The effects of diadenosine pentaphosphate (AP5A), diadenosine pentaphosphate (AP6A) and diadenosine tetraphosphate (AP4A) on the force of renal resistance vessels and aortic strips were measured using a small vessel myograph. Spectrofluorometric measurements of cytosolic free calcium concentrations ([Ca2+]i) in cultured rat vascular smooth muscle cells (VSMC) and glomerular mesangial cells (MC) were undertaken with fura2 according to established methodology.

The administration of 10 µmol/L AP4A, AP5A or AP6A significantly increased the force of isolated renal resistance vessels by 3.48 ± 0.43 mmHg (mean ± SEM; n=8), 2.14 ± 0.40 mmHg (n=12), or 2.70 ± 0.31 mmHg (n=11, each p<0.01 compared to resting tension), respectively. AP4A, AP5A or AP6A significantly increased the force of isolated aortic strips by 2.45 ± 0.57 mmHg (0.92 ± 0.13 mmHg; n=10; 2.70 ± 0.30 mmHg (n=6), or 1.48 ± 0.20 mmHg (each p<0.01 compared to resting tension), respectively. The addition of 10 µmol/L AP4A, AP5A, or AP6A significantly increased [Ca2+]i in VSMC (from 80±5 nmol/L to 114±20 nmol/L, or to 314±59 nmol/L/L, or to 332±100 nmol/L/L, each p<0.05 compared to resting value). The sustained [Ca2+]i increase after administration of AP4A, AP5A, or AP6A was 183±41 nmol/L, 143±40 nmol/L, or 148±57 nmol/L, respectively. The addition of 10 µmol/L AP4A, AP5A, or AP6A significantly increased [Ca2+]i in MC from 55±3 nmol/L (n=79) to 111±9 nmol/L, or to 106±13 nmol/L, or to 112±12 nmol/L (each p<0.05), respectively. The diadenosine pentaphosphate-induced vasoconstriction and [Ca2+]i increase was significantly reduced after administration of a specific inhibitor of P2 purinoceptors. The vasoconstricting properties of diadenosine polyphosphates are mediated by calcium influx in two different vascular beds. Therefore, it is likely that diadenosine polyphosphates may play an important role in the local regulation of contractility of vascular smooth muscle and mesangial cells and subsequently blood pressure.

The aim of this study was the identification of differentially regulated genes in rat mesangial cells (MC). The hypothesis is that MC proliferation is regulated by differential expression of proliferation-specific genes.

Using the differential display (DD) RT-PCR strategy we compared the gene expression patterns in quiescent MC in three-dimensional (3D) culture in collagen gels and proliferating MC in conventional two-dimensional (2D) culture. RNA was extracted from 2D cultures at 24, 48 and 72 hours and from 3D cultures after 6, 12, 24, 48 and 96 hours. DD RT-PCR was performed and differentially expressed bands were eluted from the gels, subcloned and used as probes for Northern analyses to confirm the differential expression. Subsequently, partial sequences were obtained and compared with the databases. We have identified two differentially regulated genes, both upregulated in the proliferating MC in 2D culture. The first gene (1.7 kb) encodes the LIM-domain protein DRAL. LIM-domains are specialized double-zinc-finger motifs involved in multiple roles as functional modifiers in protein interactions. The second gene (4.5 kb) encodes the rat type 1 collagen alpha 2 chain (COL1A2). The not yet published complete COL1A2 coding sequence was analyzed using a PCR-strategy and is reported here. It consists of 4119 nucleotides and 1372 amino acids. Comparison of the rat COL1A2 primary structure revealed a high degree of homology with other species: 90% with human, 96% with mouse and 90% with bovine COL1A2. These data demonstrate the suitability of the differential display strategy for the identification of genes differentially regulated in quiescent and proliferating mesangial cells.

Cyclic AMP production in response to stimulation by isoproterenol (0.01 mmol/L) is significantly reduced in lymphocytes of periferal blood of 14 patients with chronic renal failure (CRF) as compared with the control group of 14 healthy controls, that specifies violation of external regulatory signals transmission through adenylate cyclase system. In conditions of “breakage” of this membrane system lymphocytes appear switched-off through adenylate cyclase system. In conditions of “breakage” of this membrane system lymphocytes appear switched-off through adenylate cyclase system.
SMOKE INHIBITS BRADYKININ-INDUCED CALCIUM INFLUX IN ENDOTHELIAL CELLS
Second Department of Medicine, University Medical School of Pécs, Pécs, Hungary

Endothelial cells play a pivotal role in the control of renal hemodynamic producing nitric oxide (NO). This function can be damaged (J. Nagy et al. 1998) by smoking which accelerates the progression of renal diseases. The aim of this study was the investigation of the effect of smoking buffer (SB) on the bradykinin-induced calcium (Ca) influx, which is the first step in the NO production of the endothelial cells. Cultured pig aorta endothelial cells were used. Smoking buffer was prepared by bubbling of cigarette smoke through the buffer.

FURA-2-AM fluorescence was used to detect Ca influx. Free radical components of the SB can cause endothelial damage, that is why the protective effect of a scavanger, glutathion (GSH) was measured.

The bradykinin-induced Ca influx of the controls was taken as 100%. The effect of different dilutions of SB was investigated on bradykinin-induced Ca influx. A significant decrease of Ca influx in the SB treated cells was detected (2x dilution of SB: 14.1±16.3%, p<0.001; 4x dilution of SB: 27.1±20.4%, p<0.001). The time dependent inhibition with the 4x diluted SB was as follows (remaining Ca influx): 40 min (5.07±4.7%, p<0.001), 30 min (13.6±15.2%, p<0.001), 20 min (30.2±19%, p<0.001), 10 min (27.2±37.3%, p<0.01). GSH was protective at 2 mM (14.7±26.2 vs 54.9±25.6%, SB vs SB+GSH; p<0.01) and 4 mM (14.7±26.2 vs 74.2±13.0%, SB vs SB+GSH; p<0.05).

Our data verify that SB inhibits bradykinin-induced Ca influx of endothelial cells, which inhibition can be prevented by GSH. An ability to these results preservation of the normal hemodynamic and slowing of the progression of kidney diseases may be possible using GSH in the smoker patients.

RENA L PROXIMAL TUBULAR CELL GROWTH IS DIFFERENTIALLY MODULATED BY GROWTH FACTORS AND TYROSINE KINASE INHIBITORS
F. Ernst1, G Vargas, S Stracke, DR Jehle, F Keller, PM Jehle
Divisions of Nephrology, Universities of Ulm and Heidelberg, Germany

As renotrophic growth factors (GFs) hepatocyte growth factor (HGF), epidermal growth factor (EGF) and insulin-like growth factor-I (IGF-I) prevent acute renal failure and accelerate renal regeneration. We addressed the question whether in renal proximal tubular cells these GFs exert different mitogenic potencies and whether their effects may be differentially modulated by protein tyrosine kinase inhibitors (PTKI).

PT-1 cells, proximal tubular cells of the rabbit kidney, were cultured under defined serum free conditions and incubated with 0, 0.1, 1, 10 µM of these drugs by lactic, dehydrogenase release assay.

Benidipine and nifedipine showed significant inhibitions against the HGF (c-met) and EGF-receptor which both effects the subsequent phosphorylation of pp60src. Compared with EGF, HGF not only appeared to be a faster but also a more effective growth stimulator (4/24 h: HGF: 130%/200%; EGF: 110%/160%; p<0.01). WB and WIB revealed that HGF induced a significantly faster stimulation of its receptor-tyrosinekinase autophosphorylation and subsequent pp60src-phosphorylation than EGF. The effects of HGF and EGF on both pp60src-phosphorylation and growth stimulation could be inhibited with similar dose-dependency by TKI.

The present study indicates that the kinetic profile of signal transduction events induced by growth factors determines their mitogenic action in proximal tubular cells. Compared with EGF, the faster kinetics of HGF to induce receptor autophosphorylation and subsequent activation of pp60src may explain its superior growth-stimulating effects.
UPREGULATION OF ADENOSINE A1-RECEPTOR MRNA IN ISOLATED JUXTAGLOMERULAR CELLS, GLOMERULI AND WHOLE KIDNEY OF STREPTOZOTOCIN-INDUCED (STZ) DIABETES MELLITUS RATS

P. Benoehr; M. Albinus; C. Fleisch; T. Risler; H. Osswald
Dept. of Pharmacology, University of Tuebingen, Tuebingen, Germany

Adenosine (ADO) has been implicated in the etiology of acute renal failure of diabetic patients induced by nephrotoxic substances like radiocontrast media. Recent studies revealed that the signal-transduction chain of the ADO A1-receptor is altered in kidneys of diabetic rats (Pflueger et al., AJR 1995). In order to analyze the expression of ADO A1-receptor we measured by RT-PCR the mRNA level of ADO A1-receptor in isolated juxtaglomerular cells (JGC)(n=3), isolated glomeruli (n=3) and whole kidney (n=3) in STZ-induced diabetic and non diabetic controls. The relative amount of PCR-products was determined by Biorad Quantity One software.

After relative quantitation of mRNA we observed significantly elevated ADO A1-receptor mRNA levels (mean +/-SEM) in isolated juxtaglomerular cells (0.48 +/- 0.05 vs. 0.20 +/- 0.03), glomeruli (2.33 +/- 0.42 vs. 0.88 +/- 0.08) and whole kidney (0.54 +/- 0.06 vs. 0.26 +/- 0.03) of diabetic rats compared to controls. These data are consistent with experiments in which ADO induces enhanced vasoconstriction in STZ-rats. Also in clinical trials ADO-receptor antagonists were shown to exert renal protection against nephrotoxic substances. We conclude that the elevated gene expression of ADO A1-receptor in the kidney could be contribute to the pathophysiology of acute renal failure after nephrotoxic substances administration in conditions of established insulin-dependent diabetes mellitus.

DEATH CELL (APOPTOSIS OR NECROSIS) INDUCED BY CISPLATIN (CIS) ALONG THE NEPHRON.

Camargo, S.M.R.; Schor, N.
Nephrology Division, UNIFESP, São Paulo, Brazil.

The nephrotoxicity is a limiting factor of antineoplastic CIS. We investigated two doses of CIS (1 or 100 µM) in 4 different renal cells, IMC (mesangial cells), LLC-PK1 (proximal tubular cells), MDCK (distal tubular cells) and IMCD (inner medullar cells). Hoechst 33342 nuclei staining were employed to quantify apoptosis rate (%). DNA gel electrophoresis and TUNEL were used to confirm. Exclusion method with acridine orange and ethidium bromide were utilized to evaluate cell viability (%). IMC viability decreased with CIS 100 µM after 24h (43.75±1.2; mean±SEM, n=4), however apoptosis % did not change. With CIS 1 µM, apoptosis increased after 72h (13.8±3.4%) although viability did not change. In tubular cells, apoptosis increased with CIS 100 µM after 24 h (LLC-PK: 82.0±5.7 and MDCK: 93.5±4.4) and again, viability did not modify. With 1 µM both apoptosis and viability were maintained. IMC cells with CIS 100 µM after 48 hours have the viability decreased (28.0±4.1), apoptosis increased but less than tubular cells (27.9±7.1/24h). CIS (1µM) after 72 h increased apoptosis (20.0±6.7) with the same viability. After determining type and dose/ time-dependent death, we analyzed in LLC-PK1 and MDCK the molecular mechanism involved. We observed that caspase-3 inhibitor (z-DEVD-fmk 16 and 100µM) reduced apoptosis rate 36% in LLC-PK (160µM) and 91% in MDCK (16µM). The p53 protein seemed be rise in MDCK treated with CIS, but not LLC-PK. Western blot. We also evaluated [Ca2+]i and lipid peroxidation involvement in death mechanism from these cells. CIS did not change [Ca2+]i analyzed by fluoremetry, moreover calcium blocker channel nildeadpine did not change apoptosis rate in CIS treated cells. Lipid peroxidation did not change after the treatment. In conclusion, CIS cause damage on cells along the nephron via distinct way, apoptosis or necrosis. The data suggest that caspases are involved in apoptosis mechanism, but p53 also can be involved. [Ca2+]i and lipid peroxidation seem not to be essential to initiate apoptosis in these cells treated with CIS. Thus, it could possible, by understanding both mechanisms, apoptosis and necrosis, develop tools to interfere and shift in the death pathways.

UPREGULATION OF ADENOSINE A1-RECEPTOR MRNA IN ISOLATED JUXTAGLOMERULAR CELLS, GLOMERULI AND WHOLE KIDNEY OF STREPTOZOTOCIN-INDUCED (STZ) DIABETES MELLITUS RATS

P. Benoehr; M. Albinus; C. Fleisch; T. Risler; H. Osswald
Dept. of Pharmacology, University of Tuebingen, Tuebingen, Germany

Adenosine (ADO) has been implicated in the etiology of acute renal failure of diabetic patients induced by nephrotoxic substances like radiocontrast media. Recent studies revealed that the signal-transduction chain of the ADO A1-receptor is altered in kidneys of diabetic rats (Pflueger et al., AJR 1995). In order to analyze the expression of ADO A1-receptor we measured by RT-PCR the mRNA level of ADO A1-receptor in isolated juxtaglomerular cells (JGC)(n=3), isolated glomeruli (n=3) and whole kidney (n=3) in STZ-induced diabetic and non diabetic controls. The relative amount of PCR-products was determined by Biorad Quantity One software.

After relative quantitation of mRNA we observed significantly elevated ADO A1-receptor mRNA levels (mean +/-SEM) in isolated juxtaglomerular cells (0.48 +/- 0.05 vs. 0.20 +/- 0.03), glomeruli (2.33 +/- 0.42 vs. 0.88 +/- 0.08) and whole kidney (0.54 +/- 0.06 vs. 0.26 +/- 0.03) of diabetic rats compared to controls. These data are consistent with experiments in which ADO induces enhanced vasoconstriction in STZ-rats. Also in clinical trials ADO-receptor antagonists were shown to exert renal protection against nephrotoxic substances. We conclude that the elevated gene expression of ADO A1-receptor in the kidney could be contribute to the pathophysiology of acute renal failure after nephrotoxic substances administration in conditions of established insulin-dependent diabetes mellitus.

MESANGIAL CELLS EXPRESS THE RON ONCOGENE AND ARE ACTIVATED BY MACROPHAGE STIMULATING PROTEIN.

T Rampino, C Collesi, P Guallini, M Maggio, M Gregorini, G Socco, A Ranghino, A Gallina, C Libetta, R Tiboldo, A Dal Canton
Policlinico San Matteo and CNR, Pavia, and IRCC, Torino.

Macrophage stimulating protein (MSP) is a glycoprotein of the Scatter Factors' family that induces motility, proliferation and phagocytosis in monocytes/macrophages via its receptor, the product of ron protooncogene. Since mesangial cells (MC) and macrophages are phenotypically similar, we have studied whether MC express ron and are activated by MSP. Expression of ron mRNA was investigated by RT-PCR in SV40-transfected human MC line. MC (2x10⁵ cells/well) were cultured for 24 h and 48 h to study the effects of recombinant MSP (0, 10, 30, 50 ng/ml) on proliferation (tetrazolium method), migration (through 8-µm Transwell Costar filters), and capacity to invade a collagen matrix (Matrigel). In addition, since we found in a parallel study that human tubular cells (HK2) produce MSP, we studied proliferation and migration of MC conditioned with supernatant of cultured HK2. RT-PCR showed that MC constitutively express ron mRNA (483 bp form). MSP stimulated in a time- and dose-dependent manner MC proliferation (cell number, 13x10⁵/well after 24 h, 25x10⁵ after 48 h of culture with 50 ng/ml MSP, p<0.001), migration (7:1 cell number ratio between chamber containing 50 ng/ml MSP and chamber with no MSP and invasion (100% cells invaded MSP-loaded Matrigel). Similar results were found using HK2 supernatant to condition MC. The effects of recombinant MSP and HK2 supernatant were abrogated by a neutralizing anti-MSP antibody. These results demonstrate that MC express MSP receptor (ron) and are activated by MSP. Either circulating or as paracrine product may play a pathophysiological role in mesangio-proliferative disorders.

TWO RENAL EPITHELIAL CELL LINES EXHIBIT A DISTINCT BEHAVIOR IN RESPONSE TO A SERIES OF PROGRAMMED CELL DEATH (APOPTOSIS) INDUCERS.

Camargo, S.M.R., Campos, A.H., Segreto, H.R.C., Schor, N.
Nephrology Division, UNIFESP, São Paulo, Brazil.

It is known that apoptosis is related to specific cell line as well as depends on the type of the inducer. Thus, apoptotic responses were investigated in LLC-PK1 (pig proximal tubular cells) and MDCK (canine distal tubular cells) to six different cell death inducers. Cell cultures were maintained through standard methods, exposed individually to different stimuli. DNA agarose gel electrophoresis and Hoechst 33342 nuclei staining were used to detect DNA fragmentation and chromatin condensation, respectively. Dexmethasone (1 µM, 72-96 h), staurosporine (0.1-1.0 µM, 6 h), ionizing radiation (5 and 10 Gy, 6-24 h) and cisplatin (1-100 µM, 24-96 h) were able to promote marked and with similar degree of apoptosis in both cell lines. On the other hand, serum starvation (72-96 h) induced a significant apoptotic response only in MDCK cells, while LLC-PK1 cells did not show any DNA modification even after 144 h of serum deprivation, with necrosis ensuing thereafter. Curiously, TNF-α (100 ng/ml, 24 h) did not alter DNA patterns in both cell types. We conclude that several (but not all) cell injury triggers induce apoptotic process in epithelial tubular cells. These findings are potentially implicated in the heterogeneous response of nephron segments affected by renal aggression.

FAPESP
Abstracts

**Regulators, mediators, signalling, gene expression**

**EFFECT OF RADIATION (γ-rays) ALONE AND ASSOCIATED WITH CISPLATIN (CIS) ALONG THE NEPHRON.**
Camargo, S. M. R., Teixeira, V. P. C, Segreto, H. R. C., Schor, N. Nephrology Division, UNIFESP, São Paulo, Brazil.

The effects of γ-rays (5Gy) and CIS (1µM) on four different renal cell lines were evaluated. Cell cultures were exposed to γ-rays, CIS, and γ-rays before or after CIS. Apoptosis (APOP) and viability were evaluated in IMC (immortalized mouse mesangial cell), LLC-PK1 (pig proximal tubular cell), MDCK (Madin Distal Canine Kidney, distal tubular cell) and IMCD (inner medullary collecting duct cell). Exclusion methods with acridine orange and ethidium bromide were also utilized to evaluate cell viability (%) and Hoechst 33342 nuclei staining were employed to quantify apoptosis rate (%). Viability was not different in the four cell lines after all treatments, ranging from 76.8 to 99.5%. However, APOP increased in IMC treated with γ-rays before and after CIS (±S.E.M, (n), *vs.CIS, &vs.γ-rays, vs.CIS→γ-rays: CIS: 0.68±0.3(3), for γ-rays: 5.08±0.84(4)*, for γ-rays→CIS: 7.45±0.5(4)*, CIS→γ-rays: 8.75±2.36(3)*). LLC-PK1 treated with γ-rays before CIS showed rises in APOP rate, CIS: 2.12±0.96(6), γ-rays→CIS: 6.53±2.99(9)*, while MDCK and IMCD showed increases in APOP rate when treated with CIS before γ-rays (MDCK-CIS: 2.91±1(4), CIS→γ-rays: 11.8±1.4(4)*&$; IMCD-CIS: 3.35±0.8(4), CIS→γ-rays: 10.4±1.11(4)*&$).

Results suggest that the association between CIS and γ-rays can induce different response that depend the cell line. However, the sequence of administration of the injurious agents to LLC-PK1, MDCK and IMCD cells can modify apoptosis rate and thus, implicate in modifying dead option: APOP vs. necrosis. FAPESP

**REGULATION OF BOVINE ENDOTHELIN-CONVERTING ENZYME-1 BY HYDROGEN PEROXIDE IN BOVINE AORTIC ENDOTHELIAL CELLS.**
SL Ongil, M Saura 1, LG Santiago, G Torrecillas, ML Ongil, S M Raur, M Saura 1, C Zaragoza 1, LG Santiago, MR Puyol, CJ Lowenstein 1, DR Puyol 1.
'Div. Cardiology, Dpt. Medicine, JHMI, USA. 2Dpto. Fisiología, Universidad de Alcalá, 3Sección de Nefrología, H. Príncipe de Asturias, Madrid, Spain.

The precise role of reactive oxygen species in vascular injury due to ischemia-reperfusion is not well understood. One mechanism by which oxygen radicals may affect blood vessels is by regulating the transcription of vascular messengers. We hypothesized that reactive oxygen species may regulate endothelin converting enzyme (ECE-1) expression. Hydrogen peroxide induces a significant increase in ECE-1 mRNA (200 %), protein (400 %), and activity (200 %) in bovine aortic endothelial cells, which were measured by Northern blot, Western blot and ELISA, respectively. Catalase abolishes this effect. The increase in ECE-1 mRNA levels depends on an increased activation of the ECE-1 promoter, as demonstrated by increased luciferase activity (50 fold) when immortalized aortic endothelial cells transfected with a construct containing 682 bp of the promoter region of the gene. Serial deletions of this promoter region demonstrate that the ECE-1 promoter element that mediates this response to hydrogen peroxide is located between -444 and -216 bp. This promoter region contains a STAT/acute phase response element, and hydrogen peroxide induces the binding of STAT3/Acute phase transcription factor to this response element. Our data suggest that hydrogen peroxide may increase endothelin synthesis by regulating ECE-1, through the activation of STAT3/Acute phase factor.

**UP-REGULATION OF EXTRACELLULAR MATRIX (ECM) SYNTHESIS BY COLLAGEN I.**
MC Iglesias, MP Ruiz, R Ortega, LG Santiago, G Torrecillas, M Diez, DR Puyol.
Departamento Fisiología. Universidad de Alcalá. 1Sección de Nefrología, H. Príncipe de Asturias. Madrid, Spain.

In pathological conditions, like glomerulosclerosis, mesangial cells are embedded in an abnormal ECM. One characteristic of this matrix is the presence of interstitial collagens, such as collagen type I (COL-I), which does not appear in physiological conditions. As it has been well described that matrix proteins may influence cell behavior, present experiments were devoted to analyze the hypothesis that the presence of COL-I in the extracellular compartment might modulate the synthesis of ECM.

For that purpose, human mesangial cells (passage 3-5) were grown in COL-I or collagen IV (COL-IV) coated culture flasks, and the synthesis of different components of the TGFB system, as well as the production of some ECM proteins, were analyzed by northern blot. The more relevant results are the following (they are expressed as the ratio between the densitometric signals of the corresponding probe and that of GAPDH):

<table>
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<tr>
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<th>TGFB</th>
<th>COL-IV</th>
<th>COL-I</th>
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<tr>
<td>COL-IV</td>
<td>0.63±0.15</td>
<td>0.52±0.09</td>
<td>0.97±0.09</td>
<td>1.37±0.08</td>
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<tr>
<td>COL-I</td>
<td>1.03±0.19</td>
<td>1.03±0.02</td>
<td>2.05±0.13</td>
<td>2.05±0.10</td>
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Also decorin and L-endoglin, two components of the TGFB system were modified by the presence of COL-I. In consequence, these results confirm the up-regulation of ECM in presence of an abnormal matrix protein, a fact which may be relevant in the progression of chronic renal diseases.

**EPIDERMAL GROWTH FACTOR SIGNALING IN HUMAN MESANGIAL CELLS.**
U.F. Mondorf, A. Quipper, M. Herrero, H. Geiger
Division of Nephrology and Gastroenterology, Johann Wolfgang Goethe-Universität, Frankfurt, Germany.

Proliferation of mesangial cells plays an important role in glomerulosclerosis. Growth factors such as the epidermal growth factor (EGF) stimulate mesangial cell proliferation. While growth factor receptor signaling appears to be G protein-independent in most cell types, evidence is accumulating that EGF receptor may also involve activation of G proteins. In the present study, we have investigated signaling pathways of the EGF receptor and the possible role of pertussis toxin-sensitive G proteins (G vs. γ) in cultured human mesangial cells.

We found that EGF-induced rapid receptor autophosphorylation and tyrosine phosphorylation of other EGF receptor substrates in bovine mesangial cells transfected with a construct containing 682 bp of the promoter region of the gene. Furthermore, EGF induced an increase in the intracellular calcium concentration ([Ca²⁺]) in single Fura 2-loaded mesangial cells grown on cover slips. EGF also triggered a rapid increase in the 1,4,5-IP³ concentration. Maximum increase in 1,4,5-IP³ of 0.7 pmol/mg protein (basal value of 0.34±0.09 pmol/mg protein) was observed 5 seconds after addition of EGF. To follow that a decline upon continuation of the incubation. Thus, it appears that EGF increased [Ca²⁺] via increase in 1,4,5-IP³ production. To investigate whether EGF-induced responses are mediated by activation of pertussis toxin-sensitive G proteins, mesangial cells were treated for three hours with pertussis toxin (200 ng/ml) prior to measurement of EGF-induced increases in [Ca²⁺] and 1,4,5-IP³. Pertussis toxin pretreatment of the cells abolished EGF (200ng/ml)-induced increase of [Ca²⁺] and 1,4,5-IP³ level, whereas the vasoressin (100 nM)-induced [Ca²⁺] response remained unchanged. Our results indicate the involvement of pertussis toxin sensitive G-proteins (G vs. γ) in EGF-induced activation of PLC-γ1 and concommitant increas in [Ca²⁺].

**Effect of radiation (γ-rays) alone and associated with cisplatin (Cis) along the nephron.**
Camargo, S. M. R., Teixeira, V. P. C, Segreto, H. R. C., Schor, N. Nephrology Division, UNIFESP, São Paulo, Brazil.

The effects of γ-rays (5Gy) and CIS (1µM) on four different renal cell lines were evaluated. Cell cultures were exposed to γ-rays, CIS, and γ-rays before or after CIS. Apoptosis (APOP) and viability were evaluated in IMC (immortalized mouse mesangial cell), LLC-PK1 (pig proximal tubular cell), MDCK (Madin Distal Canine Kidney, distal tubular cell) and IMCD (inner medullary collecting duct cell). Exclusion methods with acridine orange and ethidium bromide were also utilized to evaluate cell viability (%) and Hoechst 33342 nuclei staining were employed to quantify apoptosis rate (%). Viability was not different in the four cell lines after all treatments, ranging from 76.8 to 99.5%. However, APOP increased in IMC treated with γ-rays before and after CIS (±SEM, (n), *vs.CIS, &vs.γ-rays, vs.CIS→γ-rays: CIS: 0.68±0.3(3), for γ-rays: 5.08±0.84(4)*, for γ-rays→CIS: 7.45±0.5(4)*, CIS→γ-rays: 8.75±2.36(3)*). LLC-PK1 treated with γ-rays before CIS showed rises in APOP rate, CIS: 2.12±0.96(6), γ-rays→CIS: 6.53±2.99(9)*, while MDCK and IMCD showed increases in APOP rate when treated with CIS before γ-rays (MDCK-CIS: 2.91±1(4), CIS→γ-rays: 11.8±1.4(4)*&$; IMCD-CIS: 3.35±0.8(4), CIS→γ-rays: 10.4±1.11(4)*&$).

Results suggest that the association between CIS and γ-rays can induce different response that depend the cell line. However, the sequence of administration of the injurious agents to LLC-PK1, MDCK and IMCD cells can modify apoptosis rate and thus, implicate in modifying dead option: APOP vs. necrosis. FAPESP
Fibrin formation within the crescents has been observed in crescentic glomerulonephritis. Thrombin released in glomerular injury has various biological effects besides its roles in the hemostasis. TGF-β contributes to the modulation of cell growth and extracellular matrix synthesis. We investigated TGF-β production, its role in GEC proliferation, and its modulation by thrombin in cultured rat GEC. The concentrated culture supernatants of GEC showed TGF-β activity demonstrated by bioassay using the TGF-β-dependent mink pulmonary epithelial cell line. Immunoblot analysis of the concentrated culture supernatants using anti-TGF-β antibody revealed 12.5 kDa protein, which is compatible with TGF-β. Mature TGF-β inhibited cultured GEC proliferation in a dose-dependent manner. The concentrated GEC supernatants also inhibited GEC proliferation. Reverse transcription-polymerase chain reaction showed that thrombin (0.5-5.0 U/ml) enhanced the TGF-β gene expression of GEC in a dose-dependent manner, although platelet-derived growth factor did not change it. Our data show that GEC secrete functional TGF-β, which may regulate GEC proliferation as an autocrine inhibitory cytokine, and thrombin enhances gene expression of TGF-β in GEC. These results may explain that TGF-β and thrombin participate the progression of glomerulosclerosis following GEC proliferation in crescentic glomerulonephritis.

INTERLEUKIN-1 β (IL-1 β) IS AN AUTOCRINE GROWTH FACTOR OF RAT GLOMERULAR EPITHELIAL CELLS (GEC) IN CULTURE

Hirosaki University, Hirosaki, Japan

GEC proliferation is usually observed in crescentic glomerulonephritis. However, the regulation of GEC proliferation is not fully understood. Although it is known that IL-1 β has a mitogenic effect on mesangial cells and is produced by mesangial cells, the effect of this cytokine on GEC proliferation is not known. We investigated whether cultured rat GEC could produce IL-1 β and its role in GEC proliferation. Cultured rat GEC from 24th to 36th passage were used. GEC were incubated with serum free media for 72 hours. IL-1 β level in the culture supernatant was measured with specific ELISA. GEC proliferation was evaluated with a colorimetric assay using the tetrazolium salt. IL-1 β mRNA expression by GEC was examined by reverse transcription-polymerase chain reaction (RT-PCR). Amounts of IL-1 β in the culture supernatants increased over time (24-72 hours). IL-1 β serum with Na-serum significantly stimulated IL-1 β production in a time-dependent manner. RT-PCR also demonstrated mRNA of IL-1 β which is a band of 325 base pair corresponding to predicted size in GEC. IL-1 β (0.4-40 pg/ml) showed mitogenic effect on GEC in a dose-dependent manner. Moreover, proliferation of GEC cultured in growth medium was partially inhibited with anti-IL-1 β neutralizing antibody. Our data show that IL-1 β is an autocrine growth factor for GEC and may have an important role in the regulation of GEC proliferation.
Regulators, mediators, signalling, gene expression

ANTI-INFLAMMATORY EFFECTS OF RETINOIDS ON CULTURED HUMAN MESANGIAL CELLS

Moreno-Manzano V; Sepúlveda-Muñoz JC; Lopez-Ongil S; Martínez JA; Diez-Marqués L; Rodriguez-Puyol M; Lucio-Cazaña FJ.
Department of Physiology, University of Alcala, Madrid, Spain.

5-10 µM all-trans retinoic acid (tRA) prevents the cytotoxicity of H₂O₂ in cultured human mesangial cells (CHMC) and in cultured rat mesangial cells. However we report here that a slightly higher tRA dose, 25 µM, triggers CHMC apoptosis. When we studied 9-cis retinoic acid (9-cRA) we found that: 1) 25 µM had no toxic effect 2) 10 nM inhibited CHMC proliferation to the same extent than 10 µM (tRA 3) 10 µM 9-cis RA induced twice increase in GSH than 10 µM tRA 4) 10 nM 9-cis RA had similar preventive effect on H₂O₂ toxicity than 10 µM tRA, being this effect unrelated to stimulation of antioxidant defenses nor increased catabolism of H₂O₂.

SUPPRESSION OF APOPTOSIS H₂O₂-INDUCED BY RETINOIC ACID: INHIBITION OF AP-1 SYSTEM

Moreno-Manzano V; Ishikawa Y; Lucio-Cazaña FJ; Rodriguez-Puyol M; Kitamura M.
Dept. of Medicine, UCL, London, UK. Dept. of Physiology, University of Alcala, Madrid, Spain.

All-trans-retinoic acid (tRA) induces apoptosis of various cells, whereas little is known about it’s antiapoptotic potential. In this study, we report that t-RA has the ability to inhibit hydrogen peroxide (H₂O₂)-induced apoptosis. Mesangial cells exposed to (H₂O₂) exhibited shrinkage of cytoplasm, membrane blebbing, condensation of nuclei and DNA fragmentation. Pre-treatment with t-RA (5µM) inhibited the morphologic and biochemical hallmarks of apoptosis. t-RA also inhibited apoptosis of mesangial cells triggered by pyrrolidine dithiocarbamate, whereas it did not prevent tumor necrosis factor-α-induced apoptosis. The antiapoptotic effect against (H₂O₂) was similarly observed in NRK49F fibroblasts. To explore the molecular mechanism involved in the antiapoptotic action of t-RA, it’s effects on the activator protein 1 (AP-1) pathway the crucial machinery for the H₂O₂-induced apoptosis (BBRC 240:496,1997). Norther blot analysis showed that t-RA inhibited expression of AP-1 components, c-fos and c-jun. Reporter assays showed that t-RA abrogated the H₂O₂-triggered activation of AP-1 (196 ± 21% in H₂O₂ alone, and 106 ± 7% in t-RA + H₂O₂ vs 100% in unstimulated control). These data suggest that the antiapoptotic action of t-RA is trough suppression of the AP-1-mediated apoptotic pathway.

ERYTHROPOIETIN INDUCIBLE IMMEDIATE-EARLY GENES IN HUMAN VASCULAR ENDOTHELIAL CELLS.

H Buchmayr, R Fritsche-Polanz, M Födinger, S Skoupy, W H. Hörl, G Sunder-Plassmann
Dept. of Lab. Med., Div. of Molecular Biology, and Dept. of Int. Med. III, Div. of Nephrology and Dialysis, University of Vienna, Vienna, Austria.

Patients receiving recombinant human erythropoietin (rHuEPO) may experience side-effects arising from the vascular system. The underlying mechanisms, however, are largely unknown. To elucidate downstream events following erythropoietin (EPO) receptor triggering of endothelial cells, a differential display analysis of cultured human umbilical vein endothelial cells (HUVEC) which were exposed for two hours to rHuEPO alone or a combination of rHuEPO and cycloheximide was conducted. Among 107 DNA fragments that were differentially displayed in two independent cell culture experiments, 14 corresponded to 10 genes that were upregulated by rHuEPO as confirmed in two further independent cell culture experiments using a semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) protocol. The genes coded for proteins that may be assigned to four different groups: (1) proteins implicated in the regulation of vascular functions (thromboxane-1, preproendothelin-1, 20 kDa myosin regulatory light chain, relative increase of rHuEPO induced mRNA levels: 155.2 %, p = 0.043; 112.2 %, p = 0.034; 137.6 %, p = 0.016; respectively), (2) gene products involved in gene transcription and/or translation (c-myc purine-binding transcription factor PuF, tryptophanyl-tRNA synthetase, 519 ribosomal protein; increase of mRNA levels: 126.4 %, p = 0.032; 150.9 %, p = 0.012; 134.9 %, p = 0.038; (3) subunits of mitochondrial enzymes related to energy transfer (NADH dehydrogenase subunit 6, cytochrome C oxidase subunit 1; increase of mRNA concentrations: 141.7 %, p = 0.007; 140.3 %, p = 0.01, and (4) regulators of signal transduction (protein tyrosine phosphatase G1, mono-ADP-ribosyltransferase-4; increase of transcript levels: 160.3 %, p = 0.016; 130.1 %, p = 0.166). Fifty-eight of the 107 differentially displayed cDNA fragments were nucleotide sequences without homologies to known genes. Thus, our findings provide new insights into EPO receptor triggering of human vascular endothelial cells.

PRIMARY UP-REGULATION OF THE ENDOTHELIN RECEPTOR TYPE B IN HUMAN GLOMERULONEPHRITIS (GN)

Z.I. Niemir, J. Chudek, G. Dworacki, H. Stein, R. Waldherr, S. Czekalski, & E. Ritz
University of Medical Sciences, Poznan, Silesian School of Medicine, Katowice, Poland, and University of Heidelberg, Germany.

The in situ up-regulation of endothelin (ET)-1 and ET receptor type A (ETRA) is believed to be involved in the progression of GN.

We examined the gene and protein expression of ET-1, ETRA, ETA receptor type B (ETRB) and ET-1 converting enzyme-a (ECE-1a) in 8 normal kidneys and 44 renal specimens with GN. Four patients had acute endocapillary GN, 17 had IgA-GN, 4 had membrano-proliferative GN, 8 had membranous GN, 5 had minimal change disease, and 6 had focal-segmental glomerulonephritis.

In situ hybridisation studies and RT-PCR confirmed the expression of ET-1, ETRA, ETRB and ECE-1a in the normal human kidney at the mRNA level. Mesangial cells and endothelial cells in interstitial capillaries were found to be the major source of ET-1 in control kidneys. Our results show that the glomerular production of ET-1 is sustained in non-proliferative forms of GN, while significantly reduced in marked proliferative lesions in glomeruli. Up-regulation of ET-1/ETRA in the interstitium was noted in the latter condition. However, this was accompanied by a profound increase in the tubular and interstitial expression of ETRB. The disease-induced tubular expression of ETRB and an increase in glomerular reactivity for this receptor were also the major findings in non-proliferative forms of GN. The predominant up-regulation of ETRB in the renal tissue appeared to be mediated by ET in the progression of these diseases.
CELL CYCLE REGULATION PROTEINS mRNA EXPRESSION IN CULTURED MESANGIAL CELL INFECTED WITH COXACKIEVIRUS B3

E. Menegatti, G.G. Conaldi, A. Bottelli, G. Camussi, L.M. Sena and D. Roccatello
Dipartimento di Medicina e Oncologia Sperimentale, Università di Torino, Torino e Divisione di Microbiologia Dipartimento di Scienze Cliniche e Biologiche, Università di Pavia, Varese Italy
The six group B coxackieviruses (CVBs) are highly prevalent human pathogens that cause viremia followed by involvement of different organs. Clinical and experimental evidence suggests that CVBs can induce kidney injury. In contrast to proximal tubular cells and glomerular podocytes, in which CVB infection leads to the expression of cytosolic, glomerular mesangial cells support the replication of the six CVBs but fail to develop overt cytopathologic changes. Mesangial cells continue to produce infectious progeny for numerous serial subcultures (i.e., more than 50 days). Mesangial cell proliferation is a common feature of immune-mediated glomerular diseases and often mesangial cell hyperplasia does not arrest after removal of the plasmodial challenge.
In order to evaluate the influence of CVB3 infection on mesangial cell cycle regulatory system, we analysed G1 phase and G2-M phase cyclins gene expression in mesangial cells 10 and 20 days after infection with CVB3. Mesangial cells were cultured and infected as described (J Virol 71, 9180-9187, 1997). Total RNA was extracted by guanidium thiocyanate-phenol-chloroform method and reverse transcribed. cDNA was amplified by PCR with specific primers for cyclins A, B1, D1 and E. PCR products were semi-quantified with the limiting dilution method using the ribosomal protein L7 as a housekeeping gene to normalise PCR products among samples. Band intensity was quantified by densitometric analysis. Each cyclin was expressed in noninfected mesangial cells. At day 10 a moderate increase in cyclin D1 and B1 gene expression (8 times, for cyclin D1 and 2 times for cyclin B1 respectively, compared to noninfected cells) was observed, while no increase has been found in cyclins A and E expression. At day 20 cyclin B, A and D1 mRNA were increased, and cyclin B was expressed 40 times more than control cells.
These data show that CVB3 infection is able to modulate cyclins gene expression in cultured mesangial cells.

PDGFR RECEPTOR KINASE BLOCKER AG1295 ATTENUATES INTERSTITIAL FIBROSIS IN RAT KIDNEY AFTER UNILATERAL OBSTRUCTION (UUO).

D Ludewig1, H Kosmehl2, M Sommer1, F D Böhmer1, G Stein1, 1Dept. of Intern Med. IV, 2Dept. of Pathology, Research Unit Molecular Cell Biology, Univ. of Jena, Germany
The current study was designed to investigate possible effects of AG1295 on interstitial fibrosis in rats with UUO, monitored by ED-A fibronectin expression (monoclonal antibody (mAb) 159), number of macrophages (mAb ED1), presence of myofibroblasts (mAb 1A4) and interstitial matrix deposition quantified by Sirius-Red staining and computer-aided image analysis.
Without AG1295 treatment, the Sirius-Red stained area (SRA) increased after UUO. Simultaneously, the number of macrophages (MP), the ED-A fibronectin (FN) deposition and the α-smooth muscle actin expression were elevated.

SRA MP ED-A FN
controls 6.8±1.3% 4.3±1.1 0.1±0.1
14 days UUO 19.0±1.9% 16.6±2.6 1.6±0.8
21 days UUO 23.4±1.7% 23.2±4.4 2.3±0.4
Treatment with AG1295 (12mg/kg body weight, daily i.p.) significantly reduced interstitial area and fibrosis mediators.
SRA MP ED-A FN
14 days UUO 15.7±1.9% 12.8±1.4 1.1±0.2
21 days UUO 17.0±0.7% 15.3±3.8 1.6±0.6
Conclusion: The study indicates that the PDGFR receptor kinase blocker AG1295 is able to significantly decrease interstitial fibrosis in the rat UUO model. The diminution of the early fibrosis mediators, i.e. macrophages, ED-A fibronectin and myofibroblast phenotype points to a modulated fibrosis process via blockade of PDGFR actions.

Cyclosporin A (CsA) EFFECTS ON NITRIC OXIDE PRODUCTION IN CULTURED LLC-PK1 CELLS.

R. Lima, N. Schor and E.M.S Higa.
Dept. of Nephrology and Emergency Division, UNIFESP, SP, Brazil.
CsA is an immunosuppressive agent commonly used to prevent transplant rejection, but nephrotoxicity is the major side effect which limits its use. The morphological findings are mainly at proximal tubules but the physiopathology of these effects is still unknown. Multiple vasoactive mediators such as the renin angiotensin system, thromboxane, and endothelin derived factors, like endothelin or nitric oxide (NO) have been implicated in its nephrotoxicity.
Objective: To study the effect of CsA on NO production in cultured LLC-PK1 cells. Material and methods: The cells in DMEM, 5% FCS, without phenol red were incubated during 72 hr with vehicle (control group, CTL), CsA (10mg / ml), TNF-α (50 U/ml) + INF-γ (interferon-γ 500 U/ml), used as a positive control, or CsA + TNF-α + INF-γ. After these treatments the culture medium was collected and nitrite (NO2-) analyzed by Griess method. The results were corrected by the protein harvested from these cells and measured by method of Lowry. Viability was determined in all groups by acridine orange method. Results: In CsA treated cells, the NO2-(pmoles/mg of protein) was decreased when compared to the CTL group (12.8±0.5 vs 18.8±0.6; p<0.001; both n = 8). TNF-α + INF-γ group presented a significant increase in NO2- (52.0±0.2; n = 6; p<0.05 compared to CTL). This effect was partially reversed by the simultaneous treatment with CsA (CsA + TNF-α + INF-γ = 38.8±0.3; p<0.05; n = 6). Cell viability in CTL group = 94.5 %, in CsA group = 88.5 %, in TNF-α + INF-γ group = 92.5 % and in CsA + TNF-α + INF-γ group = 90.5%.
Conclusion: Our preliminary results suggest a reduced NO production in CsA treated LLC-PK1 cells, which could be a consequence of cell damage as seen by reduced cell viability caused by this drug.
\textbf{Abstracts}

\textbf{IN VITRO DIFFERENTIATION ANTIGEN EXPRESSION ON URAEMIC MONONUCLEAR CELLS IN THE PRESENCE OF CAPTOPRIL OR LOSARTAN}  
\textsuperscript{a}Z. Zbrog, \textsuperscript{b}P. Bartnicki, \textsuperscript{c}E. Majewska, \textsuperscript{d}Z. Baj, \textsuperscript{a}M. Luciak  
\textsuperscript{a}2nd Department of Internal Medicine, \textsuperscript{b}Department of Pathophysiology and Immunology, WAM, Lodz, Poland  

The studies were aimed at the evaluation of captopril (C) and losartan (L) on in vitro differentiation antigen expression on uraemic peripheral blood mononuclear cells (PBMCN). PBMCN were isolated from 8 chronic uraemic patients on hemodialysis treatment and 8 healthy persons. The cells were cultured (72 h, 37°C) with PHA (5 µg/ml) or PMA (10 ng/ml) with C (5 µg/ml) or L (1 µg/ml). The antigen expression was measured with flow cytometry using respective monoclonal antibodies. 

\textbf{CAPTOPRIL AND LOSARTAN MODIFY MITOGEN-INDUCED PROLIFERATIVE RESPONSE OF URAEMIC MONONUCLEAR CELLS}  
\textsuperscript{a}Z. Zbrog, \textsuperscript{b}P.Bartnicki, \textsuperscript{c}E. Majewska, \textsuperscript{d}Z. Baj, \textsuperscript{a}M. Luciak  
\textsuperscript{a}2nd Department of Internal Medicine, and \textsuperscript{b}Department of Pathophysiology and Immunology, WAM, Lodz, Poland  

The studies were undertaken to determine effects of captopril (C) and losartan (L) on in vitro mitogen - induced proliferative response of uraemic peripheral blood mononuclear cells (PBMCN). PBMCN were taken from 8 chronic uraemic patients on hemodialysis treatment and 8 healthy persons. PHA- and PMA-induced proliferative responses in vitro were evaluated by \textsuperscript{3}H-thymidine incorporation in the presence of three therapeutic concentrations of C (C\textsubscript{1}-1 µg/ml, C\textsubscript{2}-2.5 µg/ml, C\textsubscript{3}-5 µg/ml) or L (L\textsubscript{1}-0.25 µg/ml, L\textsubscript{2}-0.5 µg/ml, L\textsubscript{3}-1 µg/ml). The results presented as proliferative indices were as follows (X±SEM): 

<table>
<thead>
<tr>
<th>Condition</th>
<th>C\textsubscript{1}</th>
<th>C\textsubscript{2}</th>
<th>C\textsubscript{3}</th>
<th>L\textsubscript{1}</th>
<th>L\textsubscript{2}</th>
<th>L\textsubscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uraemic</td>
<td>2.8±0.9</td>
<td>0.8±0.2</td>
<td>1.1±0.2</td>
<td>2.9±1.0</td>
<td>1.9±0.6</td>
<td>3.3±1.1</td>
</tr>
<tr>
<td>Control</td>
<td>2.7±0.7</td>
<td>1.0±0.2</td>
<td>14±0.1</td>
<td>1.8±0.3</td>
<td>1.7±0.5</td>
<td>2.4±0.7</td>
</tr>
<tr>
<td>*p&lt;0.05 in relation to PBMCN+PHA</td>
<td></td>
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It results that therapeutic concentrations of C and L depress mitogen-induced proliferative responses of uraemic and normal PBMCN.

\textbf{EFFECT OF PARATHORMONE AND VERAPAMIL ON MYOINOSITOL INCORPORATION INTO URAEMIC PERIPHERAL BLOOD MONONUCLEAR CELLS IN VITRO}  
P. Bartnicki, Z. Zbrog, J. Rysz, J. Mudyana, M. Luciak  
2nd Department of Internal Medicine, WAM, Lodz, Poland  

As myoinositol incorporation into uraemic peripheral blood mononuclear cells (PBMCN) is impaired, the studies were aimed at the possible contribution of 1-84 parathormone (PTH) and verapamil (VER) to this phenomenon. The investigations were performed on 10 chronic uraemic patients on regular hemodialysis treatment and 10 healthy persons. Incorporation of myo-[2-3H]-inositol into PBMCN was assessed in vitro following PTH (5 µg/ml; 60 min.) or PMA (10 ng/ml; 60 min.) stimulation in the presence of PTH in the concentration of 4•10^{-7}M and/or VER in the concentration of 10^{-7}M. 

The results suggest that L and C may have immunomodulatory properties.

\textbf{IgE AND INTERLEUKIN - 13 (IL-13) IN EGYPTIAN CHILDREN WITH NEPHROTIC SYNDROME (NS)}  
F. Fadel, S. El-Gohary*, A. El-Ayady, M. Francis#, and B. Mostafa. Departments of pediatrics, clinical pathology* and pathology#, Cairo University, Egypt.  

Minimal change glomerulopathy in children could be initiated by an atopic reaction and mediated through cellular immunity. Various studies demonstrated elevation of IgE and IL-4 rather than IL-13 with disease activity. In this study 68 children with NS together with 20 age and sex matched healthy controls were included. The patients were 24 with active NS before the onset of steroid treatment [ttt] (groupI), 20 active NS on steroid ttt (groupII), 13 NS steroid responsive under withdrawal (groupIII); of whom 7 relapsed during withdrawal; and 11 in remission (group IV). IgE was significantly elevated than controls value (105.7 ± 108.2 IU/ml) in minimal lesion of gpl (1413.3 ± 1431.5 IU/ml p < 0.001), gpII (686.9 ± 521.6 p < 0.01), and gp III (450.5 ± 406.0 p < 0.001), gp II (686.9 ± 521.6 p < 0.01), and gp III (450.5 ± 406.0 p < 0.001). It was still significantly elevated than controls in gp IV (805.9 ± 862.9 p < 0.001). While the levels in non-minimal cases of gsp I, II, III were not statistically significantly different from controls. This indicates that IgE can predict steroid responsiveness suggesting minimal lesion, yet itself being still elevated in those in remission represents alability/tendency of our patients to develop the active nephrotic state. IL-13 was only significantly elevated than control values (9.4 ± 1.0 pg/ml) in cases with minimal lesion active NS before the onset of steroid ttt being 10.9 ± 1.5 pg/ml (p < 0.001). We conclude that both elevated IgE and IL-13 can predict steroid responsiveness in NS suggesting minimal change glomerulopathy. And while IL-13 was only elevated during activity before the start of ttt, IgE remained elevated during ttt and also after remission.