Clin. exp. Immunol. (1991) **85**, 240–245

ADONIS 000991049100223P

Cytokine production by peripheral blood mononuclear cells in IgA nephropathy

K. N. LAI*, J. C. K. LEUNG†, P. K. T. LI* & S. F. LUI* *Department of Medicine, and †Clinical Immunology Unit, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong

(Accepted for publication 14 February 1991)

SUMMARY

The regulation of cytokine production and T cell proliferation by other cytokines is mandatory in mediating inflammatory responses but the full understanding is far from complete. We have previously reported increased production of IL-2 and IL-2 receptors (IL-2R) in IgA nephropathy. The present study was undertaken to examine other cytokine production during T cell activation in IgA nephropathy. Peripheral blood mononuclear cells (PBMC) from 17 IgA nephritic patients and 14 controls were cultured with phytohaemagglutinin and phorbol myristate acetate for 48 h for maximal cytokine production. IL-2Rs and IL-4 receptors (IL-4Rs) expressed on cultured PBMC were studied by a radioimmunoassay using monoclonal antibodies against these receptors. Although the total cellular IL-2R expression and percentages of T helper and T suppressor cells did not differ between the patients and controls, there was a significant increase in activated T helper cells expressing IL-2R in patients with IgA nephropathy. The total cellular IL-4R expression was elevated in IgA nephritic patients ($P < 0{\cdot}005$). IL-2 production by PBMC was raised in IgA nephritic patients compared with controls ($P < 0{\cdot}05$) but no difference in IL-4 or IL-6 production was observed. The interferon-gamma production by PBMC was significantly increased in patients with IgA nephropathy ($P < 0{\cdot}025$). No correlation was observed between individual cytokine levels. Our data suggest there are selective increases in cytokine production in IgA nephropathy.

Keywords IgA nephropathy peripheral blood mononuclear cells interleukins interferon-gamma cytokine receptor expression

INTRODUCTION

Cytokines are protein mediators of cell-to-cell communication important in a variety of physiologic and pathophysiologic activities. IL-2, a cytokine produced by activated helper T cells, has been shown to cause proliferation of activated T cells, induce generation of cytotoxic T cells, and stimulate production of immunoglobulins in T cell-deficient populations (Smith, 1984; Cantrell & Smith, 1984; Smith & Cantrell, 1985). IL-4 is a pleiotropic T cell-derived factor that was identified originally by its ability to enhance the proliferation of B cells stimulated with low doses of anti-μ chain antibodies (Howard *et al.*, 1982). Other than acting as B cell growth and/or differentiation factor (Defrance *et al.*, 1987), IL-4 also induces IgE production (Péne *et al.*, 1988). In addition, IL-4 acts on T lymphocytes both as a growth factor (Lee *et al.*, 1986) and in the maturation of cytotoxic T cells from their precursors (Widmer & Grabstein, 1987). IL-4 also regulates expression of the p75 chain of IL-2 receptor (IL-2R) (Loughnan & Nossal, 1989). IL-6 was first described as a 26-kD secretary protein with a variety of bioactivities, and was isolated from activated fibroblastoid cell cultures (Billiau, 1989). It is now recognized that numerous cytokines and growth factors, rather than a single mediator, are responsible for any particular biological response in the normal processes of cell growth and differentiation.

We have previously studied the T lymphocyte activation in IgA nephropathy by analysing the *in vitro* IL-2R expression and *in vivo* release of soluble IL-2R in circulation (Lai, Leung & Lai, 1988; Lai *et al.*, 1989). Our results suggested that T lymphocytes from these patients have an overproduction of IL-2 and increased activated T helper cell subset upon mitogenic stimulation. These preliminary findings have now been confirmed by other investigators (Schena *et al.*, 1989).

On the basis of possible interaction and regulation of cytokine production and T cell proliferation by other cytokines, we have examined the production of these cytokines, including interferon-gamma (IFN-γ) from peripheral blood mononuclear cells (PBMC) in IgA nephropathy stimulated with phytohaemagglutinin (PHA) and phorbol myristate acetate (PMA) to study the inter-relationship between these cytokine systems in primary IgA nephropathy.

Correspondence: Dr K. N. Lai, Reader in Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong.

MATERIALS AND METHODS

Patients and controls

We studied 17 patients with a clinical and renal immunopathologic diagnosis of primary IgA nephropathy. IgA nephropathy was diagnosed by mesangial proliferative glomerulonephritis with predominant mesangial IgA deposits and the presence of electron dense deposits in mesangium under ultrastructural examination. Systemic lupus erythematosus, Henoch–Schönlein purpura and hepatic disease were excluded by detailed clinical history, physical examination and laboratory investigations. No patient had renal insufficiency. All patients were clinically quiescent and had been free from infections or macroscopic haematuria for at least 8 weeks before the study. These patients were not on corticosteroid or immunosuppressive therapy. Fourteen age- and sex-matched healthy subjects with normal renal function and free from infection were used as donors of control PBMC.

Reagents

MR6 is a mouse IgG1 monoclonal antibody that was raised at the Royal Postgraduate Medical School, London, UK, as described (DeMaagd *et al.*, 1985). MR6 binds to a 200-kD protein associated with the human IL-4R. Addition of MR6 to cultures of T cells proliferating in response to IL-4 inhibits this response in a dose-dependent fashion (Larche, Lamb & Ritter, 1988). MR6 at a concentration of 5 μg/ml inhibits *Mycobacterium tuberculosis* extract-induced and IL-2-induced proliferation of PBMC by 75% and 88%, respectively (Larche *et al.*, 1988). MR6 at a concentration of 10 μg/ml inhibits IL-4-induced PBMC proliferation by 100% (Larche *et al.*, 1988). Anti-Tac monoclonal antibody is an antibody (clone 2A3) against the IL-2R (CD25) from Becton Dickinson, Mountain View, CA. PHA and PMA were purchased from Sigma Chemical Co. (St Louis, MO).

Cytokine production and lymphocyte proliferation

The microculture technique used has been described previously (Lai *et al.*, 1988). The PBMC from IgA nephritis patients and healthy donors were separated by density gradient. Monocytes (detected by cells bearing CD116 antigen on their cell surface) comprised less than 4% of the cell population of PBMC. Isolated PBMC were resuspended in culture medium consisting of RPMI 1640 (GIBCO, Chargrin Falls, NY) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml) at a concentration of 10^6 cells/ml. The cells were incubated at 37°C in humidified atmosphere with 5% CO_2 in the presence of PHA (10 μg/ml) and PMA (0·5 ng/ml). Preliminary experiments established that these concentrations of PHA and PMA induced maximal lymphocyte proliferation and cytokine production. The supernatants of the cultures were harvested after 48 h of incubation. These were then aliquoted, six-fold concentrated (only for IL-2 and IL-4 assays), and stored at −20°C until assayed for cytokine concentrations. Incubation times of 48 h were found to result in optimal cytokine production.

Lymphocyte proliferation was measured by thymidine uptake by the cultured PBMC. After 72 h of incubation, 0·5 μCi of ^{3}H-thymidine (Amersham International, Amersham, UK) was added to each well of the microculture plates (Costar, Cambridge, MA) and the cultures were terminated after 6 h. The incorporated radioactivity, expressed as ct/min, was determined. The thymidine uptake was calculated by (stimulated ct/min − unstimulated ct/min). Unstimulated ct/min was measured in PBMC incubated with no mitogen.

Direct immunofluorescence studies for CD antigens expressed on PBMC

The $CD4^+$ (T helper), $CD8^+$ (T suppressor), and $CD25^+$ (activated T) lymphocytes were identified by costaining with PE-conjugated anti-Leu 3a, PE-conjugated anti-Leu 2a, and FITC-conjugated anti-Tac murine monoclonal antibodies (Becton Dickinson), respectively, described previously (Lai *et al.*, 1989).

Determination of IL-2, IL-4, IL-6 and IFN-γ

ELISA kits specific for IL-2, IL-4 and IL-6 were obtained from Genzyme. These are multiple-sandwich solid-phase enzyme immunoassay using monoclonal and polyclonal antibodies against individual cytokines. Sensitivity of our assays was shown to be 50 pg/ml, 15 pg/ml, and 0·163 ng/ml for IL-2, IL-4, and IL-6, respectively. ELISA kit specific for human IFN-γ was obtained from Holland Biotechnology, Leiden, The Netherlands. These are multiple-sandwich solid-phase enzyme immunoassays using two monoclonal antibodies against IFN-γ. Units are defined according to the National Institutes for Health (NIH) human IFN-γ standard Gg-23-901-530. Sensitivity (with no concentration of supernatant) was shown to be 1 U/ml. No cross-reactivity was apparent with other cytokines including IL-1α/IL-1β or tumour necrosis factor-alpha (TNF-α). The absolute cytokine concentration was calculated by (stimulated concentration − unstimulated concentration); unstimulated concentration was measured in PBMC incubated with no mitogen.

Quantification of cellular IL-2R and IL-4R

The PBMC were incubated for 36 h with or without mitogenic stimulants before they were centrifuged at 400 ***g***. The cell count was adjusted to 1×10^6/ml in suspension medium and 100 μl of the PBMC suspension were placed in microtubes which had been coated with 3% (v/v) bovine serum albumin overnight. The tubes were then incubated for 30 min at 4°C with 100 μl of mouse anti-human IL-2R or IL-4R monoclonal antibodies at appropriate dilution. After incubation, the cells were washed thrice with HBSS and were further incubated for 30 min at 4°C with 100 μl of sheep anti-mouse IgG, ^{125}I-labelled, $F(ab')_2$ fragment, 18 μCi/μg, at appropriate dilution (Amersham International). After incubation, the cells were washed three times with HBSS and resuspended gently with a Pasteur pipette. The content of each tube was determined for radioactivity using a gamma counter. The result was expressed as percentage value of unstimulated PBMC cultured with no mitogen and was calculated by (stimulated ct/min − unstimulated ct/min) unstimulated ct/min.

Statistical analysis

The results are expressed as mean ± s.d. The data were analysed with rank sum test, Wilcoxon signed rank test, and linear regression analysis, where appropriate.

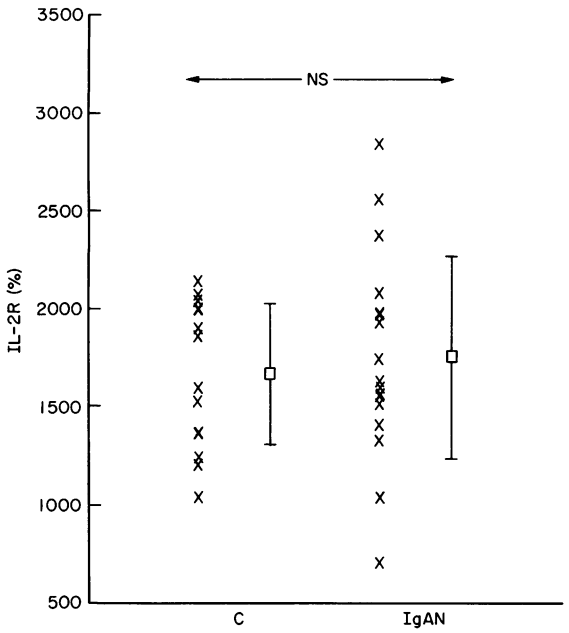

Fig. 1. IL-2 receptor (IL-2R) expression on activated PBMC from 17 patients with IgA nephropathy (IgAN) and 14 healthy control (C) subjects, cultured with phytohaemagglutinin (PHA) and phorbol myristate acetate (PMA). The results (mean ± s.d.) are expressed as percentage of basal value measured in PBMC cultured with no mitogen. NS, not significant.

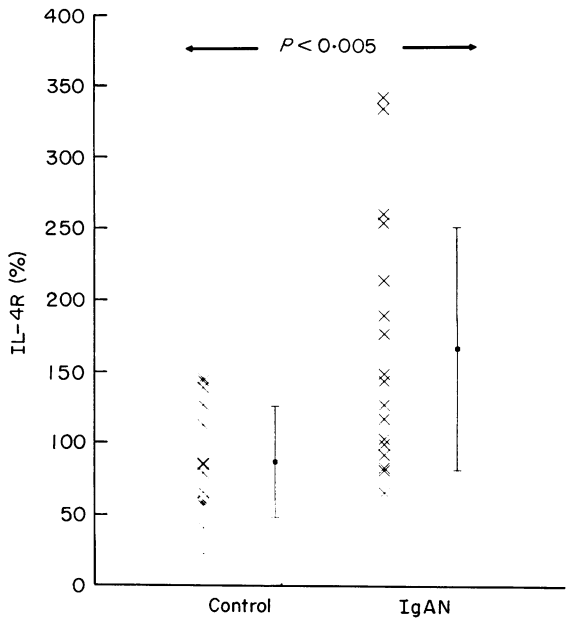

Fig. 2. IL-4 receptor (IL-4R) expression on activated PBMC from 17 patients with IgA nephropathy (IgAN) and 14 healthy control (C) subjects, cultured with phytohaemagglutinin (PHA) and phorbol myristate acetate (PMA). The results (mean ± s.d.) are expressed as percentage of basal value measured in PBMC cultured with no mitogen.

RESULTS

In preliminary studies, it was found that combination of PHA (10 μg/ml) and PMA (0·5 ng/ml) resulted in maximal stimulation of cytokine production (Lai *et al.*, 1991). Time-response studies revealed that PBMC incubated for 72 h proliferated optimally with maximal thymidine uptake. Similarly, supernatants taken from 48-h culture of PBMC stimulated with various mitogens produced maximal concentrations of cytokines and soluble IL-2R. Cellular expression of IL-2R and IL-4R was maximal at 48 h of PBMC culture.

Cellular IL-2R expression

The number of IL-2Rs on PBMC measured indirectly by radioimmunoassay is shown in Fig. 1. The mean radioactivities of unstimulated PBMC in controls and IgA nephritis patients were $4{\cdot}01 \pm 0{\cdot}72$ and $4{\cdot}21 \pm 1{\cdot}59 \times 10^3$ ct/min per 10^6 cells and increased to $68{\cdot}59 \pm 8{\cdot}61$ and $72{\cdot}00 \pm 13{\cdot}56 \times 10^3$ ct/min per 10^6 cells, respectively, with mitogenic stimulation; no difference was demonstrated between the two groups of subjects. However, with a double direct immunofluorescence technique described previously (Lai *et al.*, 1989), the IgA nephritis patients had increased activated $CD4^+$ lymphocytes (with IL-2R expressing CD25) and reduced activated $CD8^+$ lymphocytes compared with healthy controls despite the fact that the percentages of $CD4^+$ cells, $CD8^+$ cells, and CD4/CD8 ratio were not different between the patients and healthy controls (data not shown).

Cellular IL-4R expression

The number of IL-4R on PBMC measured by radioimmunoassay is depicted in Fig. 2. The mean radioactivities of unstimulated PBMC in controls and IgA nephritis patients were $4{\cdot}501 \pm 1{\cdot}25$ and $3{\cdot}40 \pm 0{\cdot}88 \times 10^3$ ct/min per 10^6 cells, respectively. Upon mitogen stimulation, the mean radioactivities of PBMC in controls and IgA nephritis patients increased to $8{\cdot}40 \pm 2{\cdot}91$ and $8{\cdot}53 \pm 2{\cdot}26 \times 10^3$ ct/min per 10^6 cells, respectively. The PBMC from IgA nephritis patients demonstrated a higher increase of IL-4R than those from healthy controls ($165{\cdot}9 \pm 84{\cdot}2\%$ *versus* $86{\cdot}9 \pm 38{\cdot}4\%$, $P < 0{\cdot}005$).

PBMC proliferation

The thymidine incorporation by unstimulated PBMC in controls and IgA nephritis patients was $0{\cdot}30 \pm 0{\cdot}09$ and $0{\cdot}28 \pm 0{\cdot}15 \times 10^3$ ct/min per 10^6 cells and increased to $160{\cdot}24 \pm 19{\cdot}19$ and $154{\cdot}94 \pm 12{\cdot}24 \times 10^3$ ct/min per 10^6 cells, respectively, with mitogenic stimulation, but no difference was demonstrated between the two groups of subjects.

Cytokine production by activated PBMC

The mean IL-2, IL-4, IL-6, and IFN-γ production by unstimulated PBMC were 50 ± 25 pg/ml, 30 ± 15 pg/ml, $0{\cdot}76 \pm 0{\cdot}32$ ng/ml, and $2{\cdot}6 \pm 1{\cdot}3$ U/ml, respectively. No difference in cytokine production by unstimulated PBMC was demonstrated between IgA nephritis patients and healthy controls. The production of these cytokines in these two groups of subjects is shown in Figs 3–6. Upon mitogen stimulation with PHA, and PMA, IgA nephritis patients had a higher level of IL-2 ($P < 0{\cdot}05$) and IFN-γ ($P < 0{\cdot}025$) production from cultured mononuclear cells compared with controls. Nonetheless, there was no difference demonstrated between the IL-4 and IL-6 production from these patients.

Correlation between the cytokine production by activated PBMC

A significant inverse correlation between lipopolysaccharide (LPS) induced IL-6 production and PHA-induced IL-2 produc-

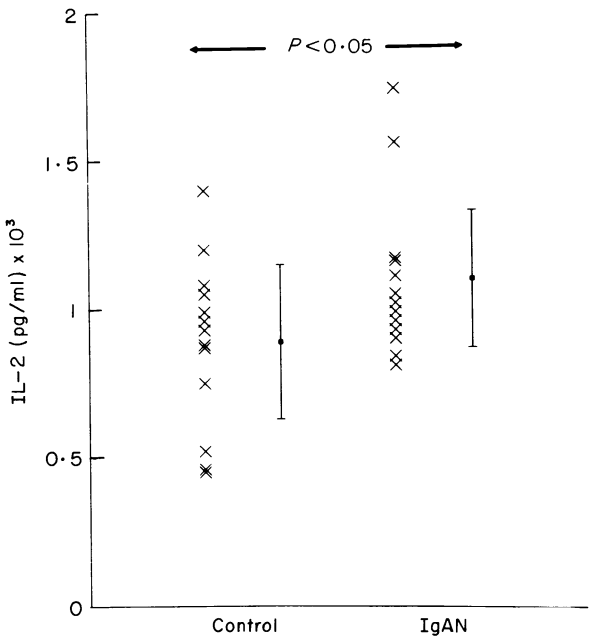

Fig. 3. IL-2 levels in supernatant taken from PBMC from 17 patients with IgA nephropathy (IgAN) and 14 healthy control (C) subjects, cultured with phytohaemagglutinin (PHA) and phorbol myristate acetate (PMA). PBMC from patients with IgAN had increased IL-2 production.

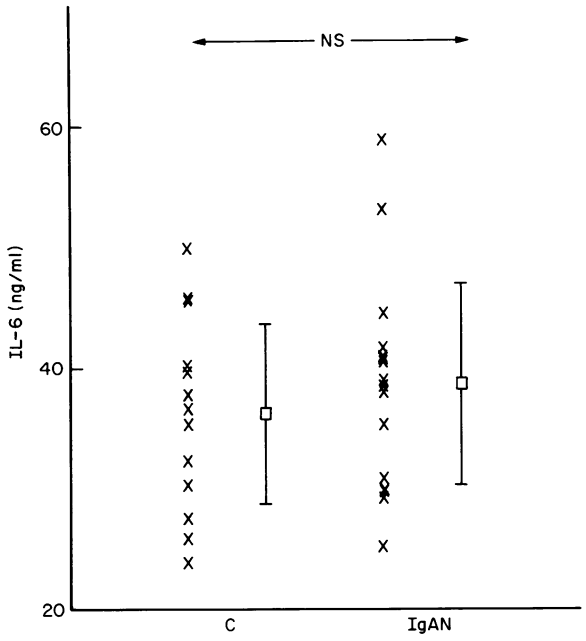

Fig. 5. IL-6 levels in supernatant taken from PBMC from 17 patients with IgA nephropathy (IgAN) and 14 healthy control (C) subjects, cultured with phytohaemagglutinin (PHA) and phorbol myristate acetate (PMA). No difference in IL-6 production from PBMC was observed between two groups of subjects.

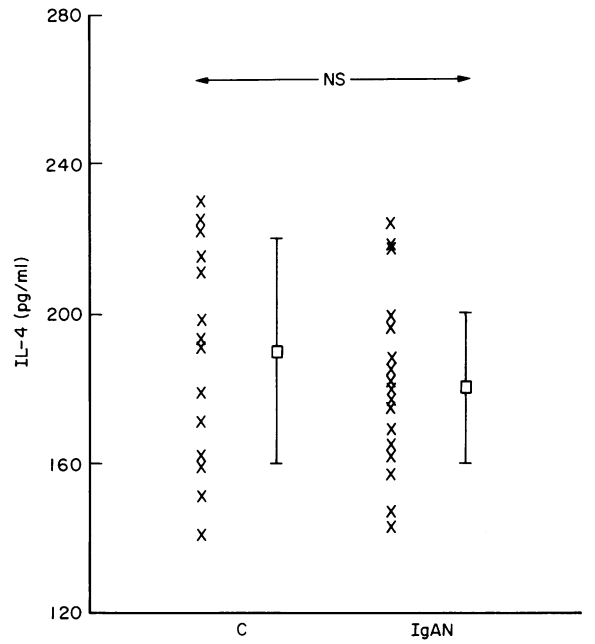

Fig. 4. IL-4 levels in supernatant taken from PBMC from 17 patients with IgA nephropathy (IgAN) and 14 healthy control (C) subjects, cultured with phytohaemagglutinin (PHA) and phorbol myristate acetate (PMA). No difference in IL-4 production from PBMC was observed between two groups of subjects.

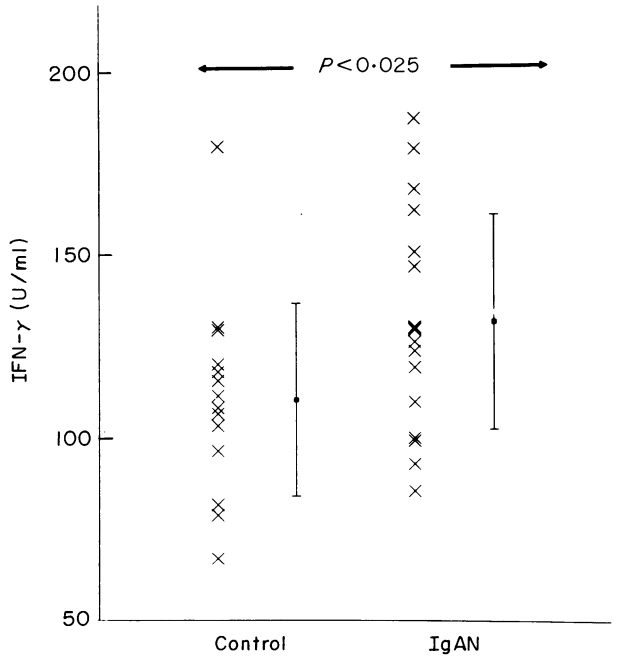

Fig. 6. Interferon-gamma (IFN-γ) levels in supernatant taken from PBMC from 17 patients with IgA nephropathy (IgAN) and 14 healthy control (C) subjects, cultured with phytohaemagglutinin (PHA) and phorbol myristate acetate (PMA). PBMC from the patients had increased IFN-γ production.

tion, and between LPS-induced IL-6 production and PHA-induced IFN-γ has recently been reported in alcoholic liver cirrhosis (Deviere *et al.*, 1989). We had shown no correlation between IL-2 and IL-6, and between IL-6 and IFN-γ in healthy subjects or IgA nephritis patients. Similarly, the production of IL-4 did not correlate with that of other cytokines.

DISCUSSION

The understanding of regulation of cytokine production and T cell proliferation by other cytokines is far from complete and available data are often contradictory. Recent studies suggest that during IL-2-induced or IL-4-induced T cell proliferation, each cytokine specifically up-regulates its own receptors (Jankovic *et al.*, 1989). IL-4 can act as T cell growth factor independently of IL-2 but the proliferative responses to IL-4 are always lower and relatively shorter lasting than those of IL-2 (Spits *et al.*, 1987). Gause *et al.* (1988) have reported that at higher concentrations the combination of IL-1 and IL-2 can induce thymocytes to produce IL-4 which then contributes to overall proliferation. A series of studies have recently revealed that IL-6 can act as an important signal for T cell proliferation and differentiation (Le & Vilcek, 1989). Preliminary data have suggested that IL-2 and IL-2R are involved in IL-6 action. Anti-Tac has been reported to block IL-6-driven proliferation of T cells partially (Garman *et al.*, 1987; Lotz *et al.*, 1988). However, the same antibody fails to affect IL-6-stimulated proliferation under different experimental conditions, suggesting the existence of an IL-2-independent pathway (Lotz *et al.*, 1988; Ceuppens *et al.*, 1988).

In the present study, we have confirmed our previous findings of increased IL-2R expression in T helper cells and raised IL-2 production by activated PBMC in patients with IgA nephropathy despite clinical quiescence. Cellular IL-4R and other cytokines involved in T cell proliferation were measured to determine the interaction between different cytokines that could mediate inflammatory responses in the pathogenesis of IgA nephropathy. IgA nephritis patients had increased cellular IL-4R expression. This was not likely to be due to an up-regulation by IL-4, as no simultaneous increase in IL-4 production was observed. It is speculated that increased IL-4R expression in IgA nephropathy could be related to increased IL-2R expression, as suggested by murine and human experiments that the IL-2R and IL-4R may co-modulate each other's expression (Spits *et al.*, 1987; Larche *et al.*, 1988). In contrast to a preliminary communication reporting increased monocyte IL-6 release during macroscopic haematuria (Ballardie *et al.*, 1990), we failed to demonstrate increased IL-6 production from PBMC that consisted of monocytes, T and B cells and monocytes only comprised $<4\%$ of PBMC. The IL-6-dependent regulatory defect suggested by Ballardie *et al.* (1990) could be related to the disease activity, since monocyte IL-6 release was raised only during synpharyngitic macroscopic haematuria and IL-6-mediated acute-phase protein responses during infection (Le & Vilcek, 1989). T cells stimulated with antigen (Kelso *et al.*, 1982) or IL-2 (Vilcek *et al.*, 1985) secrete IFN-γ, and some studies have suggested that IFN-γ acts in an autocrine manner (Simon *et al.*, 1987). We have demonstrated that of the cytokines involved in T cell proliferation, only IFN-γ and IL-2 are elevated in PBMC from patients with IgA nephropathy. We have also studied the correlation between these cytokines, as a significant inverse correlation between IL-6 and IL-2 production, and between IL-6 and IFN-γ, has recently been reported in alcoholic liver cirrhosis (Deviere *et al.*, 1989). We failed to demonstrate any correlation between different cytokine production in IgA nephritic patients or controls.

Finally, it is important to explore the pathophysiological significance of raised IFN-γ and IL-2 levels in IgA nephropathy. Two immunopathogenetic mechanisms are considered. First, IFN-γ is unique as it is the major cytokine that regulates MHC class II expression (Rosa, Cochet & Fellous, 1986; Balkwill & Burke, 1989). Polymorphism in one of the MHC class II region genes, DQ, has recently been reported in IgA nephropathy suggesting disease susceptibility genes are important in IgA nephropathy (Moore *et al.*, 1990). Second, IL-2 and IFN-γ are the two necessary cytokines in the development of cytolytic T cells that generate lymphokine-activated killer activity (Balkwill & Burke, 1989; Maraskovsky, Chen & Shortman, 1989). Increased natural killer cell activity associated with primary IgA nephropathy has been reported recently (Schena *et al.*, 1990), although their immunopathogenetic roles still remain speculative. A recent study has reported a significant elevation of serum IFN-γ in IgA nephritic patients during acute exacerbation and intraglomerular expression of MHC class II antigens in proliferative lesions in glomeruli from IgA nephritis patients (Takaeda *et al.*, 1990). These preliminary data suggest that MHC class II antigens and IFN-γ may play an important role in immune-mediated glomerular injury of IgA nephropathy (Takaeda *et al.*, 1990).

Our study confirms the hyperactivity of T helper cells in IgA nephropathy and selective increase in cytokine production by PBMC is observed. Further studies are warranted to investigate the pathophysiological significance of these abnormalities in cytokine production.

ACKNOWLEDGMENTS

This study was supported by a grant from the Croucher Foundation and we thank Dr M. Larche and Dr M. A. Ritter, Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London, for providing the monoclonal antibody MR6.

REFERENCES

BALKWILL, F.R. & BURKE, F. (1989) The cytokine network. *Immunol. Today*, **10,** 299.

BALLARDIE, F.M., BAKER, P., O'DONOGHUE, D.J. & VOSE, B.M. (1990) Cytokine IL-6 regulation of autoimmunity in IgA nephropathy. In *Abstracts of the XIth International Congress of Nephrology, July 15–20, Tokyo* p. 44.

BILLIAU, A. (1989) Interferons and inflammation. *J. Interferon Res.* **7,** 559.

CANTRELL, D.A. & SMITH, K.A. (1984) The interleukin T-cell system: a new cell growth model. *Science*, **224,** 1312.

CEUPPENS, J.L., BAROJA, M.L., LORRE, K., VAN DAMME, J. & BILLIAU, A. (1988) Human T cell activation with phytohemagglutinin. The function of IL-6 as an accessory signal. *J. Immunol.* **141,** 3868.

DEFRANCE, T., VANBERVLIET, B., AUBRY, J.P., TAKEBE, Y., ARAI, N., MIYAJIMA, A., YOKOTA, T., LEE, F., ARAI, K., DE VRIES, J.E. & BANCHEREAU, J. (1987) B cell growth promoting-activity of recombinant human interleukin-4. *J. Immunol.* **139,** 1135.

DEMAAGD, R.A., MACKENZIE, W.A., SCHUURMAN, H.-J., RITTER, M.A., PRICE, K.M., BROEKHUIZEN, R. & KATER, L. (1985) The human thymus microenvironment: heterogeneity detected by monoclonal anti-epithelial cell antibodies. *Immunology*, **54,** 745.

DEVIERE, J., CONTENT, J., DENYS, C., VANDENBUSSCHE, P., SCHANDENE, L., WYBRAN, J. & DUPONT, E. (1989) High interleukin-6 serum levels and increased production by leucocytes in alcoholic liver cirrhosis. Correlation with IgA serum levels and lymphokines production. *Clin. exp. Immunol.* **77,** 221.

GARMAN, R.D., JACOBS, K., CLARK, S. & RAULET, D. (1987) B-cell-stimulatory factor 2 functions as a second signal for interleukin 2 production by mature murine T cells. *Proc. natl Acad. Sci. USA*, **84,** 7629.

GAUSE, W.C., TAKASHI, T., MOUNTZ, J.D., FINKELMAN, F.D. & STEINBERG, A.D. (1988) Activation of $CD4^-$, $CD8^-$ thymocytes with IL 4 vs IL 1+IL 2. *J. Immunol.* **141,** 2240.

HOWARD, M., FARRAR, J., HILFIKER, M., JOHNSON, B., TAKATSU, K., HAMAOKA, T. & PAUL, W.E. (1982) Identification of a T cell derived B cell growth factor distinct from interleukin 2. *J. exp. Med.* **155,** 914.

JANKOVIC, D.L., GIBERT, M., BARAN, D., OHARA, J., PAUL, W.E. & THEZE, J. (1989) Activation by IL-2, but not IL-4, up-regulates the expression of the p55 subunit of the IL-2 receptor on IL-2- and IL-4-dependent T cell lines. *J. Immunol.* **142,** 3113.

KELSO, A., GLASEBROOK, A.L., KANAGAWA, O. & BRUNNER, K.T. (1982) Production of macrophage-activating factor by T lymphocytes clones and correlation with other lymphokine activities. *J. Immunol.* **129,** 550.

LAI, K.N., LEUNG, J.C.K., LAI, F.M. & TAM, J.S. (1989) T-lymphocyte activation in IgA nephropathy: serum soluble interleukin 2 receptor levels, interleukin 2 production, and interleukin 2 receptor expression by cultured lymphocytes. *J. clin. Immunol.* **9,** 485.

LAI, K.N., LEUNG, J.C.K. & LAI, F.M. (1988) In vitro study of expression of interleukin-2 receptors in T-lymphocytes from patients with IgA nephropathy. *Clin. Nephrol.* **30,** 330.

LAI, K.N., LEUNG, J.C.K., LI, P., LARCHE, M. & RITTER, M.A. (1991) The effect of blockade of interleukin 2 receptors and interleukin 4 receptors on cytokine production. *APMIS*, (In press).

LARCHE, M., LAMB, J.R. & RITTER, M.A. (1988) A novel T-lymphocyte molecule that may function in the induction of self-tolerance and MHC-restriction within human thymic microenvironment. *Immunology*, **64,** 101.

LARCHE, M., LAMB, J.R., O'HEHIR, R.E., IMAMI-SHITA, N., ZANDERS, E.D., QUINT, D.E., MOQBEL, R. & RITTER, M.A. (1988) Functional evidence for a monoclonal antibody that binds to the human IL-4 receptor. *Immunology*, **65,** 617.

LE, J. & VILCEK, J. (1989) Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase response. *Lab. Invest.* **61,** 588.

LEE, F.K., YOKOTA, Y., OTSUKA, T., MEYERSON, P., VILLARET, D., COFFMAN, R., MOSMANN, T., RENNICK, D., ROEHM, N. & SMITH, C. (1986) Isolation and characterization of a mouse interleukin cDNA clone that expresses BSF-1 activities and T cell, and mast cell stimulating activities. *Proc. natl Acad. Sci. USA*, **83,** 2061.

LOTZ, M., JIRIK, F., KABOURIDIS, P., TSOUKAS, C., HIRANO, T., KISHIMOTO, T. & CARSON, D.A. (1988) B cell stimulating factor 2/interleukin 6 is a costimulant for human thymocytes and T lymphocytes. *J. exp. Med.* **167,** 1253.

LOUGHNAN, M.S. & NOSSAL, G.J.V. (1989) Interleukin 4 and 5 control expression of IL-2 receptor on murine B cells through independent induction of its two chains. *Nature*, **340,** 76.

MARASKOVSKY, E., CHEN, W.F. & SHORTMAN, K. (1989) IL-2 and IFN-gamma are two necessary lymphokines in the development of cytolytic T cells. *J. Immunol.* **143,** 1210.

MOORE, R.H., HITMAN, G.A., LUCAS, E.Y., RICHARDS, N.T., VENNING, M.C., PAPIHA, S., GOODSHIP, T., FIDLER, A., AWAD, J., FESTENSTEIN, H., CUNNINGHAM, J. & MARSH, F.P. (1990) HLA DQ region gene polymorphism associated with primary IgA nephropathy. *Kidney Int.* **37,** 991.

PÉNE, J., ROUSSET, F., BRIERE, F., CHRETIEN, I., BONNEFOY, J.Y., SPITS, H., YOKOTA, T., ARAI, N., ARAI, K., BANCHEREAU, J. & DE VRIES, J.E. (1988) IgE production by normal human lymphocytes is induced by interleukin-4 and suppressed by interferons gamma and alpha and prostaglandin E_2. *Proc. natl Acad. Sci. USA*, **85,** 6880.

ROSA, F.M., COCHET, M.M. & FELLOUS, M. (1986) Interferon and major histocompatibility complex genes: a model to analyse eukaryotic gene regulation? *Interferon*, **7,** 47.

SCHENA, F.P., MASTROLITTI, G., JIRILLO, E., MUNNO, I., PELLEGRINO, N., FRACASSO, A.R. & AVENTAGGIATO, L. (1989) Increased production of interleukin-2 and IL-2 receptors in IgA nephropathy. *Kidney Int.* **33,** 875.

SCHENA, F.P., OTTOLENGHI, A., COLIZZI, M., SCIVITTARO, V., ANTONACI, S. & POLIGNANO, A. (1990) Natural killer cell subsets and NK cytotoxicity in primary IgA nephropathy. In *Abstracts of the XIth International Congress of Nephrology, July 15–20, Tokyo* p. 337.

SIMON, M.M., HOCHGESCHWENDER, U., BRUGGER, U. & LONDOLFO, S. (1987) Monoclonal antibodies to interferon-gamma inhibit interleukin-2-dependent induction of growth and maturation in lectin/antigen-reactive cytotoxic T lymphocyte precursors. *J. Immunol.* **136,** 2755.

SMITH, K.A. (1984) The determinants of T cell growth. *Symp. fundam. Cancer Res. Collect. Pap.* **37,** 185.

SMITH, K.A. & CANTRELL, D.A. (1985) Interleukin 2 regulates its own receptors. *Proc. natl Acad. Sci. USA*, **82,** 864.

SPITS, H., YSSEL, H., TAKEBE, Y., ARAI, N., YOKOTA, T., LEE, F., ARAI, K., BANCHEREAU, J. & DE VRIES, J.E. (1987) Recombinant interleukin 4 promotes the growth of human T cells. *J. Immunol.* **139,** 1142.

TAKAEDA, M., YOKOYAMA, H., ABE, T., GOSHIMA, G., YOSHIMURA, M. & TOMOSUGI, N. (1990) Intraglomerular expression of MHC-class II and Ki-67 antigens and serum gamma-interferon levels in IgA nephropathy. In *Abstracts of the XIth International Congress of Nephrology, July 15–20, Tokyo* p. 334.

VILCEK, J., HENRIKSEN-DESTEFANO, D., SIEGEL, D., KLION, A., ROBB, R.J. & LE, J. (1985) Regulation of IFN-gamma induction in human peripheral blood cells by exogenous and endogenously produced interleukin 2. *J. Immunol.* **135,** 1851.

WIDMER, M.B. & GRABSTEIN, K.H. (1987) Regulation of cytolytic T-lymphocyte generation by B-cell stimulatory factor. *Nature*, **326,** 795.