Interleukin-13 inhibits inducible nitric oxide synthase expression in human mesangial cells

Marta SAURA,* Rosa MARTÍNEZ-DALMAU,* Adrian MINTY,† Dolores PÉREZ-SALA* and Santiago LAMAS*‡

*Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid, Spain, and †Sanofi Recherche, Labège, France

The synthesis of nitric oxide in inflammatory situations requires the expression of an inducible isoform of nitric oxide synthase (iNOS). Human mesangial cells (HMC) express an iNOS enzyme after exposure to multiple co-stimuli. In this study we have observed that while tumour necrosis factor- α , interleukin (IL)-1 β , interferon- γ and bacterial lipopolysaccharide (LPS) were unable to significantly induce NO synthesis when used alone, they induced an evident stimulation of NO synthesis when used in various combinations. A mixture of the three cytokines (CM) and LPS resulted in a 10–15-fold stimulation of NO synthesis over control values which started to be significant after 16 h. The addition of IL-13, a cytokine with anti-inflammatory properties,

inhibited CM/LPS-induced NO synthesis in a concentration-dependent manner. A marked inhibitory effect (60–65%) could be observed when HMC were treated with IL-13 (10 ng/ml) 24 h before, at the same time as, or even 4 h after the addition of CM/LPS. This inhibitory effect was still significant (25%) when IL-13 was added 16 h after CM/LPS. Northern analysis showed that IL-13-mediated iNOS inhibition was closely correlated with the suppression of iNOS mRNA expression. These results identify IL-13 as a powerful regulatory tool for the inhibition of NO synthesis in human cells, a property which may be pathophysiologically relevant in NO-related inflammatory processes.

INTRODUCTION

In the past decade nitric oxide (NO) has been identified as a biological messenger molecule which participates in many physiological and pathophysiological processes [1]. Its synthesis is achieved by a family of enzymes, the nitric oxide synthases (NOSs), which convert the amino acid L-arginine into L-citrulline and NO. The expression and regulation of each of the three isoforms which comprise this family, i.e. neuronal NOS, endothelial NOS and inducible NOS (iNOS), is distinct and complex [2]. Inflammation is one of the pathophysiological states in which NO seems to play a central role. NO has been implicated in processes such as microbial killing, septic shock, leucocyte adhesion and diabetes [3]. The molecular and cellular mechanisms responsible for NO-mediated responses are beginning to be explored. The generation of potentially toxic free radical species such as peroxynitrite (ONOO⁻) and/or the inhibition of key regulatory enzymes may be invoked as active participants in inflammatory events where a potential role for NO has been encountered [4]. A common feature of these situations is the expression of iNOS in the specific tissue being studied. This NOS isoform is mainly regulated at the level of transcription and does not require calcium for its activation. This scenario creates interest not only for the study of the mechanisms involved in these processes but also in the search for endogenous or exogenous negative regulators of induced NO synthesis in inflammation. To date, several cytokines/growth factors as well as other compounds have been shown to negatively modulate induced NO synthesis. Thus interleukins -4, -8 and -10, transforming growth factors, epidermal growth factor, fibroblast growth factors, insulin-like growth factor-1, macrophage deactivating factor, macrophage stimulating protein, platelet-derived growth factor, osteopontin and dexamethasone [4,5] have been found to inhibit stimulated NO synthesis in different

species and cell types. In keeping with the search for new regulatory tools of this pathway, we were recently able to establish a role for an antioxidant, pirrolidin dithiocarbamate, as an inhibitor of NO generation, using rat mesangial cells as a model [6]. Mesangial cells are modified vascular smooth muscle cells which are interspersed among the capillaries of the renal glomerulus, serving functions of architectural support and regulation of macromolecular trafficking and glomerular filtration [7]. They express iNOS under the influence of cytokines and microbial wall products such as lipopolysaccharide (LPS), and the regulation of this effect has been studied in cells of rat origin [8,9]. The involvement of NO in diseases such as glomerulonephritis has been described [10]. The investigation of the stimulatory pathways and modes of inhibition of NO synthesis in this cell type is, therefore, relevant.

IL-13 is a 132-amino-acid novel lymphokine which was isolated from activated human T lymphocytes and is expressed in the T helper subset 2 (TH-2) lymphocyte population [11]. This cytokine has been shown to display anti-inflammatory properties. Some of its actions include the modulation of monocyte and B cell function, suppression of cytotoxicity mediated by monocytes/macrophages and inhibition of the production of pro-inflammatory cytokines [12]. We postulated that IL-13 might participate in the regulation of NO synthesis in human cells, as NO generation is an important phenomenon in inflammatory situations. Results herein reported demonstrate that IL-13 negatively regulates induced NO synthesis in human mesangial cells.

MATERIALS AND METHODS

Materials

Recombinant human tumour necrosis factor- α (rhTNF- α ; 9.8×10^6 units/mg) was a gift from Knoll Pharmaceuticals

Abbreviations used: HMC, human mesangial cells; NO_x, sum of NO₂⁻ + NO₃⁻; iNOS, inducible nitric oxide synthase; IL, interleukin; TNF- α , tumour necrosis factor- α ; IFN- γ , interferon- γ ; CM, cytokine mixture (rhTNF- α + rhIL-1 β + rhIFN- γ ; the prefix rh denotes recombinant human); LPS, lipopolysaccharide.

‡ To whom correspondence should be addressed, at: Departamento de Estructura y Función de Proteínas, Centro de Investigaciones Biológicas, Velázquez 144, 28006 Madrid, Spain

(Whippany, NJ, U.S.A.); recombinant human interferon- γ (rhIFN- γ ; 6.9×10^7 units/mg) was from Hoffmann-La Roche; recombinant human IL-1 β (rhIL-1 β ; 5×10^7 units/mg) was from Boehringer (Mannheim, Germany); recombinant human IL-4 (rhIL-4; 10^8 units/mg) was a gift from Dr. A. de la Hera (Centro de Investigaciones Biológicas, Madrid, Spain); and recombinant human IL-13 (rhIL-13) was from Sanofi Recherche (Labège, France). Cell culture media and fetal calf serum were from Gibco; media supplements and Dulbecco's modified Eagle's medium devoid of Phenol Red were from Bio-Whittaker (Walkersville, MD, U.S.A.); cell culture plates were from Costar (Cambridge, MA, U.S.A.); [α - 32 P]dCTP (3000 Ci/mmol) was from Amersham; nitrate reductase was from Boehringer. Antibodies for cell characterization were obtained from local sources. All other reagents, including LPS (*Escherichia coli* strain 026:B6), were from Sigma (St. Louis, MO, U.S.A.).

Cell culture

Human mesangial cells (HMC) were obtained from normal cadaver kidneys which were unsuitable for transplantation. Initial primary cultures were obtained using a technique of graded sieving. Groups of cells with the morphological characteristics of mesangial cells were subcloned and expanded. Characterization was performed as follows: (a) morphological criteria with elongated appearance; (b) staining with anti-Thy 1.1; (c) staining with anti-vimentin; (d) staining with anti-myosin; and (e) absence of staining with anti-(factor VIII). The cells were cultured in 100 mm plates or 12-well plates in 5% CO₂ and fed every 48 h with RPMI 1640 supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin (100 units/ml) and streptomycin (100 μ g/ml). The potential toxicity of reagents for HMC was ruled out by exclusion of Trypan Blue evaluated by optical microscopy (> 90% of cells were viable) and of propidium iodide using flow cytometry analysis (> 90% viable).

Incubation procedure

On the day of the experiment, cells were washed twice with RPMI devoid of Phenol Red, as this compound may interfere with NO₂⁻ fluorometric measurement, and left in their usual medium except for the absence of serum and Phenol Red. Confluent plates of HMC were incubated with the different reagents as described for each experiment. At the end of the experiment, supernatants were collected for NO₂⁻ measurements and stored at -80 °C. In some cases, cells from these same plates were used for RNA isolation as described below.

NO₂⁻ and NO_x (NO₂⁻ + NO₃⁻) determinations

The procedure for NO₂⁻ measurement was based on the fluorescent reaction of NO₂⁻ with 2,3-diaminonaphthalene using a method described previously [13]. In brief, 50 μ l of a 0.05 mg/ml solution of 2,3-diaminonaphthalene prepared in 0.62 M HCl was added to 500 μ l of sample and 500 μ l of water. After a 10 min incubation in the dark at room temperature, the reaction was stopped with 29.4 μ l of 5 M NaOH. Fluorescence was determined in a Perkin-Elmer LS 50B fluorometer using excitation and emission wavelengths of 365 and 450 nm respectively. Samples of RPMI 1640 that had not been exposed to mesangial cells were assayed for background levels of NO₂⁻, and these values were subtracted from the values measured in the cell culture media. In a subset of experiments, NO_x (NO₂⁻ + NO₃⁻) was determined according to published methods [14].

RNA isolation and Northern blot analysis

Total cellular RNA was isolated from HMC using the guanidinium thiocyanate/phenol/chloroform method [15], size-fractionated by electrophoresis (10 μ g/lane) through denaturing 1% agarose/0.66 M formaldehyde gels, transferred to Hybond hybridization transfer membranes (Amersham) and UV-cross-linked before hybridization as previously described [16]. A 1.8 kb fragment of the cDNA encoding the human hepatic iNOS isoform (a gift from Dr. David Geller, University of Pittsburgh, PA, U.S.A.) [17] was labelled with [α - 32 P]dCTP using a commercial kit for random hexamer labelling (Boehringer Mannheim) and used as a probe for Northern analysis. Hybridization was performed at 42 °C for 12–16 h. Membranes were then washed at final stringency conditions of 0.2 $\times$ SSC/0.1% SDS at 55 °C and exposed on Kodak X-AR film, using an intensifying screen. In order to correct for uneven lane loading and to confirm that differences in mRNA expression were specific for iNOS, every blot was denuded and rehybridized with a β -actin probe [18]. Densitometric analysis was performed on an Apple scanner using Image 1.3.1 program (NIH, Bethesda, MD, U.S.A.) or on phosphorimager equipment (Molecular Dynamics, Sunnyvale, CA, U.S.A.). Results are expressed in arbitrary units as the ratio of iNOS/ β -actin expression.

Statistical analysis

Each experimental condition was performed in triplicate within each experiment, except for Northern analysis. Comparisons were made by analysis of variance followed by Dunnett's modification of the *t* test where there was a common control, or by the unpaired two-tailed Student's *t* test for other comparisons. This test was used whenever the number of total observations allowed it and after performing a Kolmogorov-Smirnov test to confirm the normal distribution of the data. When the number of observations was insufficient to perform adequate parametric tests, non-parametric tests were employed (Kruskal-Wallis). The level of statistical significance was defined as *P* < 0.05.

RESULTS

HMC synthesize NO after induction by multiple co-stimulatory signals

Because of the scarcity of reports concerning the activation of iNOS in HMC, we first characterized the behaviour of these cells under potential conditions of iNOS induction. For this purpose several cytokines and LPS, known to induce the synthesis of NO in other systems, were used alone and in all possible combinations. Exposure of HMC to rhTNF- α (100 ng/ml), rhIL-1 β (100 units/ml), rhIFN- γ (100 units/ml) or LPS (10 μ g/ml) independently did not induce the activation of iNOS, as measured by NO₂⁻ accumulation. Some combinations of two or three cytokines or of one or two cytokines plus LPS induced significant activation of iNOS, although the maximal response (10–15-fold) was achieved with the combination of the three cytokines tested plus LPS (Figure 1). For subsequent experiments the cytokine mixture (CM) TNF- α + IL-1 β + IFN- γ in the presence of LPS (CM/LPS) was employed.

Incubation of stimulated HMC (+CM/LPS) with two different NOS antagonists, N^G-methyl-L-arginine (500 μ M, 24 h) and N^w-nitro-L-arginine (500 μ M, 24 h), significantly blunted CM/LPS-induced NO production (80% and 70% inhibition respectively) (*n* = 2), thus supporting the notion that NO generated in these cell treatments was L-arginine-dependent.

Figure 1 Effect of cytokines, LPS and their combinations on NO production in HMC

Cells were incubated for 24 h in the presence of vehicle, rhTNF- α (100 ng/ml), rhIFN- γ (100 units/ml), rhIL-1 β (100 units/ml) and LPS (10 μ g/ml) either alone or in different combinations, as shown. Bar graphs represent the fold stimulation over control NO $_2^-$ levels. Shown are means $\pm$ S.E.M. from a representative experiment out of three.

Figure 2 Effect of rhIL-13 on the time course of NO production in HMC treated with CM/LPS

Cells were treated with a combination of rhTNF- α (100 ng/ml), rhIFN- γ (100 units/ml), rhIL-1 β (100 units/ml) and LPS (10 μ g/ml) for the indicated periods in the absence (open circles) or presence (closed circles) of 10 ng/ml rhIL-13. Data represent means $\pm$ S.E.M. of NO $_2^-$ determinations from two independent experiments, each performed in triplicate. Where error bars are not visible they are smaller than data point. * $P < 0.05$ versus absence of rhIL-13.

IL-13 inhibits the synthesis of NO induced by CM/LPS in HMC

We next tested whether IL-13, a known negative regulator of pro-inflammatory signals, could modulate the synthesis of NO. Figure 2 shows the time course of NO production by HMC stimulated with CM/LPS in the presence or absence of IL-13. The inhibitory effect of IL-13 could be seen from the moment at which NO generation in response to CM/LPS became detectable. IL-13 inhibited the synthesis of NO induced by CM/LPS in a dose-dependent fashion (Figure 3). A threshold effect was observed with 0.1 ng/ml IL-13, the IC $_{50}$ was 0.87 ± 0.03 ng/ml ($n = 3$) and a maximal effect was observed with 10 ng/ml (75 %

Figure 3 Effect of different concentrations of rhIL-13 on NO production in HMC treated with CM/LPS

Cells were treated with a combination of rhTNF- α (100 ng/ml), rhIFN- γ (100 units/ml), rhIL-1 β (100 units/ml) and LPS (10 μ g/ml) for 24 h in the absence or presence of rhIL-13 at the indicated concentrations. Data represent means $\pm$ S.E.M. of NO $_2^-$ determinations from one representative experiment out of three, each performed in triplicate. Where error bars are not visible they are smaller than data point. * $P < 0.05$ versus absence of rhIL-13.

inhibition relative to the effect of CM/LPS without IL-13). Higher doses of IL-13 (100 ng/ml) did not result in a greater inhibitory effect of induced NO synthesis. In order to exclude a potential effect of IL-13 on the oxidation of NO $_2^-$ to NO $_3^-$ [19] which could account for part of the inhibitory effect of IL-13 on NO $_2^-$ accumulation, we determined NO $_x$ in three different experiments in which HMC were treated with vehicle, IL-13 (10 ng/ml, 24 h), CM/LPS (24 h at concentrations given above) and CM/LPS + IL-13 (24 h). NO $_2^-$, expressed as a percentage of NO $_x$, was similar for all experimental conditions: vehicle, 25.6 ± 4.8 %; IL-13, 27.4 ± 6.9 %; CM/LPS, 25.7 ± 1.5 %; CM/LPS + IL-13, 30.8 ± 1.3 %, thus implying that IL-13 was not selectively modulating nitrite oxidase activity in this cell type. Because of the high concentration of NO $_3^-$ salt in RPMI (100 mg/l), the latter subset of experiments was performed in Dulbecco's modified Eagle's medium without Phenol Red (concentration of NO $_3^-$ salt, 0.1 mg/l).

Figure 4 Time course of the IL-13-mediated inhibition of NO synthesis in HMC stimulated with CM/LPS

Cells were incubated for 24 h in the absence or presence of rhTNF- α (100 ng/ml), rhIFN- γ (100 units/ml), rhIL-1 β (100 units/ml) and LPS (10 μ g/ml) (CM/LPS). rhIL-13 (10 ng/ml) was added to cells either 24 h before CM/LPS (48 h total exposure time), together with CM/LPS (24 h total exposure time) or at several time points after the addition of CM/LPS (20, 16, 12 and 8 h total exposure times). Data represent means $\pm$ S.E.M. of NO $_2^-$ determinations from three different experiments, each performed in triplicate. Where error bars are not visible they are smaller than data point. * $P < 0.05$ versus control; † $P < 0.05$ versus CM/LPS.

Figure 5 Effect of different concentrations of IL-13 on iNOS mRNA expression in HMC stimulated with CM

The top panel shows Northern blot analysis of total RNA (10 µg/lane) from HMC probed with a 1.8 kb fragment of human hepatic iNOS cDNA. Expression of rat β-actin in the same membrane is shown in the middle panel, and the densitometric analysis of the iNOS Northern blot corrected for β-actin expression is shown below. A 4.5 kb transcript was visible in stimulated cells. Cells were treated with vehicle (CT), IL-13 (10 ng/ml; 24 h) and a combination of rhTNF-α (100 ng/ml), rhIFN-γ (100 units/ml) and rhIL-1β (100 units/ml) (CM) for 24 h in the absence and presence of rhIL-13 (24 h) at the indicated concentrations. Representative data from one of two independent experiments are shown.

We were also interested in establishing the temporal pattern of inhibition exerted by IL-13. As shown in Figure 4, IL-13 was able to significantly inhibit the CM/LPS-mediated induction of NO synthesis when added before (62% inhibition), at the same time as (61% inhibition) or even after the co-stimulatory mixture (64, 50, 38 and 25% inhibition when IL-13 was added 4, 8, 12 and 16 h after CM/LPS respectively). Thus IL-13 was able to inhibit induced NO synthesis over a wide temporal window.

It has been shown that IL-13 shares some biological actions with IL-4, another cytokine with important regulatory effects on cells of the immune system. As NO synthesis and iNOS expression have been shown to be modified by this cytokine in murine macrophages [20], we decided to investigate whether IL-4 could also have an effect similar to that of IL-13 in the modulation of NO synthesis in HMC. Inclusion of rhIL-4 (10 ng/ml) during stimulation of HMC with CM/LPS for 24 h abrogated the synthesis of NO to a similar degree as with rhIL-13 (~60%). However, in two independent experiments in which IL-4 was added for 8 h (16 h after the inductive stimuli), it failed to significantly inhibit NO synthesis induced by CM/LPS stimulation: vehicle, 1.71 ± 0.38 ; CM/LPS, 19.83 ± 0.93 ; CM/LPS + IL-4, 16.82 ± 0.76 nmol/10⁶ cells ($P < 0.05$ vehicle versus CM/LPS and CM/LPS + IL-4). Simultaneous addition of both cytokines did not result in a further degree of inhibition ($n = 2$; results not shown).

Figure 6 Effect of rhIL-13 on the time course of iNOS mRNA expression in HMC treated with CM/LPS

The top panel shows Northern blot analysis of total RNA (10 µg/lane) from HMC probed with a 1.8 kb fragment of human hepatic iNOS cDNA. Expression of rat β-actin in the same membrane is shown in the middle panel, and the densitometric analysis of the iNOS Northern blot corrected for β-actin expression is depicted below. Cells were treated with vehicle (CT), rhIL-13 alone (10 ng/ml; 24 h) or a combination of rhTNF-α (100 ng/ml), rhIFN-γ (100 units/ml), rhIL-1β (100 units/ml) and LPS (10 µg/ml) for the indicated periods in the absence or presence of rhIL-13 (10 ng/ml). Representative data from one of two independent blots are shown.

IL-13 inhibits iNOS mRNA expression in HMC in a dose-dependent fashion

In order to gain insight into the molecular mechanisms involved in the inhibitory effect of IL-13 on induced NO synthesis, we performed Northern analysis to monitor the changes in iNOS mRNA expression. As shown in Figure 5, exposure of HMC to CM resulted in the appearance of a single 4.5–4.6 kb transcript which corresponds to iNOS mRNA. This message was absent from both vehicle- and IL-13-treated HMC. The addition of IL-13 blunted CM-induced iNOS mRNA expression in a concentration-dependent manner, which was correlated closely with the dose-response curve of NO synthesis (Figure 3), as may be inferred from densitometric analysis (Figure 5, bottom panel). Essentially the same results were obtained when CM/LPS was used as the inductive stimulus (not shown). We were also interested in defining the temporal course of inhibition by IL-13 of iNOS mRNA expression. An inhibitory effect of IL-13 could be seen after just 8 h of co-treatment, which was the earliest time point at which a visible iNOS transcript in response to CM/LPS could be detected (results not shown). IL-13 inhibition of iNOS mRNA expression was clearly visible after 12 or 24 h of simultaneous exposure of HMC to IL-13 + CM/LPS (Figure 6).

DISCUSSION

Our results indicate that IL-13 is a cytokine that is capable of regulating the synthesis of NO in HMC. To our knowledge, this study is the first to establish a role for IL-13 in inhibition of NO synthesis in a human cell type. Furthermore, it confirms previously reported observations concerning the requirement for several co-stimulatory signals in order to induce NO synthesis in HMC [21]. This contrasts with the mode of stimulation observed in other species such as rat [22] and cow [23], where only one cytokine or LPS is required to induce NO synthesis. This pattern of induction in human cells seems to be conserved across tissues according to data reported for hepatocytes [24], retinal pigmented epithelial cells [25] and osteoblast-like cells [26]. It might be speculated that this differential resistance to induction of NO synthesis in the human species is the result of a pattern of response to inflammation which has been fine-tuned during evolution.

Inhibition of NO synthesis by IL-13 has been reported in granulocyte/monocyte colony-stimulating factor-activated, LPS-induced murine peritoneal macrophages [27] and in stimulated mouse bone-marrow-derived macrophages [28]. However, the mechanism of this effect has not been elucidated. IL-13 itself is expressed in a rat model of glomerulonephritis [29], and recently its ability to induce 15-lipoxygenase in human blood monocytes has been reported [30]. Our data demonstrate an inhibitory effect of this lymphokine in human cells and provide a basis for its use as a regulatory tool in the inducible pathway mediating NO synthesis. Of particular interest was the observation that IL-13 was able to inhibit NO production not only when added before or at the same time as the inductive stimuli, but also when added several hours after cells had been induced. A similar effect was encountered with pirrolidin dithiocarbamate in rat mesangial cells [6], but IL-13 has the additional interest of being an endogenous modulator and therefore potentially employable by the organism in inflammatory situations where concerted intervention by the different cytokines might be occurring.

IL-4 shares many features with IL-13 with regard to biological activity, receptor structure and signal transduction pathways [31,32], and has been reported to inhibit NO synthesis in murine macrophages [33,34] and immune spleen cells [35]. A similar inhibitory effect was observed in the present study in stimulated HMC co-treated with rhIL-4. In another recent report, IL-4 stimulated L-arginine-dependent cyclic GMP production in human monocytes activated by IFN- γ [36]. This discrepancy may be related to the specific regulation of iNOS in different cell types. However, while studies of IL-4 inhibition of NO synthesis in murine macrophages and in HMC suggest that it is only effective at the initial stages of induction of iNOS [20], IL-13 shows a wider temporal window of intervention, thus making it more valuable as a potential therapeutic tool.

The abrogation of stimulated NO synthesis by different inhibitory cytokines does not seem to proceed through the same mechanisms. Hence transforming growth factor- β decreases the expression of iNOS protein by post-transcriptional mechanisms in murine macrophages [37] but not in rat vascular smooth muscle cells [38], whereas IL-4 reduces iNOS mRNA expression levels, at least at late time points of stimulation, without destabilizing the message [20]. Our study shows that IL-13 is able to inhibit significantly iNOS mRNA expression when cells are stimulated and co-treated with this cytokine. However, additional studies are required to establish whether this effect is due to mRNA destabilization or to the inhibition of transcription of new mRNA. Little information is available concerning the post-receptor events that take place after IL-13 is added to cells, and

only general speculation is possible at present. One possible mechanism is modulation of the JAK-STAT pathways triggered by such factors as IFN- γ [39]. Unfortunately, the requirement for multiple co-stimulatory signals to induce NO synthesis in human cells precludes a clear-cut approach to establish the effect of IL-13 on the individual pathways triggered by each isolated stimulus. A recent report described the activation of the IL-4-dependent transcription factor nuclear factor-IL4 by IL-13 [40]. Analysis of the published promoter region of the human iNOS gene [41] does not show an apparent consensus binding sequence for this factor, although it does not exclude its presence in an undetermined region of the 5'-flanking sequence. Nevertheless, no data have been reported to our knowledge with regard to the signal transduction mechanisms or transcription factors involved in the inhibitory effect of IL-13 on NO synthesis. Besides its effects on iNOS mRNA expression, IL-13 could be also modulating NO₂⁻ accumulation through modification of iNOS cofactor levels. In this regard, there are reports describing the modulation of tetrahydrobiopterin levels by activating and deactivating cytokines [42] in other cell types, suggesting that this might be an important mechanism for regulation of NO synthesis. Whether IL-13 is able to modify intracellular tetrahydrobiopterin synthesis in HMC is an attractive hypothesis which remains to be explored. Nevertheless, we believe that suppression of iNOS mRNA levels should be considered, according to our data, as a central mechanism of inhibition of NO synthesis in HMC.

In summary, this study establishes a role for IL-13 as a negative modulator of NO synthesis in a human cell type. Furthermore, it illustrates the complex regulation of iNOS by the cytokine network. The negative regulation of NO synthesis may be one of the means used by IL-13 to exert an anti-inflammatory action. In this context, IL-13 may represent an endogenous regulator of the inflammatory response evoked by mesangial cells in human diseases such as glomerulonephritis.

We are grateful to Dr. G. Giménez and Dr. L. Boscá for helpful comments, and to Dr. Manuel Rodríguez Puyol and Dr. Diego Rodríguez Puyol for their advice and support. We thank Dr. Daniel Caput and Dr. Pascual Ferrara from Sanofi Recherche for providing rhIL-13, Dr. Antonio de la Hera for providing rhIL-4, and Dr. David Geller for the human hepatic iNOS cDNA. We are indebted to the transplant co-ordination team of Hospital 12 de Octubre, Madrid, Spain, for supplying us with kidneys unsuitable for transplantation. This work is supported by a grant from the Spanish Ministry of Education and Science (DGICYT PB 93-0044).

REFERENCES

- Nathan, C. (1992) *FASEB J.* **6**, 3051–3064
- Nathan, C. and Xie, Q.-w. (1994) *J. Biol. Chem.* **269**, 13725–13728
- Moncada, S. and Higgs, A. (1993) *N. Engl. J. Med.* **329**, 2002–2012
- Laskin, J. D., Heck, D. E. and Laskin, D. L. (1994) *Trends Endocrinol. Metab.* **5**, 377–382
- Bogdan, C., Röllinghoff, M., Vodovotz, Y., Xie, Q.-w. and Nathan, C. (1994) in *Immunotherapy of Infections* (Masihi, N., ed.), pp. 37–54, Marcel Dekker, Inc., New York, Basel, Hong Kong
- Saura, M., López, S., Rodríguez Puyol, M., Rodríguez Puyol, D. and Lamas, S. (1995) *Kidney Int.* **47**, 500–509
- Mené, P., Simonson, M. S. and Dunn, M. J. (1989) *Physiol. Rev.* **69**, 1347–1425
- Pfeilschifter, J., Kunz, D. and Muhl, H. (1993) *Nephron* **64**, 518–525
- Raj, L. and Shultz, P. J. (1993) *J. Am. Soc. Nephrol.* **3**, 1435–1441
- Cattell, V., Cook, T. and Moncada, S. (1990) *Kidney Int.* **38**, 1056–1060
- Minty, A., Chalou, P., Derocq, J. M., Dumont, X., Guillemot, J. C., Kaghad, M., Labit, C., Leplatois, P., Liauzun, P., Miloux, B., Minty, C., Casellas, P. G. L., Lupdker, J., Shire, D., Ferrara, P. and Caput, D. (1993) *Nature (London)* **362**, 248–250
- Zurawski, G. and de Vries, J. E. (1994) *Immunology Today* **15**, 19–26
- Misko, T. P., Schilling, R. J., Salvemini, D., Moore, W. M. and Currie, M. G. (1993) *Anal. Biochem.* **214**, 11–16
- Schmidt, H. H. W., Warner, T. D., Nakane, M., Förstermann, U. and Murad, F. (1992) *Mol. Pharmacol.* **41**, 615–624
- Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159

- 16 Lamas, S., Michel, T., Collins, T., Brenner, B. M. and Marsden, P. A. (1992) *J. Clin. Invest.* **90**, 879–887
- 17 Geller, D. A., Lowenstein, C. J., Shapiro, R. A., Nussler, A. K., Di Silvio, M., Wang, S. C., Nakayama, D. K., Simmons, R. L., Snyder, S. H. and Billiar, T. R. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3491–3495
- 18 Alonso, S., Minty, A., Bourlet, Y. and Buckingham, M. (1986) *J. Mol. Evol.* **23**, 11–22
- 19 Kim, Y.-M. and Lancaster, J. R., Jr. (1993) *FEBS Lett.* **332**, 255–259
- 20 Bogdan, C., Vodovotz, Y., Paik, J., Xie, Q.-w. and Nathan, C. (1994) *J. Leukocyte Biol.* **55**, 227–233
- 21 Nicolson, A. G., Haites, N. E., McKay, N. G., Wilson, H. M., MacLeod, A. M. and Benjamin, N. (1993) *Biochem. Biophys. Res. Commun.* **193**, 1269–1274
- 22 Pfeilschifter, J. and Schwarzenbach, H. (1990) *FEBS Lett.* **273**, 185–187
- 23 Marsden, P. A. and Ballermann, B. J. (1990) *J. Exp. Med.* **172**, 1843–1852
- 24 Nussler, A. K., Di Silvio, M., Billiar, T. R., Hoffman, R. A., Geller, D. A., Selby, R., Madariaga, J. and Simmons, R. L. (1992) *J. Exp. Med.* **176**, 261–264
- 25 Goureau, O., Hicks, D. and Courtois, Y. (1994) *Biochem. Biophys. Res. Commun.* **198**, 120–126
- 26 Ralston, S. H., Todd, D., Helfrich, M., Benjamin, N. and Grabowski, P. S. (1994) *Endocrinology (Baltimore)* **135**, 330–336
- 27 Doyle, A. G., Herbein, G., Montaner, L. J., Minty, A. J., Caput, D., Ferrara, P. and Gordon, S. (1994) *Eur. J. Immunol.* **24**, 1441–1445
- 28 Doherty, T. M., Kastelein, R., Menon, S., Andrade, S. and Coffman, R. L. (1993) *J. Immunol.* **151**, 7151–7160
- 29 Lakkis, F. G. and Cruet, E. N. (1993) *Biochem. Biophys. Res. Commun.* **197**, 612–618
- 30 Nassar, G. M., Morrow, J. D., Jackson Roberts, L., II, Lakkis, F. G. and Badr, K. F. (1994) *J. Biol. Chem.* **269**, 27631–27634
- 31 Zurawski, S. M., Vega, F., Jr., Huyghe, B. and Zurawski, G. (1994) *EMBO J.* **12**, 2663–2670
- 32 Smerz-Bertling, C. and Duschl, A. (1995) *J. Biol. Chem.* **270**, 966–970
- 33 Liew, F. Y., Li, Y., Severn, A., Millott, S., Schmidt, J., Salter, M. and Moncada, S. (1991) *Eur. J. Immunol.* **21**, 2489–2494
- 34 Cenci, E., Romani, L., Mencacci, A., Spaccapelo, R., Schiaffella, E., Puccetti, P. and Bistoni, F. (1993) *Eur. J. Immunol.* **23**, 1034–1038
- 35 Al-Ramadi, B. K., Meissler, J. J., Jr., Huang, D. and Eisenstein, T. K. (1992) *Eur. J. Immunol.* **22**, 2249–2254
- 36 Kolb, J. P., Paul-Eugene, N., Damais, C., Yamaoka, K., Drapier, J. C. and Dugas, B. (1994) *J. Biol. Chem.* **269**, 9811–9816
- 37 Vodovotz, Y., Bogdan, C., Paik, J., Xie, Q.-w. and Nathan, C. (1993) *J. Exp. Med.* **178**, 605–613
- 38 Perrella, M. A., Yoshizumi, M., Fen, Z., Tsai, J., Hsieh, C., Kourembanas, S. and Lee, M. (1994) *J. Biol. Chem.* **269**, 14595–14600
- 39 Sadowski, H. B., Shuai, K., Darnell, J. E., Jr. and Gilman, M. Z. (1993) *Science* **261**, 1739–1744
- 40 Köhler, I., Alliger, P., Minty, A., Caput, D., Ferrara, P., Höll-Neugebauer, B., Rank, G. and Rieber, E. P. (1994) *FEBS Lett.* **345**, 187–192
- 41 Chartrain, N. A., Geller, D. A., Koty, P. P., Sitrin, N. F., Nussler, A. K., Hoffman, E. P., Billiar, T. R., Hutchinson, N. I. and Mudgett, J. S. (1994) *J. Biol. Chem.* **269**, 6765–6772
- 42 Schoedon, G., Schneemann, M., Blau, N., Edgell, C. J.-S. and Schaffner, A. (1993) *Biochem. Biophys. Res. Commun.* **196**, 1343–1348