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Complete prevention of the clinical expression of adjuvant-induced arthritis in rats by cyclosporin-A and lobenzarit: The regulation of lymph node cell populations and cytokine production

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Abstract. A single dose of either cyclosporin-A (CsA) or lobenzarit (CCA) given with an arthrogenic adjuvant completely prevented expression of experimental adjuvant arthritis in rats. The aim of this study was to understand how these drugs prevented the arthritis expression by studying the popliteal lymph nodes draining the arthritic joints at various times after adjuvant injection. Neither drug affected the proliferation in popliteal lymph nodes at the time arthritis was normally expressed, however, there was a marked change in the types of cells present. Immunofluorescence assays showed a reduction in the proportion of $CD4^+$ cells, while the proportion of B-lymphocytes was almost doubled. This coincided with a marked elevation in the ability of these cells to produce interleukin (IL)-6. At the same time production of other cytokines (IL-2, tumour necrosis factor (TNF) and interferon (IFN)-γ) was not greatly affected. However, one day after adjuvant injection IL-2 and IFN-γ production was reduced. In vitro experiments showed that IL-6 production by lymphoid cells was relatively unaffected by CsA and CCA but IL-2, TNF and IFN-γ were suppressed by CsA. The results indicate that CsA and CCA may modify the response to the arthritic adjuvant by specifically inhibiting IL-2, TNF and IFN-γ production at the time of adjuvant injection. The lack of inhibition of IL-6 by these drugs reveals it may not play a key role in the initiation of this model of chronic inflammation.

Key words: Arthritis – Cyclosporin – Lobenzarit – Cytokines and lymphocytes

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Introduction

The events which initiate and maintain chronic inflammatory diseases such as rheumatoid arthritis are poorly understood. Inflammatory cytokines released from activated leucocytes have been identified as mediators of this underlying process. Regulating the cells which produce inflammatory cytokines is a promising area of drug research. The beneficial effect of inhibiting just one of the inflammatory cytokines, tumour necrosis factor (TNF) α, with antibodies in rheumatoid arthritis patients indicates the key role TNFα plays in maintaining chronic inflammation [1]. In addition many of the currently used disease modifying drugs have also been shown to regulate activated leucocytes and the inflammatory cytokines they produce [2, 3].

The fungal cyclic peptide, cyclosporin A (CsA), has been used extensively to promote the survival of organ grafts and is widely used in the treatment of chronic inflammatory disorders such as rheumatoid arthritis [4, 5]. Lobenzarit (CCA), a synthetic compound structurally resembling mefenamic acid, is a novel disease modifying anti-rheumatic drug widely used in Japan. Extensive in vitro studies had led to the belief that both of these drugs are effective because they inhibit T lymphocytes. However the action of CsA and CCA may not only be dependent on selective depletion of T cells [6, 7], but also suppress accessory cell [8, 9] and B lymphocyte functions [10–12]. CsA and CCA are effective at suppressing arthritis in animal models of the disease [13–16]. We have also found that CsA treatment not only reduces the severity of disease, but treatment during the induction period also prevented development of adjuvant arthritis and rendered

these animals resistant to further challenge with the same arthritogen [16]. This study was designed to better understand how both drugs completely prevented arthritis expression by studying the popliteal lymph nodes draining the arthritic joints at various time after adjuvant injection. The effects of CsA and CCA on the numbers, types (e.g. B, T cells subsets) and the production of important inflammatory mediators by these cells were analysed.

Since both drugs completely inhibited development of arthritis, we initially hypothesised that these drugs would completely suppress the immune response to the adjuvant. Surprisingly, our findings indicated that CsA and CCA modified, rather than suppressed, immune responses to the arthritogenic adjuvant.

Materials and methods

Drugs

CsA was a gift from Sandoz Australia. CCA was obtained from the Institute of Drug Technology Australia Ltd. Australia.

Adjuvant disease

Adjuvant arthritis was induced as previously described [14]. In brief, on day O, groups of male Dark Agouti (~ 250 g) rats from the University of Adelaide Animal Services were injected subdermally near the tail base with 100 µl of finely ground, heat-killed, delipidated mixed human strains of *Mycobacterium tuberculosis* dispersed in squalane at a concentration of 5 mg/ml. The poly arthritis on day 14 after adjuvant injection was assessed by scoring each paw for disease severity as previously described [14].

Drug administration

Co-administration of CsA (40 mg/kg) or CCA (40 mg/kg) with the adjuvant was achieved by grinding the drugs (100 mg/ml) in the adjuvant. In some experiments the adjuvant was given alone on day 0 and CsA, made up in 1 ml of saline, was injected subcutaneously from days −1 to 5 at a dose of 10 mg/kg into the hind flank of the animal.

Detection of CsA in the blood

Blood levels of CsA were measured with an immunoassay ("Cyclotrac" SP RIA – Incstar Corp., MI, USA) used routinely to monitor CsA levels as low as 0.1 µM in transplantation patients by Dr Ray Morris at the Queen Elizabeth Hospital, Woodville, South Australia.

Cell isolation

Peripheral blood mononuclear (PBM) cells were isolated as previously described [2]. Popliteal lymph node (PLN) were removed immediately after the rats were killed by cervical dislocation and kept in cold Hank's buffered saline. The lymph nodes were passed through a fine wire mesh and the single cell suspensions washed in Hank's buffered saline. The PBM and PLN cells were then resuspended in RPMI-1640 medium.

Cytokine production (ex vivo)

2×10^5 PBM and PLN cells were incubated in a final volume of 250 ul RPMI medium supplemented with 10% foetal calf serum, 50 IU/ml penicillin and 50 U/ml streptomycin. These cells incubated in media alone or stimulated with either 5 ug/ml lipopolysaccharide (LPS) (*Escherichia coli* 0111 : B4, Sigma Chem Co, St Louis, MO, USA) or 5 µg/ml Concanavalin A (Con A) (Sigma Chem Co.). After 24 h the supernatants were sampled and assayed for the various cytokines. The in vitro effect of CsA on PBM, PLN and adherent peritoneal (AP) cells isolated from normal rats was investigated in the same manner with the addition of CsA at various concentrations. Samples of supernatants were either assayed immediately or stored at −70 °C for up to one month before assay.

Thymidine incorporation

Proliferation of cells was assessed by measuring ^{3}H-thymidine incorporation as previously described [17]. Briefly, 2×10^5 isolated lymph node cells were incubated in 250 µl of media with 0.5 µCi ^{3}H-thymidine in each well of a 96 well microtitre tray. After 24 h the incorporated thymidine was precipitated onto glass filters with 5% (w/v) trichloroacetic acid using an automated cell harvester. The precipitated ^{3}H was counted on a Beckman scintillation counter.

Assays for cytokines

The following assays were carried out as described [18]: IL-1 using the IL-1 sensitive A375 cell line; TNF using the TNF-sensitive cell line L929; IL-6 using 7TD1 hybridoma cells; and IL-2 using the CTLL-2 cell line. The A375 cell line is also sensitive to TNF (α and β) but only at levels more than ten fold higher than that detected in the samples tested. All other assays were found to be very specific for the particular cytokine to be measured (recombinant human IL-1, IL-2, IL-4, IL-6, TNF(α and β), IFN-γ and IFN-α were tested). To assay IFN-γ, an enzyme-linked immuno-assay kit specific for rat IFN-γ was used (Holland Biotechnology, The Netherlands) that measured levels as low as 1 unit (0.25 ng)/ml.

Assay for PGE_2

A competitive radioimmunoassay able to detect levels of PGE_2 as low as 2 picoMolar was used as described [18].

Monoclonal antibodies

Mark 1 mAb's [19], which bind to cells with immunoglobulin on their surface (mainly B lymphocytes), were a gift from Prof. H. Bazin, Brussels, Belgium. R73 mAb/s [20], which bind to cells with $\alpha\beta$ receptors (a sub-population of T lymphocytes), were a gift from Dr. T. Hunig, Wurzburg, Germany. OX6 (binding to cells expressing MHC II), OX8 (binding to CD8 + ve cells), and W3/25 (binding to CD4 + ve cells) mAb's have all been previously described [21–23].

Immunofluorescence assays

Isolated rat lymph node cells were stained with mAb in an immunofluorescence assay using methods described previously [24].

Statistical analysis

Statistical significance values were calculated using a Student's t-test [25]. We chose $p < 0.05$ as a significant statistical difference.

Results

The effect of CsA on the expression of adjuvant-induced arthritis and associated cachexia

Weight loss and onset of disease were prevented by a single dose of CsA or CCA administered admixed with the arthritogenic adjuvant (given at day 0) (Table 1). Alternatively, subcutaneous administration of CsA every second day from day −1 to day 13 or from day −1 to day 7 was equally effective at preventing disease and weight loss. Blood levels of CsA following administration of a single dose of CsA in the adjuvant were also measured. Levels of 0.31 μM were detected on day 3 and 0.37 μM on day 7 post injection. These levels fell to 0.1 μM on day 14. Levels of 0.2 μM or more are reported in rheumatoid arthritic patients receiving CsA therapy [26].

The effect of CsA and CCA treatments on lymphoid cell populations

Table 2 shows the yields of mononuclear cells isolated from rats 14 days after they were injected in the tail base with either adjuvant alone or a mixture of adjuvant plus CsA or CCA. The results show that the adjuvant injection resulted in a more than 3-fold increase in the numbers of PLN cells isolated. Neither CsA nor CCA affected the numbers of cells isolated despite completely preventing the arthritic disease. Similar results were obtained when the CsA was administered subcutaneously every second day on days −1 to 14 (a total of 7 doses). CsA and CCA did not have any effect on the enhanced uptake of ^{3}H-thymidine by PLN cells induced by adjuvant injection. The increased cell recoveries were associated with increased ^{3}H–thymidine uptake showing that cell proliferation, rather than lymphocyte recruitment, mainly contributed to the node enlargement.

PLN cells were isolated from four groups of rats (untreated, adjuvant alone, adjuvant with CsA and adjuvant with CCA) on day 14. Sub populations of cells were labelled with monoclonal antibodies and compared by flow cytometric analysis (Table 3). The most marked changes induced by treatment with both drugs were in the proportion of cells staining with Mark 1 antibody (mostly B cells). Although adjuvant alone had little effect on the proportions of these cells, CsA and CCA treatments induced approximately double the proportion of Mark 1 positive cells. This increase coincided with a less marked decrease in the proportions of cells staining with W3/25 (CD4 + ve cells) and R73 (α/β T cells) antibodies. The proportion of OX6 positive (MHC class II) cells was higher in animals receiving adjuvant and was further elevated in animals treated with CsA or CCA.

Cytokine production by lymph node cells ex vivo after treatment in vivo

The effect of CsA and CCA treatment on the ability of PLN cells to produce a number of cytokines is represented in Table 4. The levels of all cytokines (except IL-6) produced by unstimulated cells were below the detectable limits of our assays. Stimulation with mitogen Con A or LPS induced secretion of all cytokines.

With Con A stimulation CsA and CCA treatments significantly reduced the ability of PLN cells to produce IFN-γ and IL-2 at day 1 ($p < 0.05$). Lower levels of TNF were also detected at day 1. On day 14 both drug treatments markedly increased the ability of these cells to produce IL-6 ($p < 0.05$). IFN-γ and IL-2 production was not markedly different from untreated controls at day 14. TNF release was lower but only statistically significant differences were noted in the CCA treated group.

With LPS stimulation we noted a significant reduction in IL-1 produced by both drug treatments on day 14. Conversely, IL-6 production was markedly stimulated on Day 14 similar to that noted with Con A stimulation. We noted no significant change in TNF activity produced (no data shown) and IL-2 production could not be detected in any of the groups following LPS stimulation.

Effects of CsA on secretion of cytokines and PGE_2 by leucocytes in vitro

Cytokine production was also assessed following stimulation of normal PLN, PBM and AP cells in vitro with either Con A or LPS in the presence of graded concentrations of CsA or CCA. Table 5 shows the concentrations of CsA and CCA calculated to inhibit cytokine

Table 1. The effect of CsA and CCA treatments on the induction of arthritis and weight change at day 14 by a mycobacterial adjuvant (Adj) injection in male Dark Agouti rats.

Treatment	Number of rats	Severity of arthritis[1]	Weight change (gm)[2]
None	20	0	+32.6 ± 4.2
Adj	31	7.85 ± 0.70	−21.5 ± 3.0
Adj + CsA[3]	32	0	+25.4 ± 2.4
Adj + CsA s.c. (Days −1 to 7)[4]	5	0	+32.2 ± 7.8
Adj + CsA s.c. (Days −1 to 13)[4]	9	0	+17.3 ± 5.3
Adj + CCA[3]	8	0	+29.5 ± 5.9

[1] Mean ± SEM scored 14 days after inoculating adjuvant in tail base. The total score per rat was calculated by tallying the score for each rear paw (maximum 4 each) and front paw (maximum 3 each). [2] The mean ± standard error of the weight change over 14 days. [3] 40 mg/Kg CsA or CCA admixed with the adjuvant injected in the tail. [4] 10 mg/Kg CsA injected subcutaneously every second day.

Table 2. Yield and uptake of ^{3}H-thymidine of cells isolated from the popliteal lymph nodes on day 14 when arthritic disease is expressed.

Treatment	Yield of cells $\times 10^5$ per node	Uptake of ^{3}H-thymidine (dpm/10^5 cells)
Normal	7.1 ± 0.1[1]	346 ± 58
Adjuvant alone	23.4 ± 5.8**	9.800 ± 987**
Adjuvant + CsA	25.6 ± 7.3**	12.800 ± 1.270**
Adjuvant + CCA	32.7 ± 8.1**	11.600 ± 1.380**

[1] Mean ± SEM of at least 6 experiments. ** $p < 0.005$ when compared to normal (non arthritic) rats.

production by 50%. IFN-γ and IL-2 production by Con A-stimulated PLN and PBM cells were particularly sensitive to CsA treatment, with ED_{50}'s of about 10 nM or less. Con A stimulated TNF production by PLN and PBM cells and this was also inhibited by CsA in vitro (EDF_{50}'s < 100 nM). By contrast, IL-6 production was only inhibited by relatively high concentrations of the drug (ED_{50}'s > 1 μM). Production of IL-1 by LPS-stimulated PBM cells was inhibited at moderate concentrations of CsA (ED_{50} = 147 nM), but TNF and IL-6 production were only affected by much higher concentrations of CsA (> 1 μM). Cytokine production by AP cells was resistant to CsA at the concentrations tested. IL-6 production was also unaffected by CsA. We found that CsA was toxic to these cells only at concentrations greater than 10 mM as assessed by trypan blue exclusion.

PGE_2 production by PBM and AP cells was inhibited by CsA treatment (Table 5). Con A-stimulated PBM cells were most sensitive to the effects of CsA. Per cell, LPS stimulated PBM cells produced approximately 4 times more PGE_2 than Con A-stimulated PBM cells and 10 fold more than LPS stimulated AP cells.

At the concentrations tested CCA had little effect on any of the mediators tested with all cell types.

We found that CsA and CCA did not have any direct effect on the biological or immunological assays used to measure the mediators.

Discussion

The complete prevention of adjuvant induced arthritis by CsA and CCA suggests that both drugs affect key processes involved in the initiation of arthritis. CsA need not be given in close juxtaposition to the arthritogen, and its antiarthritic action is unlikely to involve the interaction and modification of the mycobacterial antigen(s) [27] thought to initiate the disease. CsA's effects are likely to be due to its effect on leucocytes involved in the generation of the arthritis. Although adjuvant-induced arthritis in rats and rheumatoid arthritis in humans are not identical, they share many clinical and pathological features. Both diseases are examples of immune mediated chronic inflammation in which T lymphocytes play a central role in both establishing and sustaining chronic inflammation [5, 28] and, the adjuvant arthritis model in rats has proved a reliable model for the evaluation of drugs used in rheumatoid arthritis.

The effect on lymph node cell populations

The effectiveness of CsA and CCA was not due to a general inhibition of lymphocyte activation by the processed mycobacterial antigen(s). The enlargement of the regional lymph nodes draining the hind feet of rats with active disease was also seen in successfully treated rats. This failure of CsA and CCA to reduced PLN cell proliferation during the stages of disease expression was somewhat surprising since CsA [29, 30] and CCA [7] are thought to inhibit events underlying lymphocyte proliferation. The increased numbers of cells appeared to be due to B cell proliferation. While these findings are also consistent with reports of enhanced B cell responses in CsA treated mice [31] and elevated IgG levels in CsA-immunosuppressed transplant recipients

Table 3. Popliteal lymph node (draining the rear feet) cell subpopulations 14 days after mycobacterial adjuvant injection (Adj) ± cyclosporin (CsA) or lobenzarit (CCA) in rats.

Antibody	Normal (no Adj)	Adj	Adj + CsA in tail	Adj + CsA s.c. day 1 to 7	Adj + CCA in tail
OX6	20.6 ± 1.3[1]	28.3 ± 1.9**	36.4 ± 2.1**	34.4 ± 1.3*	36.5 ± 2.0**
MHC Class II^+	(14)[2]	(23)	(23)	(4)	(4)
OX6	27.5 ± 2.8	24.4 ± 1.8	26.1 ± 1.1	25.3 ± 3.7	25.4 ± 2.3
$CD8^+$	(7)	(14)	(14)	(4)	(4)
W3/25	62.4 ± 0.8	56.9 ± 1.9*	46.3 ± 2.0**	49.2 ± 1.2*	50.3 ± 1.6*
$CD4^+$	(11)	(15)	(14)	(4)	(4)
Mark I	15.4 ± 1.2	13.3 ± 1.6	24.8 ± 2.7**	26.9 ± 2.2**	26.9 ± 1.7**
B cells	(7)	(6)	(8)	(4)	(4)
R73	72.7 ± 1.3	70.8 ± 1.1	59.6 ± 1.8**	66.2 ± 1.5	65.2 ± 1.7*
α/β T cells	(7)	(8)	(8)	(4)	(4)

[1] Percentage of cells staining positive with the monoclonal antibody ± SEM as determined by fluorescent activated cells sorting analysis.
[2] Number of animals per group. * and ** significance is $p < 0.05$ and $p < 0.005$ respectively. for (i) Adj (no treatment) animals compared to normal animals and (ii) Adj + CsA treated animals compared to Adj (adjuvant alone injected) animals.

Table 4. Cytokine production by lymph node cells ex vivo during the early stages of disease initiation (day 1) and when arthritic diseases is expressed (day 14). Cells isolated from the popliteal nodes of Dark Agouti rats with or without mycobacterial adjuvant (Ad) ± Cyclosporin A (CsA) or Lobenzarit (CCA).

	Stimulus					
	Con A			LPS		
Cytokine[1]	IFN-γ	IL-2	TNF	IL-6	IL-1	IL-6
Group						
Normal	3.81 ± 1.13	35.5 ± 8.1	92.5 ± 1.8	59 ± 10	4.8 ± 2.3	63 ± 22
Day 1[2]						
Adj	24.3 ± 5.0	86.0 ± 16.6	51.0 ± 16.7	15600 ± 8560	2.3 ± 1.3	482 ± 212
Adj/CsA	6.75 ± 0.78*	27.8 ± 10.4*	27.0 ± 7.3	25300 ± 3520	< 2	298 ± 138
Adj/CCA	10.91 ± 5.7*	31.1 ± 17.9*	30.8 ± 10.8	20700 ± 3790	< 2	598 ± 289
Day 14						
Adj	5.58 ± 1.39	50.0 ± 0.9	10.9 ± 2.87	1450 ± 280	13.6 ± 0.86	669 ± 71
Adj/CsA	3.60 ± 0.20	42.0 ± 14.1	5.53 ± 2.75	3660 ± 460*	3.59 ± 1.23*	3180 ± 727*
Adj/CCA	5.09 ± 1.91	38.9 ± 10.7	5.81 ± 1.91*	4410 ± 380*	< 2*	3520 + 189*

[1] ng/10^5 cells of IFNγ measured in a immunoassay and units/ml per 10^6 cells biological activity IL-2, IL-1, TNF, IL-6 measured using CTLL, A375, L929, or 7TD1 cells respectively. Mean ± SEM of at least 4 animals. [2] Post-injection with adjuvant. * $p < 0.05$ compared to Adj treated control. Levels of cytokines released following 48 h stimulation of 2×10^6 popliteal lymph node cells with 5 ug/ml of either Con A or lipopolysaccharide (LPS).

[32], CCA has been reported to directly inhibit B cell responses [11].

CsA and CCA may no longer be present in amounts sufficient to influence cell functions at the time of PLN proliferation (day 14). The therapeutic effects of both drugs may occur during the stage of disease initiation and rely on the suppression of IL-2 and IFN-γ that was observed one day following adjuvant injection. The suppression of these cytokines would explain the substantial reduction in the proportion of cells which are αβ T lymphocytes and express CD4 antigen. The functioning of this cell sub population seems to be essential for disease expression since treatment of experimental arthritis with anti-CD4 mAB also suppresses the disease [28, 33].

The effects on inflammatory mediator production

The reduction in the ability of PLN cells to produce IL-2 and IFN-γ suggests that node lymphocytes of the T helper type 1 class [34] are preferentially affected by CsA treatment. It also shows that these T helper type 1 responses are crucial for initiating and possible maintaining adjuvant arthritis. We have no complementary data relating to the production of those cytokines (IL-4, IL-5, IL-10) that characterise the T helper type 2 cells which promote humoral immunity to determine if they are similarly affected. The elevated B cell proliferation and IL-6 production observed on day 14 might suggest that this cell population is unaffected by both drugs.

The effects of CsA treatment on cytokine production by PLN cells during the early phase of disease initiation (day 1) were consistent with the effect of CsA on these rat cells seen in vitro. Inhibition of IL-2 and IFN-γ but not IL-6 production by CsA is the same as that reported using human PBM cells in vitro [35]. Overall, our in vitro findings indicate that in contrast to IL-2, TNF and IFN-γ production, IL-6 production by all types of cells tested is relatively unaffected by CsA. CsA's lack of inhibition of IL-6 contrasts to recent reports indicating that CsA

Table 5. The differential effects of CsA and CCA in vitro on the production of IL-1, IL-2, IL-6, TNF, IFNγ and PGE_2 by rat popliteal lymph node (PLN) cells, peripheral blood mononuclear (PBM) cells and adherent peritoneal (AP) cells stimulated with 5 ug/ml Con A or LPS for 24 h.

	Cell type (Stimulus)			
Mediator[1]	PLN (Con A)	PBM (Con A)	PBM (LPS)	AP (LPS)
CsA				
IL-2	5.8 ± 3.5[2]	8.1 ± 5.7	NM[3]	NM
IL-1	NM	NM	147 ± 57	3,300 ± 900
IL-6	6,930 ± 520	1,590 ± 530	7,130 ± 133	1,030 ± 120
TNF	85 ± 12	19 ± 7.8	411 ± 117	1,350 ± 521
IFNγ	11.2 ± 2.2	5.9 ± 1.1	NM	NM
PGE_2	NM	9.9 ± 4.1	2,930 ± 250	> 10,000
CCA				
For IL-2, IL-1, IL-6 and TNF	> 100,000	> 100,000	> 100,000	> 100,000

[1] The release of mediators into the supernatant was measured using the appropriate biological or immunological assay (see methods). [2] The concentration (ED_{50}) in nanomoles per litre of drug which reduced the activity of mediator present in supernatant after 24 h by 50%. Each value represents the mean ± SEM from at least 4 experiments. [3] NM = the level produced was too low to accurately calculate an ED_{50} value (less than 2 units/ml biological activity, 1 unit (0.2 ng)/ml IFNγ or 2 picoMolar PGE_2).

reduced IL-6 levels in the serum [36] and synovial fluid [37, 38] in arthritic rats. While serum and synovial levels may be good indicators of inflammatory disease activity this study is concerned with IL-6 production by isolated lymphoid cells that may act within the lymph nodes draining sites of chronic inflammation. IL-6 production in a variety of other tissues is stimulated by the other inflammatory cytokines (eg IL-2, IFNγ, TNF) elevated during adjuvant arthritis. The reduction in IL-6 others have reported may be due to CsA's inhibition of these cytokines rather than a direct effect on IL-6 itself.

The inhibition of in vitro TNF production by CsA was variable. The method we used for assaying TNF production will detect both TNF-α (mainly produced by macrophages and monocytes) and TNF-β (mainly produced by lymphocytes). Con A is primarily a stimulator of lymphocytes and of TNF-β production, whereas LPS, a stimulator of monocytes and macrophages, should preferentially stimulate TNF-α production. We found that Con A-stimulated TNF production was more sensitive to CsA than the LPS-stimulated TNF production. This probably indicates that CsA more effectively inhibits TNF-β, rather than TNF-α, production.

The pattern of inhibition of PGE_2 production by CsA was similar to that seen for TNF indicating that PGE_2 production by lymphocytes (ConA stimulated PBM) was more sensitive to CsA than PGE_2 production by monocytes and macrophages (LPS stimulated PBM and AP). The importance of inhibiting PGE_2 production by lymphoid cells in disease is uncertain since Con A stimulated PBM produced far less PGE_2 per cell than LPS stimulated PBM and AP. This is consistent with the report that CsA had little effect on production of this eicosanoid in patients with rheumatoid arthritis [39].

Our in vitro data shows that CsA has little direct effect on the production of IL-1, TNF and IL-6 by peritoneal macrophages. However, IFN-γ is a stimulator of macrophage function, including IL-1, TNF and IL-6 production [40]. thus, its inhibition by CsA [41] and CCA may result in a decrease in the responsiveness of these cells in vivo. This indirect inhibition of macrophage functions, for example, the reduction of lymphocyte-derived IFN-γ observed on day 1, may be a major disease-suppressing effect of CsA.

In contrast to CsA, we found that CCA had little effect on the production of any of the cytokines in vitro. Other reports indicate that CCA will not inhibit production of IL-6 [42] and, there is conflicting evidence on its ability to inhibit IL-2 [7, 11] and IL-1 production [9, 43]. If the implications of our in vitro findings are correct, then either CCA is a pro-drug or its effects are indirectly due to other important effects on the immune system [44].

This study has identified crucial processes in the initiation of chronic inflammation that are sensitive to the actions of CsA and CCA. These drugs appear to selectively inhibit key activities of lymphocytes which modify rather than completely suppress the immune response to the arthritic adjuvant.

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