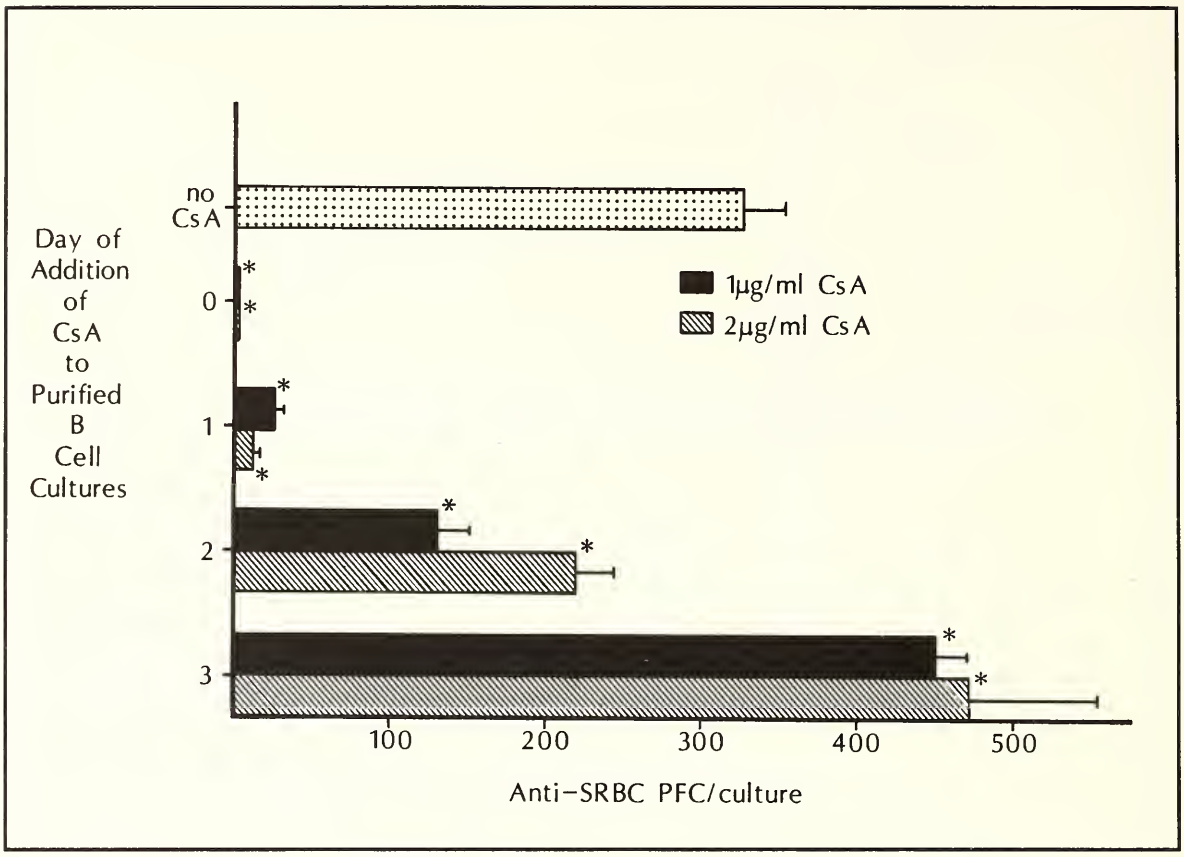

Figure 5. Purified B cells are sensitive to CsA-induced inhibition during the first 48 hours of culture. Purified B cells were cultured at a concentration of 1.5 x 10^6 cells/well along with 20% CM-1 and SRBC. CsA at concentrations of either 1 μg/ml or 2 μg/ml was added to three sets of triplicate wells on day 0, 1, 2, and 3 of culture. On day 5, the average number of PFC per culture well for each experimental condition ± SEM was determined and represented as described in Figure 1. The purified B cells did not produce any PFC, when cultured without the addition of CM-1.
* $p < 0.001$ compared with control cultures without CsA.

the cultures either on day 0, 1, 2, or 3 before harvest on day 5. Inhibition of the PFC response was virtually complete, when the drug was added during the first 24 hours, and only partially complete, when the drug was added after 48 hours (Table 1). A dose of 1 μg/ml appeared to be less suppressive at 48 hours (45%) than did a dose of 2 μg/ml (69%). When added on day 3 (72 hrs after initiation of the culture), CsA had no effect on PFC formation and, in some experiments, even appeared to enhance numbers of PFC. In order to assess the CsA sensitivity of splenocytes primed to SRBC *in vivo*, spleen cells of mice injected three days before sacrifice with a sub-immunogenic dose of SRBC were cultured together with additional SRBC in the standard PFC culture system. Again, CsA was added on day 0, 1, 2 and 3 of culture. In this case, the *in vivo* antigen-primed splenocytes were sensitive to CsA-induced inhibition only when added simultaneously with the antigen on day 0 (Table 1). In these cultures, no statistically significant inhibition of the five day PFC response was observed,when the CsA was added at any time following the initiation of the culture.

Sensitivity of mixed B and T cell cultures to CsA inhibition in the PFC response. In order to assess the sensitivity of mixed populations of naive T and B cells toward CsA, enriched B cells plus enriched T cells were cultured together at a ratio of 3:1 with antigen.

CsA was added at various time intervals following initiation of culture (Fig. 2). Although neither the B cell nor T cell populations alone were capable of mounting a significant anti-SRBC PFC response, the mixed culture produced large numbers of PFC. This PFC response was inhibited almost completely by both 1 μg/ml and 2 μg/ml CsA added immediately or up to 48 hours after initiation of culture. By 72 hours into the culture period, the PFC response was completely refractory to inhibition induced by 1 μg/ml CsA. The larger dose of 2 μg/ml was able to induce significant suppression in these 72-hour cultures (62%).

Sensitivity of mixed B and T cell cultures to CsA in the presence of T cell lymphokines. Because IL-2 production has been shown to be inhibited by CsA (Shevach, 1985), an attempt was made to bypass this effect of the drug on T cells by supplying the mixed B and T cell cultures with exogenous IL-2 in supernatants from Con A stimulated rat splenocytes (CM-1). These supernatants contained significant levels of IL-2 as measured by their ability to maintain the growth of the IL-2 dependent CTLL-2 T cell line (data not shown). The IL-2 rich CM-1 was added either with or without CsA to mixed cultures of B and T cells on the first day of culture with SRBC. Although the addition of 12% CM-1 alone resulted in a significant enhancement of the PFC response, the simultaneous addition of CsA completely inhibited *in vitro* PFC production (Fig. 3).

Sensitivity of B cells stimulated with antigens and lymphokines to CsA. To evaluate the effect of cyclosporine on B cells in the absence of T cells, positively selected, highly purified B cells were cultured with antigen plus lymphokine rich supernatants in the presence of increasing doses of CsA. Although these B cells alone in the presence of antigen were unable to produce PFCs, the addition of 25% CM-1 containing T cell replacing factors enabled the antigen stimulated B cells to become antibody forming cells (Fig. 4). The addition of CsA at concentrations between 250 ng/ml and 2000 ng/ml on day 0 of culture, however, completely inhibited the plaque forming ability of the antigen-lymphokine stimulated B cells. A concentration as low as 60 ng/ml still inhibited the B cells by 75%. In order to assess whether or not the CsA-induced inhibition of B cells followed the same temporal pattern of sensitivity that was observed in cultures containing both T and B cells, CsA was added to the positively-selected, purified B cells upon initiation of the cultures or at 24, 48, or 72 hours of the culture period. The pattern of inhibition observed repeatedly in these cultures was very similar to that seen in the whole spleen or B and T cell cultures. A representative experiment is shown in Figure 5. Nearly complete inhibition of the B cell response occurred when CsA was added at the time of culture or 24 hours later, and partial inhibition was observed at 48 hours. In this experiment, adding CsA at 72 hours appeared to somewhat enhance the PFC-forming ability of the purified B cells. In general, in all primary cultures tested, CsA-induced inhibition of the PFC response was nearly 100% through the first 24 hours of culture. The level of inhibition at 48 hours was more variable from experiment to experiment, ranging from approximately 30% to 80%. Addition of CsA on day 3 resulted in even more variable results, giving either mild suppression, no effect, or enhancement.

DISCUSSION

The *in vivo* antibody response to T-dependent antigens has been found to be exquisitely sensitive to cyclosporine A, particularly in the early, sensitization phase of a primary response (Thomson, 1983; Borel, *et al.,* 1976). In this study, the effects of CsA in B cells in an *in vitro* plaque-forming cell response of murine splenocytes to the T-dependent antigen, sheep red blood cells, was examined. Some of the drug-induced inhibition was ap-

parently due to direct inhibition of B cells, independent of CsA effects on lymphokine production of T cells.

Cyclosporine-induced inhibition of the *in vitro* PFC response to SRBC was found to parallel the pattern of inhibition observed *in vivo*. CsA inhibited the formation of antibody-forming cells in a dose dependent manner, when added at physiologically relevant levels upon initiation of the culture. Murine blood levels of 1-3 μg/ml CsA can be readily achieved and are successful in suppressing graft rejection and other immune reactions (Manca, *et al.,* 1985; Attridge, *et al.,* 1982; Borel, 1988; Halloran, *et al.,* 1988). Although this level is a physiological concentration that corresponds to blood peak levels, this level is likely to persist much longer in the lymphatic organs as well as other sites (Borel, 1988). In our experimental system, a concentration of 0.125 μg/ml CsA caused about 50% inhibition of the PFC response, when added at the time of culture, and the PFC response of naive splenocytes was completely inhibited at both 1 μg/ml and 2 μg/ml. The ability of CsA to inhibit the antibody response to an antigen, however, diminished over time. Although CsA added 24 hours following initiation of the culture was still highly immunosuppressive beyond 24 hours, the responding lymphocytes become increasingly more refractory to CsA-induced inhibition. Paavonen, *et al.* (1981) obtained similar results, when human blood leukocytes were exposed to the mitogens, PWM or protein A of *Staphylococcus aureus* Cowan and CsA. Non-toxic levels of CsA were effective in inhibiting the proliferative response, intracellular Ig synthesis, and the release of Ig, if present throughout the 6-day culture period. If added on day 2, however, the inhibitory effect was partial; on day 4, no inhibitory effect was observed. The temporal sensitivity of immune cells toward CsA both *in vitro* and *in vivo* has been observed in several other systems, and, in general, immune responses are more strongly inhibited, when the drug is administered shortly before, during, or after exposure to an antigen or other stimulus (Thomson, 1983). The present study demonstrates that the *in vitro* antibody response to the antigen SRBC shows a pattern of temporal sensitivity to CsA very similar to that observed in other systems, whereby the drug appears to affect an early stage of lymphocyte activation. Furthermore, the inhibitory effect of CsA on antigen-stimulated lymphocytes is not simply a toxic one, since no inhibition of PFC numbers was seen when CsA was added on the third day of culture, and the cells were harvested two days later. This observation was further substantiated by the finding that lymphocytes taken from animals primed three days earlier with a very low, sub-immunogenic dose of SRBC were not sensitive to *in vitro* CsA-induced immunosuppression, when added at any time other than day 0. Not only does this result indicate that CsA is non-toxic *in vitro*, but it also indicates that at least some of the early, CsA-sensitive, activation steps had occurred *in vivo* following SRBC priming and before re-exposure to antigen and CsA *in vitro*.

Enriched B and T cells cultured at a 3:1 ratio and stimulated with SRBC were inhibited by the addition of CsA in a pattern similar to that of whole splenocyte cultures. Again, complete inhibition was observed when CsA was added during the first 24 hours. A more profound inhibition occurred when the drug was added at 48 hours than was observed in the whole splenocyte culture.

CsA may mediate immunosuppression of the antibody response to T-dependent antigens primarily through an inhibition of T cell activation, either through inhibition of IL-2 production or IL-2 responsiveness of the T cell. Although the susceptibilty of T cells to CsA seems clear, the reported ability of the drug to inhibit B cells has been more controversial. The B cell response to certain activation stimuli, such as LPS, appears to be drug resistant, whereas activation by other stimuli appears to be sensitive (Lillhoj, *et al.,* 1984;

Shevach, 1985; Kunkl and Klaus, 1980; White, *et al.,* 1979). Klaus and Kunkle (1983) claimed that B cells responding to T-dependent antigens were not sensitive to CsA and that inhibition of antibody responses was due to the loss of T cell help. This study shows, however, that IL-2 rich supernatants of concanavalin A stimulated rat spleen are unable to alleviate, even partially, the immunosuppression induced by CsA in the mixed B/T lymphocyte cultures even though the added lymphokines significantly increased the total PFC capacity of the cultured cells.

Indeed, B cells alone, in the presence of Con A-induced T cell lymphokines, are highly sensitive to CsA. Positively selected, highly purified B cell cultures (98% Ig^+) supplied with lymphokine-rich supernatants and antigen were capable of producing large numbers of PFC toward SRBC, but this capacity was severely inhibited in the presence of even very low doses of CsA. Thus, although CsA may prevent antigen-activated T cells from producing Il-2 and other lymphokines on which B cells are dependent, the results discussed here indicate that SRBC-activated B cells are themselves highly sensitive to CsA. In this system, B cells alone are apparently slightly more sensitive to CsA than whole splenocyte cultures. Whereas 125 ng/ml CsA inhibited the splenocytes by 50%, the same concentration of CsA inhibited the lymphokine-antigen stimulated B cells by over 90%. The temporal pattern of inhibition in the B cell plus lymphokine cultures was very similar to that observed in the mixed B and T cell culture. Dongworth and Klaus (1982) also found that the proliferative response to anti-μ stimulated murine B cells could still be inhibited significantly by CsA even after 48 hours in culture. In later studies, small, resting murine B cells stimulated with anti-μ plus B cell stimulatory factor (IL-4) to proliferate were also shown to be sensitive to the drug, whereas induction of Ia expression on the same cells by this T cell factor was CsA resistant (O'Gara, *et al.,* 1986). Muraguchi, *et al.* (1983) also reported that human B lymphocytes stimulated with anti-μ appear to be similarly sensitive to CsA-induced inhibition, although significant inhibition only occurred during the first twenty-four hours of culture. Indeed, these investigators reported that human B cells treated with low doses of anti-μ were unable to proliferate in the presence of CsA for the first 24 hours of culture in the presence of the T cell lymphokine, B cell growth factor (BCGF). Our findings, using non-mitogenic, antigen specific stimulation of B cells to produce antibody, confirm the results of other investigators, who used agents that polyclonally activate cells through the B cell antigen receptor. In contrast, the activation of B cells by mitogenic T independent-1 (TI-1) antigens is apparently not particularly susceptible to inhibition by CsA, possibly because these activators override or bypass the CsA sensitive step(s) in the B cell activation process (Shevach, 1985). Originally, the failure of CsA to induce inhibition in nude mice stimulated to produce antibody against LPS, a potent polyclonal B cell activator, was taken as evidence that B cells were not sensitive to CsA (Borel, *et al.,* 1977). Apparently, naive murine B cells responding to a T-dependent antigen are highly sensitive to CsA, and perhaps even for a longer period than T cells. Mitogen-stimulated T cells are sensitive to CsA-induced inhibition only during the first few hours after exposure to either Con A or PHA (Kay and Benzie, 1983). Of course, T cells, like B cells stimulated with Ag, may be sensitive for a longer period than those stimulated with powerful, polyclonal activators. Unexpectedly, in a number of experiments, significant enhancement of the PFC response was observed, when CsA was added on the third day of culture. Cyclosporine has also been observed to enhance *in vitro* CTL activity, when added late to already activated killer T cells (Hess, 1985). CsA has also been shown to have an immune enhancing effect *in vivo* by several investigators, when administered over a brief period around the time of immunization (Klaus and Kunkl, 1983; Behforouz, *et al.,* 1986;

Thomson, *et al.,* 1983; Korczek, *et al.,* 1986). The mechanism of CsA-induced enhancement is unclear but may be an important physiological effect of this drug. CsA may operate as an immunomodulatory rather than strictly as an immunosuppressive drug. In the B cell, the early uptake of CsA may interfere with the differentiation of the Ag stimulated B cell on its way to immunoglobulin synthesis by a yet unknown mechanism (Borel and Gunn, 1986). Although the present investigation was confined to the study of an *in vitro* antibody response to only one T-dependent Ag, SRBC, for at least this antigen, B cells are highly sensitive to CsA-induced inhibition for a period extending through the first 48 hours of culture, independent of any inhibition which might be exerted on the T cell. CsA-induced inhibition of naive B cells may actually be more the rule rather than the exception, and the T-independent mitogenic antigens, which stimulate B cells in the presence of CsA, may simply bypass the early, CsA-sensitive sequence of events, which must occur in B cells activated by non-mitogenic antigens. The *in vitro* model presented here provides an additional system in which the entire mechanism of CsA immunosuppressive action may be investigated more closely and, perhaps, an aid in our understanding of early B cell activation by antigen.

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