Clin Exp Immunol 1999; **116**:410–414

Characterization of phenotype and cytokine profiles of T cell lines derived from vitreous humour in ocular inflammation in man

M. MUHAYA, V. L. CALDER, H. M. A. TOWLER, G. JOLLY, M. MCLAUCHLAN & S. LIGHTMAN *Department of Clinical Ophthalmology, Institute of Ophthalmology, University College London, London, UK*

(Accepted for publication 23 February 1999)

SUMMARY

Intermediate uveitis (IU) and Fuchs' heterochromic cyclitis (FHC) are two chronic ocular inflammatory disorders. They differ considerably in ocular morbidity, which is higher in IU. T cell lines were derived from the vitreous humour (VH) and peripheral blood (PB) of 10 patients with IU and four patients with FHC. There was a predominance of $CD8^+$ in all the lines. However, there was a significantly higher percentage of $CD4^+$ T cells in the T cell lines derived from VH of IU ($32{\cdot}0 \pm 8{\cdot}6\%$) compared with FHC patients ($19{\cdot}2 \pm 8{\cdot}9\%$) ($P = 0{\cdot}04$). The VH-derived T cell lines (VDTC) produced significantly higher levels of IL-2, interferon-gamma (IFN-γ) and IL-10, but not IL-4, compared with PB-derived T cell lines (PBDTC) in both entities. There was significantly higher IL-2 production by VDTC from IU when compared with FHC patients (1810 ± 220 pg/ml *versus* 518 ± 94 pg/ml; $P = 0{\cdot}009$), which could account for the more aggressive clinical features of this condition. In contrast IL-10 production was significantly higher by the VDTC from FHC compared with IU patients. The high IL-10 production by T cells infiltrating VH of FHC patients could down-regulate the inflammatory responses, thereby contributing to the benign clinical course seen in these patients. The accumulation of T cells with differing cytokine profiles in the VH suggests an important role for these cytokines in the pathogenesis of these chronic uveitides.

Keywords cytokines vitreous humour T cells uveitis IL-10

INTRODUCTION

Intermediate uveitis (IU) and Fuchs' heterochromic iridocyclitis (FHC) are two chronic uveitic entities. IU refers to intra-ocular inflammation involving predominantly the vitreous humour (VH) and the peripheral retina. FHC, as the name implies, involves inflammation of the iris and ciliary body. Most patients with IU present with blurred vision and/or floaters as a result of vitritis, i.e. cellular infiltration of the VH and retinal oedema. In contrast, FHC may initially go unnoticed by the patient, who may present to the ophthalmologist several years later with the complications of chronic inflammation such as cataract or glaucoma.

The VH is infiltrated by inflammatory cells, particularly T cells, in both IU and FHC [1,2]. There are significant differences in the clinical course between FHC and IU in that retinal oedema, particularly in the macula area which is a major cause of visual loss in uveitis, occurs frequently in IU [3–5] but is rare in FHC, occurring only after cataract surgery [6,7]. FHC is therefore a benign chronic uveitis in contrast to IU, which is a disease with a higher ocular morbidity. An additional intriguing difference is the vitritis, which responds well to steroids in IU whereas these have no effect in FHC.

It has been widely accepted that T cells play a predominant role in uveitis [8–10]. We have demonstrated that the predominant cellular phenotypes in the aqueous humour (AH) of 10 patients with FHC was $CD8^+$ T cells with few B cells present [11]. In contrast, the predominant phenotype in the AH of patients with IAU, a more aggressive anterior uveitis, was $CD4^+$ T cells.

The classification of T helper cells into Th1 and Th2 based on their cytokine production patterns is well established [12–16]. More recently, cells other than $CD4^+$ T cells, including $CD8^+$ T cells, monocytes, natural killer (NK) cells, B cells, eosinophils, mast cells, basophils and other cells, have been shown to be capable of producing Th1-associated (type 1) and Th2-associated (type 2) cytokines [17]. Type 1 cytokines include IL-2, interferon-gamma (IFN-γ), IL-12 and tumour necrosis factor-alpha (TNF-α), while type 2 include IL-4, IL-5, IL-10, and IL-13. In humans, however, it has been shown that IL-10 is produced by both Th1 and Th2-type cells [18]. In general, type 1 cytokines favour the development of cell-mediated immunity, whereas type 2 cytokines favour a humoral immune response.

Correspondence: Professor Susan Lightman, Department of Clinical Ophthalmology, Institute of Ophthalmology, University College London, Bath Street, London EC1V 9EL, UK.

Some of the T cells infiltrating the VH in both FHC and IU are activated and are likely to be, at least in part, responsible for the pathological findings in the ocular structures. The importance of the VH in the pathology of IU is demonstrated by the fact that vitrectomy performed for complications of IU has been advocated as potentially having an anti-inflammatory effect in patients who failed to respond to periocular corticosteroids or cryotherapy [19].

Since T cells mediate their effects via cytokines it is highly relevant to determine which cytokines these cells produce. The number of cells obtained from VH samples is usually very small, making characterization of cellular phenotypes and cytokine profile difficult to undertake. This problem has been largely overcome by establishing mitogen-driven T cell lines. In this study the T cell population from the VH of patients with FHC and IU was isolated and expanded with phytohaemagglutinin (PHA), a T cell mitogen. Cytokine analysis of the VH and the potential production of cytokines by these T cell lines after non-specific stimulation has highlighted the differences in the immunopathological process between these two disease entities. In addition, the cytokine profiles of the fresh VH were determined.

PATIENTS AND METHODS

Patients

Four FHC (age 35–45 years, mean age 38 years) and 10 idiopathic IU patients (age 31–68 years, mean age 48 years), including two patients with pars planitis, a subtype of IU, were identified for the study. The indication for vitrectomy in the FHC patients was troublesome floaters due to the VH debris and in the IU patients ranged from VH biopsy to exclude masquerade syndromes to vitrectomy to control the inflammatory process. None of the IU patients was on disease-modifying drugs at the time of sampling, even though four of them had been treated with systemic steroids previously. None of the FHC patients was ever treated with steroids.

VH samples

Undiluted VH (ranged from 200 μl to 1000 μl) was collected during vitrectomy and was immediately spun at 4°C at 400 ***g*** for 10 min. The supernatants were aliquoted and kept at −70°C for subsequent ELISA. The cell pellets were washed twice with complete human medium comprising RPMI 1640 Dutch modification (buffered for 5% CO_2 and not requiring the addition of HEPES) supplemented with 10% heat-inactivated AB^+ human serum, gentamycin (50 μg/ml), 200 μM L-glutamine, 1 mM sodium pyruvate and 1% non-essential amino acids (all Sigma Chemical Co., Poole, UK).

The cell pellet was then transferred to a 24-well plate (Corning Costar, High Wycombe, UK) containing 1 ml of complete human medium containing 20 U/ml of recombinant IL-2 (Biotest Folex). Phytohaemagglutinin (PHA-P; Sigma) was added at 1 μg/ml and the cells were incubated in humidified 5% CO_2 in air at 37°C. An additional 1 ml of human medium containing IL-2 was added after 3 days. After a week irradiated (30 Gy) autologous feeders were added (1:1 ratio) with PHA and the human medium and IL-2 was replaced every 3 days. This cycle was repeated for all the lines until 2×10^5 cells were obtained. Almost all the samples required four to five restimulations to expand to this number.

PB lymphocytes

Fresh heparinized blood (20 ml) was obtained from the patients simultaneously with the VH samples and the lymphocytes were isolated by density centrifugation using Ficoll–Hypaque [20] (Pharmacia, Aylesbury, UK) and cultured with PHA and IL-2 as for the VH at 2×10^5 per ml. The remaining fresh PB mononuclear cells (PBMC) were frozen in liquid nitrogen for later use as autologous feeder cells. The PB lymphocytes that were kept in culture in each cycle were approx. 2×10^5/ml in 25 ml, making a total of 5 million cells. As the numbers increased with each cycle, the excess cells were removed and frozen in liquid nitrogen. The cell concentration at the end of preparation of the T cell lines was maintained at 2×10^5/ml. The PB lymphocytes were expanded in a similar number of restimulations as the VH lymphocytes. This was to ensure that the PB-derived T cell lines (PBDTC) underwent the same number of cycles and treatment as the VH-derived T cell lines (VDTC).

Measurement of IL-2, IL-4, IL-10 and IFN-γ by ELISA

To determine the ability of the cells to produce cytokines, the cells from VH and PB were stimulated without the addition of IL-2 in two separate wells at 2×10^5 in 2 ml and incubated in humidified 5% CO_2 in air at 37°C for 72 h. Another well containing an equal number of feeder cells alone with similar addition of PHA as a negative control was set up to rule out the possibility of cytokine production by these cells. The supernatants were harvested at 24, 48 and 72 h after stimulation with PHA and subsequently kept at −70°C until analysed by ELISA.

The following cytokines were quantified in the fresh VH supernatant and the cell culture supernatant using commercially prepared sandwich ELISAs (Quantikine, R&D Systems, Oxford, UK) where the samples were assayed in duplicate. The minimal detection levels as determined by the manufacturer for the cytokines were as follows: IL-2 > 7·0 pg/ml; IL-4 > 0·090 pg/ml (Hypersensitive Quantikine); IL-10 > 1·5 pg/ml and IFN-$\gamma \geq$ 3·0 pg/ml.

Phenotypic analysis

After 72 h of restimulating T cells with PHA, the dead autologous feeder cells were removed by centrifuging with Ficoll–Hypaque and the viable cells eluted and subsequently washed with RPMI. The viable cells were then washed twice in PBS containing 0·2% sodium azide (Sigma) and 0·2% bovine serum albumin (BSA; Sigma) and the cell pellets resuspended in 30 μl FACS buffer containing the appropriate directly conjugated antibodies (Becton Dickinson, Oxford, UK). The cells were phenotyped using the following surface markers: $CD3^+$, $CD4^+$ and $CD8^+$. The percentages of $CD4^+$ and $CD8^+$ T cells in the lines were determined by the percentages of CD4 and CD8 staining of the $CD3^+$ cells. Flow cytometry was carried out using the FACScan flow cytometer (Becton Dickinson) equipped with a 15-mW argon laser and filter settings for FITC (530 nm), PE (585 nm), and PerCP emitting in the deep red (> 650 nm) were used. Only live cells were gated for cell size by forward scatter and granularity by side scatter, and significant numbers of dead cells were not seen.

Statistical analysis

The differences between the two patient groups were analysed using the non-parametric Mann–Whitney *U*-test. $P < 0{\cdot}05$ was considered significant.

RESULTS

Cytokine level in fresh VH supernatants

IL-10 was detected in two out of four FHC patients with a mean level of 8·9 ± 15·9 pg/ml (mean ± s.d.) and in five out of 10 IU

Table 1. Level of cytokine secretion by T cell lines from vitreous humour (VH) and peripheral blood (PB) of patients with Fuchs' heterochromic cyclitis (FHC) and intermediate uveitis (IU)

Cytokine level, pg/ml	IL-2		IL-4		IL-10		IFN-γ	
	VH	PB	VH	PB	VH	PB	VH	PB
FHC								
24	1358 ± 793**	293·8 ± 100·5	19·05 ± 19·9	12·92 ± 14·79	107·4 ± 98·4	43·6 ± 25·5	†1367 ± 332**	278·8 ± 87·7
48	518·1 ± 188·5*	114·5 ± 77·5	13·04 ± 14·59	19·2 ± 21·9	†237·2 ± 80·9*	26·6 ± 26·7	947 ± 458*	441 ± 265
72	194·0 ± 156·9*	37·9 ± 28·4	11·86 ± 13·2	12·33 ± 14·0	65·48 ± 12·36*	25·1 ± 12·76	605·8 ± 141·3*	171·3 ± 65
IU								
24	1874 ± 318*	919 ± 744	21·26 ± 12·95	17·06 ± 14·02	55·7 ± 13·4	17·88 ± 16·24	912 ± 303	617 ± 772
48	††1810 ± 622**	453 ± 341	21·76 ± 14·92	14·41 ± 9·37	60·9 ± 36·1	34·4 ± 52·4	951 ± 405	604 ± 671
72	1307 ± 825*	194·9 ± 175·4	16·79 ± 10·69	19·9 ± 12·69	60 ± 29·4*	11·18 ± 9·37	849 ± 378*	345 ± 291

Non-parametric Mann–Whitney test was used to compare the level of cytokine secretion by T cell lines (i) between the VH and PB in each patient group (*) and (ii) in the VH between the two patient groups (†).

*$P < 0·05$; **$P < 0·01$.

†$P < 0·05$; ††$P < 0·01$.

patients (22·3 ± 48·0 pg/ml). There was no significant difference in the IL-10 levels between FHC and IU patients. IL-2, IL-4 and IFN-γ were not detected in the VH supernatants in either patient group.

T cell line phenotypes

There was a predominance of $CD8^+$ T cells in all the lines. Interestingly the percentage of $CD8^+$ T cells was significantly higher in the cultures derived from VH of FHC (77·8 ± 9·2) patients compared with those derived from VH of IU (64·6 ± 8·3) patients ($P = 0·04$). In contrast, there was a significantly higher percentage of $CD4^+$ T cells in the VH derived from IU (32·0 ± 8·6) compared with FHC (19·2 ± 8·9) ($P = 0·04$).

Cytokines in culture supernatants in T cell lines from VH and PB following non-specific stimulation with PHA

All the T cell lines derived from the VH of both groups of patients produced significantly higher levels of IL-2, IL-10 and IFN-γ compared with PBDTC. In contrast, there was no selective increase in IL-4 production by VDTC compared with PBDTC, where IL-4 was detected at relatively low levels (< 40 pg/ml) in all the lines.

The IL-2 level was significantly higher in the VDTC from IU when compared with FHC patients at 48 h (1810 ± 220 pg/ml *versus* 518·1 ± 94·2 pg/ml; $P = 0·009$) (Table 1). The level of IL-10 was higher in the VDTC compared with PBDTC at 48 h ($P = 0·03$) and 72 h ($P = 0·04$) in FHC and at 72 h in IU ($P = 0·04$). The level of IL-10 was highest at 48 h after stimulation in both FHC and IU, being significantly higher in the VH lines in FHC (237·2 ± 80·9 pg/ml) compared with IU lines (60·9 ± 36·1 pg/ml) ($P = 0·03$).

DISCUSSION

In this study we have quantified the cytokines present in the fresh VH reflecting the *in vivo* environment and the *ex vivo* cytokine production by T cell lines derived from the VH and the PB of patients with FHC and IU. The comparison between these two situations is important, as *in vivo* the T cells are influenced by multiple factors affecting their effector functions and analysis of the *in vivo* sample is a snapshot of only one time point.

In the fresh VH supernatants, IL-10, an immunoregulatory cytokine mainly produced by monocytes and T cells and known to inhibit the expression of inflammatory and haematopoietic cytokines as well as its own expression [21,22], was detected in only half the number of patients in each group. In addition no IL-2, IL-4 or IFN-γ were detected. The failure to detect these cytokines may be due to the fact that VH was taken at one time point from patients whose eyes were stable clinically.

The predominant T cell phenotype after prolonged culture with multiple passages of expansion with PHA of all the lines was predominantly $CD3^+CD8^+$. However, the percentage of $CD4^+$ was significantly higher in the VDTC from IU than those from FHC patients. The PB-derived T lymphocytes, which contain more $CD4^+$ T cells at the initial phase, however, were found to have higher percentages of $CD8^+$ T cells after longer periods of culture. This suggests either that the culture conditions were preferential for $CD8^+$ T cells over $CD4^+$ T cells, or that the $CD8^+$ T cells were more responsive to PHA compared with $CD4^+$ T cells.

An interesting finding is the selective high production by VDTC compared with the PBDTC of all the cytokines except IL-4 in both patient groups. The profoundly higher level of cytokine production by a similar number of cells from the VH compared with PB suggests a larger proportion of cytokine-producing cells localized in the VH. The low level of IL-4 production by both the VH-derived and PBDTC suggests little role if any for this cytokine in the pathogenesis of ocular inflammation in either FHC or IU.

Interestingly, in this study the VDTC were capable of producing significantly increased levels of type 1 cytokines IFN-γ and IL-2, which were not detected in any of the fresh VH supernatants. This could be due to the presence within the VH of inhibitory factors such as transforming growth factor-beta (TGF-β) [23,24], alpha-melanocyte-stimulating hormone (α-MSH) [25] and neuropeptides [26], which are immunosuppressive in nature. In the absence of these factors, *ex vivo*, the ability to produce these cytokines was revealed. Circumstantial evidence for the presence of inhibitory factors in the vitreous is our finding of poor initial stimulation of the T cells when the cell pellets were not thoroughly washed with medium before being put into culture.

As the production of IL-2 is an important determinant of the

magnitude of the T cell-dependent response [27], the higher production of IL-2 by VDTC from IU patients may be consistent with the more aggressive clinical course of IU patients compared with FHC patients. As IL-2 is produced by activated T cells, it may suggest that the T cells isolated from IU patients are initially more activated compared with T cells isolated from FHC patients. However, due to low numbers of cells, we were unable to phenotype the cells prior to culture. After being in tissue culture for several weeks, expression of T cell activation markers such as CD25 in these lines was likely to be very high and would not therefore reflect the true initial state of activation of these T cells. Previous flow cytometric studies in which intraocular T cells from AH and VH have been examined for expression of activation markers have reported increased levels of T cell activation [11,28].

We suggest that activated T cells play a predominant role in the pathogenesis of IU based on these data. This is a significant finding, as the inflammation in IU responds well to steroids, which are known to block the expression and action of most cytokines. Moreover, cyclosporin A, which prevents transcription of IL-2 and other cytokines at the cellular level [29], is usually effective in controlling the inflammation in IU [30–32].

The source of IL-2 could be from either $CD4^+$ or $CD8^+$ T cells. However, using intracellular staining it was reported that $CD4^+$ T cells are the major producers of IL-2 compared with $CD8^+$ T cells [33]. The percentage of $CD4^+$ T cells was significantly higher in the T cell lines derived from the VH of IU patients.

IL-10 is known to be an important down-regulator of macrophage functions and suppresses production of proinflammatory cytokines by activated monocytes and macrophages [34,35]. The significantly higher level of IL-10 in the T cell culture supernatant from VH in FHC patients compared with culture supernatants of IU patients may reflect similar *in vivo* behaviour of these cells. IL-10 has also been shown to contribute to ocular immune privilege [36]. Interestingly, it has been detected at a significantly lower level in the AH of uveitis patients compared with those with no intraocular inflammation (Muhaya *et al*. unpublished data). Further evidence for a down-regulatory role for IL-10 is suggested by a recent study in which it was detected at a significantly higher level in the AH of FHC compared with idiopathic anterior uveitis (IAU), another form of anterior uveitis with a more aggressive clinical picture [11].

The important role of IL-10 in FHC, as suggested by these results, may explain the steroid non-responsiveness of uveitis in FHC. Steroids have been shown to increase IL-10 protein and mRNA expression in the serum in multiple sclerosis patients with acute relapse by Gayo *et al*. (unpublished data). In addition, *ex vivo* steroids were shown to increase IL-10 secretion, increase intracellular IL-10 mean fluorescent intensity and the percentage of IL-10-expressing cells. These data suggest that the beneficial effect of steroids in the control of disease activity may be due to their capacity to increase the spontaneous expression of IL-10. Hence in FHC, as IL-10 is already produced at a relatively high level, the further immunosuppressive effects of steroid are not apparent.

In conclusion, the T cells infiltrating the VH in FHC and IU patients and their effector cytokines are important in the pathogenesis of intraocular inflammation. IL-10, a down-regulatory cytokine, was produced in higher quantity by the T cells derived from VH patients with FHC, a benign form of uveitis. Its therapeutic benefits have been shown in several clinical situations such as rheumatoid arthritis [37], Crohn's disease [38] and inflammatory bowel disease [39,40]. Hence the information obtained from this study is important in planning future therapeutic strategies for intraocular inflammation. It may be possible in the near future to use slow release devices already available for intraocular use [41] to allow chronic administration of IL-10 into the eye, thus avoiding the well known side-effects of steroids that are the mainstay of treatment for intraocular inflammation.

REFERENCES

1 Nolle B, Eckardt C. Cellular phenotype of vitreous cells in intermediate uveitis. Dev Ophthalmol 1992; **23**:145–9.
2 Davis J, Solomon D, Nussenblatt R. Immuncytohistochemical staining of vitreous cells: indications, techniques, and results. Ophthamol 1992; **99**:250–6.
3 Smith R, Godfrey W. Complications of chronic cyclitis. Am J Ophthalmol 1976; **82**:277–82.
4 Welch R, Maumanee A, Whalen A. Peripheral posterior segment inflammation, vitreous opacities and edema of the posterior pole. Arch Ophthalmol 1960; **64**:540–9.
5 Kimura S, Hogan M. Chronic cyclitis. Trans Am Ophthalmol Soc UK 1963; **61**:397–413.
6 Liesegang T. Clinical features and prognosis in Fuchs' uveitis syndrome. Arch Ophthalmol 1982; **100**:1622–6.
7 Jones N. Fuchs' heterochromic uveitis: an update. Surv Ophthalmol 1993; **37**:253–72.
8 Chan C, Mochizuki M, Palestine A *et al*. Kinetics of T-lymphocyte subsets in the eyes of Lewis rats with experimental autoimmune uveitis. Cell Immunol 1985; **96**:430–4.
9 Nusenblatt RB, Scher I. Effects of cyclosporine on T-cell subsets in experimental autoimmune uveitis. Invest Ophthalmol Vis Sci 1985; **26**:10–14.
10 Lightman S, Chan C Immunopathology of ocular inflammatory disorders. In: Lightman S, ed. Immunology of eye diseases. London: Kluwer Academic, 1989:87–89.
11 Muhaya M, Calder V, Towler H, Shaer B, McLauchlan M, Lightman S. Characterisation of T cells and cytokines in the aqueous humour of patients with Fuchs' heterocromic cyclitis and idiopathic anterior uveitis. Clin Exp Immunol 1998; **111**:123–8.
12 Cher D, Mossman T. Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated by Th1 clones. J Immunol 1987; **138**:3688–94.
13 Mosmann T, Coffman R. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989; 7:145–73.
14 Mosmann T, Cherwienski H, Bond M, Giedlin M, Coffman R. Two types of murine helper T cell clones. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986; **136**:2348–57.
15 Romagnani S. Human Th1 and Th2 subsets: doubt no more. Immunol Today 1991; **12**:256–7.
16 Sher A, Coffman R. Regulation of immunity to parasites by T cells and T cell derived cytokines. Annu Rev Immunol 1989; **10**:385–409.
17 Sad S, Marcotte R, Mosmann TR. Cytokine-induced differentiation of precursor mouse CD8+ T cells into cytotoxic CD8+ T cells secreting Th1 or Th2 cytokines. Immunity 1995; **2**:271–9.
18 Del Prete G, De Carli M, Almerigogna F. Human IL-10 is produced by both helper type 1 (Th1) and type 2 (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 1993; **150**:353–60.
19 Mieler WF, Will BR, Lewis H, Aaberg TM. Vitrectomy in the management of peripheral uveitis. Ophthalmol 1988; **95**:859–64.
20 Boyum A. Separation of blood leucocytes' granulocytes and lymphocytes. Tissue Antigens 1974; **4**:269–74.
21 Casatella M, Meda L, Bonora S, Ceska M, Constantin G. Interleukin-10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leucocytes. Evidence for an autocrine role of

tumour necrosis factor and IL-1 in mediating the production of IL-8 triggered by lipopolysaccharide. J Exp Med 1993; **178**:2207–711.
22 Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. Annu Rev Immunol 1993; **11**:165–90.
23 Cousins S, McCabe M, Danielpour D, Streilein J. Identification of transforming growth factor-beta as an immunosuppressive factor in aqueous humour. Invest Ophthalmol Vis Sci 1991; **32**:2201–11.
24 Streilein JW, Wilbanks GA, Cousins SW. Immunoregulatory mechanisms of the eye. J Neuroimmunol 1992; **39**:185–200.
25 Taylor AW, Streilein JW, Cousins SW. Identification of alpha-melanocyte-stimulating-hormone as a possible immunoregulatory factor in aqueous humour. Curr Eye Res 1992; **12**:1199–206.
26 Ferguson T, Fletcher S, Herndon J, Griffith T. Neuropeptides modulate immune deviation induced via the anterior chamber of the eye. J Immunol 1995; **155**:1746–56.
27 Smith K. Interleukin-2: inception, impact and implications. Science 1988; **240**:1169–76.
28 Deschenes J, Char D, Kaleta S. Activated T lymphocytes in uveitis. Br J Ophthalmol 1988; **72**:82–87.
29 Cross S, Halden N, Leonardo M *et al*. Functionally distinct NF-kappa B binding sites in the immunoglobulin kappa and IL-2 receptor alpha chain genes. Science 1989; **244**:466–9.
30 Nussenblatt RB, Palestine AG, Chan CC. Cyclosporin A therapy in the treatment of intraocular inflammatory disease resistant to systemic corticosteroids and cytotoxic agents. Am J Ophthalmol 1983; **96**:275–82.
31 Towler H, Whiting P, Forrester J. Combination low dose cyclosporin A steroid therapy in chronic intracocular inflammation. Eye 1990; **4**:514–20.
32 Lightman S. Uveitis: management. Lancet 1991; **338**:1501–4.
33 Andersson U, Andersson A, Lindfors A, Wagner K, Moller G, Heusser C. Simultaneous production of IL-2, IL-4 and IFN-gamma by activated human blood lymphocytes. Eur J Immunol 1990; **20**:1591–6.
34 Fiorentino D, Zlotnik A, Vieira P, Mossman T, Howard M, Moore K, O'Garra A. IL-10 acts on the antigen presenting cell to inhibit cytokine production by Th1 cells. J Immunol 1991; **146**:3444–51.
35 Enk A, Angeloni V, Udey M, Katz S. Inhibition of Langerhans cell antigen presenting function by IL-10. A role for IL-10 in induction of tolerance. J Immunol 1993; **151**:2390–8.
36 Li XY, D'Orazio LT, Niederkorn JY. Role of Th1 and Th2 cells in anterior chamber-associated immune deviation. Immunol 1996; **89**:34–40.
37 Van Roon J, van Roy J, Gmelig Meyling F, Lafeber F, Bijilsma J. Prevention and reversal of cartilage degradation in rheumatoid arthritis by IL-10 and IL-4. Arthritis Rheum 1996; **39**:829–35.
38 Van Deventer SJ, Elson CO, Fedorak RN. Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. Crohn's Disease Study Group. Gastroenterol 1997; **113**:383–9.
39 Powrie F, Leach M, Mauze S, Menon S, Caddle LB, Coffman RL. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. Immunity 1994; **1**:553–62.
40 Kuhn R, Lohler J, Rennick D, Rajewski K, Muller W. Interleukin-10 deficient mice develop chronic enterocolitis. Cell 1993; **75**:263–74.
41 Martin DF, Parks DJ, Mellow SD *et al.* Treatment of cytomegalovirus retinitis with an intraocular sustained-release ganciclovir implant. A randomized control clinical trial. Arch Ophthalmol 1994; **112**:1531–9.