Clin Exp Immunol 1995; 100:475–479

Increased IL-2, IL-4 and interferon-gamma (IFN-γ) in steroid-sensitive nephrotic syndrome

T. J. NEUHAUS*§, M. WADHWA‡, R. CALLARD† & T. M. BARRATT*§ *Divisions of Clinical Sciences (*Medical Unit) and Cell and Molecular Biology (†Cellular Immunology Unit), Institute of Child Health, London, ‡Division of Immunobiology, National Institute for Biological Standards and Control (NIBSC), Potters Bar, and §Renal Unit, Great Ormond Street Hospital for Children, London, UK*

(Accepted for publication 28 February 1995)

SUMMARY

We investigated the production of cytokines by peripheral blood mononuclear cells (PBMC) and serum cytokine concentrations in children with steroid-sensitive idiopathic nephrotic syndrome (SSNS). PBMC from patients off treatment were collected during remission and relapse and cultured in medium alone or stimulated with calcium ionophore plus phorbol myristate acetate. Control PBMC were taken from healthy age-matched children. IL-2 was measured by bioassay, IL-4 by immunoradiometric assay, and IL-8 and IFN-γ by ELISA. After 24 h culture without stimulation, IL-2, IL-4 and IFN-γ were not detectable in the supernatant in any of the children. After stimulation, the supernatant concentrations of IL-2 (median 172 U/ml at 24 h) and IL-4 (160 pg/ml at 24 h; 210 pg/ml at 72 h) were significantly increased in relapse compared with remission (IL-2 37 U/ml; IL-4 65 pg/ml and 60 pg/ml) and controls (IL-2 69 U/ml; IL-4 40 pg/ml and 40 pg/ml) ($P < 0{\cdot}05$). The concentration of IFN-γ was not significantly increased in relapse compared with remission and controls (600, 325, and 145 U/ml, respectively, at 72 h). IL-8 concentrations were similar in relapse, remission and controls with stimulation (median 32, 40 and 40 ng/ml, respectively) and without (30, 17 and 10 ng/ml). IL-2 was not detectable in serum, but IL-4, IL-8 and IFN-γ were measurable in about half the patients, both in relapse and remission, though were virtually undetectable in controls. We conclude that relapse of SSNS in children is associated with T lymphocyte activation with release of IL-2, IL-4 and IFN-γ.

Keywords steroid-sensitive nephrotic syndrome children IL-2 IL-4 interferon-gamma

INTRODUCTION

Most children with primary nephrotic syndrome respond to corticosteroid treatment (steroid-sensitive nephrotic syndrome (SSNS)) and have minimal-change histology (MCNS) on biopsy [1]. The disorder is believed to be a consequence of T lymphocyte dysfunction although, by definition, cellular glomerular infiltrates are not present. Relapses are often triggered by a viral upper respiratory tract infection or an allergic event, consistent with the hypothesis of an immune pathogenesis. Several *in vivo* and *in vitro* immunological abnormalities have been associated with SSNS [2]. Furthermore, measles infection, which is known to inhibit T lymphocyte function [3], is associated with the induction of remission [4].

T lymphocyte activation has been observed in SSNS using MoAbs against T lymphocyte surface markers [5–7]. Experimental data suggest that proteinuria in SSNS is mediated by a circulating factor(s) produced either by activated peripheral blood mononuclear cells (PBMC) or T lymphocytes [8–11]. Further evidence of an immune pathogenesis of SSNS arises from the clinical response to immunosuppressive treatment, and the increased incidence of atopy in affected children [12,13].

These findings suggest a role for cytokines, secreted by activated T lymphocytes or monocytes, in the pathogenesis of SSNS. So far only a few studies of cytokines in children with SSNS have been reported, comparing children in relapse with normal controls. IL-1 and IL-2 in supernatants of phytohaemagglutinin (PHA)-stimulated PBMC were reported to be increased in relapse of MCNS [14]. Concentrations of IL-4 and IL-8 were found to be elevated in serum [15,16]. In urine, tumour necrosis factor-alpha (TNF-α) did not differ between relapse of SSNS and controls [17]. However, a comparative analysis of cytokine concentrations in children in remission with those in relapse has not been reported.

In this study we have investigated the *in vitro* production by PBMC and serum concentrations of IL-2, IL-4, IL-8 and

Correspondence: Professor T. M. Barratt, Medical Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.

© 1995 Blackwell Science

IFN-γ in children with SSNS off treatment, comparing children in relapse with those in remission.

PATIENTS AND METHODS

Patients

To study the *in vitro* production of cytokines by PBMC, eight children with SSNS were investigated in remission (seven males, one female; median age 11·5 years, range 6–16 years); four had undergone renal biopsy and all showed MCNS. Another seven children with SSNS were examined in early relapse (five males, two females; median age 10·5 years, range 9–17 years); the five that had had a biopsy all showed MCNS. In addition, nine healthy controls (two males, seven females; median age 14·2 years, range 6·5–19·5 years) were studied. There were no significant differences in age between the three groups.

For cytokine measurement in serum, 15 further children with SSNS (12 males, three females; median age 11·5 years, range 6·5–17 years) were investigated in remission and subsequent early relapse (median interval 4 weeks). Seven had MCNS on biopsy. Twelve further age-matched healthy controls (six males, six females; median age 12·5 years, range 10–15 years) were examined.

At the time of the investigation the patients were off immunosuppressive treatment, which is known to inhibit cytokine production [18–21]. Children with a raised temperature or overt clinical infection were excluded.

All patients had been in remission for at least 1 month, defined as negative or trace protein on urine dipstick testing and urine albumin/creatinine ratio in the normal range ($< 0{\cdot}1$ mg/mg). Relapse was defined as 2 + on dipstick for 2 days and a urine albumin/creatinine ratio $\geqslant 1{\cdot}0$ mg/mg. Patients were examined early in a relapse (day 3 or 4 after onset of proteinuria) to disentangle primary immunological effects from secondary consequences of the nephrotic state itself.

The study was approved by the local Ethical Committee.

Collection of serum and in vitro *cytokine production*

Serum. Blood (10 ml) was collected from a cubital vein, centrifuged and the serum immediately stored at −70°C until required.

Supernatant. Blood (20 ml) was collected in sterile tubes containing 400 U preservative-free heparin. The blood was diluted with an equal amount of RPMI 1640 (Gibco, Life Technologies, Paisley, UK), containing glutamine (2 mM), Hepes (25 mM), gentamicin (50 μg/ml) and 5% heat-inactivated fetal calf serum (FCS). Autologous serum was not used. PBMC were isolated over Ficoll–Paque (Pharmacia, Milton Keynes, UK) and washed three times. The viability of the cells was checked by trypan blue. The cells were resuspended at 1×10^6 cells/ml in RPMI 1640, supplemented with 2 mM of extra glutamine and 10% FCS. Cells were then cultured in medium alone or with 0·1 μg/ml of A23187 calcium ionophore (Sigma, Poole, UK) plus 10 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma) in flat-bottomed wells (2 ml/well) at 37°C in a humidified atmosphere of 5% CO_2. Culture supernatants were collected after 24 and 72 h and immediately stored at −70°C.

Cytokine assays

IL-2 was measured using a bioassay based on the IL-2-dependent mouse cytotoxic lymphocyte cell line CTLL-2 [22]. Briefly, duplicate samples were extensively diluted (so that the proliferation of CTLL-2 cells was unaffected by calcium ionophore and PMA) in 96-well plates to a final volume of 50 μl per well.

CTLL-2 cells (50 μl, 10^5/ml) were added to each well and incubated at 37°C for 24 h. Tritiated thymidine was then added to each well and after 4 h the cells were harvested onto filter mats. Cell proliferation was evaluated by measuring the radioactivity incorporated into DNA by scintillation counting. A titration of IL-2 standard calibrated against the WHO first international standard for human IL-2 (86/504; NIBSC, Potters Bar, UK) was incubated in each assay. The amount of IL-2 in the samples was calculated using parallel line analysis. The assay sensitivity was 0·1 U/ml, equivalent to 7·6 pg/ml.

IL-4 was measured using a two-site immunoradiometric assay (IMRA) [23]. The plates were coated with purified MoAb 1A6-10 (10 μg/ml in PBS, 200 μl/well; NIBSC) overnight at 4°C. The wells were washed twice with PBS containing 5% w/v skimmed milk powder (PBS/milk) and the remaining protein binding sites were blocked with PBS/milk for 30 min at room temperature. Samples (200 μl/well, neat or diluted) were incubated at room temperature for 3 h. Titration of the interim reference reagent for IL-4 (89/508; NIBSC) was incubated in each assay. The plates were washed four times with PBS/milk and then incubated with ^{125}I-labelled MoAb 7A3-3 (200 μl/well containing 250 000 ct/min; NIBSC) for 2 h at room temperature. The plates were finally washed five times with PBS/milk and the radioactivity bound to the wells was estimated in a gamma counter. The assay sensitivity was 10 pg/ml.

IL-8 was measured using a two-site ELISA. The plates were coated with purified MoAb DMC-7 (5 μg/ml in PBS, 100 μl/well; Monsanto, St Louis, MO, USA) overnight at 4°C. Non-specific binding sites were blocked with 0·5% bovine serum albumin (BSA) in PBS for 0·5 h at room temperature. Samples (100 μl/well, neat or diluted) were incubated at room temperature for 3 h. Titration of the interim reference reagent for IL-8 (89/520; NIBSC) was incubated in each assay. Bound IL-8 was detected with the biotinylated sheep polyclonal antibody (100 μl/well, 1:1000 diluted in PBS/BSA; NIBSC) and incubated for 2–3 h. Streptavidin-biotinylated horseradish peroxidase (Amersham, Aylesbury, UK; 100 μl/well, diluted 1:5000 in PBS/BSA) was added for 1 h at room temperature. Enzyme substrate (TMB 1 mg/ml, pH 5·0) was added (100 μl/well), the reaction stopped after 5 min with 50 μl of 2 M H_2SO_4 and absorbance read at 450 nm, using an ELISA reader. The sensitivity of the assay was 20 pg/ml.

IFN-γ was measured by a commercially available ELISA (AMS Biotechnology, Whitney, Oxon, UK) [24], using the MoAbs 1-D1K and 7-B6–1, respectively, as the capturing and detecting reagents. A titration of the IFN-γ standard (88/606; NIBSC) was incubated in each assay. The reaction of biotin-streptavidin-alkaline phosphatase was visualized with the substrate p-NPP (1 mg/ml, pH 9·8), and the absorbance read at 405 nm after 30–60 min developing time. The sensitivity of the assay was 0·5 U/ml.

IL-2, secreted early upon stimulation, was measured after 24 h of stimulation, whereas IFN-γ, secreted late, was analysed after 72 h. IL-4 and IL-8 were measured after 24 h and 72 h [25].

Statistical analysis

The primary hypothesis to be tested was that cytokine concentrations in relapse were higher than in remission.

Table 1. Cytokine concentrations in supernatant produced by stimulated peripheral blood mononuclear cells (PBMC)

	Relapse (Rel) $n = 7$	Remission (Rem) $n = 8$	Controls (Con) $n = 9$	ANOVA	*P* value* Rel *versus* Rem	Rel *versus* Con
IL-2 (U/ml)						
24 h	172 (28–240)	37 (< 0·1–144)	69 (2–149)	< 0·05	< 0.05	< 0·05
IL-4 (pg/ml)						
24 h	160 (80–450)	65 (< 10–130)	40 (< 10–170)	< 0·01	< 0·05	< 0·01
72 h	210 (150–900)	60 (< 10–200)	40 (< 10–200)	< 0·01	< 0·05	< 0·01
IL-8 (ng/ml)						
24 h	32 (24–42)	40 (26–50)	40 (22–60)	NS	NS	NS
72 h	38 (26–40)	37 (28–50)	40 (20–60)	NS	NS	NS
IFN-γ (U/ml)						
72 h	600 (135–650)	325 (16–850)	145 (11–700)	NS	NS	NS

Results are median (range in parentheses).
* No significant differences between remission and controls.

The data were expressed as median and analysed by non-parametric tests. For cytokines in supernatants, differences between all three groups were determined using the non-parametric one-way analysis of variance (ANOVA, Kruskal–Wallis). If ANOVA was statistically significant ($P < 0·05$), individual differences were analysed by Wilcoxon rank sum test.

For cytokines in serum, differences between paired samples in remission and relapse were determined using the non-parametric Wilcoxon signed rank test. Comparisons between remission and controls, and relapse and controls were analysed by Wilcoxon rank sum test.

RESULTS

Supernatant

IL-2, IL-4 and IFN-γ were not detectable in any supernatants from PBMC cultured for 24 h without stimulation. IL-8 concentration was slightly greater in relapse (median 30 ng/ml) compared with remission (17 ng/ml) and controls (10 ng/ml), but without reaching statistical significance (ANOVA, $P = 0·10$).

In supernatants from PBMC stimulated with calcium ionophore/PMA, IL-2 and IL-4 production was significantly greater ($P < 0·05$) in relapse compared with remission and controls (Table 1). No difference was found between remission and controls. IFN-γ production was not significantly increased in relapse (ANOVA, $P = 0·11$). IL-8 production was similar in relapse, remission and controls, and the concentrations were slightly greater than without stimulation. One patient was examined in remission and subsequent relapse: IL-2 (8 *versus* 240 U/ml), IL-4 (110 *versus* 450 pg/ml at 24 h; 60 *versus* 900 pg/ml at 72 h) and IFN-γ (300 *versus* 600 U/ml) were markedly increased in relapse, whereas IL-8 was not different (28 *versus* 26 ng/ml at 24 h; 36 *versus* 40 ng/ml at 72 h).

Serum

In SSNS one-third to two-thirds of the patients had detectable concentrations of serum IL-4, IL-8 and IFN-γ (Fig. 1). In normal controls serum concentrations of IL-4, IL-8 and IFN-γ were undetectable except for two children, one of whom had measurable IL-4 and the other IL-8. For each cytokine, there was a statistically significant increase ($P < 0·05$) in relapse and/or remission compared with controls. There was no difference in serum concentrations between relapse and remission for IL-4 and IFN-γ; patients with high concentration in relapse had also high concentrations in remission. In addition, the patients with high IL-4 concentration also had high IFN-γ concentration (both in relapse and remission). Serum IL-8 was significantly greater in relapse ($P < 0·05$). The three patients with detectable IL-8 in remission (140, 300 and 400 pg/ml, respectively) all had higher concentrations in relapse (400, 400 and 1900 pg/ml). In contrast to these cytokines, no IL-2 was detected in serum from either normal controls or SSNS patients.

DISCUSSION

In this study, we have shown that the production of IL-2 and IL-4 by calcium ionophore/PMA-stimulated PBMC was significantly greater in relapse of SSNS than in remission or age-matched controls. In contrast, the production of IFN-γ and IL-8 did not significantly differ between remission, relapse and controls. IL-2, IL-4 and IFN-γ were not detectable in any supernatants of resting PBMC (cultured for 24 h, but not stimulated). The observed increase in IL-2 production is consistent with one study in children with SSNS showing increased IL-2 concentrations in the supernatant of stimulated PBMC in relapse [14].

Activated T lymphocytes are the predominant source of IL-2, IL-4 and IFN-γ [25]. T lymphocytes in early relapse of SSNS were more easily activated upon stimulation, indicating that resting T lymphocytes (or T lymphocyte subsets) are up-regulated. In our series all patients with overt infection were excluded and all were analysed early in relapse to avoid the effects of the nephrotic state itself. Cyclosporin A, an effective treatment of SSNS in children [26], inhibits IL-2, IL-4 and IFN-γ synthesis specifically as well as subsequent activation of T lymphocytes [20]. However, it remains unclear whether this

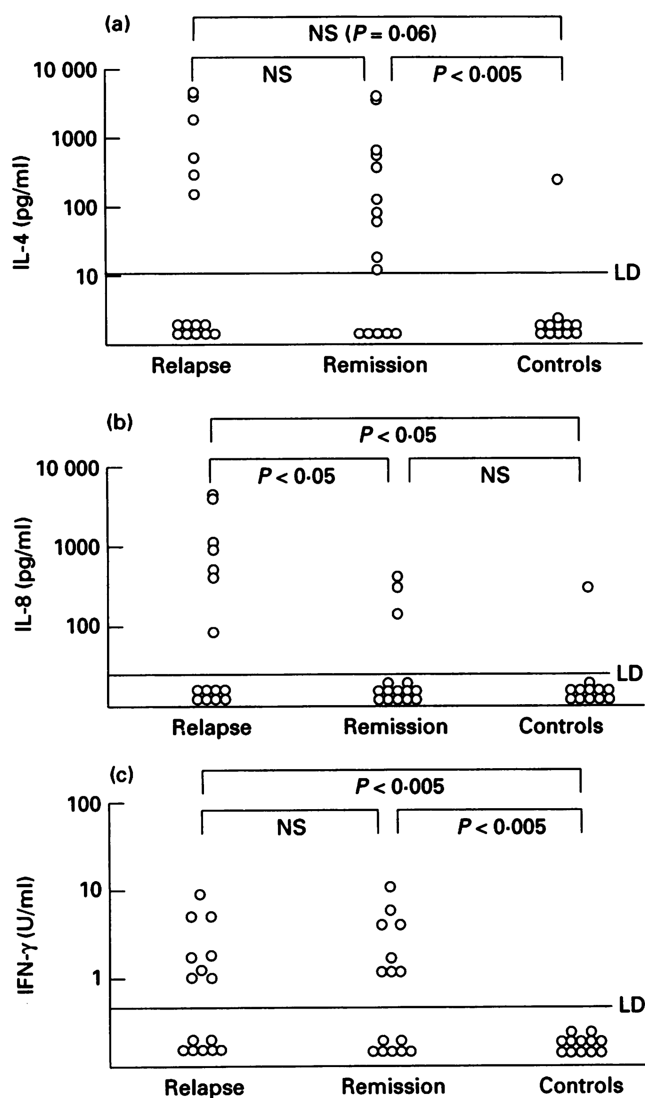

Fig 1. Serum cytokine concentrations. (a) Serum IL-4 concentration. LD, Limit of detection. (b) Serum IL-8 concentration. (c) Serum IFN-γ concentration.

up-regulation and activation of T lymphocytes is a primary event of SSNS. A secondary effect of a clinically unrecognized viral infection or other trigger cannot be ruled out.

Serum IL-2 was not detectable in any of the children, possibly because it had been bound to soluble IL-2 receptor [28]. In addition, despite increased *in vitro* production of IL-4 by stimulated PBMC in relapse *versus* remission, the circulating serum concentrations were not different. The reason for this finding is unclear; similar to IL-2, soluble IL-4 receptor might bound excess circulating IL-4. In contrast, serum IL-8 concentrations were increased in relapse, although IL-8 production by PBMC was not different. IL-8 is not only produced by T lymphocytes [21] and monocytes [27], but also by non-immune cell types including endothelial cells, fibroblasts, mesangial cells and renal tubular cells [29,30]. The clinical significance of raised serum cytokine concentrations in relapse of SSNS remains unclear, as about one half of the patients in relapse had undetectable concentrations of IL-4, IL-8 and IFN-γ. In addition, serum IL-4 and IFN-γ were not different in relapse and remission.

IL-4 is an essential cofactor for IgE production and subsequent allergic reactions [31]. In SSNS, both incidence of atopy [12,13] and serum IgE concentration [13,14,32] are raised. The increased IL-4 production in relapse might, at least in part, account for these findings.

Is there a direct relation between increased production of cytokines and proteinuria? Animal experiments suggest a role for circulating factors produced either by activated PBMC or by T lymphocytes in proteinuria in SSNS/MCNS [8–11]. However, these factors have not been characterized. IL-2 seems to be an unlikely candidate. Despite increased concentration in supernatant, IL-2 was not detectable in serum, and proteinuria is a very rare side effect of pharmacological doses of IL-2 administered to patients with malignancies [33,34].

The effect of IL-4 and IFN-γ on proteinuria in SSNS/MCNS is not known. However, the finding of similar circulating serum concentrations of IL-4 and IFN-γ in remission and subsequent relapse suggests no direct effect either on disease activity or on proteinuria.

Elevated serum concentrations of IL-8 in children with relapse of SSNS have been reported [16]. In the present study, only half of the patients in relapse had elevated IL-8 concentrations in serum, and none in supernatant compared with remission and controls. Increased serum IL-8 concentrations (in a similar range as in SSNS) have been reported in various non-nephrotic conditions, including cystitis and pyelonephritis [35]. IL-8 is produced by normal renal mesangial [29] and cortical tubular [30] cells. However, no local glomerular expression of IL-8 was found by immunohistochemistry in three adults with MCNS [36].

A correlation between diseases and cytokine concentrations, particularly if 'routinely' measured, is difficult to elucidate. The often wide range of cytokine concentrations of normal healthy subjects is difficult to establish; the spuriously high concentrations, particularly evident on use of immunoassays (matrix effects), overlap with the concentrations observed in diseased individuals. Since cytokines usually act locally they may not be detected in serum and other body fluids, as they may not pass into the circulation or if they do, they may be complexed with their soluble receptors or other antagonistic molecules. Many diseases are episodal and it is possible that the cytokine analysis has been performed in the stage of disease which does not truly reflect the pathogenesis or episode-linked disease activity.

We conclude that early relapse of SSNS in children is associated with T lymphocyte activation. However, cytokines of activated T lymphocytes such as IL-2, IL-4, IL-8 and IFN-γ are unlikely to exert a direct effect on proteinuria. The relationship between T lymphocytes and proteinuria is still unclear.

ACKNOWLEDGMENTS

T.J.N. was supported by the Karl Reiser and Heinrich Paur Fund, Zurich, and by the Jubiläumsstiftung Rentenanstalt, Zurich. We thank Paula Dilger (NIBSC) for her technical expertise.

REFERENCES

1 International Study of Kidney Disease in Children. The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. J Pediatr 1981; **98**:561–4.

2 Schnaper HW. The immune system in minimal change nephrotic syndrome. Pediatr Nephrol 1989; **3**:101–10.
3 Joffe MI, Rabson AR. Dissociation of lymphokine production and blastogenesis in children with measles infections. Clin Immunol Immunopathol 1978; **10**:335–43.
4 Blumberg RW, Cassady HA. Effect of measles on the nephrotic syndrome. Am J Dis Child 1947; **73**:151–68.
5 Fiser RT, Arnold WC, Charlton RK *et al.* T-lymphocyte subsets in nephrotic syndrome. Kidney Int 1991; **40**:913–6.
6 Kobayashi K, Yoshikawa N, Nakamura H. T-cell subpopulations in childhood nephrotic syndrome. Clin Nephrol 1994; **41**:253–8.
7 Hulton SA, Shah V, Byrne MR *et al.* Lymphocyte subsets and interleukin 2 receptor expression in childhood nephrotic syndrome. Pediatr Nephrol 1994; **8**:135–9.
8 Maruyama K, Tomizawa S, Shimabukuro N *et al.* Effect of supernatants derived from T lymphocyte culture in minimal change nephrotic syndrome on rat kidney capillaries. Nephron 1989; **51**:73–76.
9 Yoshizawa N, Kusumi Y, Matsumoto K *et al.* Studies of a glomerular permeability factor in patients with minimal-change nephrotic syndrome. Nephron 1989; **51**:370–6.
10 Koyama A, Fujisaki M, Kobayashi M *et al.* A glomerular permeability factor produced by human T-cell hybridomas. Kidney Int 1991; **40**:453–60.
11 Tanaka R, Yoshikawa N, Nakamura H *et al.* Infusion of peripheral blood mononuclear cell products from nephrotic children increases albuminuria in rats. Nephron 1992; **60**:35–41.
12 Meadow SR, Sarsfield JK. Steroid-responsive nephrotic syndrome and allergy: clinical studies. Arch Dis Child 1981; **56**:509–16.
13 Groshong T, Mendelson L, Mendoza S *et al.* Serum IgE in patients with minimal-change nephrotic syndrome. J Pediatr 1973; **83**:767–71.
14 Saxena S, Mittal A, Andal A. Pattern of interleukins in minimal-change nephrotic syndrome of childhood. Nephron 1993; **65**:56–61.
15 Cho BS, Lee CE, Pyun KH. Studies on the role of interleukin-4 and Fc RII in the pathogenesis of minimal change nephrotic syndrome. J Korean Med Sci 1992; **7**:343–8.
16 Garin EH, Blanchard DK, Matsushima K *et al.* IL-8 production by peripheral blood mononuclear cells in nephrotic patients. Kidney Int 1994; **45**:1311–7.
17 Ozen S, Saatci U, Tinaztepe K *et al.* Urinary tumor necrosis factor levels in primary glomerulopathies. Nephron 1994; **66**:291–4.
18 Boumpas DT, Anastassiou ED, Older SA *et al.* Dexamethasone inhibits human interleukin 2 but not interleukin 2 receptor gene expression *in vitro* at the level of nuclear transcription. J Clin Invest 1991; **87**:1739–47.
19 Mori A, Yamamoto K, Dohi M *et al.* Interleukin-4 gene expression in human peripheral blood mononuclear cells. Int Arch Allergy Appl Immunol 1991; **95**:282–4.
20 Emmel EA, Verweij CL, Durand DB *et al.* Cyclosporin A specifically inhibits function of nuclear proteins involved in T cell activation. Science 1989; **246**:1617–20.
21 Zipfel PF, Bialonski A, Skerka C. Induction of members of the IL-8/NAP-1 gene family in human T lymphocytes is suppressed by cyclosporin A. Biochem Biophys Res Commun 1991; **181**:179–83.
22 Wadhwa M, Bird C, Tinker A *et al.* Quantitative biological assays for individual cytokines. In: Balkwill FR, ed. Cytokines: a practical approach. Oxford: IRL Press, 1991:309–30.
23 Bird C, Wadhwa M, Thorpe R. Development of immunoassays for human IL-3 and IL-4, some of which discriminate between different recombinant DNA derived molecules. Cytokine 1991; **3**:562–7.
24 Andersson G, Ekre HPT, Alm G *et al.* Monoclonal antibody two-site ELISA for human IFN-γ. J Immunol Methods 1989; **125**:89–96.
25 Paliard X, De Waal Malefijt R, Yssel H *et al.* Simultaneous production of IL-2, IL-4, and IFN-γ by activated human $CD4^+$ and $CD8^+$ T cell clones. J Immunol 1988; **141**:849–55.
26 Niaudet P, Broyer M, Habib R. Treatment of idiopathic nephrotic syndrome with cyclosporin A in children. Clin Nephrol 1991; **35** (Suppl. 1):31–6.
27 Schrader JM, Mrowietz V, Morita E *et al.* Purification and partial biochemical characterization of a human monocyte-derived, neutrophil-activating peptide that lacks interleukin 1 activity. J Immunol 1987; **139**:3474–83.
28 Rubin LA, Nelson DL. The soluble interleukin-2 receptor: biology, function and clinical application. Ann Intern Med 1990; **113**:619–27.
29 Kusner DJ, Luebbers EL, Nowinski RJ *et al.* Cytokine- and LPS-induced synthesis of interleukin-8 from human mesangial cells. Kidney Int 1991; **39**:1240–8.
30 Schmouder RL, Strieter RM, Wiggins RC *et al.* *In vitro* and *in vivo* interleukin-8 production in human renal cortical epithelia. Kidney Int 1992; **41**:191–4.
31 Vercelli D, Geha RS. Regulation of IgE synthesis in man. J Clin Immunol 1989; **9**:75–83.
32 Meadow SR, Sarsfield JK, Scott DG *et al.* Steroid-responsive nephrotic syndrome and allergy: immunological studies. Arch Dis Child 1981; **56**:517–24.
33 Rosenberg SA, Lotze MT, Muul LM *et al.* A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high dose interleukin-2 alone. N Engl J Med 1987; **316**:889–97.
34 Hisanaga S, Kawagoe H, Yamamoto Y *et al.* Nephrotic syndrome associated with recombinant interleukin-2. Nephron 1990; **54**:277–8.
35 Jacobson SH, Hylander B, Wretlind B *et al.* Interleukin-6 and interleukin-8 in serum and urine in patients with acute pyelonephritis in relation to bacterial-virulence-associated traits and renal function. Nephron 1994; **67**:172–9.
36 Wada T, Yokoyama H, Tomosugi N *et al.* Detection of urinary interleukin-8 in glomerular disease. Kidney Int 1994; **46**:455–60.