# CYTOKINES IN DIABETIC NEPHROPATHY

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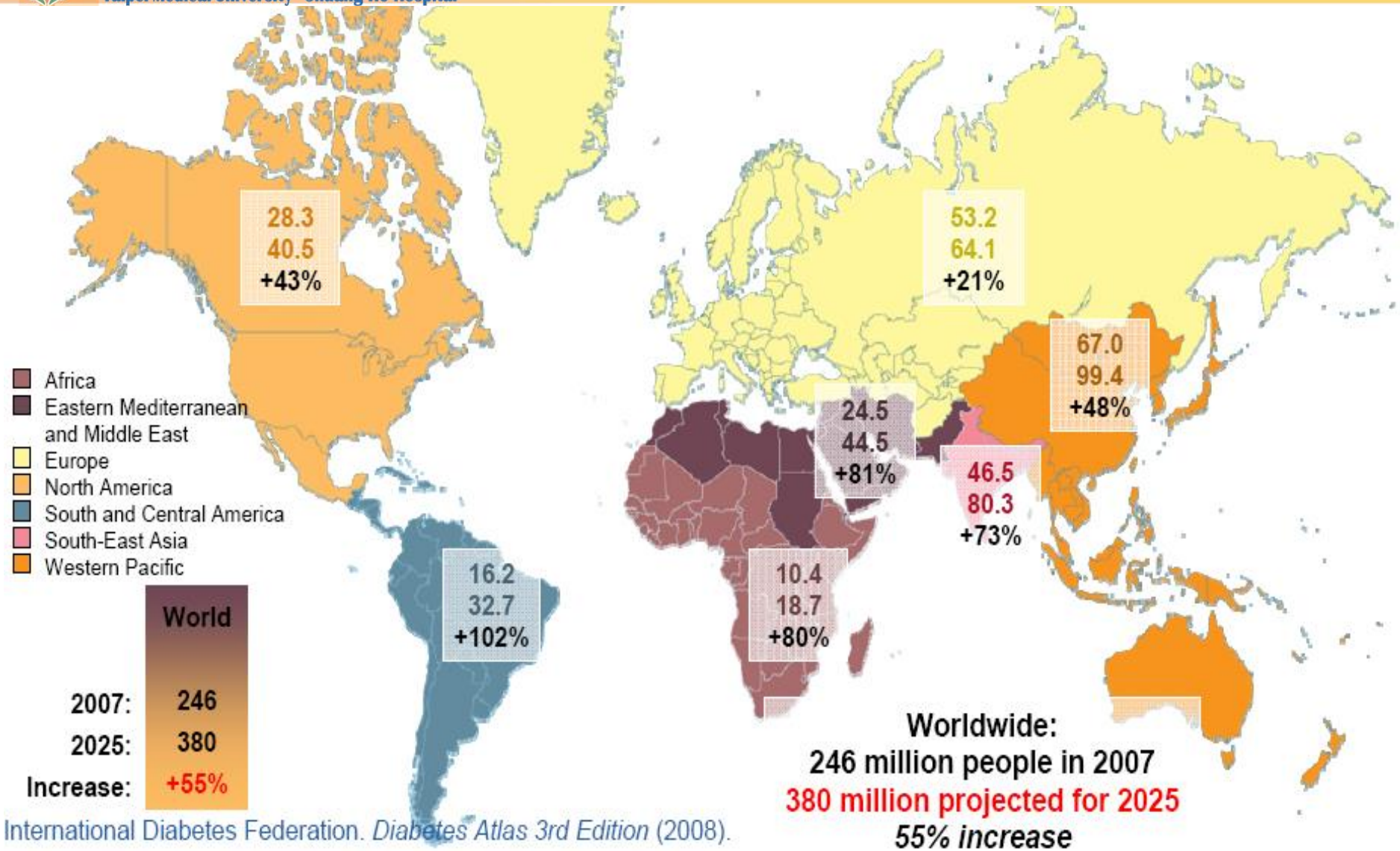
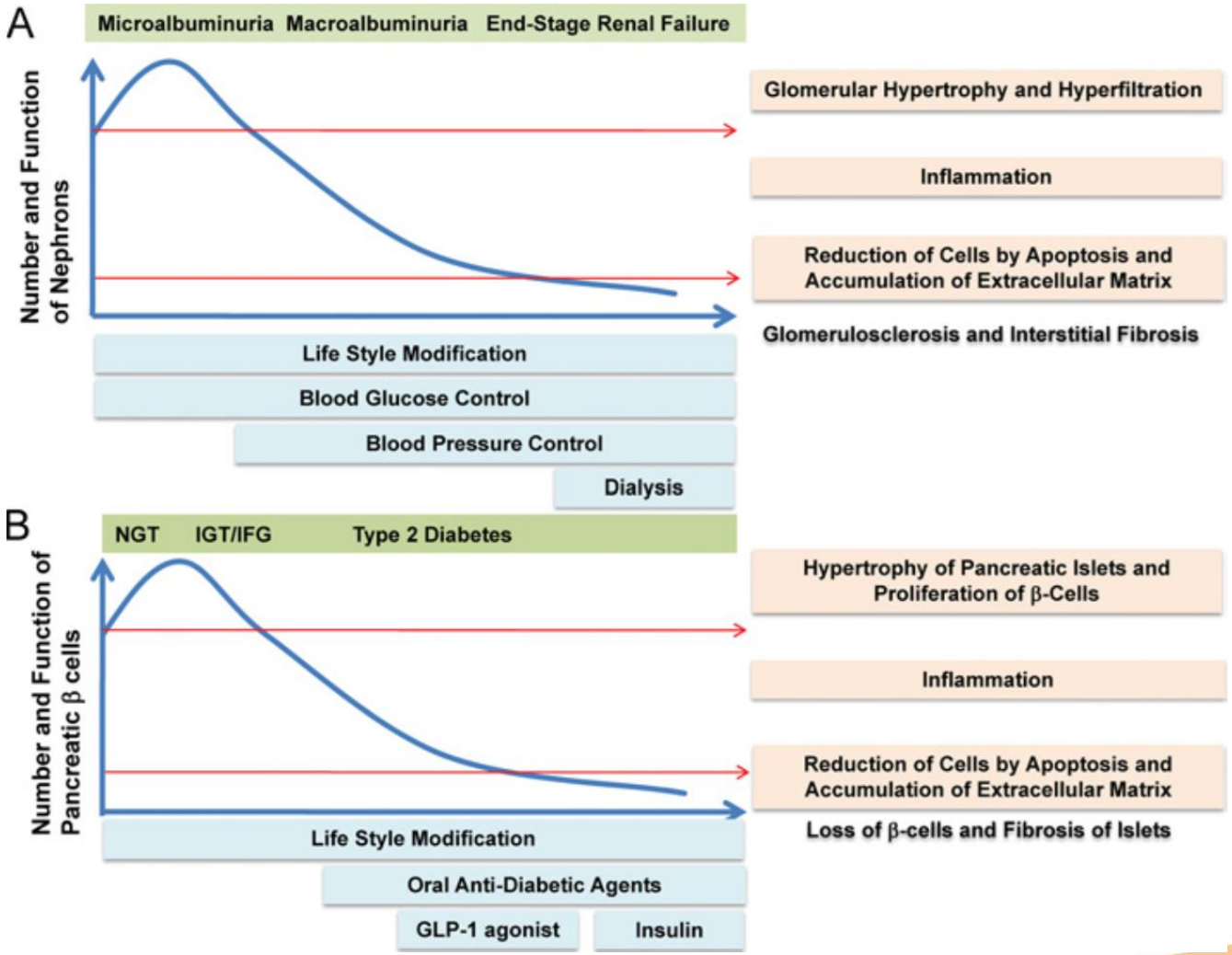



International Diabetes Federation. *Diabetes Atlas 3rd Edition* (2008).

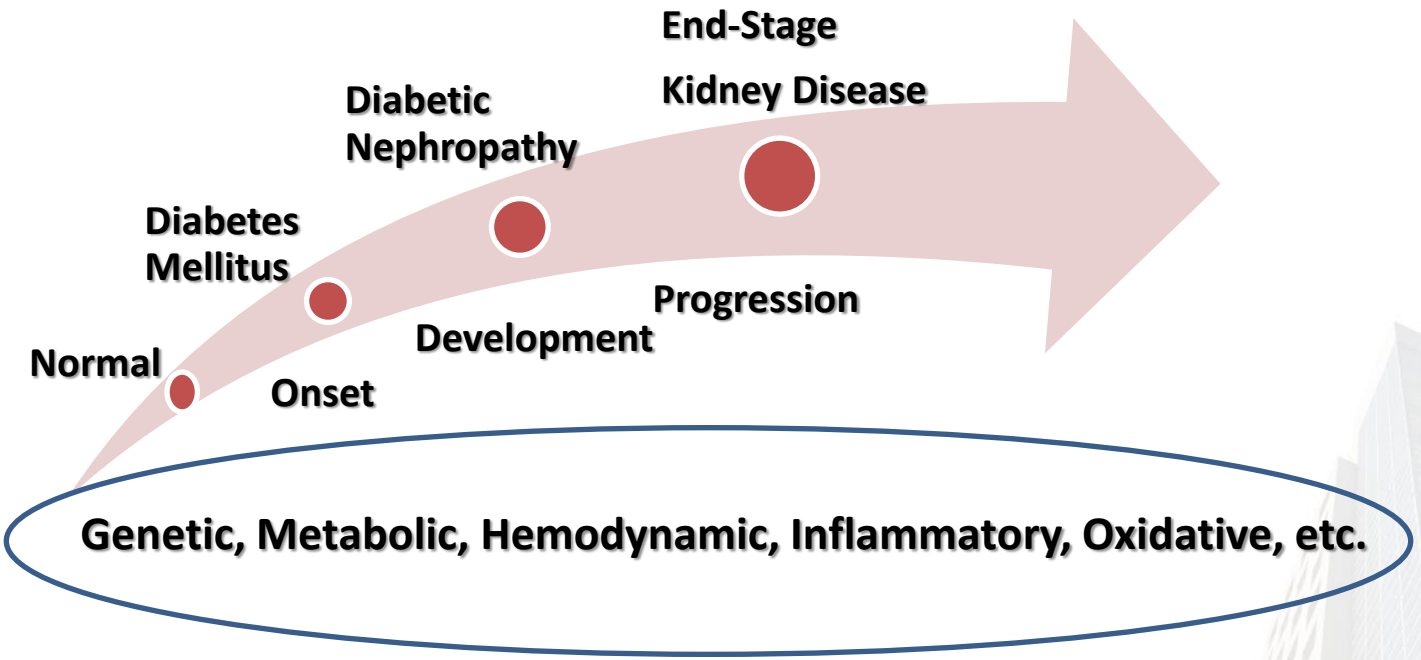
***International Diabetes Federation 2008***

# Natural History and Disease Course of Type 2 Diabetes Mellitus and Diabetic Nephropathy



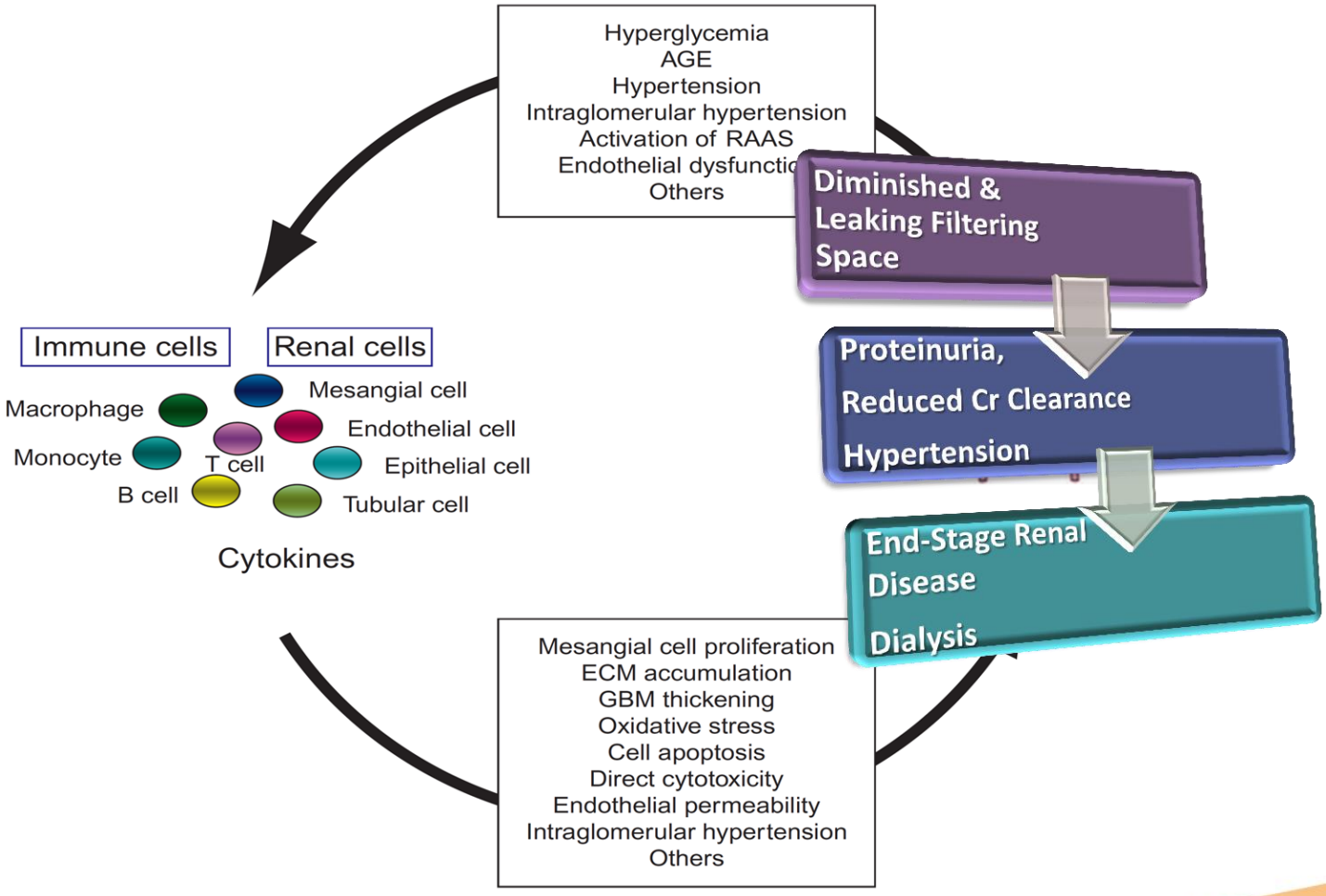

*Clinical Science (2013) 124, 139–152*

# Risk Factors for Diabetes (DM) and Diabetic Nephropathy (DN)




*Lin YF. et al. Advances in Clinical Chemistry Vol 56, 2012*

# Interactions Between Cytokines and Diabetic Kidney Disease




*Lin YF. et al. Advances in Clinical Chemistry Vol 56, 2012*

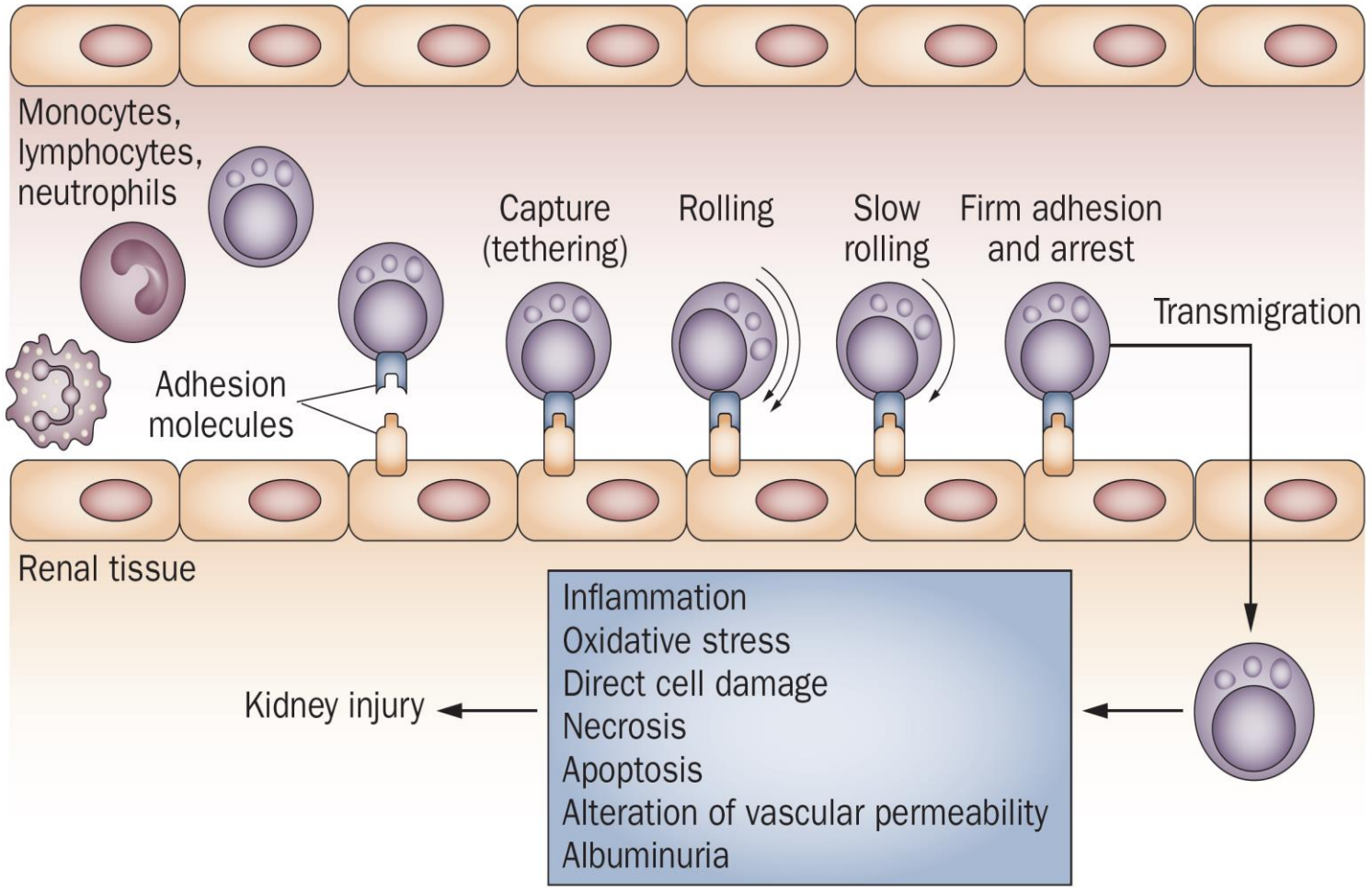
# Leukocyte Recruitment and Involvement in the Process of Diabetic Nephropathy

| Cell Type | Adhesion Molecules, Chemokines | Products | Proposed Role |
|---|---|---|---|
| Monocytes, macrophages | ICAM-1, MCP-1 | Nitric oxide, reactive oxygen species, IL-1, TNF-$\alpha$, complement factors, metalloproteinases, PDGF, TGF-$\beta$ | Endothelial damage, induction of fibroblast and mesangial cell proliferation |
| T lymphocytes | LFA-1/ICAM-1, RANTES | IFN-$\gamma$, TNF-$\alpha$ | Activation of endothelial cells and macrophages |
| Neutrophils | Mac-1 | Superoxide anion, hydrogen peroxide | Endothelial damage |

**ICAM-1, intercellular adhesion molecule-1; MCP, monocyte chemoattractant protein-1; RANTES, regulated on activation, normal T cell exposed and secreted.**

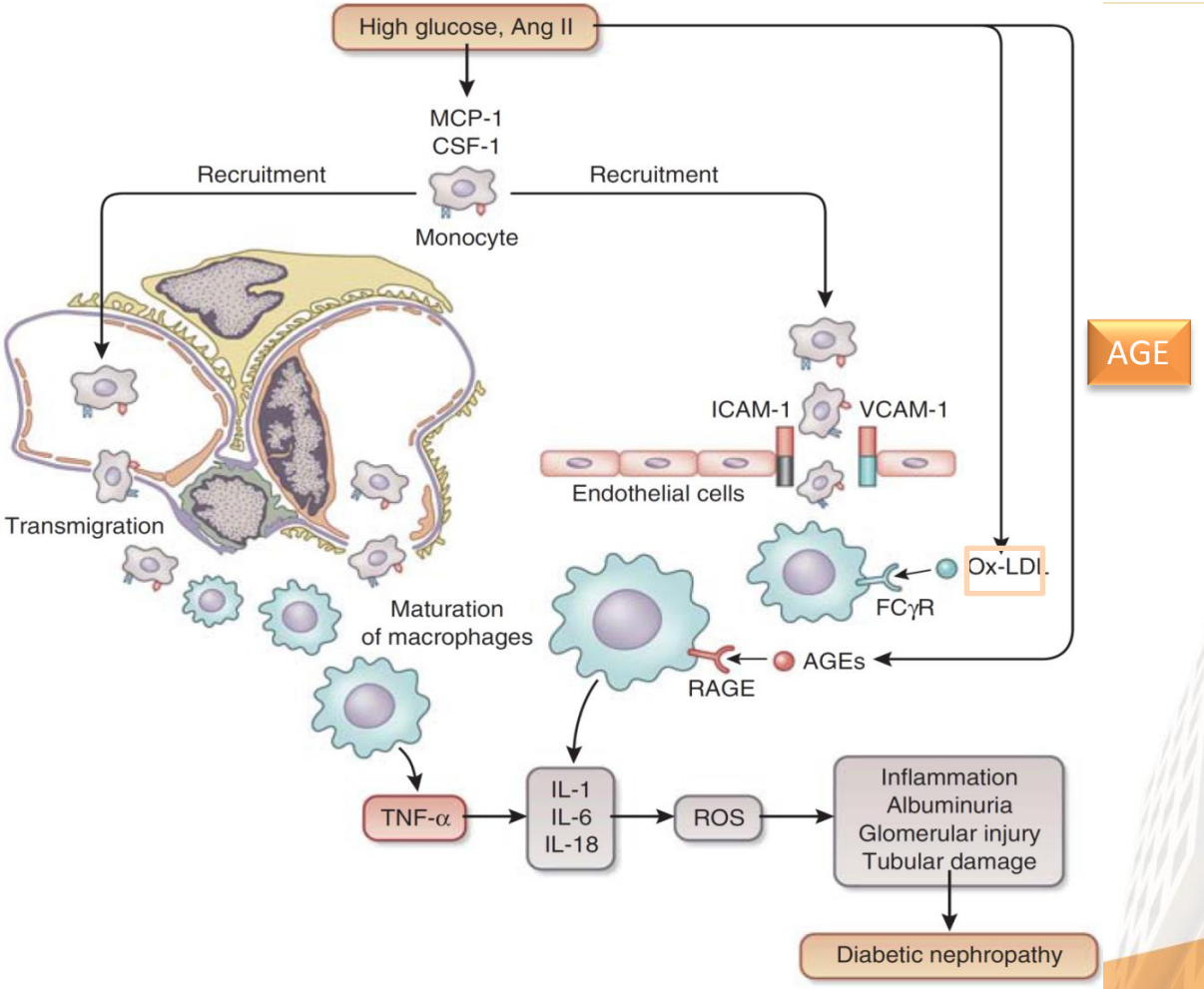
*J Am Soc Nephrol 17: 368–377, 2006*

# Leukocyte Infiltration in Diabetic Nephropathy




*Nat. Rev. Nephrol. 7, 327–340 (2011);*

# Sequence of Events After Leukocyte Activation in Diabetic Nephropathy



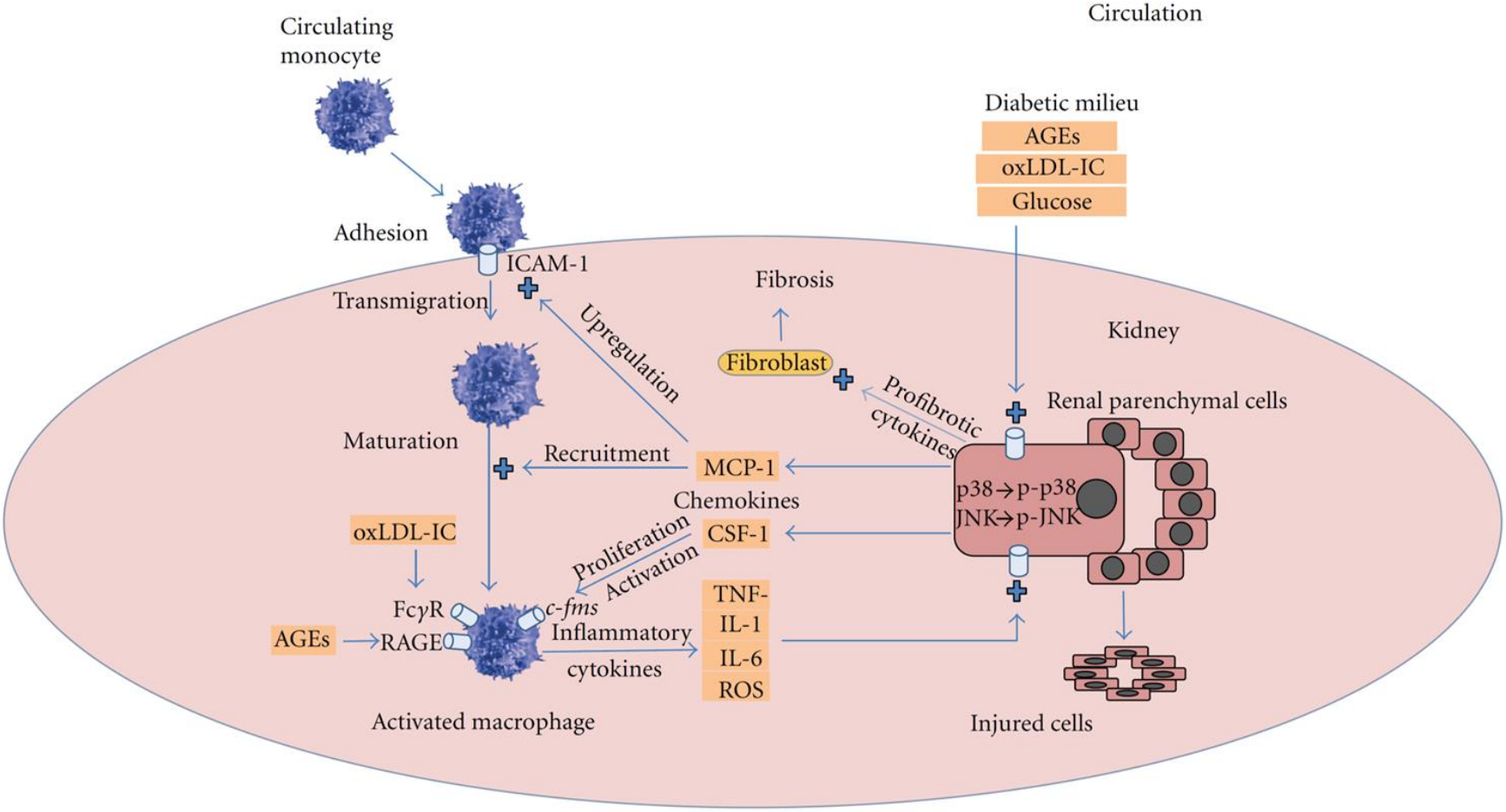

*Kidney International (2015) 88, 662–665*

# Inflammatory Cytokines in Diabetic Nephropathy

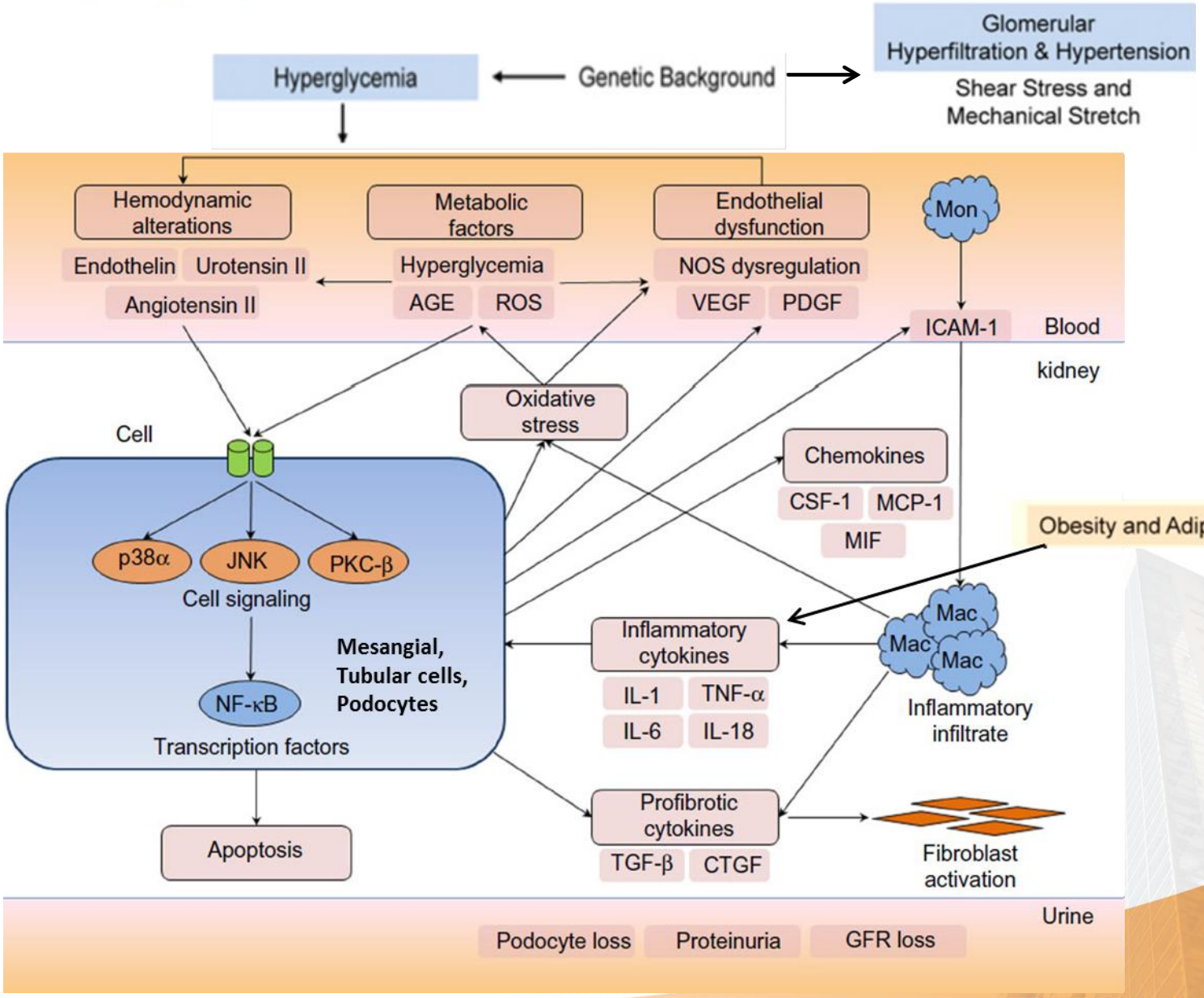
- Chemokines and their receptors
  - CCL2 (MCP-1) and its receptor CCR2
  - CX3CL1 (fractalkine) and its receptor CX3CR1
  - CCL5 (RANTES) and its receptor CCR5
- Adhesion molecules
  - Intercellular adhesion molecule 1
  - Vascular cell adhesion protein 1
  - Endothelial cell-selective adhesion molecule
  - E-selectin (CD62E)
  - α- Actinin 4
- Transcription factors
  - Nuclear factor kB
- Inflammatory cytokines
  - IL-1, IL-6 and IL-18
  - Tumor necrosis factor

*Nat. Rev. Nephrol. 7, 327–340 (2011);*

# The Inflammatory Amplification Loop in Diabetic Nephropathy



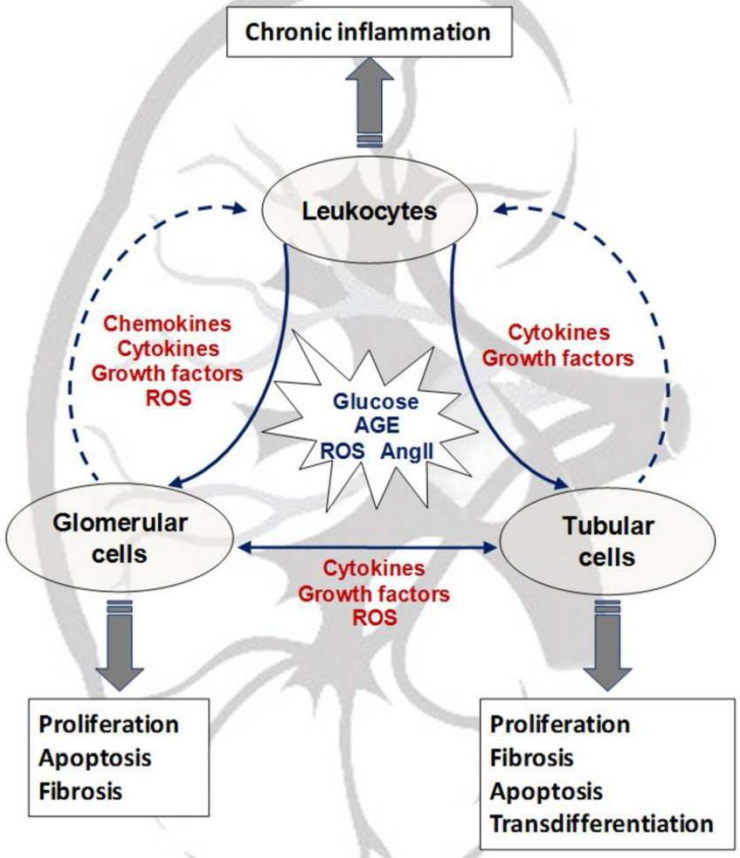

*Mediators of Inflammation Volume 2012, Article ID 146154*




*Clinical Science (2013) 124, 139–152.*

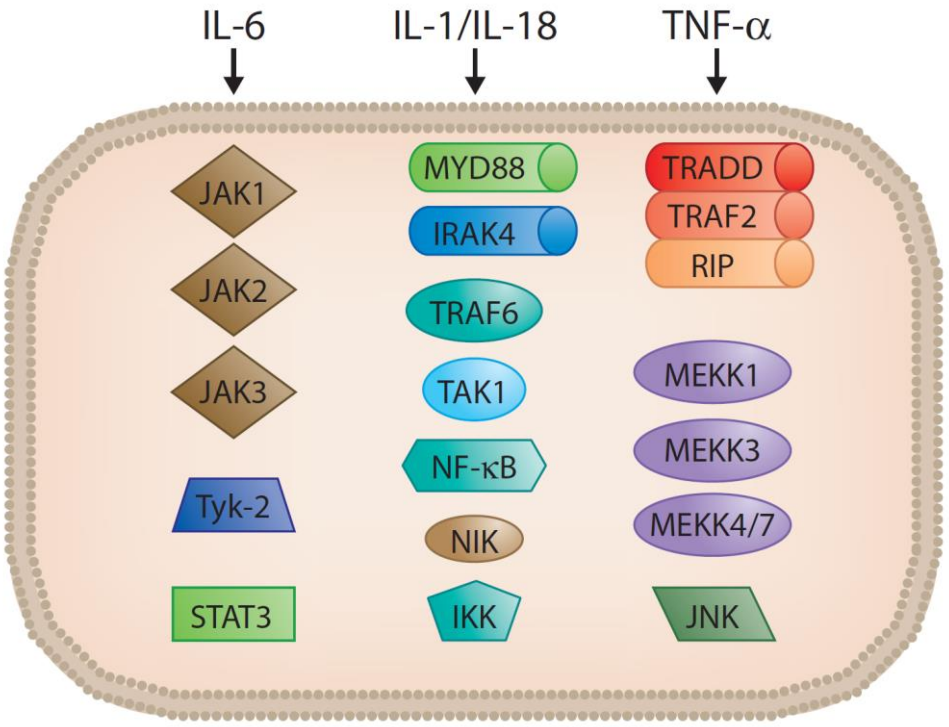
*International Journal of Nephrology and Renovascular Disease. 2014;7:361-381.*

# Overview of Role of Inflammation in Diabetic Nephropathy




Am. J. Physiol Renal Physiol, Vol.297, No.5, 2009

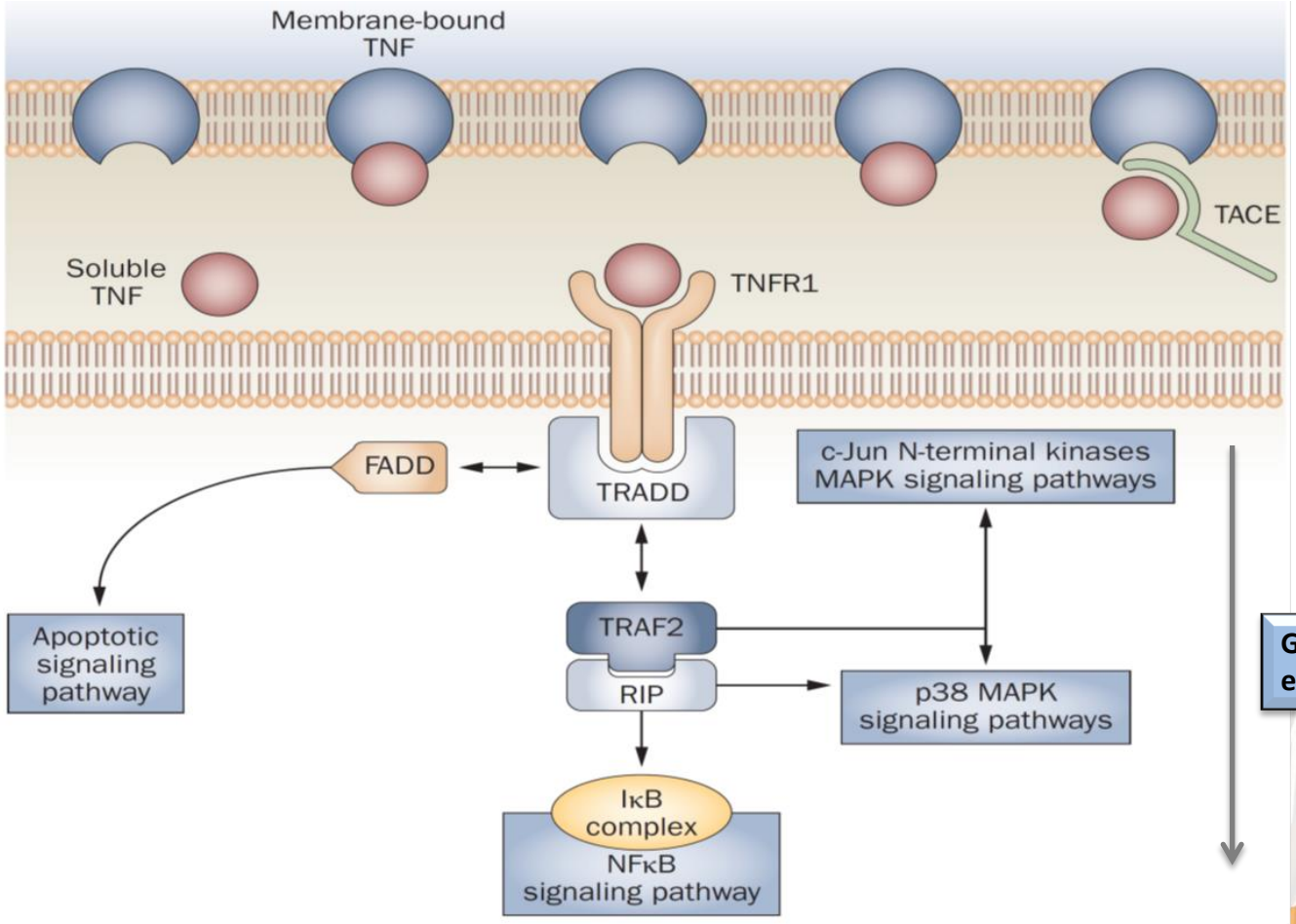
# Intracellular Cytokine-Associated Signaling Pathways




JAK, Janus kinase;
Tyk, Tirosine kinase;
STAT, signal transducer and activator of transcription;
MYD88, myeloid differentiation factor-88;
IRAK, IL receptor–associated kinase;
TRAF, TNF receptor–associated factor;
TAK, TGF- –associated kinase;
NIK, NF- B–inducing kinase;
IKK, inhibitor of NF- B kinase;
TRADD, TNF receptor–associated death domain;
RIP, receptor interacting protein;
MEKK, mitogen-activated protein kinase/Erk kinase kinase;
JNK, c-Jun N-terminal kinase

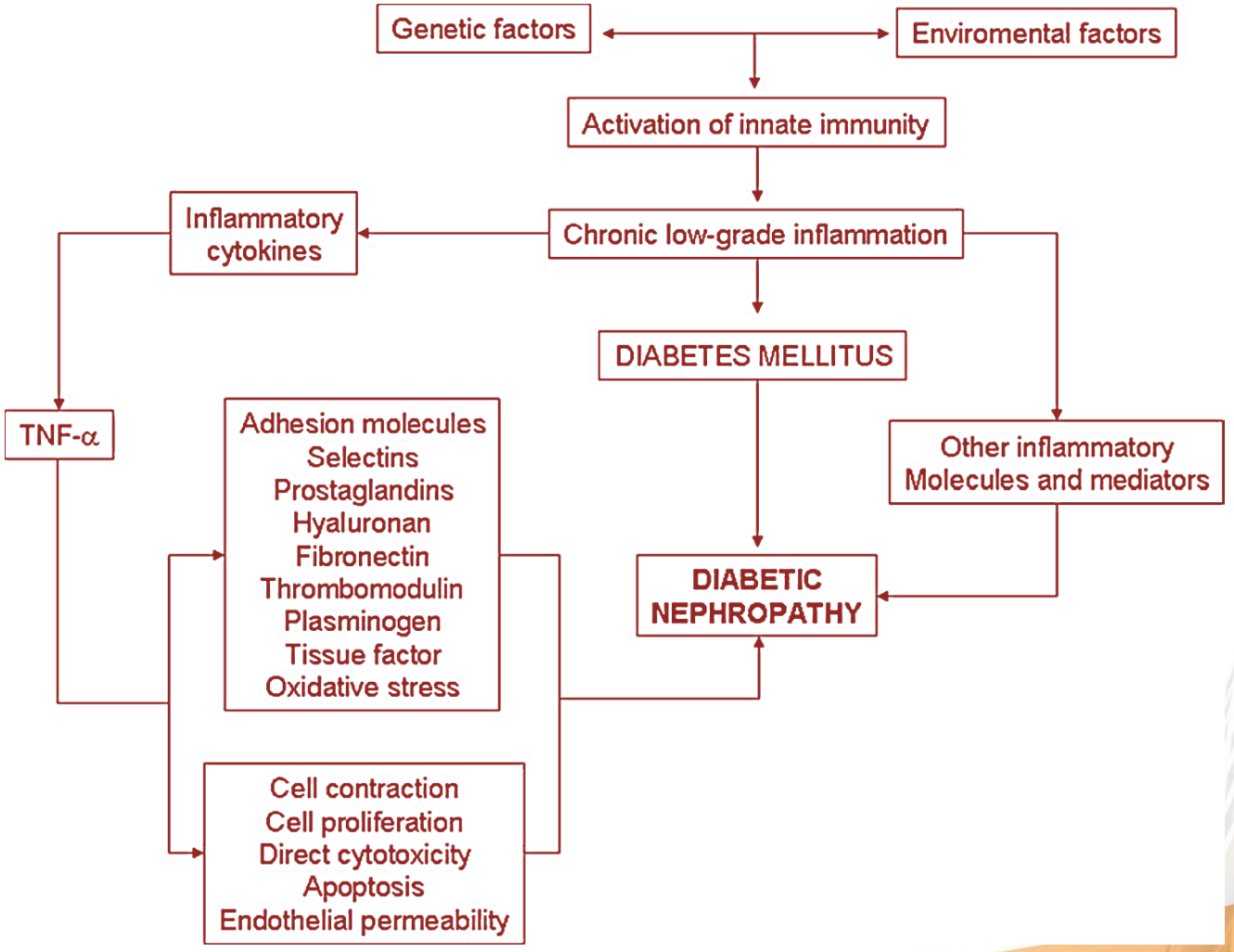
*J Am Soc Nephrol 19: 433–442, 2008*

# TNF Signaling Cascades play a Pivotal Role in Diabetic Nephropathy




*Nat. Rev. Nephrol. 7, 327–340 (2011);*

# Targets of TNF α in Pathogenesis of Diabetic Nephropathy



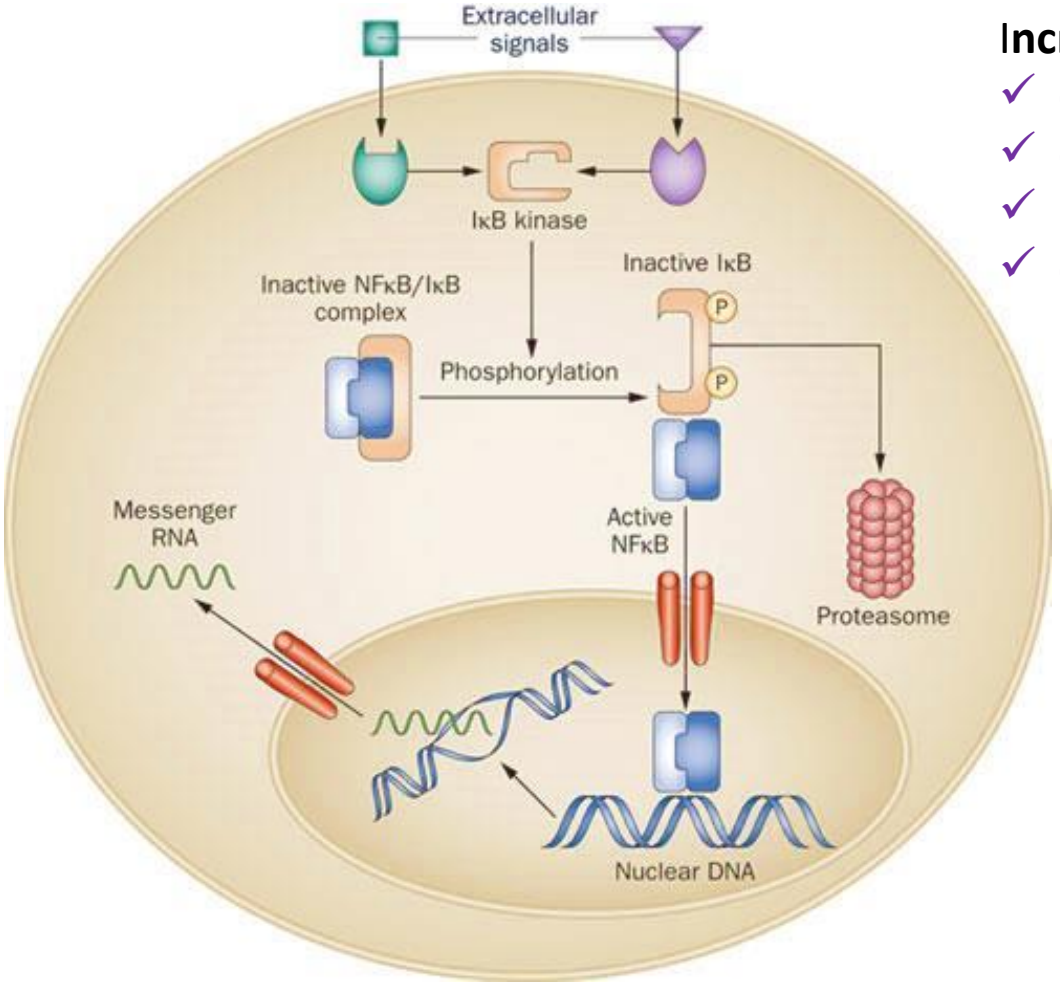

*Cytokine & Growth Factor Reviews 20 (2009) 165–173*

# Role of Tumor Necrosis Factor-α in Diabetic Nephropathy

- ***Disrupt the balance between vasoconstrictor and vasodilator substances*** (adenosine, nitric oxide, prostaglandins, platelet activating factor, endothelin-1).
- ***Contraction of mesangial cells.***
- Alteration in the **protein permeability barrier** of the glomerulus.
- Activation of the production of ***chemoattractant factors for neutrophils and monocytes.***
- Stimulation of the ***production of plasminogen-activator inhibitor type-1 and tissue factor*** by mesangial and endothelial cells.
- ***Induction of apoptosis*** mediated by the TNF-a receptor p55-associated death domain (TRADD), the Fas-associated death domain (FADD) and the insulin-like growth factor binding protein-3.

***Cytokine & Growth Factor Reviews 20 (2009) 165–173***

# Role of NFκB Signaling Pathways in Diabetic Nephropathy

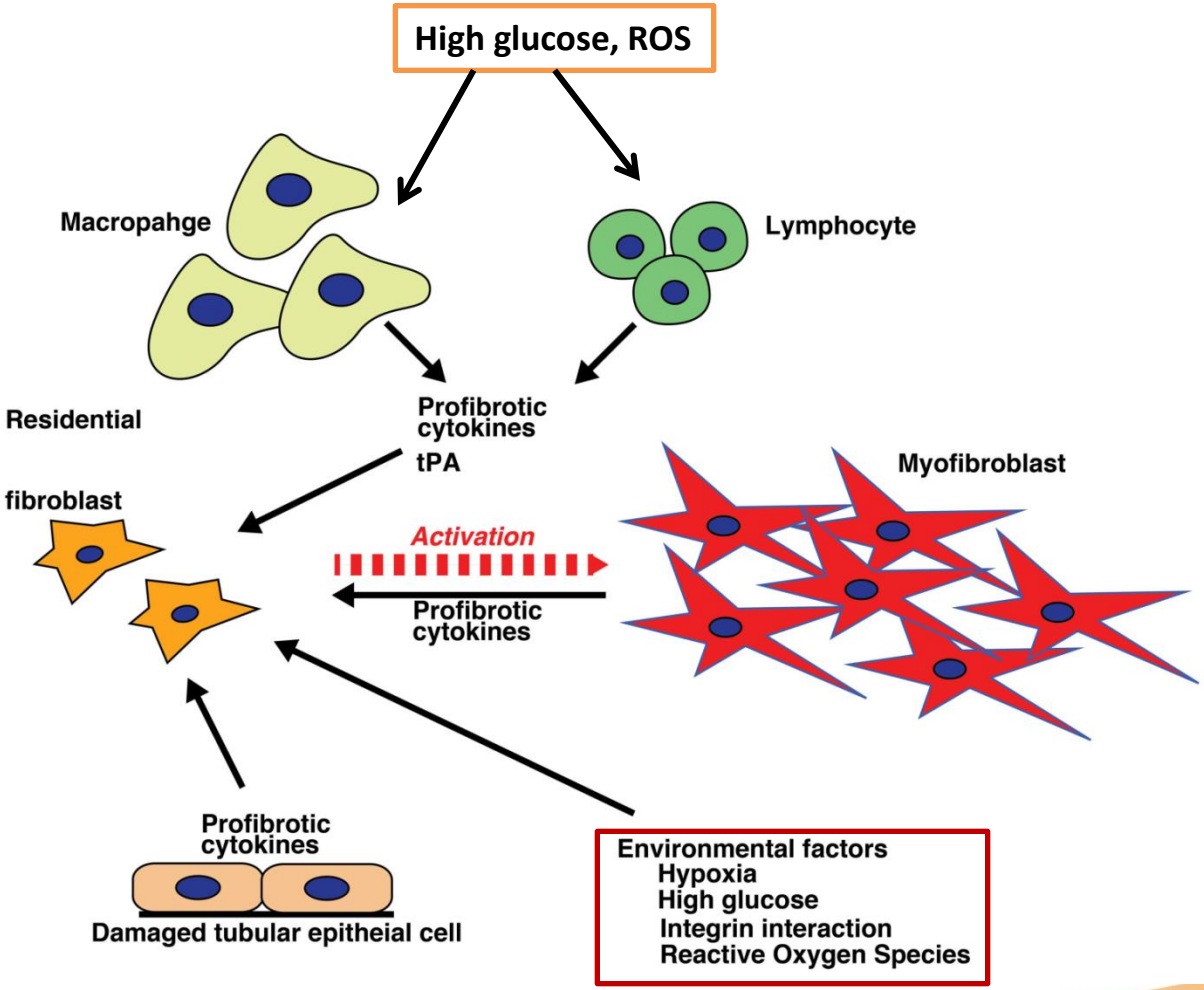



**Increased transcription of target genes**

- ✓ **chemokines,**
- ✓ **effector molecules of immunity,**
- ✓ **inflammatory cytokines, and**
- ✓ **cell adhesion molecules**

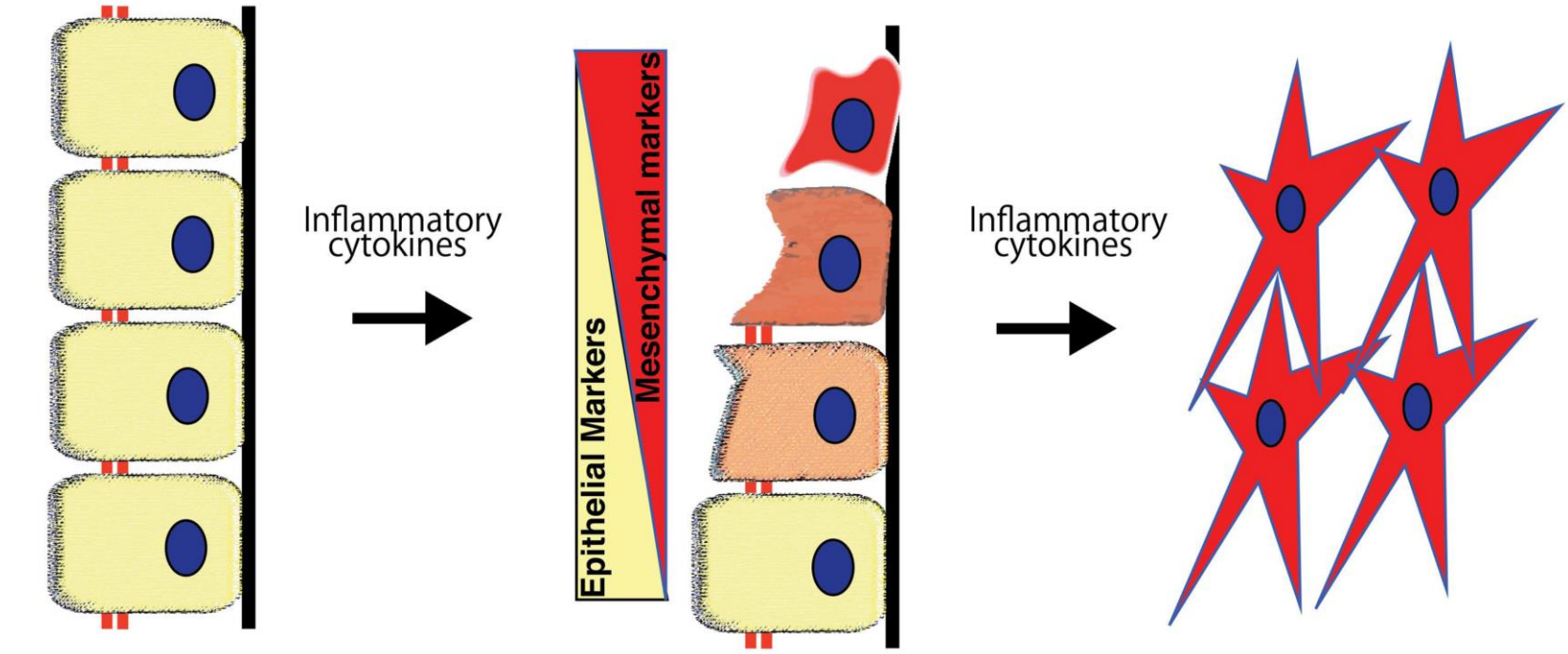
*Nat. Rev. Nephrol. 7, 327–340 (2011);*

# Activation of Fibroblasts by Inflammatory Cytokines




*Front. Endocrinol., 06 February 2013*

# Process of Epithelial-to-Mesenchymal Transition Mediated by Inflammatory Cytokines



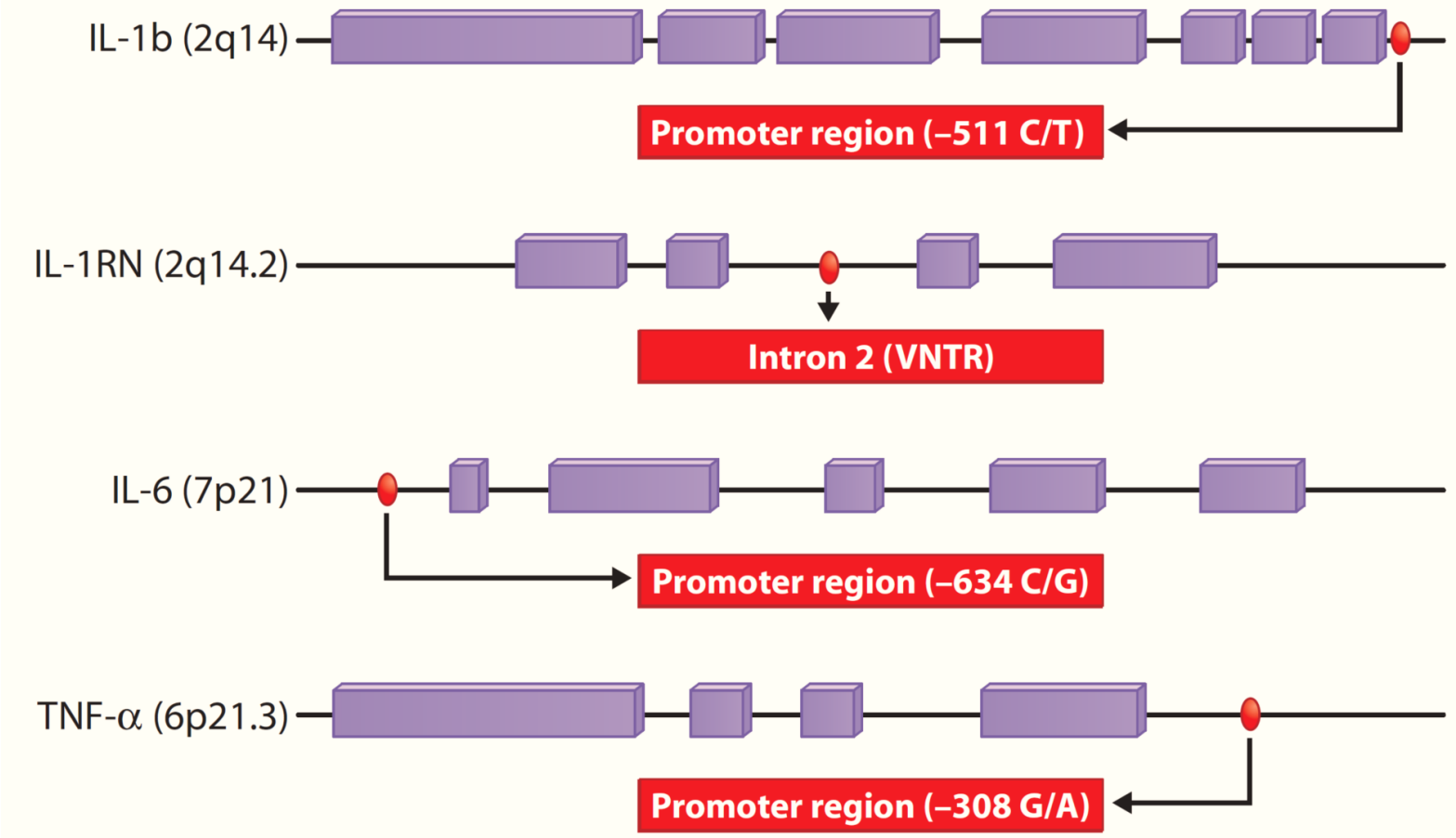

*Front. Endocrinol., 06 February 2013*

# Candidate Genes for Diabetic Nephropathy

| Gene Class | Gene | Location | Loci | Population | Phenotype | Reference |
|---|---|---|---|---|---|---|
| Cytokines and growth factors | Adiponectin | 3q | ADIPOQ | Danish, Finnish, French | Type 1 DN | (33) |
| | IGF-1 | 12q23.2 | IGF1 | White | Type 1 DN | (30) |
| | IGF-binding protein 1 | 7p14 | IGFBP1 | White | Type 2 DN | (51) |
| | TGF-β receptor II | 3p24.1 | TGFβR2 | White | Type 1 DN | (30) |
| | TGF-β receptor III | 1p22.1 | TGFβR3 | White | Type 1 DN | (30) |
| Extracellular matrix components | Collagen type IV, α I | 7q32.1 | COL4AI | White | Type 1 DN | (30) |
| | Laminin, α 4 | 6q21 | LAMA4 | White | Type 1 DN | (30) |
| | Laminin, γ 1 | 1q25.3 | LAMCI | White | Type 1 DN | (30) |
| Matrix metalloproteinases and dipeptidases | Tissue inhibitor of metalloproteinase 3 | 22q12.3 | TIMP3 | White | Type 1 DN | (30) |
| | Matrix metalloproteinase 9 | 20q13.12 | MMP9 | White | Type 1 DN | (30) |
| | Carnosinase | 18q22.3 | CNDP1 | White | Type 2 DN | (41,42) |
| Transcription factors | HNF1B1/transcription factor 2, hepatic (MODY5) | 17q12 | HNF1B1/TCF2 | White | Type 1 DN | (30) |
| | Neuropilin 1 | 10p11.22 | NRPI | White | Type 1 DN | (30) |
| | Protein kinase C β 1 | 16p12.1 | PRKCBI | White | Type 1 DN | (30) |
| | SMAD, mothers against DPP homolog 3 | 15q22.33 | SMAD3 | White | Type 1 DN | (30) |
| | Upstream transcription factor 1 | 1q23.3 | USFI | White | Type 1 DN | (30) |
| Renal function and renin angiotensin system components | Angiotensin II receptor, type 1 | 3q24 | AGTR1 | White | Type 1 DN | (30) |
| | Aquaporin 1 | 7p14.3 | AQP1 | White | Type 1 DN | (30) |
| | B-cell leukemia/lymphoma 2 (bcl-2) proto-oncogene | 18q21.33 | BCL2 | White | Type 1 DN | (30) |
| | Catalase | 11p13 | CAT | White | Type 1 DN | (30) |
| | Glutathione peroxidase 1 | 3p21.3 | GPXI | White | Type 1 DN | (30) |
| | Lipoprotein lipase | 8p21.3 | LPL | White | Type 1 DN | (30) |
| | Cytochrome b, α polypeptide | 16q24.3 | p22phox | White | Type 1 DN | (30) |
| | Angiotensin-converting enzyme | 17q23 | ACE | White | Type 1 DN, Type 2 DN | (60,61,64,65) |
| Inflammatory factors | Engulfment and cell motility factor | 7p14 | ELMO1 | Japanese, Black | Type 2 DN | (23,46–48) |
| Endothelial function and oxidative stress | Nitric oxide synthase 3 | 7q36.1 | NOS3 | Japanese, White | DN, Type 1 DN | (54–58) |
| | Superoxide dismutase 2 | 6q25 | SOD2 | Caucasian, Korean, Japanese | Type 1 DN, Type 2 DN | (66–68) |
| Lipid metabolism | Apolipoprotein E | 19q | ApoE | White | Type 1 DN, Type 2 DN | (69,70) |

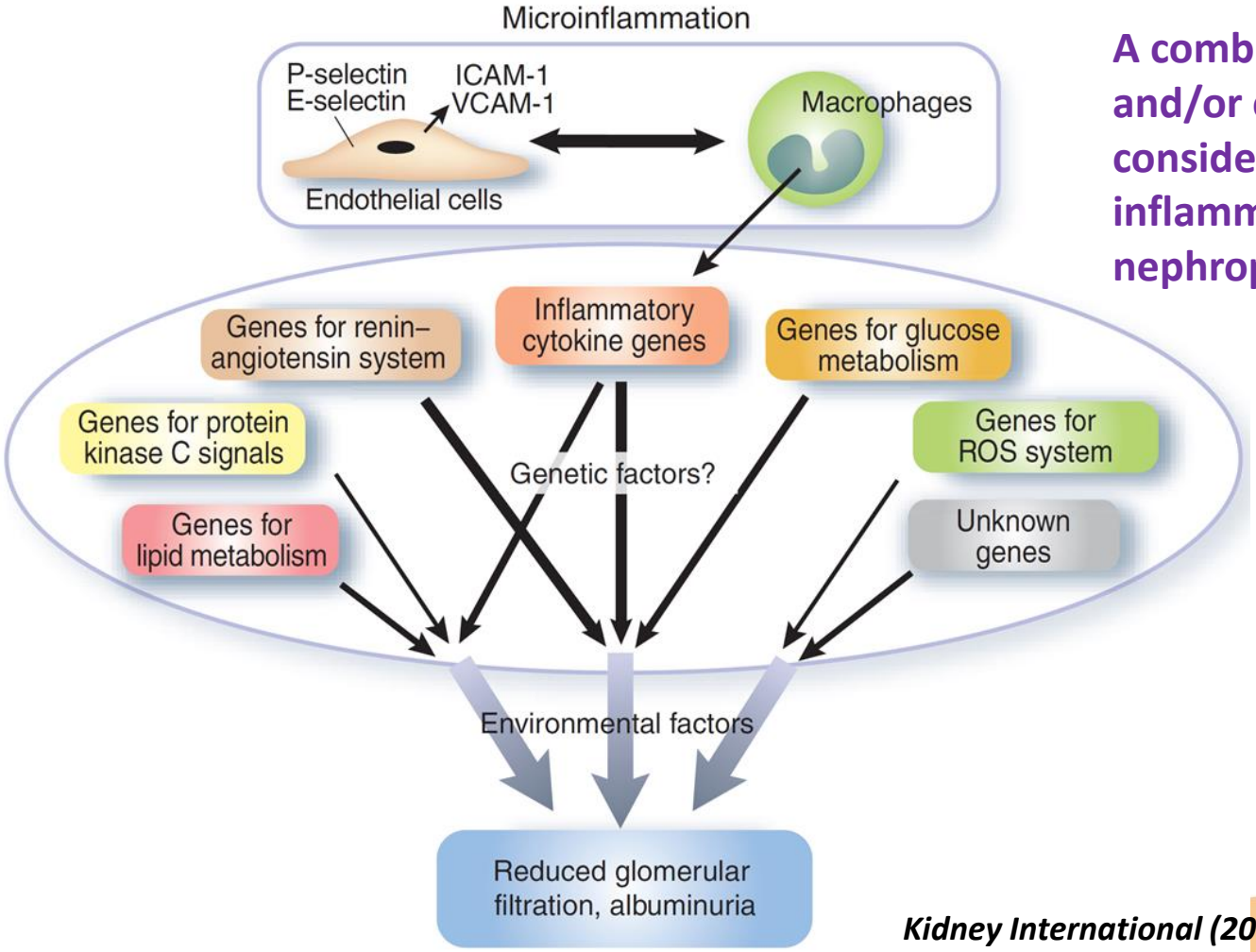
*Clin J Am Soc Nephrol 2: 1306–1316, 2007*

# Inflammatory Cytokine Genetic Polymorphisms Implicated in Diabetic Nephropathy




*J Am Soc Nephrol 19: 433–442, 2008*

# Combination of Gene and/or Environmental Factors in Diabetic Nephropathy

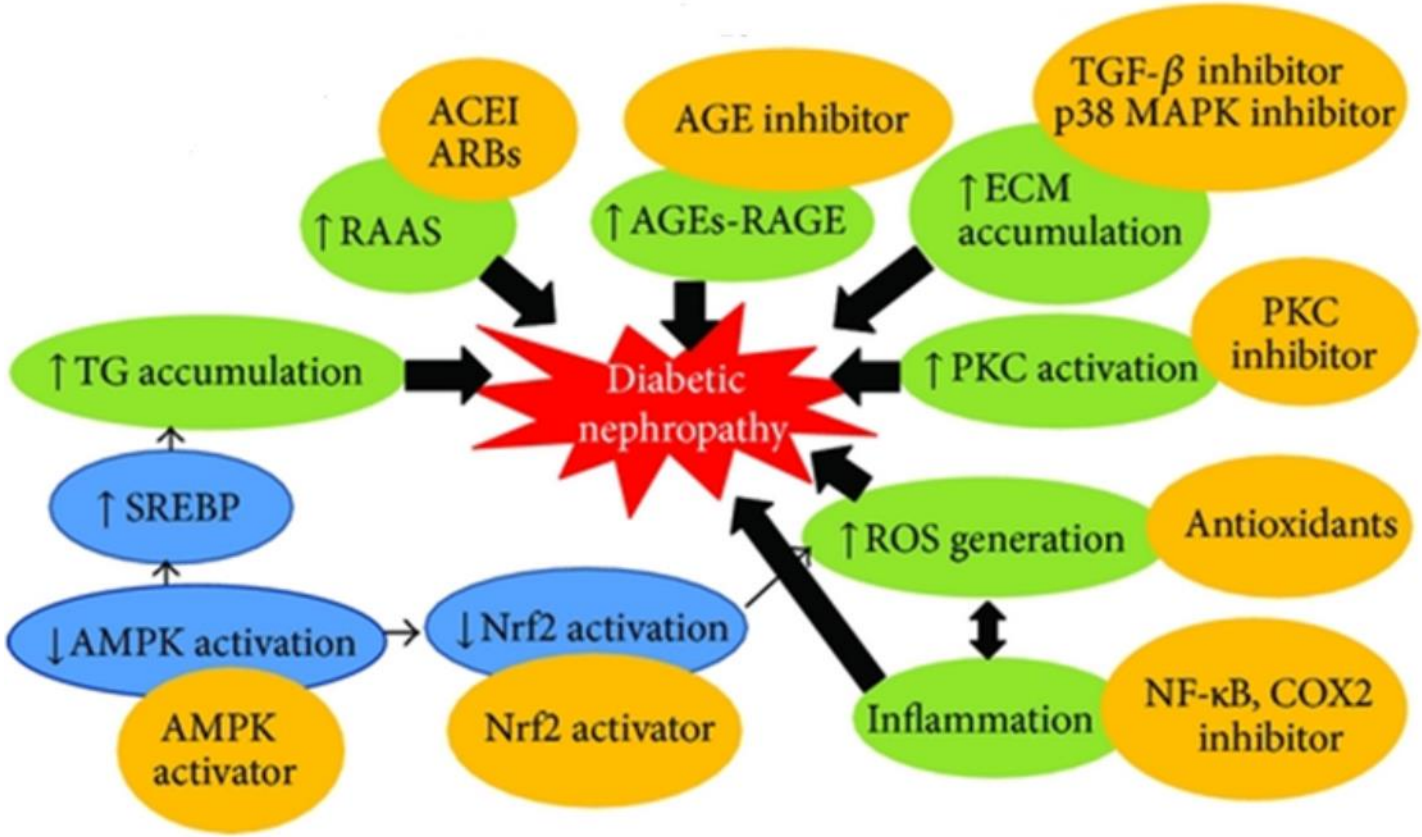



**A combination of multiple genetic and/or environmental factors is considered to contribute to micro-inflammation and diabetic nephropathy**

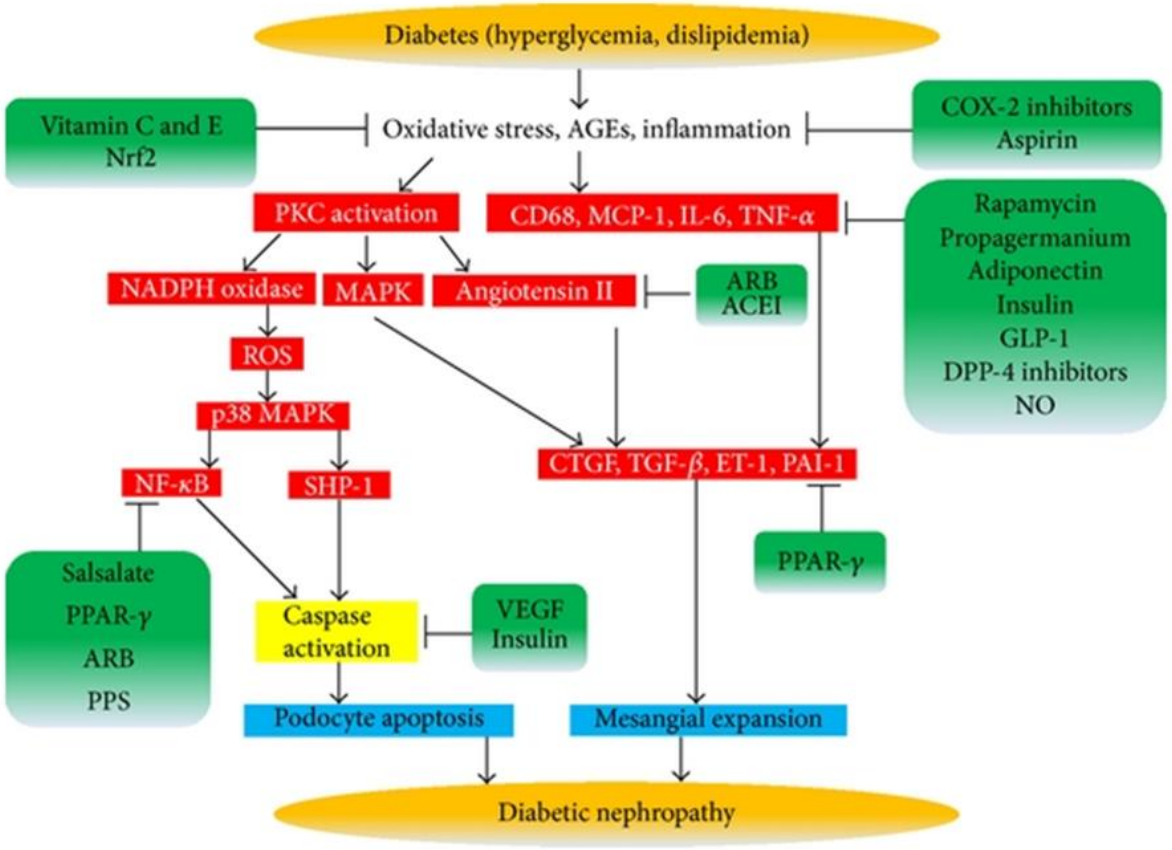
*Kidney International (2008) 74, 413–415*

# Therapeutic Implications of Cytokines in Diabetic Nephropathy

# Mechanism Based Targeted Therapy in Diabetic Nephropathy



# Mechanism Based Targeted Therapy in Diabetic Nephropathy




1. NFkB and MCP-1 inhibitor (Spironlactone, Bardoxolone)
2. TNF-$\alpha$ inhibitor (Pentoxifylline, exogenous insulin)
3. Adipokines (adiponectin)
4. Inhibition of ICAM, PAI and NF-$\kappa$ B (PPAR-$\gamma$ agonist, Pioglitazone)
5. HMG-CoA Reductase Inhibitors
6. mTOR inhibitors (Rapamycin)
7. Aspirin and COX-2 Inhibitors
8. Inhibition of PKC Activation
9. GLP-1 and DPP-IV Inhibitors (linagliptin)

*Journal of Diabetes Research, 2013*

## Therapeutic Targeting of Cytokines in Diabetic Nephropathy

| Agents | Actions | Therapeutic outcome |
|---|---|---|
| Chimeric anti-TNF-α antibody (infliximab) | Inhibition of TNF-α | Reduction of albuminuria |
| Soluble TNF-α receptor fusion protein | Inhibition of TNF-α | Prevention of sodium retention<br>Prevention of renal hypertrophy |
| Pentoxifylline | Inhibition of TNF-α<br>Modulation of IFN γ, IL-1β, IL-6 | Reduction of albuminuria and proteinuria[a] |
| Neutralizing anti-TGF-β antibody | Inhibition of TGF-β | Attenuation of renal hypertrophy<br>Suppression of renal fibrosis<br>Reduction of albuminuria |
| Soluble type III TGF-β receptors (betaglycan) | Inhibition of TGF-β | Reduction of the deposition of extracellular matrix components<br>Suppression of mesangial matrix expansion<br>Reduction of albuminuria<br>Amelioration of renal damage |
| TGF-β inhibitor (SMP-534) | Inhibition of p38 signaling<br>Inhibition of TGF-β signal transduction | Reduction of the deposition of extracellular matrix components<br>Suppression of mesangial matrix expansion<br>Reduction of albuminuria |
| Suppressors of cytokine signaling (SOCS) deliver | Inhibition of JAK/STAT/SOCS | Reduction of proinflammatory cytokines<br>Suppression of glomerular hypertrophy, mesangial matrix expansion, tubular atrophy |

[a] Human; others, experimental data.

*Lin YF. et al. Advances in Clinical Chemistry Vol 56, 2012*

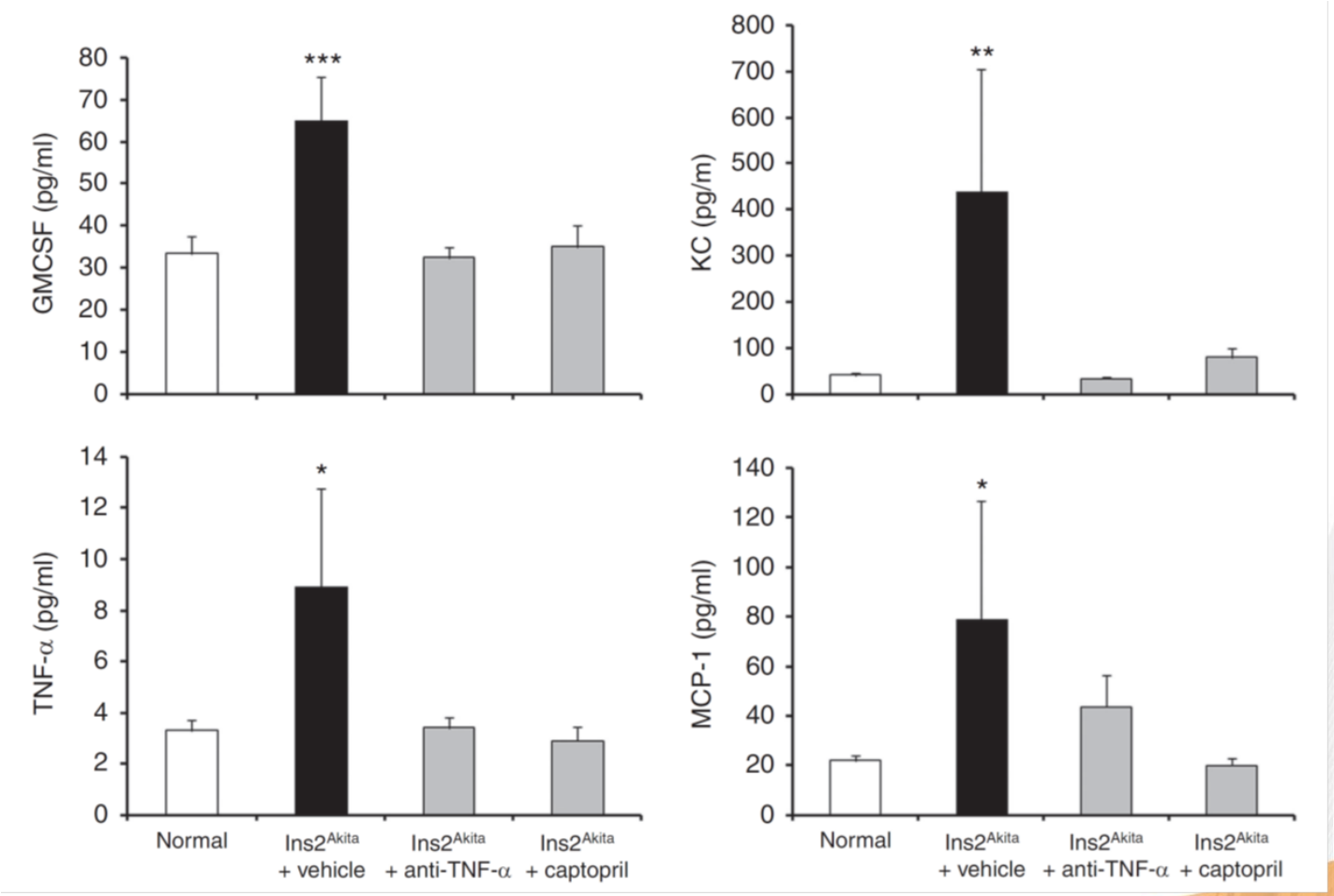
# Anti-TNF-α Strategies for Treatment of Diabetic Nephropathy

- Soluble TNF-a receptor fusion protein (experimental data)
  - Reduction of urinary TNF-a excretion
  - Prevention of sodium retention
  - Prevention of renal hypertrophy
- Chimeric monoclonal antibody against TNF-a (experimental data)
  - Reduction of urinary TNF-a excretion
  - Amelioration of urinary albumin excretion
- **Pentoxifylline (experimental and clinical data)**
  - Reduction of the renal over-expression of inflammatory cytokine genes
  - Reduction of urinary TNF-a excretion
  - Reduction of albuminuria and proteinuria
  - Reduction of the urinary excretion of N-acetyl-b-glucosaminidase

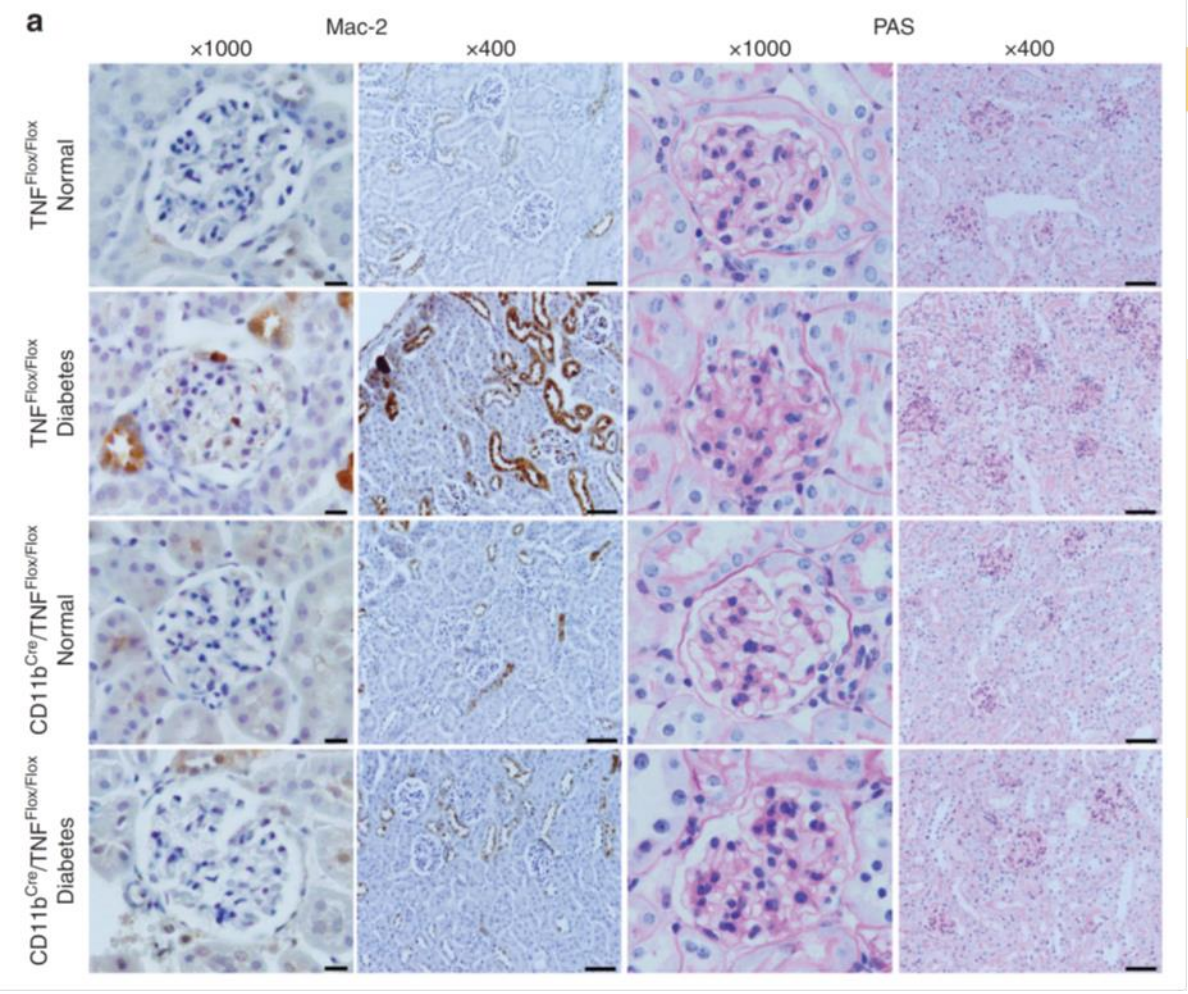


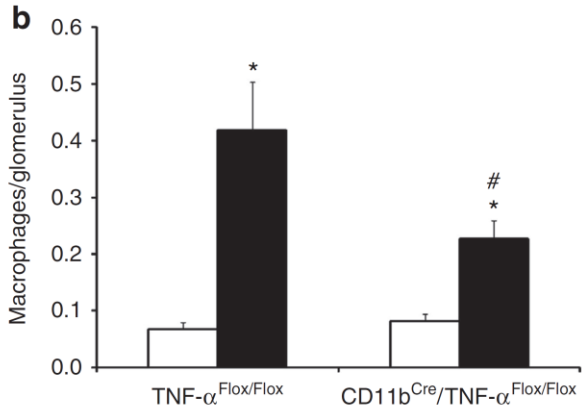
*Cytokine & Growth Factor Reviews 20 (2009) 165–173*

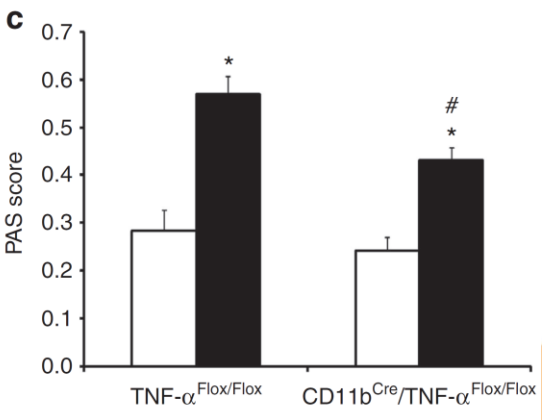
# TNF-α inhibition decreases plasma inflammatory cytokines in diabetic mice




*Kidney Int. 2015 Oct;88(4):722-33*



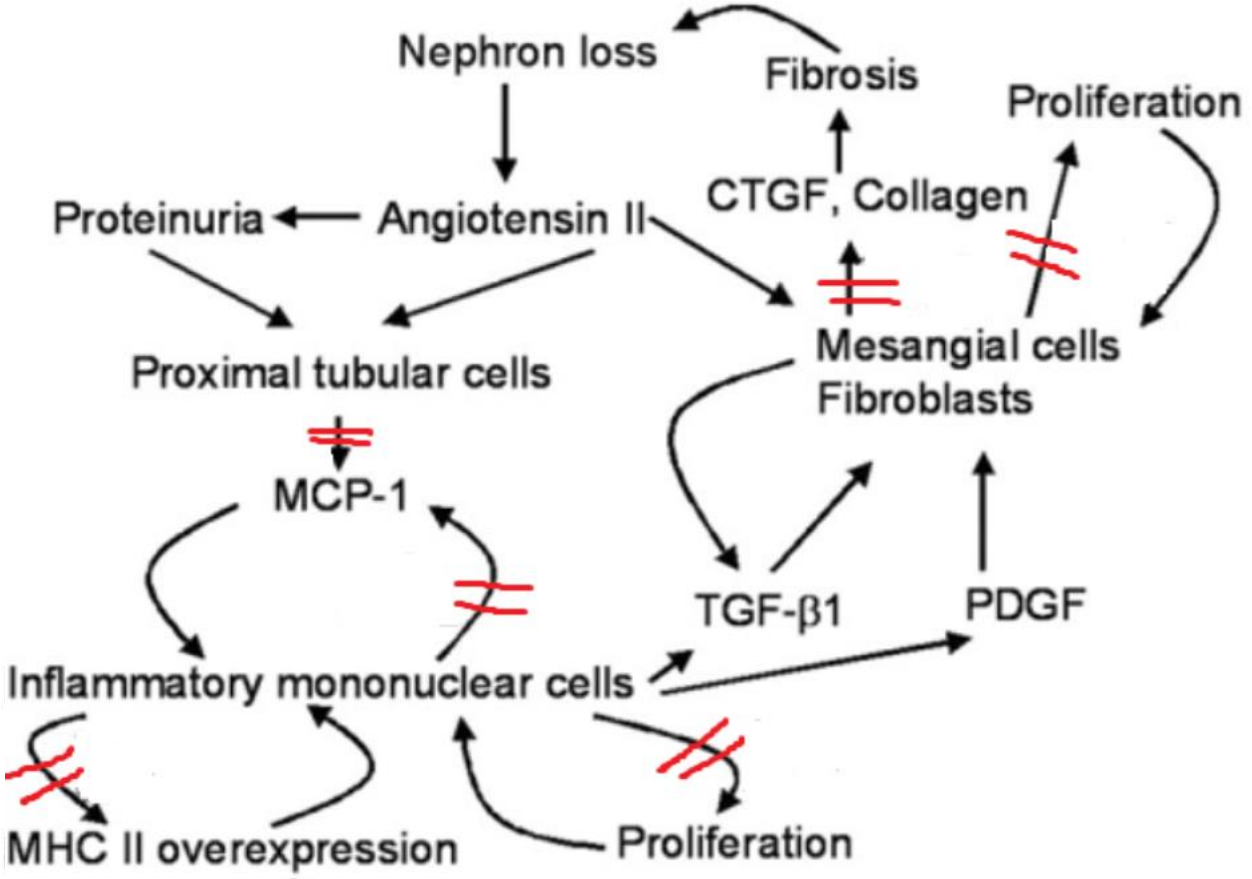




**Selective tumor necrosis factor-α (TNF-α) depletion in macrophages**

- **Reduces macrophage recruitment and**
- **Improves kidney histological changes in diabetic mice**

***Kidney Int. 2015 Oct;88(4):722-33***

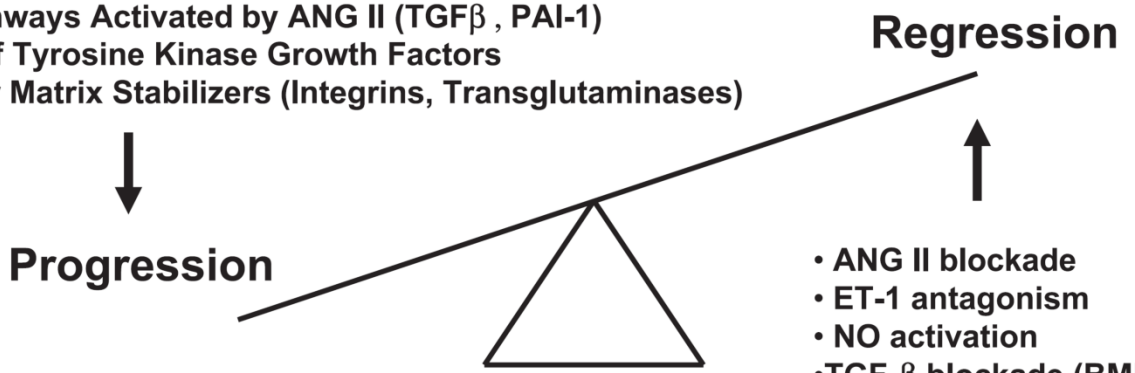
# Actions of Pentoxiphylline Potentially Relevant for its Beneficial Renal Effects in Diabetic Nephropathy




*J Clin Med Asso 2005 Vol 68, No 3*

# Progression and Regression of Renal Fibrosis are Dynamic Processes under the Control of Pro-fibrotic and Anti-fibrotic Systems

- Inflammation
- Reactive Oxygen-Nitrogen Species
- Vasoactive Peptides (ANG II, ET-1)
- Fibrotic Pathways Activated by ANG II (TGFβ , PAI-1)
- Receptors of Tyrosine Kinase Growth Factors
- Extracellular Matrix Stabilizers (Integrins, Transglutaminases)




- ANG II blockade
- ET-1 antagonism
- NO activation
- TGF-β blockade (BMP-7)
- Hepatic Growth Factor

- Antioxidants
- Blockade of fibrogenic growth factors
- Matrix destabilization
  (blockers of integrins, transglutaminase 2)
- Matrix degradation
  (MMP activation)
- Inhibition of agents associated with ANG II
  (PAI-1, Aldosterone)

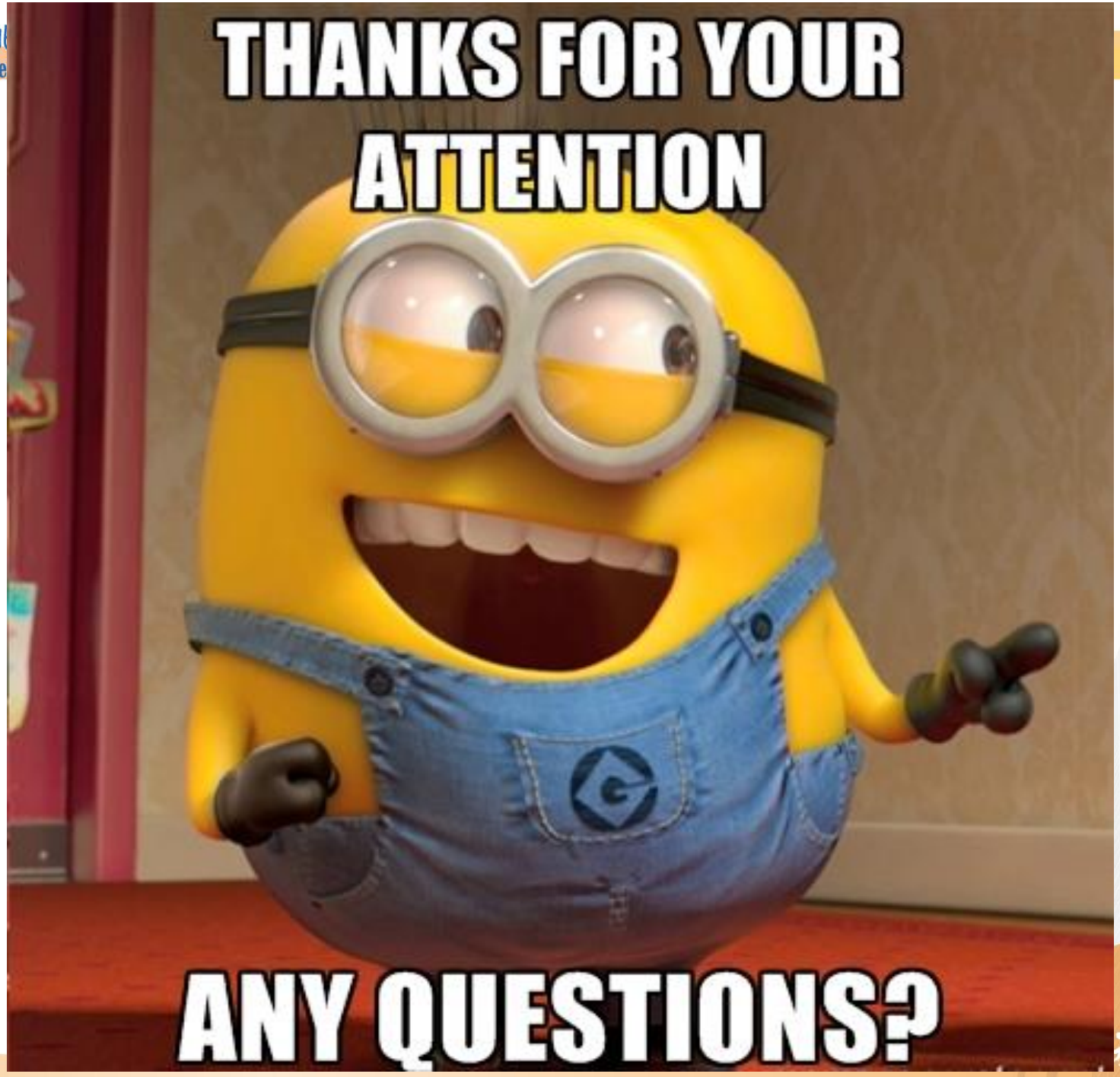
AJP-Renal Physiol. Vol 289, August 2005

# Take Home Message

- Developing **ideal therapeutic agents** that effectively blunt the development and progression of DN is an important issue for physicians.
- **Inflammation especially cytokines exert an important role** in the pathogenic complexity of development and progression during DN process.
- **Genetic variations** may also participate in the susceptibility to initiation, progression, and/or therapeutic response to DN.
- The **role of cytokines** in DN becoming a new era for novel therapeutic interventions that may benefit DN patients.
- Further clinical trials are in need to examine such potential strategies in establishing remission or even regression of DN.

# Author Contributions

- Yuh-Feng Lin, M.D. Ph.D., Graduate Institute of Clinical Medicine, Taipei Medical University, Taipei, Taiwan; Division of Nephrology, Department of Medicine, Shuang-Ho Hospital, Taipei, Taiwan
- Huey-Kang Sytwu
- Chia-Chao Wu


THANKS FOR YOUR ATTENTION
ANY QUESTIONS?