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Differential control of mesangial cell proliferation by interferon-gamma

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SUMMARY

Rat mesangial cells were shown to be sensitive to recombinant interferon-gamma (IFN-γ). IFN-γ reduced thymidine uptake by these cells and inhibited cell proliferation. Incubation of the cells with 1000 U/ml IFN-γ decreased thymidine uptake by up to 64% and cell numbers were decreased by 17%. The effects of IFN-γ were dose and time dependent and were partially reversible by the anti-IFN-γ monoclonal antibody DB-1. This lymphokine did not reduce incorporation of RNA and protein precursors however. Measurements of ^{3}H-uridine and ^{3}H-leucine incorporation indicated significant increases in RNA and protein synthesis (37% and 45%, respectively) on a per cell basis. The mitogenic effects of IL-1 and platelet-derived growth factor (PDGF) were also susceptible to IFN-γ-mediated inhibition but the mitogenic response to epidermal growth factor (EGF) was much less sensitive. We conclude that while IFN-γ may act to modulate the mitogenic signals provided by some factors such as IL-1 and PDGF, the response to EGF appears to be unaffected.

Keywords mesangial cytokines lymphokines growth

INTRODUCTION

Mesangial hypercellularity is observed in a variety of glomerular disorders. In some forms of immune-mediated glomerulonephritis, this is due to proliferating mesangial cells as well as the presence of an inflammatory infiltrate (Sterzel & Pabst, 1982; Hooke *et al.*, 1984; Hooke, Gee & Atkins, 1987; Nolasco *et al.*, 1987). Glomerular function may be affected directly by this hypercellularity or by changes in mesangial cell physiological activities. These cells can influence glomerular function by their contractile response (Ardaillou, 1987) and via the secretion of various biologically active substances including prostaglandins, thromboxane, interleukin-1 (IL-1), interleukin-6 (IL-6), platelet-derived growth factor (PDGF) and basement membrane proteins (Lovett & Larsen, 1988; Webber *et al.*, 1987; Shultz *et al.*, 1988; Schlondorff, 1987; Horii *et al.*, 1989; Ishimura *et al.*, 1989). It is therefore imporant to understand the interactions between the leucocytic infiltrate and the mesangial cell in relation to the pathogenesis of glomerulonephritis.

It has been reported recently that IL-1, IL-6, PDGF, epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) stimulate mesangial cell proliferation *in vitro* (Lovett, Ryan & Sterzel, 1983a; Abboud, Saunders & Knauss, 1987; Schultz *et al.*, 1988; Horii *et al.*, 1989; Doi *et al.*, 1989). PDGF, IL-6 and EGF are produced within the kidney and IL-1 is produced by many cells such as fibroblasts and endothelial cells and it has been proposed as an autocrine growth factor for mesangial cells (Lovett *et al.*, 1986). Conventionally, however, IL-1 is regarded as a product of macrophages that have been activated by T lymphocytes. The macrophage infiltrate accompanying glomerulonephritis is thus a potential source of large amounts of IL-1 as the other major component of the leucocytic infiltrate is the T lymphocyte.

The most potent macrophage activating signal provided by the T cell is interferon-gamma (IFN-γ). This lymphokine is also involved in the control of cell division, cell differentiation and tumour cell proliferation (Tsujisaki *et al.*, 1987; Friesel, Komoriya & Maciag, 1987; Schmiegel *et al.*, 1988; Warner, Friedman & Libby, 1989). It acts via a specific cell surface receptor and has been demonstrated to block the mitotic activities of PDGF, EGF and fibroblast growth factor on fibroblasts (Hosang, 1988). The mesangial cell itself has been stimulated to MHC class II antigen expression by IFN-γ and to be inhibited from incorporating thymidine (Martin *et al.*, 1989).

We confirm here the inhibitory effects of rat IFN-γ on a rat mesangial cell line grown in serum containing culture medium and show that the mitogenic activities of PDGF and IL-1 are also abrogated by it. The data on EGF-mediated growth, on the other hand, suggest resistance to IFN-γ. The anti-proliferative action of IFN-γ is not accompanied by a general metabolic decline, as RNA and protein synthesis were not inhibited. This lymphokine is therefore a potentially important factor in modulating the mitogenic signals produced by the inflammatory infiltrate associated with glomerulonephritis.

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MATERIALS AND METHODS

Cell preparation

Glomeruli were isolated from Sprague–Dawley rat kidneys by mechanical sieving as described previously (Glasgow, Hancock & Atkins, 1981). Mesangial cells were obtained by a standard method using collagenase digestion (Lovett *et al.*, 1983b) and small metal rings for isolating colonies of cells. Cells were maintained in RPMI 1640 (GIBCO, Grand Island, NY) with 20% heat-inactivated fetal calf serum (FCS; Flow Laboratories, Rickmansworth, UK), 20 mM HEPES buffer, and 100 U/ml penicillin and 100 μg/ml streptomycin, using plastic tissue culture flasks (Flow Laboratories) in a humidified 5% CO_2 atmosphere at 37°C. The medium was changed every 3 days and subcultures were performed weekly. For these experiments, cells between the 10th and 25th passage of a cloned cell line were used, although the cloned cells have continued to grow after 100 passages without any signs of senescence.

The mesangial nature of the cells was confirmed by accepted criteria (Striker & Striker, 1985). Cells were grown in insulin-containing medium (5 μg/ml) then washed twice in HBSS and incubated with angiotensin II. Contractions were detected by phase contrast microscopy. The cells were stained with monoclonal antibodies to rat common leucocyte antigen and rat Ia common antigen using the OX1 and OX6 reagents (courtesy of Dr A. Williams, Oxford, UK) human anti-vimentin antibody (Dakopatts, Glostrup, Denmark) by a peroxidase staining reaction as described previously (Hancock, Becker & Atkins, 1982). Polyclonal antibodies were also used to look for factor VIII staining (Dakopatts) and fibronectin (Beringwerke, Marburg, Germany). For the latter reagent, cold acetone fixation was used instead of the periodate–lysine–paraformaldehyde reagent used for the other antibodies. The cells reacted with anti-vimentin and anti-fibronectin antibodies but no positive reactions were obtained with OX1, OX6 or factor VIII antibody, excluding contamination by leucocytes or endothelial cells. The cells were also tested for their sensitivity to 10 μg/ml mitomycin-C and 100 μg/ml aminonucleoside puromycin. To exclude contamination by fibroblasts, the cells were grown in MEM (Eagles') with D-valine substituted for L-pavaline (Sigma Chemical Co., St Louis, MO) and with FCS that had been dialysed against three changes of normal saline (Gilbert & Migeon, 1975).

The *Limulus* assay (Sigma) was used to determine endotoxin levels in culture medium and reagents. No significant contamination was observed at the detection level of 0·1 ng/ml.

Reagents

EGF was from Sigma (purified from mouse submaxillary gland, tissue culture grade). Recombinant rat IFN-γ and anti rat-IFN-γ monoclonal antibody (DB-1) were kindly provided by Dr P. H. van der Meide (Rijswijk, The Netherlands). The anti-human B lymphocyte (CD22) monoclonal antibody (PHM14) produced in our laboratory was used as an isotype control. Recombinant human IFN-γ was the generous gift of Biogen Research Corp. (Cambridge, MA). Recombinant human PDGF was from Genzyme (Boston, MA). Recombinant human IL-1α was kindly provided by Hoffmann–La Roche (Nutley, NJ).

Thymidine uptake measurements

Mesangial cells were added to 96-well flat-bottomed microtitre trays in 20% FCS–RPMI 1640. Different numbers of cells were added to establish rapid growth or confluence. Preliminary experiments determined the appropriate times when these conditions were attained. After 24 h the medium was changed to RPMI 1640 with varying concentrations of FCS, with or without addition of the different growth factors/lymphokines. Controls for experiments involving PDGF included addition of acetic acid dilutions as this is the vehicle for this cytokine. An 18-h ^{3}H-thymidine pulse (0·5 μCi/well) was given prior to harvesting 2, 4 and 6 days after addition of the growth factors/lymphokines. For harvesting the medium was removed, the cells washed twice with warm phosphate-buffered saline (PBS) and then dissolved in 100 μl 0·2 *N* NaOH. This solution was neutralized with HCl and scintillation counting was performed (Wallac Rackbeta Counter). Replicates of six wells were used for all measurements.

Cell counts

In order to assess the effects of growth factors on cell numbers, experiments were conducted in 24-well 2-ml trays (Disposable Products, Adelaide, Australia). Thirty-thousand cells were pre-incubated in the wells for 24 h, washed with warm RPMI 1640 and new medium added (supplemented with the different growth factors/lymphokines). The cells were harvested at intervals by trypsinization and counted in a Coulter counter (Model DN; Coulter Electronics, Harpenden, UK).

DNA, RNA and protein synthesis

The measurement of tritiated precursor incorporation into macromolecules as distinct from cellular uptake was performed by using TCA precipitation as described by Cavender & Edelbaum (1988). Briefly, this depends on separating the non-incorporated soluble precursor molecules by washing from the precipitated macromolecules which include DNA, RNA and polypeptide chains. Hence only those precursors which are used to produce these larger molecules are measured. Thymidine (2 μCi/ml, 23 Ci/mM, Amersham), uridine (4 μCi/ml, 29 Ci/mM, Amersham) and leucine (10 μCi/ml, 140 Ci/mM, Amersham) incorporation was used as an index of DNA, RNA and protein synthesis, respectively.

Cell cycle and DNA content

Single cell suspensions were prepared by incubation with 0·1% trypsin (Commonwealth Serum Laboratories, Melbourne, Australia) and 0·02% EDTA in PBS. The cells were stained with propidium iodide as described by Krishan (1975). Briefly, the cells were incubated for 20 min at 4°C with DNAse (10 μg/ml; Worthington Biochemical Corp., Freehold, NJ) and were fixed for 30 min at 4°C with 50% methanol. After washing with PBS, the cells were incubated for 30 min at 37°C with RNAse (1 mg/ml; type 1A, Sigma Chemical Co.) in PBS. The cells were resuspended in propidium iodide solution (50 μg/ml in 0·1% sodium citrate). After 20 min of incubation in the dark on ice, they were mixed and passed through a 50-μm mesh and the fluorescence measured within 2 min on an EPICS 752 flow cytometer (Coulter Electronics). The EPICS argon laser was set to a wavelength of 488 nm, at a power of 600 mW for excitation of the PI dye. The analysis of cell cycle based on DNA content was based on the PARA1 software package (Coulter Electro-

nics). This program assumes Gaussian distributions for Go/G1 and G2+M cells and calculates the percentages of these cells and then S phase cells by subtraction from the total.

RESULTS

Effects of growth factors on mesangial cells

EGF is a known mesangial cell mitogen (Abboud *et al.*, 1987) and this was confirmed in our experiments by its capacity to stimulate thymidine uptake by mesangial cells growing rapidly in the presence of FCS or in confluent cultures (Fig. 1). These results were confirmed in separate experiments by counting cell numbers, as wells with added EGF contained more cells than control cultures (65 000 *versus* 43 000 cells/well in 24-well plates with 3 ng/ml EDG after 3 days; $P < 0.001$). Similarly, PDGF was mitogenic as expected (Fig. 1). Human recombinant IL-1 caused increases in thymidine uptake compared with controls when added to confluent cultures (Fig. 1), but, surprisingly, gave no significant increases in rapidly growing cultures (data not shown). In the former experiments, IL-1 was not added until the cells were visibly confluent and the rate of thymidine incorporation had decreased. The effects of IL-1 on rapidly growing cells were tested in separate experiments which involved release of cells from serum deprived conditions (20 000 cells in 0·5% FCS for 2 days) by addition of 5% FCS and IL-1 and a further culture period of 3 days. These results confirm than the assay system can detect growth signals additional to those provided by the FCS in the culture medium.

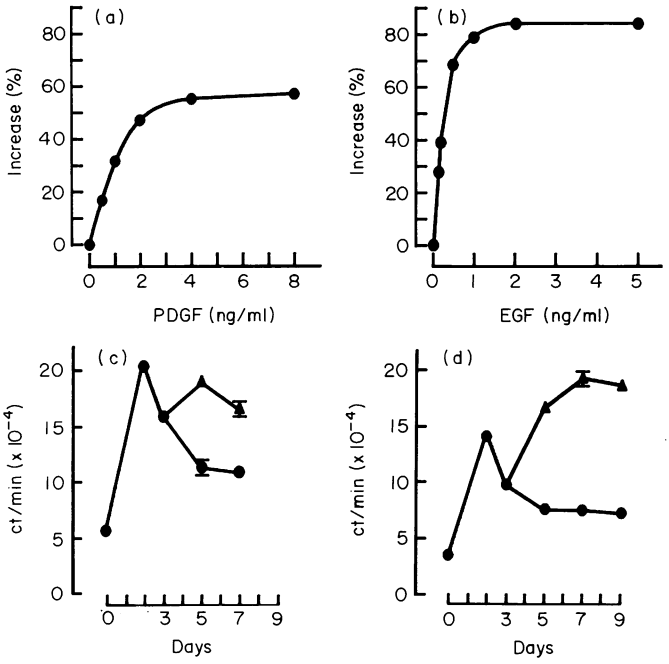

Fig. 1. Effect of various growth factors on thymidine uptake in rat mesangial cells. A, B, 5000 cells were pre-incubated in 20% FCS for 24 h and then serum deprived for 2 days in medium with 0·5% FCS. They were then incubated for 2 days with various doses of PDGF (or appropriate acetic acid dilution) in medium with 1% FCS (a) or EGF in medium with 5% FCS (b); (c), (d), 20 000 cells were incubated for 3 days in medium with 0·5% FCS and were then exposed to 5% FCS (day 0). After 3 days of incubation, the medium was changed to 5% FCS with or without IL-1 (1000 U/ml) (c) or EGF (1 ng/ml) (d). The results are expressed as percentage of increase of control in (a) and (b) (actual ct/min values (mean ± s.e.m.) for control and plateau responses for a and b were 32 590 ± 782 *versus* 51 503 ± 878 and 666 929 ± 933 *versus* 122 182 ± 3642, respectively) and the mean ± s.d. of six wells in (c) and (d) (error bars were mostly too short to be included). Symbols in (c) are: ●, medium alone; ▲, IL-1. Symbols in (d) are: ●, medium alone; ▲, EGF.

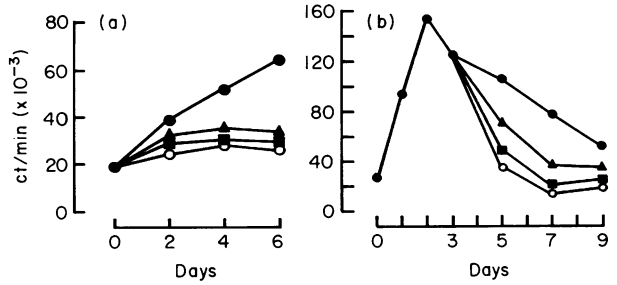

Fig. 2. Effects of IFN-γ or thymidine uptake in rat mesangial cells: (a) 5000 rapidly growing cells were pre-incubated in 20% FCS for 24 h and then given various doses of IFN-γ; (b) 20 000 cells were maintained for 3 days in medium with 0·5% FCS, then cultured in medium with 5% FCS up to Day 3 to achieve confluence. The cells were then exposed to various doses of IFN-γ in medium with 5% FCS. Medium and IFN-γ were replaced at 2-day intervals. The results are expressed as the mean of six wells in each point. ●, medium alone; ▲, IFN-γ 10 U/ml; ■, IFN-γ 100 U/ml; ○, IFN-γ 1000 U/ml.

Inhibitory effects of IFN-γ on mesangial cells

In contrast to the stimulatory effects by the known mitogenic factors (EGF, IL-1 and PDGF), addition of recombinant rat IFN-γ caused profound inhibition of thymidine uptake by mesangial cells. This effect was dose and time dependent (Fig. 2) and was seen when the cells were growing rapidly or when they had reached confluence.

The following experiments were performed to confirm the specificity of this observation. Human recombinant IFN-γ when added to the cultures resulted in only marginal levels of inhibition at comparable doses, for example reaching 12% inhibition at day 6 using 1000 U/ml in confluent cultures but caused 47% inhibition of rapidly growing human fibroblast (HE9 cell line) at day 2. Rat IFN-γ when added to rat skin fibroblasts (5th to 7th passage) cultures yielded very similar responses to mesangial cells (33% inhibition at day 6, $P < 0.01$) but did not inhibit human fibroblasts (data not shown). The specificity of the response was further explored by addition of the known anti-rat IFN-γ monoclonal antibody DB-1 which partially reversed the inhibitory effects of rat IFN-γ (see Table 1).

It is possible that thymidine uptake is not an accurate index of DNA synthesis (Hosang, 1988) and hence further studies were performed to determine the effects of IFN-γ on mesangial cell proliferation. These consisted of direct cell counting and determinations of thymidine incorporation into macromolecules by using TCA precipitation. Direct cell counting revealed an inhibitory effect of IFN-γ at day 3 using cultures of rapidly growing cells. This effect was dose dependent, a 17% reduction was observed with 1000 U/ml. In contrast, addition of 1 ng/ml EGF induced a 68% increase in cell numbers. TCA precipitation experiments yielded identical results to thymidine uptake studies when IFN-γ dose–response curves were compared on a

Table 1. Blocking of IFN-γ activity by specific antibody

	Thymidine uptake (ct/min)		
	Without antibody	DB-1	PHM14
Experiment 1			
IFN-γ (U/ml)			
0	84000 ± 3479	109937 ± 3482	98076 ± 2906
50	36920 ± 416 (44%)	80097 ± 936 (73%)	47693 ± 1252 (49%)
Experiment 2			
IFN-γ (U/ml)			
0	66467 ± 1711	83570 ± 1297	74415 ± 562
50	24719 ± 1064 (37%)	54147 ± 1751 (65%)	30253 ± 1053 (41%)

IFN-γ or medium was pre-incubated at 37°C for 1 h with a specific antibody, DB-1 (12·5 μg/ml), or with an isotype control antibody, PHM14. It was then added to 5000 cells and thymidine uptake was measured after 3 days. Means ± s.e.m. of six wells are shown, figures in parentheses are percentage values relative to controls without IFN-γ.

Table 2. Effect of IFN-γ on ^{3}H-uridine and leucine incorporation in confluent cells

	Exp. 1	Exp. 2	Exp. 3	Increase (%)
Uridine incorporation (ct/min per 10^5 cells)				
Control	136 ± 7	218 ± 8	227 ± 7	
IFN-γ	217 ± 4*	267 ± 7†	294 ± 9*	37
Leucine incorporation (ct/min per 10^5 cells)				
Control	8663 ± 354	7257 ± 91	6202 ± 544	
INF-γ	12771 ± 170*	10240 ± 257*	9126 ± 458*	45

Confluent cultures in 24-well plates containing medium with 5% FCS were maintained for 3 days with or without 1000 U/ml IFN-γ. Direct cell counts were then performed and results are expressed as means ± s.e.m. of six wells. *$P < 0.001$, †$P < 0.05$ *versus* control. Percentage values are means of the three experiments shown.

percentage basis (data not shown), confirming that the inhibitory effects of IFN-γ were not just directed at a cellular 'thymidine pool' phenomenon.

Analogous experiments were performed on rapidly growing cells with tritiated uridine and tritiated leucine. These indicate that RNA and protein synthesis were not inhibited by IFN-γ, in fact, there was a small increase in the amount of leucine incorporation on a per well basis (12–22% at days 1, 2 and 3). Experiments in large wells and confluent cells showed a larger and significant increase (37% and 45%, for RNA and protein synthesis, respectively) when these parameters were expressed on a per cell basis (see Table 2).

Interaction of IFN-γ with mesangial cell mitogens

In view of the reported ability of IFN-γ to inhibit PDGF- and EGF-induced proliferation of fibroblast and smooth muscle cell cultures (Hosang, 1988; Warner *et al.*, 1989), it was interesting to determine the interactions of these cytokines in this cell system, as mesangial cells are of mesenchymal origin and resemble smooth muscle cells (Striker & Striker, 1985). Addition of IFN-γ to cultures stimulated with PDGF yielded inhibitory profiles remarkably similar to those of control cultures which depended solely on FCS for growth stimulation (Fig. 3). Cultures stimulated with EGF were also inhibited, but proportionally this effect was greatly reduced (Fig. 3). It is clear from this comparison that there was less inhibition when EGF was present and that this cytokine appeared to partially reverse the activity of IFN-γ. IL-1-induced proliferation was affected in a similar manner to that observed for PDGF, being highly sensitive to the inhibitory activity of IFN-γ (See Fig. 3).

DISCUSSION

We have demonstrated that IFN-γ inhibits thymidine uptake and incorporation into a rat mesangial cell line. This confirms a previous report of the inhibitory action of this lymphokine on rat mesangial cells (Martin *et al.*, 1989). Earlier work on mesangial cells has emphasized the mitogenic activities of various cytokines (EGF, PDGF and IGF-1) and the lymphokines IL-1 and IL-6, so the description of an inhibitory factor is of interest as it may act as a regulatory substance, especially in the context of the inflammatory infiltrates observed in glomeru-

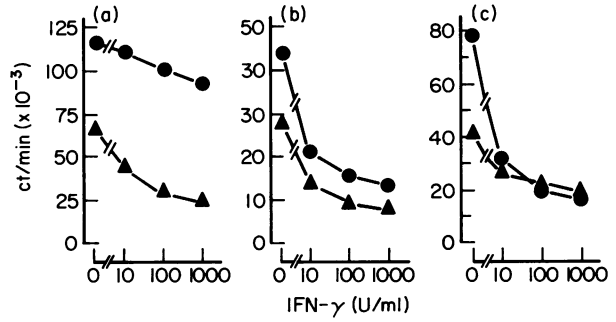

Fig. 3. Effect of IFN-γ on EGF-, PDGF- or IL-1-induced proliferation of rat mesangial cells. Five-thousand cells (a, b) or 20 000 cells (c) were pre-incubated for 24 h in 96-well plates with medium containing 20% FCS. (a) the rapidly growing cells were then changed to medium with 5% FCS and incubated with EGF (1 ng/ml) and/or various doses of IFN-γ for 3 days; (b) the cells were then maintained for 2 days under serum deprived conditions in 0·5% FCS. The cultures were then changed to medium with 1% FCS and PDGF (1 ng/ml) and/or various additions of IFN-γ, for two days; (c) the cells were also changed to 0·5% FCS for 3 days. They were then allowed to reach confluence after a further change to 5% FCS for 3 days. Finally, a 2-day incubation with IL-1 (1000 U/ml) and/or IFN-γ was performed. Values for thymidine uptake are shown as the mean of six wells in each case. Symbols are: ▲, IFN-γ alone in a, b, c; ●, IFN-γ + EGF in a; IFN-γ + PDGF in b; IFN-γ + IL-1 in c.

lonephritis. Thus the influx of T lymphocytes as well as macrophages into the kidney would be expected to lead to IFN-γ and IL-1 production, initially at the same time, as both lymphokines are produced in response to early immune activation events (Durum, Schmidt & Oppenheim, 1985; MacDonald, 1986).

The observation that IFN-γ inhibits mesangial cell thymidine uptake is in keeping with the known anti-proliferative effects of this lymphokine on a wide range of cells (Nickoloff *et al.*, 1984; Friesel *et al.*, 1987; Gajewski & Fitch, 1988; Warner *et al.*, 1989). It is also known to affect a variety of specific biochemical events in different cell types (Tsujisaki *et al.*, 1987; Morganelli & Guyre, 1988; Taetle & Honeysett, 1988; Suzuki *et al.*, 1989). In considering the potential net effect of this cytokine on mesangial cells *in vivo*, however, its capacity to activate macrophage production of IL-1 must be weighed. Thus the direct inhibitory action of IFN-γ would appear to be competing with the mitogenic action of IL-1. The *in vitro* data presented here would indicate that in this particular case the inhibitory effect ought to be dominant.

The bulk of these studies involved thymidine uptake measurements which are presumed to provide an index of the cell's proliferative status. However, as it is possible that thymidine pool size changes and other metabolic aspects of this compound may be affected independently of those involved in cell division (Hosang, 1988), we sought to establish that uptake was in fact a good determinant of incorporation into macromolecules and a correlate of cell division. This was achieved by the demonstration that TCA precipitation studies gave exactly parallel results to those achieved with uptake measurements and that cell counting confirmed the mitotic or inhibitory potential of the cytokine/lymphokine in question.

The specificity of the inhibitory reaction seen with the IFN-γ preparation is determined by a number of factors. The lymphokine is a recombinant protein and would therefore be expected to be free of other lymphokines; the most likely contaminant in this preparation is endotoxin and this was not found in significant amounts by the *Limulus* assay, neither did large doses of LPS (up to 100 μg/ml) have comparable inhibitory activities to those observed with IFN-γ (data not shown; maximum inhibition obtained with LPS was 30%). The well-known species restriction of this lymphokine applied to these experiments; lymphokines and cells of human and rat origins had the appropriate restricted reactivity for each other and the use of the rat anti-IFN-γ monoclonal antibody DB-1 caused significant (but not complete) reversal of IFN-γ inhibition. Complete inhibition is not necessarily expected in this type of experiment as the antibody is in competition with a high affinity solid phase (cell surface) receptor for a few days. The described species specificity data also argues against the presence of a non-specific toxic factor.

The rat mesangial cells responded to the mitogens PDGF, EGF and IL-1 as expected and as is the case with other cell types such as fibroblasts and smooth muscle cells for example (Carpenter & Cohen, 1979; Kimball, Fisher & Persico, 1988; Warner *et al.*, 1989). Our results suggest that IL-1 serves as a mitogen in confluent as opposed to rapidly growing cultures. This might mean that IL-1 does not provide an additional mitogenic signal to rapidly growing cells, or that there is optimal production of it under these conditions. The finding that EGF and PDGF were able to elicit extra growth from these cells does, however, indicate that some cells were available for further stimulation. Rapidly growing mesangial cells have been shown to produce IL-1 mRNA whereas cells maintained in serum-free medium did not do so (Lovett & Larsen, 1988). The particular cell line used in our studies has been shown to produce equivalent levels of IL-1α mRNA under conditions of rapid and confluent growth (D. Paterson, personal communication). This observation, together with our inability to elicit extra growth by adding IL-1 to rapidly growing cultures, suggests that it is the cell's capacity to respond to this factor rather than its ability to make it which changes when confluence is attained.

The question of whether IL-1-mediated mitogenic activity occurs with confluent *versus* actively growing cells is contentious. IL-1 has been described elsewhere as an autocrine growth factor acting exclusively on rapidly growing cells and potentiating the activity of PDGF (Lovett *et al.*, 1983a, 1986). However, Horii *et al.* (1989) showed, as we have, stimulation by IL-1 when cells from confluent cultures were used. The differing conclusions may hinge on the definition of rapid growth.

Experiments to determine the interaction of these mitogenic factors with IFN-γ indicated that the PDGF response was as sensitive to inhibition as was the growth of these cells in normal FCS-containing medium, where PDGF is probably the dominant growth factor (Ross, Raines & Bowen-Pope, 1986). The proliferative response to IL-1 was also highly sensitive to IFN-γ. As both PDGF and IL-1 are produced by mesangial cells and may be considered as putative autocrine growth factors, it is relevant that their synthesis by endothelial cells is inhibited by IFN-γ (Suzuki *et al.*, 1989). The latter lymphokine may therefore act to block the mitogenic stimulus provided by IL-1 and PDGF at the transcriptional level. However, IFN-γ promotes IL-1 gene transcription in macrophages (Collart *et al.*, 1986), so it remains to be established whether the inhibitory effects observed *in vitro* with mesangial cells can be confirmed *in*

vivo. The inhibitory effect of IFN-γ on the EGF response was much less obvious; indeed, the subtraction of the control response values leaves the impression that it was not affected at all and suggests that the mitogenic factors operate through different routes in this cell line. A previous study of the inhibitory effects of IFN-γ on human fibroblasts concluded that both EGF and PDGF responses were sensitive to this lymphokine (Hosang, 1988) although more IFN-γ was required to achieve 50% inhibition of the EGF response than the PDGF response.

IFN-γ acts not only to inhibit cell proliferation but also regulates various cell products such as MHC class II molecules in many cell types, collagen matrix desposition and IL-1 production. It is therefore feasible that mesangial cells can respond to it also by alterations in the production of matrix proteins and other known secretory products such as IL-1. Indeed, MHC class II molecules production has recently been described following stimulation by IFN-γ of rat mesangial cells (Martin *et al.*, 1989). Our results indicate that total protein synthesis as measured by ^{3}H-leucine is increased despite marked inhibition of cell division. This implies a switch from production of proteins involved in cell division to others of unknown function. Such functions may be geared to secretory molecules such as IL-1, PGE2 (as is the case for IL-1-activated mesangial cells) and matrix components such as fibronectin and collagen.

Work is in progress to determine the production of these components by mesangial cells in response to IFN-γ. We are also exploring the interactions of macrophages with mesangial cells *in vitro* and *in vivo* following exposure to this lymphokine.

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REFERENCES

ABBOUD H.E., SAUNDERS, C. & KNAUSS, T.C. (1987) Effect of epidermal growth (EGF) on cultured mesangial cells. *Kidney Int.* **31,** 184 (Abstract).

ARDAILLOU, R., SRAER, J., CHANSEL, D., ARDAILLOU, N. & SRAER, J.D. (1987) The effects of angiotensin II on isolated glomeruli and cultured glomerular cells. *Kidney Int.* **31,** (Suppl. 20), S74.

CARPENTER, G. & COHEN, S. (1979) Epidermal growth factor. *Annu. Rev. Biochem.* **48,** 193.

CAVENDER, D.E. & EDELBAUM, D. (1988) Inhibition by IL-1 of endothelial cell activation induced by tumor necrosis factor or lymphotoxin. *J. Immunol.* **141,** 3111.

COLLART, M.A., BELIN, D., VASSALLI, J.D., DE KOSSODO, S. & VASSALLI, P. (1986) Interferon gamma enhances macrophage transcription of TNF/cachectin, Interleukin-1 and urokinase genes which are controlled by short lived repressors. *J. exp. Med.* **164,** 2113.

DOI, T., STRIKER, L.J., ELLIOT, S.J., CONTI, F.G. & STRIKER, G.E. (1989) Insulin like growth factor-1 is a progression factor for human mesangial cells. *Am. J. Pathol.* **134,** 395.

DURUM, S.K., SCHMIDT, J.A. & OPPENHEIM, J.J. (1985) Interleukin 1: an immunological perspective. *Annu. Rev. Immunol.* **3,** 263.

FRIESEL, R., KOMORIYA, A. & MACIAG, T. (1987) Inhibition of endothelial cell proliferation by gamma interferon. *J. cell. Biol.* **104,** 689.

GAJEWSKI, T.F. & FITCH, F.W. (1988) Anti-proliferative effect of IFN-γ in immune regulation. I. IFN-γ inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. *J. Immunol.* **140,** 4245.

GILBERT, S.F. & MIGEON, B.R. (1975) D-valine as a selective agent for normal human and rodent epithelial cells in culture. *Cell,* **5,** 11.

GLASGOW, E.F., HANCOCK, W.W. & ATKINS, R.C. (1981) The technique of glomerular culture. In *Techniques for Nephropathology* (ed. by D. Allen & J. Dowling) p. 87, CRC Press, Boca Raton, FL.

HANCOCK, W.W., BECKER, G.J. & ATKINS, R.C. (1982) A comparison of fixatives and immunohistochemical techniques for use with monoclonal antibodies to cell surface antigens. *Am. J. clin. Pathol.* **78,** 825.

HOOKE, D.H., HANCOCK, W.W., KRAFT, N. & ATKINS, R.C. (1984) Monoclonal antibody analysis of glomerular hypercellularity in human glomerulonephritis. *Clin. Nephrol.* **22,** 163.

HOOKE, D.H., GEE, D.C. & ATKINS, R.C. (1987) Leukocyte analysis using monoclonal antibodies in human glomerulonephritis. *Kidney Int.* **31,** 964.

HORII, Y., MURAGUCHI, A., IWANO, M., MATSUDA, T., HIRAYAMA, T., YAMADA, H., FUJI, Y., DOHI, K., ISHIKAWA, H., OHMOTO, Y., YOSHIZAKI, K., HIRANO, T., KISHIMOTO, T. (1989) Involvement of IL-6 in mesangial proliferative glomerulonephritis. *J. Immunol.* **143,** 3949.

HOSANG, M. (1988) Recombinant interferon inhibits the mitogenic effect of platelet-derived growth factor at a level distal to the growth factor receptor. *J. cell. Physiol.* **134,** 396.

ISHIMURA, E., STERZEL, R.B., BUDDE, K. & KASHGARIAN, M. (1989) Formation of extracellular matrix by cultured rat mesangial cells. *Am. J. Pathol.* **134,** 843.

KIMBALL, E.S., FISHER, C. & PERSICO, F.J. (1988) Potentiation of Balb/3T3 fibroblast proliferative response by interleukin-1 and epidermal growth factor. *Cell. Immunol.* **113,** 341.

KRISHAN, A. (1975) Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining. *J. cell. Biol.* **66,** 188.

LOVETT, D.H. & LARSEN, A. (1988) Cell cycle-dependent interleukin 1 gene expression by cultured glomerular mesangial cells. *J. clin. Invest.* **82,** 115.

LOVETT, D.H., RYAN, J.L. & STERZEL, B. (1983a) Stimulation of rat mesangial cell proliferation by macrophage interleukin 1. *J. Immunol.* **131,** 2830.

LOVETT, D.H., STERZEL, R.B., KASHGARIAN, M. & RYAN, J.L. (1983b) Neutral proteinase activity produced *in vitro* by cells of the glomerular mesangium. *Kidney Int.* **23,** 342.

LOVETT, D.H., SZAMEL, M., RYAN, J.L., STERZEL, R.B., GEMSA, D. & RESCH, K. (1986) Interleukin 1 and the glomerular mesangium. I. Purification and characterization of a mesangial cell-derived outgrowth factor. *J. Immunol.* **136,** 3700.

MACDONALD, H.R. (1986) T-cell activation. *Annu. Rev. Cell Biol.* **2,** 231.

MARTIN, M., SCHWINZER, R., SCHELLEKENS, H. & RESCH, K. (1989) Glomerular mesangial cells in local inflammation. Induction of the expression of MHC class II antigens by IFN-gamma. *J. Immunol.* **142,** 1887.

MORGANELLI, P.M. & GUYRE, P.M. (1988) IFN-γ plus glucocorticoids stimulate the expression of a newly identified human mononuclear phagocyte-specific antigen. *J. Immunol.* **140,** 2296.

NICKOLOFF, B.J., BASHAM, T.Y., MERIGAN, T.C. & MORHENN, V.B. (1984) Antiproliferative effects of recombinant gamma interferon on cultured human keratinocytes. *Lab. Invest.* **51,** 697.

NOLASCO, F.E.B., CAMERON, J.S., HARTLEY, B., COELHO, A., HILDRETH, G. & REUBEN, R. (1987) Intraglomerular T cells and monocytes in nephritis: study with monoclonal antibodies. *Kidney Int.* **31,** 1160.

ROSS, R., RAINES, E.W. & BOWEN-POPE, D.F. (1986) The biology of platelet derived growth factor. *Cell,* **46,** 155.

SCHLONDORFF, D. (1987) The glomerular mesangial cell: an expanding role for a specialized pericyte. *FASEB J.* **1,** 272.

SCHMIEGEL, W.H., CAESAR, J., KALLHOFF, H., GRETEN, H., SCHREIBER, H.W. & THIELE, H.G. (1988) Antiproliferative effects exerted by recombinant human tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) on human pancreatic tumour cell lines. *Pancreas,* **3,** 180.

SHULTZ P.J., DICORLETO, P.E., SILVER, B.J. & ABBOUD, H.E. (1988)

Mesangial cells express PDGF mRNAs and proliferate in response to PDGF. *Am. J. Physiol.* **255,** F674.

STERZEL, R.B. & PABST, R. (1982) The temporal relationship between glomerular cell proliferation and monocyte infiltration in experimental glomerulonephritis. *Virchows Arch. [Cell Pathol].* **38,** 337.

STRIKER, G.E. & STRIKER, L.J. (1985) Biology of disease. Glomerular cell culture. *Lab. Invest.* **53,** 122.

SUZUKI, H., SHIBANO, K., OKANE, M., KONO, I., MATSUI, Y., YAMADA, K. & KASHIWAGI, H., (1989) Interferon-modulates messenger RNA levels of c-sis (PDGF-B chain), PDGF-A chain, and IL-1b genes in human vascular endothelial cells. *Am. J. Pathol.* **134,** 35.

TAETLE, R., HOENYSETT, J.M. (1988) Gamma-interferon modulates monocyte/macrophage transferrin receptor expression. *Blood,* **71,** 1590.

TSUJISAKI, M., IGARASHI, M., SAKAGUCHI, K., EISINGER, M., HERLYN M. & FERRONE, S. (1987) Immunochemical and functional analysis of HLA class II antigens induced by recombinant immune interferon on normal epidermal melanocytes. *J. Immunol.* **138,** 1310.

WARNER, S.J.C., FRIEDMAN, G.B. & LIBBY, P. (1989) Immune interferon inhibits proliferation and induces 2′-5′-oligoadenylate synthetase gene expression in human vascular smooth muscle cells. *J. clin. Invest.* **83,** 1174.

WERBER, H.I., EMANCIPATOR, S.N., TYKOCINSKI, M.L. & SEDOR, J.R. (1987) The Interleukin 1 gene is expressed by rat glomerular mesangial cells and is augmented in immune complex glomerulonephritis. *J. Immunol.* **138,** 3207.