Clin Exp Immunol 1994; 95:519–524

# Increased cyclosporine sensitivity of T cells from cord blood compared with those from the adult

R. M. MCDOUALL, A. J. SUITTERS, H. SMITH, M. H. YACOUB & M. L. ROSE *Transplant Immunology, Heart Science Centre, National Heart and Lung Institute at Harefield Hospital, Harefield, UK*

*(Accepted for publication 23 November 1993)*

## SUMMARY

Despite the increasing numbers of paediatric transplants performed, little is known about the immune responses of T lymphocytes in human neonates. Here we have compared the effects of cyclosporine on the phytohaemagglutinin (PHA) response of immature (cord) and mature (adult) lymphocytes using the following parameters of activation: (i) proliferation, measured by $^3$H-thymidine uptake; (ii) expression of cell surface IL-2 receptor; (iii) release of IL-2 into the supernatant. Cyclosporine was added to cultures of PHA-stimulated lymphocytes at doses ranging from 5 to 5000 ng/ml. The proliferative response of cord lymphocytes was considerably more sensitive to cyclosporine at each dose, so that 50% inhibition was achieved by 6 ng/ml and 21·5 ng/ml doses of cyclosporine on cord and adult lymphocytes, respectively. Expression of the IL-2 receptor by PHA-activated T cells and their subsets was assessed by flow cytometry. Cyclosporine inhibited IL-2 receptor expression to a significantly greater degree in cord CD4 and CD8 cells (49·7% and 70·1%) than in adults (17·9% and 30·0%). Biologically active IL-2 release was measured using the IL-2-dependent cell line CTLL-2. Cyclosporine at doses 50–5000 ng/ml produced 80–99% inhibition of both cord and adult responses. However, at very low doses (5 ng/ml) cyclosporine produced 69·3% inhibition of cord lymphocytes, compared with 42·0% of adult lymphocytes. These results suggest that the T cells of neonates are considerably more sensitive to cyclosporine than are adult T cells.

**Keywords** cyclosporine neonatal T cells

## INTRODUCTION

Despite the world-wide increase in numbers of paediatric transplants, relatively few studies have investigated the immune responses of immature human T lymphocytes, and none to date has investigated the effect of cyclosporine on immature T cells. In contrast, ontogeny and development of the B cell/immunoglobulin system from birth to 5 years of age have been quite extensively investigated [1,2].

The few studies of immature T cell responses reported have shown conflicting results. Early work of Billingham *et al.* [3] showed that T cell recognition of self is not developed until well after birth in mice, suggesting development of the human T recognition may similarly be delayed. However, this appears not to be the case. Studies of human fetal thymocytes [4] and fetal blood lymphocytes [5] showed proliferative responses to phytohaemagglutinin (PHA) to be present before birth.

Studies which have directly compared the proliferative response of cord and adult cells to PHA have concluded that cord cells proliferate as well as [6] or better than [7] adult cells. Similarly, the level of induction of the IL-2 receptor (IL-2R) on T lymphocytes as a result of PHA [6,8,9] or concanavalin A (Con A) [8,10] stimulation appears to be similar between cord and adult T cells, as does the duration of IL-2R expression [9].

One of the most useful ways to assess T cell responses is to measure production of biologically active IL-2, since IL-2 drives proliferation of CD4 T cells and allows maturation of cytotoxic CD8 T cell precursors [11]. A number of workers have reported that cord T cells produce as much or more IL-2 as adult cells [6,10,12,13].

Cyclosporine (CsA), the potent immunosuppressive drug, inhibits transcription of messenger RNA for IL-2 at pharmacological doses *in vitro* [14]. Here we have directly compared the sensitivity of cord and adult lymphocytes to CsA using a well established *in vitro* model of T cell activation, the response to PHA.

Correspondence: Dr Marlene Rose, Transplant Immunology, Heart Science Centre, Harefield Hospital, Harefield, Middlesex UB9 6JH, UK.

## MATERIALS AND METHODS

*Cells*

Umbilical cord blood was removed immediately after delivery and collected in heparin. The mononuclear cells were isolated by Lymphoprep (Nycomed (UK) Ltd, West Midlands, UK) density centrifugation, washed in RPMI 1640 (GIBCO, Paisley, UK), frozen in 10% DMSO (BDH, Merck, Poole, UK) and

stored in liquid nitrogen until required. Mature peripheral blood lymphocytes (PBL) were separated from heparinized blood samples from normal, healthy adult volunteers and separated as above. Both cord and adult lymphocytes were resuspended at a final concentration of $1 \times 10^6$ cells/ml in complete medium: RPMI 1640, 10% heat-inactivated human AB serum (Sigma, Poole, UK), supplemented with 2 mM L-glutamine (GIBCO) and 150 U/ml penicillin/streptomycin (GIBCO). Experiments comparing cord and adult cells were performed on the same days.

*Measurements of contaminating maternal cells in the cord samples*

Before any experiments the Kleihauer's stain was used to assess percentage contamination of maternal cells in the cord blood samples. Whole blood smears were made from every cord blood sample, air dried for 5–6 h and fixed in 80% ethyl alcohol (BDH). Blood smears were wrapped in cling-film and stored at −20°C before staining. Control blood smears were prepared from a mixture of cord and adult blood.

After defrosting, the slides were stained in acid buffer (24·7 ml 0·2 M $Na_2HPO_4$ + 75·3 ml 0·1 M citric acid; pH 3·2) for 2 min, washed in tap water and counterstained in 1% eosin (BDH) for 1 min. The slides were air-dried and mounted in DPX mounting medium (BDH). Adult haemoglobin was eluted from the erythrocytes by incubation in acid buffer so that the fetal haemoglobin alone was stained by the eosin. Percentage contamination of maternal cells was counted. Any samples with more than 10% maternal contamination were not used.

*PHA stimulation*

Proliferative responses of both cord and adult lymphocytes were measured by incubation of the cells at $0{\cdot}5 \times 10^6$ cells/ml with 2 μg/ml PHA (Sigma). Triplicate wells of round-bottomed 96-well plates (Nunc, Roskilde, Denmark) were set up and pulsed with 1 μCi $^3$H-thymidine (Amersham, Aylesbury, UK) 24 h before harvesting. Plates were incubated at 37°C and 5% $CO_2$. $^3$H-thymidine incorporation was assessed at various times and measured in a β-scintillation counter (LKB/Wallac, Milton Keynes, UK). Control wells had no PHA added.

*Measurement of IL-2 receptors on cell surface after PHA stimulation*

Cells were cultured at $1 \times 10^6$ cells/ml in complete medium (as above) plus 2 μg/ml PHA in 25-cm$^2$ flasks (Falcon, Oxford, UK). The flasks were incubated at 37°C, 5% $CO_2$ for various times. At each time point 2 ml resuspended cells were removed and a cell pellet obtained by centrifugation. The pellet was resuspended in PBS containing 0·01% sodium azide (Sigma), split into six tubes (approx. $5 \times 10^5$ cells/tube) and stained with MoAbs (Becton Dickinson, Oxford, UK) at predetermined optimal concentrations. The cells were analysed using double-labelling methods: 10 μl FITC-labelled MoAb against either T cells (CD3) or their subsets (CD4 and CD8) were added to 10 μl PE-labelled MoAb against either Class II (HLA-DR) or IL-2R (CD25). Cells were incubated 15 min, 4°C in the dark; washed with 2 ml PBS azide and centrifuged for 5 min, 300 ***g***. The pellet was resuspended and fixed with 50 μl 3% formaldehyde (BDH). The cells were further incubated for 5 min, 4°C and diluted with 150 μl Isoton II (Coulter, Hialeah, FL) before acquiring on the flow cytometer (Becton Dickinson). Control flasks had no PHA added. The percentage of IL-2R-bearing cells at the start of the experiment and at each time point was calculated.

*Release of IL-2*

The amount of IL-2 released into the supernatant by PHA-stimulated cord and adult lymphocytes was measured by bioassay using the IL-2-dependent murine cytotoxic cell line, CTLL-2 (European Collection of Animal Cell Cultures, Salisbury, UK, kindly supplied by Professor R. Lechler, RPMS, London, UK). The CTLL-2 cell line was cultured in RPMI 1640 supplemented with 2 mM L-glutamine, 150 U/ml penicillin/streptomycin, 10% heat-inactivated fetal calf serum (FCS; GIBCO) and 10 U/ml human recombinant IL-2 (rIL-2) (Boehringer, Mannheim, Germany). The cells were washed three times 24 h before the bioassay and cultured in medium, but without the addition of rIL-2.

The supernatant from the 2-ml cell suspension, removed for the measurement of IL-2R from the above flasks, was frozen and stored at −70°C until use. Briefly, 100 μl supernatant from each time point were added to 100 μl CTLL-2 cell suspension at $1 \times 10^4$ cells/ml in triplicate wells of round-bottomed, 96-well plates, incubated 24 h at 37°C, 5% $CO_2$, and pulsed with 1 μCi $^3$H-thymidine for 16 h. Plates were harvested and counted on a β-scintillation counter. The amount of IL-2 released was read off against a calibration curve and expressed as units IL-2 produced. As above, control flasks had no PHA added, and the amount of IL-2 at the start of the experiment was calculated.

*Effect of cyclosporine on cord and adult lymphocytes*

The above experiments of (i) response to PHA, (ii) percentage of cells expressing the IL-2R, and (iii) amount of IL-2 released were repeated in the presence or absence of PHA as before, and with the addition of varying doses of CsA (Sandoz, Basel, Switzerland). The CsA was dissolved in 100% ethanol (EtOH) (BDH) and further diluted in complete medium to give final concentrations of 5, 50, 500 and 5000 ng/ml when added at the start of each experiment. Control plates and flasks had either medium alone or medium plus vehicle (EtOH). Percentage inhibition by CsA was calculated for each experiment.

*Statistical analysis*

Statistical analysis was performed by Student's *t*-test.

## RESULTS

Initially it was sought to establish the kinetics of cell activation in response to PHA measured as cell proliferation, expression of the IL-2R, and secretion of IL-2 in the absence of CsA.

*Response to PHA*

Ten experiments were performed comparing cord and adult lymphocyte proliferation to PHA. Cells were cultured for up to 5 days, and $^3$H-thymidine incorporation was assessed at 1, 2, 3, 4 and 5 days. The pooled results (Fig. 1) show that the peak proliferative response of the cord lymphocytes occurred 1 day earlier (day 3) than the response of adult lymphocytes (day 4). The degree of proliferation varied enormously between individuals, being greater in the cord lymphocytes in four cases and in adult lymphocytes in six cases.

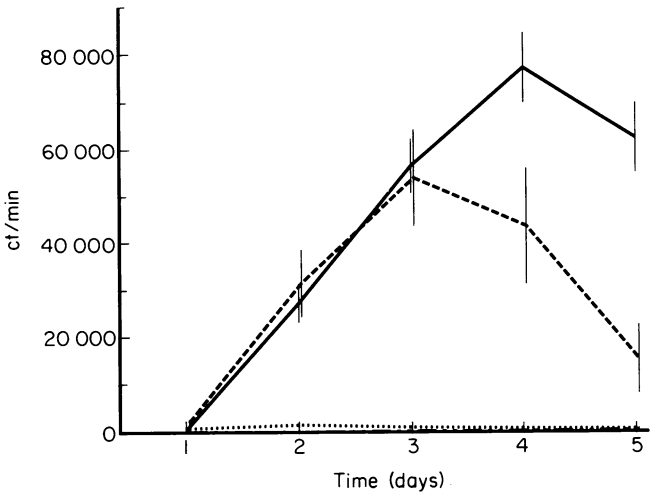


**Fig. 1.** Mean values (±s.e.m.) of the proliferative response of cord and adult lymphocytes to phytohaemagglutinin (PHA), measured by $^3$H-thymidine uptake over a period of 5 days ($n=10$ for each group). ——, Adult+PHA; — - - —, Adult−PHA; - - - - -, cord+PHA; . . . . . , cord−PHA.

In all experiments control wells with no PHA added showed no proliferation above background.

*Measurement of IL-2 receptor*

Six experiments were performed to assess the time for maximum increase of IL-2R (CD25) expression on cord (Fig. 2a) and adult (Fig. 2b) T cells and their subsets following PHA stimulation. In initial experiments, flasks were incubated and samples removed at 1, 2, 3, 4 and 5 days. In subsequent experiments, samples were removed at 12 h and 24 h and 4 days. In all six experiments the percentage of T cells (CD3) and both subsets (CD4 and CD8) expressing the IL-2R was still increasing at 4 days, correlating with the proliferative response. In five of these six experiments a smaller percentage of cord T cells (CD3) and their subsets (CD4 and CD8) expressed IL-2R than adult T cells. Thus at day 4 a mean of 62·0% of cord and 85·3% of adult CD3 cells were expressing the IL-2R ($P=0·01$).

There was no response in the absence of PHA.

*Release of IL-2*

Three experiments were performed measuring the IL-2 release after 1, 2, 3, 4 and 5 days. In all three cases the peak IL-2 release was maximum after 1 day, and subsequently tailed off completely to pre-PHA-stimulation levels. Three more experiments were performed with additional harvest times of time 0, 6, 12, 15 and 24 h and 4 days. These experiments confirmed that the maximum IL-2 release occurred at 24 h following PHA stimulation in cord and adult lymphocytes.

In four of the six experiments the release of IL-2 from cord lymphocytes greatly exceeded that from adult lymphocytes. An example of this is shown in Fig. 3. Overall, however, there was no significant difference in IL-2 levels between cord and adult cells.

Controls not stimulated with PHA did not release IL-2.

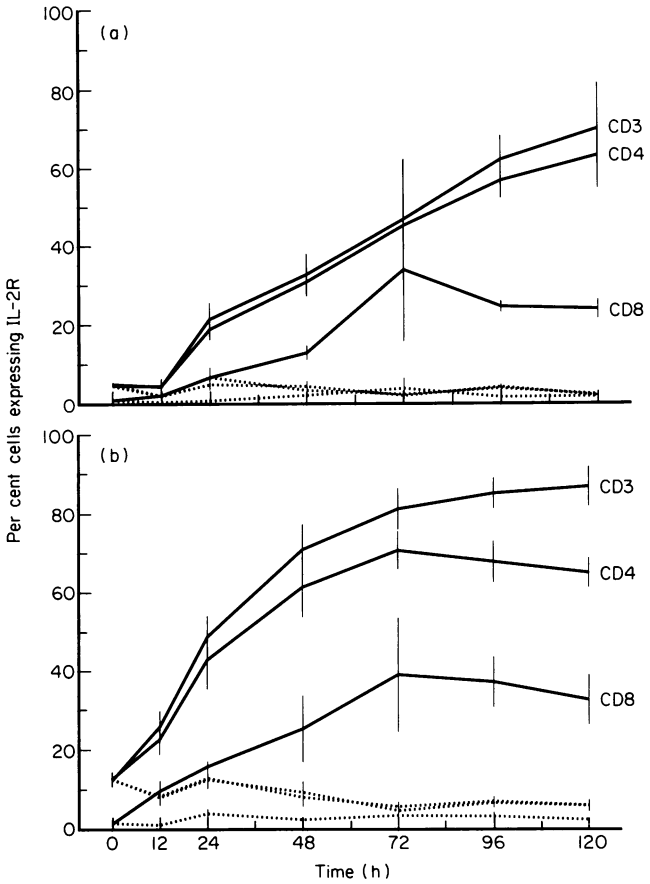


**Fig. 2.** (a) Mean values (±s.e.m.) of expression of IL-2R on cord lymphocytes in the presence or absence of phytohaemagglutinin (PHA), measured over a period of 120 h (5 days). Cells were dual labelled with FITC-antibody against CD3, CD4 or CD8 and PE-antibody against CD25. Results are expressed as per cent CD3, CD4 or CD8 also expressing CD25 ($n=6$). ——, Cord+PHA; . . . . . , cord−PHA. (b) Mean values (±s.e.m.) of expression of IL-2R on adult lymphocytes in the presence or absence of PHA, measured over a period of 120 h (5 days). Cells were dual labelled with FITC-antibody against CD3, CD4 or CD8 and PE-antibody against CD25. Results are expressed as per cent CD3, CD4 or CD8 also expressing CD25 ($n=6$). ——, Adult+PHA; . . . . . , adult−PHA.

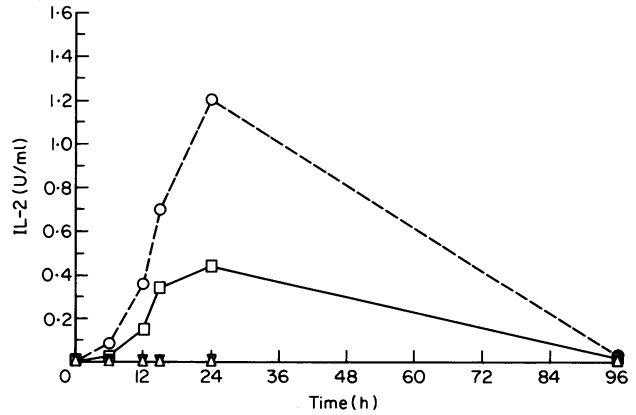


**Fig. 3.** Release of IL-2 into the supernatant by cord and adult lymphocytes in one experiment following phytohaemagglutinin (PHA) stimulation, measured over a period of 96 h (4 days). □, Adult+PHA; △, adult−PHA; ○, cord+PHA; ▽, cord−PHA.

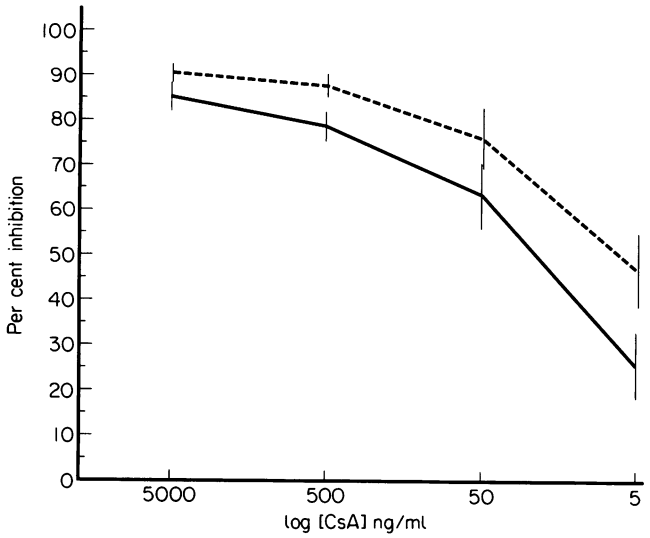


**Fig. 4.** Mean values (±s.e.m.) of the inhibitory effect of cyclosporine (CsA) on cord and adult lymphocyte proliferation 4 days following phytohaemagglutinin (PHA) stimulation. Figures are expressed as per cent inhibition caused by varying doses of CsA. Fifty per cent inhibition was achieved by 6 ng/ml and 21·5 ng/ml doses of CsA on cord and adult lymphocytes respectively ($n=6$). - - - - -, Cord; ——, adult.

*Effect of cyclosporine—response to PHA*

Six experiments were performed to assess the inhibitory effect of CsA on the proliferative response of cord and adult lymphocytes. In all cases the cord lymphocytes were considerably more sensitive to CsA at all four doses tested, especially at the low dose of 5 ng/ml ($P=0·084$). Thus comparing the percentage inhibition at peak proliferation (day 4), 50% inhibition was achieved by 6 ng/ml and 21·5 ng/ml doses of CsA on cord and adult lymphocytes, respectively (Fig. 4). The vehicle alone had no effect on proliferation, the results being the same as medium.

As before, control plates without PHA showed no proliferation.

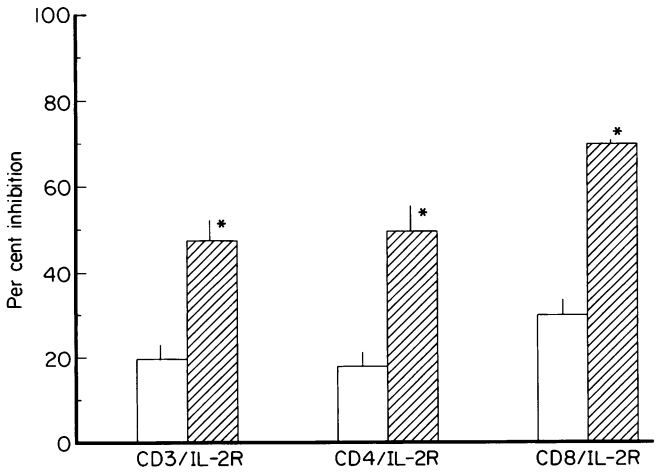


**Fig. 5.** Mean values (±s.e.m.) of the inhibitory effect of cyclosporine (CsA) on IL-2R expression by cord and adult T cells 4 days following phytohaemagglutinin (PHA) stimulation. Figures are expressed as per cent inhibition of cells expressing the IL-2R caused by CsA at 50 ng/ml ($n=3$). *$P=0·05$ for CD3 cells and $P<0·025$ and $P<0·005$ for CD4 and CD8 subsets respectively. □, Adult ± s.e.m.; ■, cord ± s.e.m.

*Effect of cyclosporine—expression of IL-2 receptors*

The effect of CsA on expression of IL-2R on T cells and their subsets was assessed in three experiments using CsA at a dose of 50 ng/ml (Fig. 5). The percentage of cells expressing IL-2R was measured at time 0 h, 12 h, 24 h and day 4. As with the proliferative response, percentage inhibition was calculated at each time point. Figure 5 shows the percentage inhibition on day 4 (at the time of peak proliferation) from these three experiments. Inhibition by CsA was significantly greater in cord T cell (CD3) populations (47·5%) than in adults (19·7%) ($P=0·05$), with a significantly greater degree of inhibition in both cord CD4 and CD8 subsets (49·7% and 70·1%) than in adults (17·9% and 30·0%) ($P<0·025$ and $P<0·005$ respectively).

There was no increase in IL-2R expression in cultures not containing PHA.

*Effect of cyclosporine—release of IL-2*

The effect of CsA on IL-2 release was assessed in four experiments, three as above for the study of IL-2R expression plus one extra. Measurements were made at time points 0, 12, 15 and 24 h and 4 days. The percentage inhibition by CsA was calculated for all four doses of CsA, at the peak time for IL-2 release, which was 24 h (Fig. 6). At this time the vehicle alone caused variable inhibition (0%, 25%, 13% and 37%) compared with medium. The results from the four experiments were pooled and the per cent inhibition caused by CsA was calculated as per cent inhibition of the response in the presence of vehicle. In all cases, and at all doses, CsA produced a greater inhibitory effect on cord lymphocytes than adults (Fig. 6). At doses 50–5000 ng/ml both cord and adult lymphocytes were inhibited by 80–99%. However, at very low doses (5 ng/ml) CsA produced considerably greater inhibition of cord lymphocytes (69·3%) compared with adult lymphocytes (42·0%), ($P=0·082$) (Fig. 6).

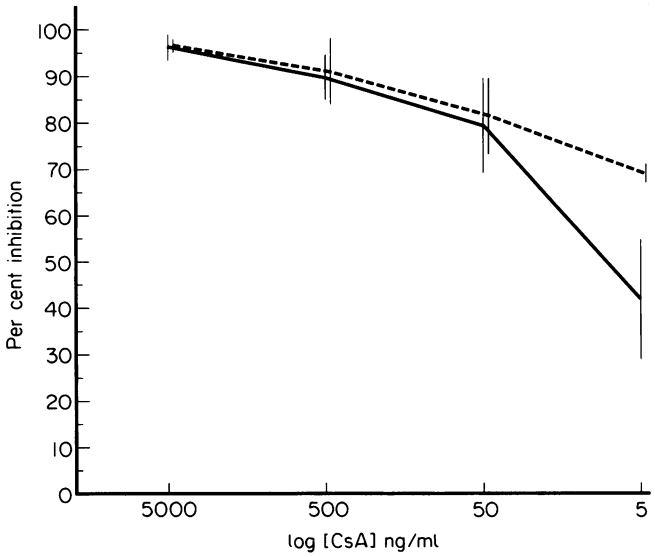


**Fig. 6.** Mean values (±s.e.m.) of the inhibitory effect of cyclosporine (CsA) on release of IL-2 into the supernatant by cord and adult lymphocytes 24 h following phytohaemagglutinin (PHA) stimulation. Figures are expressed as per cent inhibition caused by varying doses of CsA. CsA at 5 ng/ml induced 69·3% inhibition of cord lymphocytes compared with 42·0% of adult lymphocytes ($n=4$). - - - - -, Cord; ——, adult.

There was no IL-2 release in control flasks with PHA omitted.

## DISCUSSION

The main aim of this study was to investigate the inhibitory effects of CsA on cord lymphocytes compared with adults, as an increasing number of paediatric and neonatal transplants are being performed, and relatively little is known about the immune responses of neonatal T cells. We have investigated the proliferative response to PHA because it is a well established *in vitro* model of T cell activation and is inhibited by physiological doses of CsA [15]. We have used three assays with which to assess this response: proliferation, cell surface expression of IL-2R, and secretion of IL-2 into the supernatant. The initial experiments were performed to compare the basic responses of cord and adult lymphocytes to PHA and to establish optimal conditions for such assays so that the effects of CsA could be measured when responses were at their greatest.

The initial experiments confirmed that the proliferative response of PHA-stimulated cord lymphocytes was comparable to that of adults. It has been shown that IL-2R appear within 4–6 h after PHA stimulation in normal adult cells, and that receptor number peaks after 48–72 h [16]. After this it is reported that the number of IL-2R progressively decreases unless there is further stimulation by re-exposure to PHA or stimulating antigen [16]. In our system IL-2R expression was first measured after 12 h, by which time an increase in expression on both cord and adult T cells was observed. We found that the percentage of cells expressing the IL-2R was still increasing after 4 or 5 days, which paralleled the proliferative response as measured by $^3$H-thymidine incorporation. This finding was consistent with that of Yokoi *et al.* [8], who also reported that T cells from both cord blood and adults expressed Tac antigen on stimulation with various stimuli in parallel with their proliferation. In our system 2 μg/ml PHA was used, whereas Depper *et al.* [16] used only 0·5 μg/ml, which might account for the shorter duration of IL-2R expression they observed. In agreement with our findings, Nelson *et al.* [9] reported cord and adult lymphocytes to show increasing expression of IL-2R up to 5 days post-PHA-stimulation, decreasing by day 7. We found that a smaller percentage of cord T cells and their subsets expressed the IL-2R than did adult cells.

The maximum IL-2 production in both cord and adult lymphocytes occurred 24 h after PHA stimulation. This is in agreement with Miyawaki *et al.* [13], who reported maximum IL-2 production in both cord and adult lymphocytes between 24 h and 48 h following PHA stimulation. In our study the activated cord lymphocytes released more IL-2 into the supernatant than adults, in agreement with Fairfax & Borzy [7]. The overall ratio of CD4/CD8 is higher in newborns than in adults [17], so it has been suggested that either cord blood has a greater number of cells capable of producing IL-2, or else that cord cells do produce more IL-2 per cell than adults [7].

In conclusion, our results, along with those of others [7,13], show that there appears to be a fully competent IL-2 system at birth.

CsA is a potent immunosuppressive drug, its major side effects being those of nephrotoxicity and hypertension. Clearly, it would be advantageous to use it at sub-nephrotoxic doses, provided such doses are still immunosuppressive. In agreement with many others (reviewed by Hess *et al.* [15]), we found that CsA inhibited, in a dose-dependent manner, proliferation of PHA-stimulated lymphocytes, expression of the IL-2R and release of IL-2 into the supernatant. In all three assays the cord lymphocyte responses were inhibited considerably more than adults. Inhibition of expression of IL-2R was significantly different ($P < 0{\cdot}05$). Inhibition of proliferation and IL-2 release was most evident at low CsA doses (5–50 ng/ml), although the confidence limits did not reach 95% ($P = 0{\cdot}08$ in both cases).

The question whether CsA inhibits expression of cell surface IL-2R has been controversial. Miyawaki *et al.* [18] demonstrated that CsA did not prevent the expression of Tac antigen on mitogen-stimulated human T cells, although Bettens *et al.* [19] reported that CsA inhibited the expression of IL-2R on PHA-stimulated lymphocytes. A report by Weir *et al.* [20] suggests that CsA, at concentrations of 300 ng/ml and 600 ng/ml, only modestly inhibited IL-2R expression following PHA stimulation. This is in contrast with our own findings, though this group used quite low concentrations of PHA (0·33 μg/ml) and only stimulated for 24 h. The recent findings of Li *et al.* [21] showed that therapeutic doses of CsA (100 ng/ml) did inhibit expression of IL-2R. In our study the percentage inhibition was calculated at day 4 when proliferation was maximal, and at a dose of 50 ng/ml. We found substantial inhibition of IL-2R on T cells and both subsets by CsA, the inhibition being significantly greater on cord T cell subsets. Thus cord T cells showed 47·5% inhibition compared with 19·7% for adult T cells.

The results from the measurement of IL-2 release by cord and adult lymphocytes show that cord lymphocytes are considerably more sensitive to CsA at the low dose range of 5–50 ng/ml compared with adults. This suggests that neonates may be able to tolerate low trough levels better than adults. Our results are consistent with reports by Tsao *et al.* [22], who found an age-related difference in lymphocyte CsA sensitivity in pigs, with neonates being more sensitive to CsA than mature pigs.

Recent advances have been made in our understanding of the precise mechanisms of action of CsA on T cells. CsA inhibits transcription of IL-2 by preventing the activation of specific transcription factors such as NF-AT and NF-IL2A [23]. It binds to its intracellular receptor, cyclophillin, and the target for this drug/receptor complex is the $Ca^{++}$-dependent calmodulin-regulated phosphatase calcineurin [24]. Recent *in vitro* experiments strongly suggest that calcineurin is a key enzyme in the T cell signal transduction cascade [25]. Thus over-expression of calcineurin in Jurkat cells renders them more resistant to the effects of CsA and augments both NF-AT- and NF-IL2A-dependent transcription.

Two previous reports [26,27] found considerable variation between the inhibitory effect of CsA on the proliferative response to PHA of different individuals. The possibility exists that this observation plus the current observations of sensitivity of cord lymphocytes may reflect the amount of calcineurin present in an individual's T cells. It would therefore be valuable to test directly for calcineurin content and relate it to CsA sensitivity. The possibility of an age-related difference in calcineurin content of T cells might make it possible to test lymphocytes from paediatric cases without the present requirement for large amounts of blood.

We therefore suggest that CsA can probably be used in neonates at lower doses than those used in adults to inhibit expression of IL-2R and to prevent IL-2 release.

## ACKNOWLEDGMENTS

We wish to thank Sister Romney and the staff of the Delivery Suite at Northwick Park Hospital for their help with collecting cord blood samples. We also thank Professor Robert Lechler for supplying the CTLL-2 cell line for the bioassay.

## REFERENCES

1 Wu LYF, Lawton AR, Cooper MD. Differentiation capacity of cultured B lymphocytes from immunodeficient patients. J Clin Invest 1973; **52**:3180–9.
2 Anderson U, Bird AG, Britton S, Palacios R. Humoral and cellular immunity in humans studied at the cell level from birth to two years of age. Immunol Rev 1981; **57**:5–38.
3 Billingham RE, Brent L, Medawar PB. 'Actively acquired tolerance' of foreign cells. Nature 1953; **172**:603–6.
4 Kay HEM, Doe J, Hockley A. Response of human foetal thymocytes to phytohaemagglutinin (PHA). Immunology 1970; **18**:393–6.
5 Rayfield LS, Brent L, Rodeck CH. Development of cell-mediated lympholysis in human foetal blood lymphocytes. Clin Exp Immunol 1980; **42**:561–70.
6 Kawano Y, Noma T, Yata J. Analysis of decreased autologous mixed lymphocyte reaction of cord blood lymphocytes: with special reference to production of and response to interleukin-2 (IL-2). Asian Pac J Allerg Immunol 1984; **2**:49–55.
7 Fairfax CA, Borzy MS. Interleukin-2 production, proliferative response and receptor expression by cord blood mononuclear cells. J Clin Lab Immunol 1988; **27**:63–67.
8 Yokoi T, Miyawaki T, Yachie A, Ohzeki S, Tanaguchi N. Discrepancy in expression ability of Tac antigen and Ia determinants defined by monoclonal antibodies on activated or cultured cord blood T lymphocytes. J Immunol 1982; **129**:1441–5.
9 Nelson DL, Kurman CC, Fritz ME, Boutin B, Rubin LA. The production of soluble and cellular interleukin-2 receptors by cord blood mononuclear cells following *in vitro* activation. Pediatr Res 1986; **20**:136–9.
10 Wilson CB, Westall J, Johnston L, Lewis DB, Dower SK, Alpert AR. Decreased production of interferon-gamma by human neonatal cells. J Clin Invest 1986; **77**:860–7.
11 Smith KA. The interleukin-2 receptor. Ann Rev Cell Biol 1989; **5**:397–425.
12 Hayward AR, Kurnick J. Newborn T cell suppression: early appearance, maintenance in culture, and lack of growth factor suppression. J Immunol 1981; **126**:50–53.
13 Miyawaki T, Seki H, Taga K, Sato H, Taniguchi N. Dissociated production of interleukin-2 and immune gamma-interferon by phytohaemagglutinin stimulated lymphocytes in healthy infants. Clin Exp Immunol 1985; **59**:505–11.
14 Elliott JF, Lin Y, Mizel SB, Bleackley RC, Harnish DG, Paetkau V. Induction of interleukin-2 messenger RNA inhibited by cyclosporine A. Science 1984; **226**:1439–41.
15 Hess AD, Esa AY, Colombani PM. Mechanisms of action of cyclosporine: effect on cells of the immune system and on subcellular events in T cell activation. Transpl Proc 1988; **20**:29–40.
16 Depper JM, Leonard WJ, Krönke M *et al.* Regulation of interleukin 2 receptor expression: effects of phorbol diester, phospholipase C and reexposure to lectin or antigen. J Immunol 1984; **133**:3054–61.
17 Foa R, Giubellino MC, Fierro MT, Lusso P, Ferrando ML. Immature T lymphocytes in human cord blood identified by monoclonal antibodies: a model for the study of the differentiation pathway of T cells in humans. Cell Immunol 1984; **89**:194–201.
18 Miyawaki T, Yachie A, Ohzeki S, Nagaoki T, Taniguchi N. Cyclosporin A does not prevent expression of Tac antigen, a probable TCGF receptor molecule, on mitogen-stimulated human T cells. J Immunol 1983; **130**:2737–42.
19 Bettens F, Walker C, Bonnard GD, de Weck AL. Effect of cyclosporin A on the early activation of human T helper lymphocytes: inhibition of RNA-synthesis and modification of the expression of activation antigens. Immunobiol 1985; **170**:434–47.
20 Weir MR, Peppler R, Gomolka D, Handwerger BS. Additive inhibition of afferent and efferent immunological responses of human peripheral blood mononuclear cells by verapamil and cyclosporine. Transpl 1991; **51**:851–7.
21 Li B, Sehajpal PK, Subramaniam A, Joseph A, Stenzel KH, Suthanthiran M. Inhibition of interleukin 2 receptor expression in normal human T cells by cyclosporine. Transpl 1992; **53**:146–51.
22 Tsao PW, Diaz RJ, Radde IC *et al.* Age-related differences in the effects of cyclosporine on lymphocyte intracellular free calcium. Transpl 1991; **52**:345–9.
23 Lin CS, Boltz RC, Siekierka JJ, Sigal NH. FK-506 and cyclosporin A inhibit highly similar signal transduction pathways in human T lymphocytes. Cell Immunol 1991; **133**:269–84.
24 Liu J, Farmer JD Jr, Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin–cyclosporin A and FKBP–FK506 complexes. Cell 1991; **66**:807–15.
25 Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature 1992; **357**:695–7.
26 Manca F, Carozzi S, Kunkl A *et al.* Individual sensitivity to cyclosporine *in vitro* can be detected in kidney failure patients. Transpl Proc 1985; **17**:1265–7.
27 Sjöberg O, Tötterman TH. Variations in inhibition by cyclosporine A of the proliferative response, using different individuals and T cell stimulators. Transpl Proc 1986; **18**:40–41.