Glomerular-podocytic-tubular crosstalk in IgA nephropathy

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Background

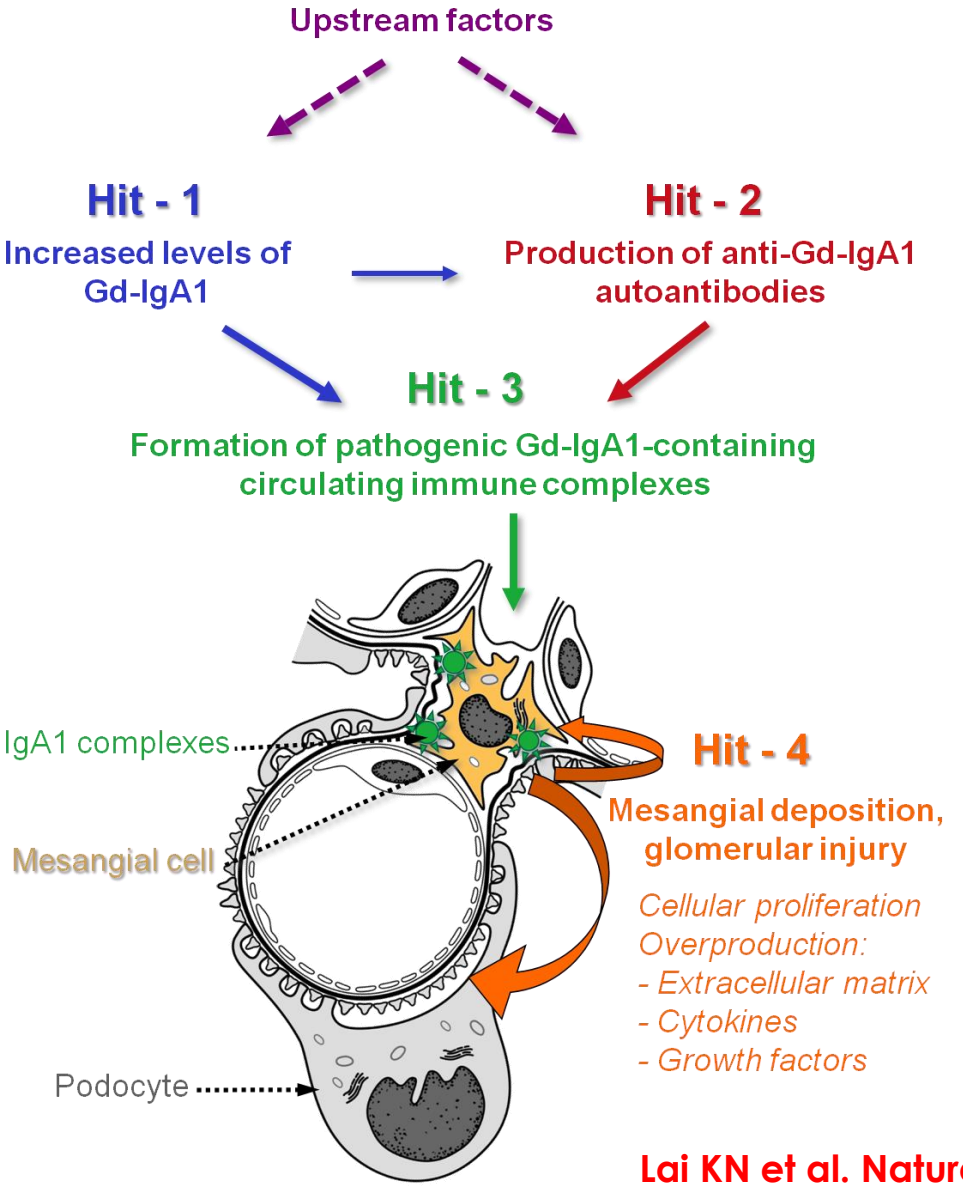
- IgA nephropathy (IgAN) is the most common glomerulonephritis worldwide and is also an important cause of kidney failure. The disease is characterized by the deposition of "pathogenetic" polymeric immunoglobulin A (IgA) in the glomerular mesangium.

- Podocytes hold a strategic position in the regulation of trafficking between the mesangial and tubulointerstitial compartments of the nephron. Injury to podocytes leads to heavy proteinuria, glomerulosclerosis, and loss of kidney function.

- Podocytopenia and increased urinary podocyte number are associated with proteinuria and poor prognosis in IgA nephropathy

- The degree of tubular injury determine the long-term prognosis in IgAN

Pathogenesis Model of IgA nephropathy With Multi-hit Pathways



Lai KN et al. Nature Review Disease Primers 2016

Podocyte injury in IgAN

Nephrol Dial Transplant (2009) 24: 62–72
doi: 10.1093/ndt/gfn441
Advance Access publication 6 August 2008

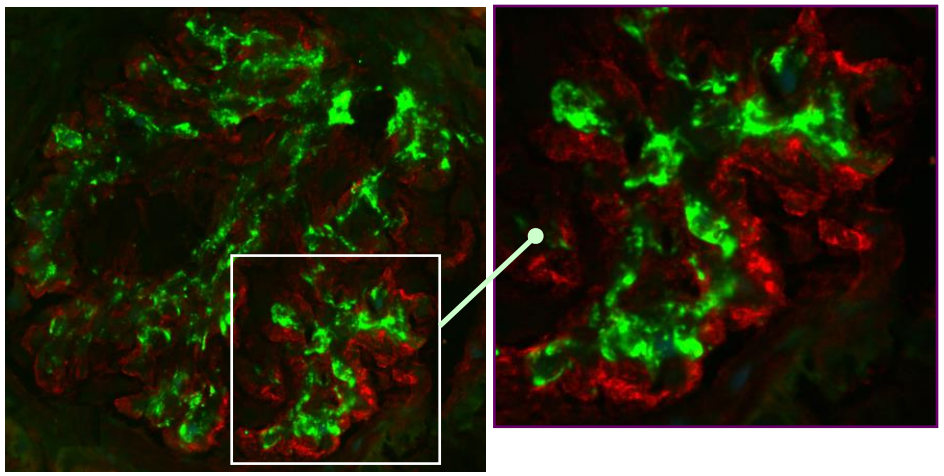
NDT
Nephrology Dialysis Transplantation

Original Article

Podocyte injury induced by mesangial-derived cytokines in IgA nephropathy

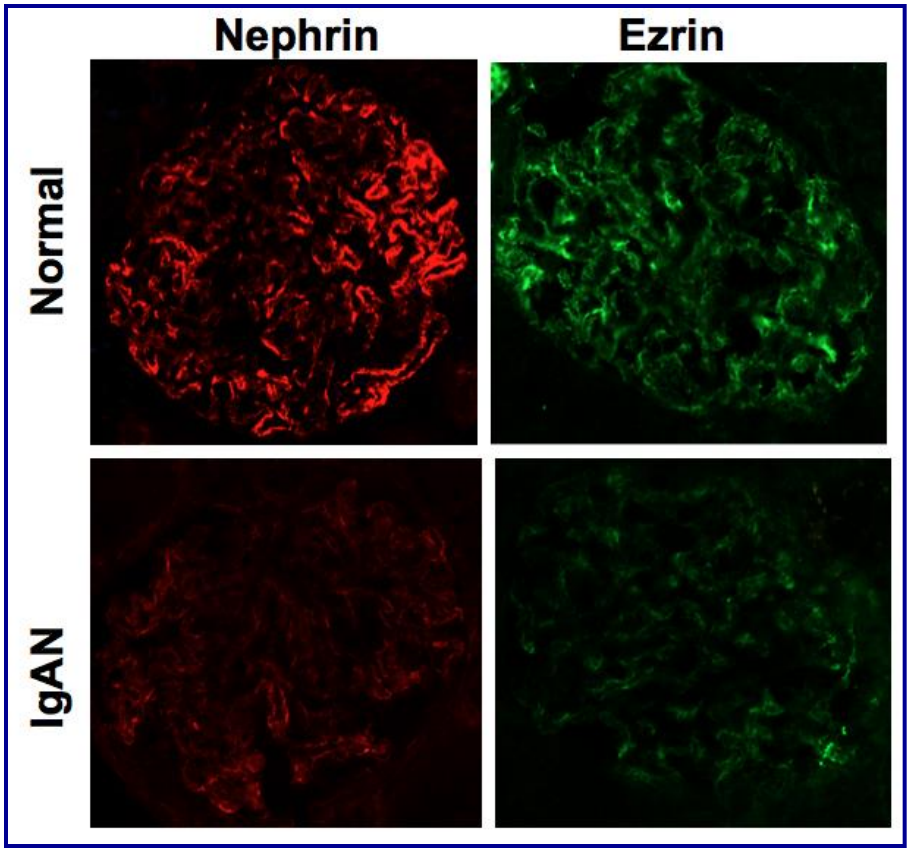
Kar Neng Lai[1], Joseph C. K. Leung[1], Loretta Y. Y. Chan[1], Moin A. Saleem[2], Peter W. Mathieson[2], Ka Ying Tam[1], Jing Xiao[1], Fernand M. Lai[3] and Sydney C. W. Tang[1]

	FcαR	ASGPRH1	ASGPRH2	pIgR	TfR	Fcα/μR	GAPDH
Amplicon size	415bp	448bp	1072bp	245bp	601bp	702bp	452bp
Podocyte	-	-	-	-	+++	-	+++
HT29	-	+	-	++	+++	-	+++
HepG2	-	++++	+++	+	+++	-	+++
HMC	-	-	-	-	+++	-	+++
PBMC	++	+	-	-	+++	+	+++
U937	+++	w+	-	-	+++	-	+++
neg ctl	-	-	-	-	-	-	-



- Podocytes did not express mRNA for any known IgA receptors except the transferrin receptor.
- The absence of known IgA receptors in podocytes was confirmed by distinctly separate immunostaining of IgA (green) and nephrin (red) in renal biopsy of IgAN.

IgA-HMC media down-regulate podocytic proteins expression



	Urine Protein (g/day)	GFR (creatinine clearance) (ml/min/1.73m^2)
Nephrin	r = -0.5457 p = 0.0086**	r = 0.4817 p = 0.0232*
Ezrin	r = -0.4424 p = 0.0393*	r = 0.6167 p = 0.0022**
Podocin	r = -0.5128 p = 0.0147*	r = 0.6434 p = 0.0012**
Synaptopodin	r = -0.2470 p = 0.2677	r = 0.1308 p = 0.5619

- IgA conditioned medium prepared from IgAN patients down-regulated the expression of podocytic proteins including nephrin, erzin, podocin and synaptopodin in cultured podocytes.

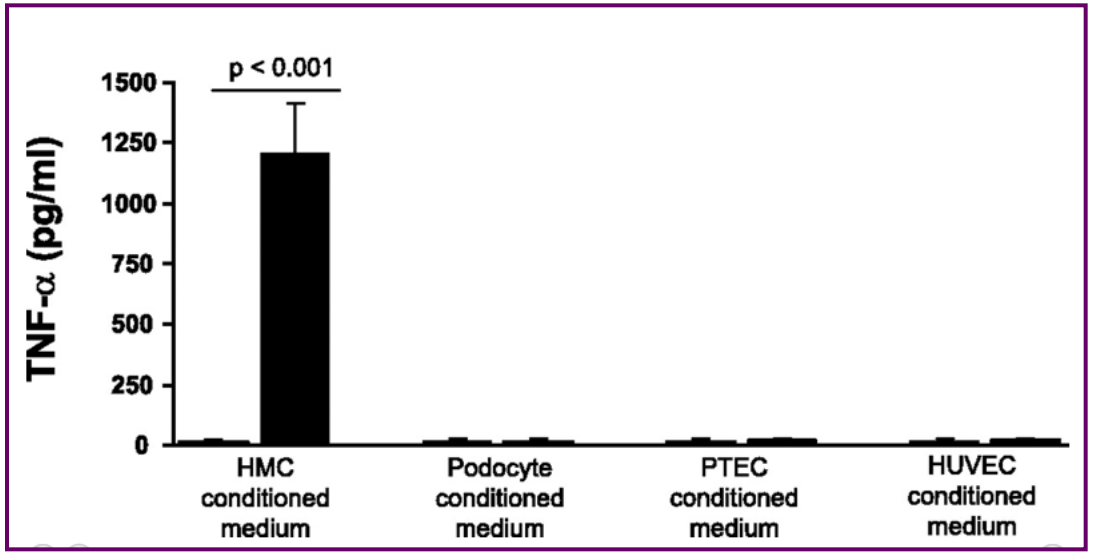
- Interestingly, the mRNA expression of nephrin, erzin correlated with the degree of proteinuria and creatinine clearance.

Glomerulo-podocytic cross-talk existed in IgAN *via* humoral factors

Am J Physiol Renal Physiol 294: F945–F955, 2008.
First published February 6, 2008; doi:10.1152/ajprenal.00423.2007.

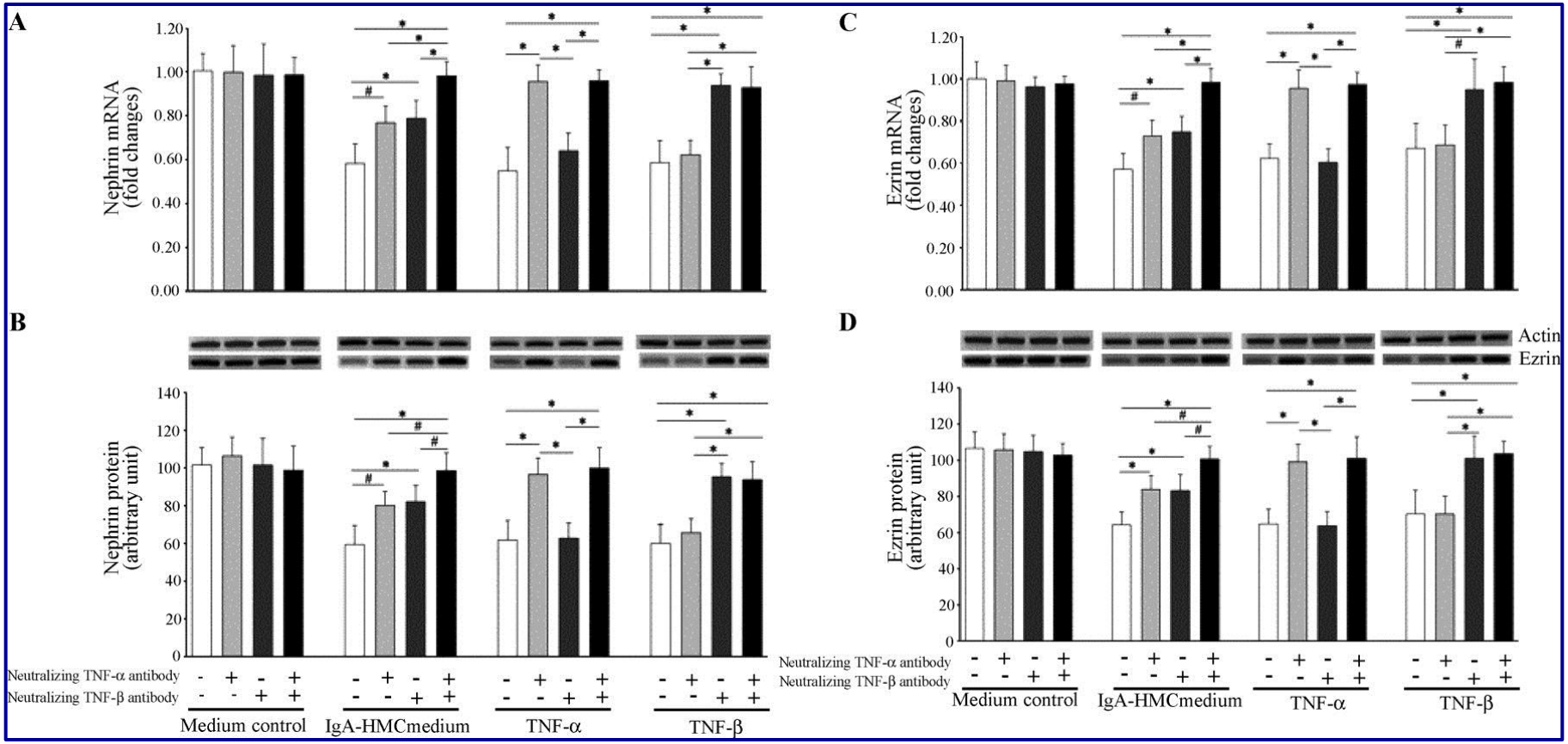
Activation of podocytes by mesangial-derived TNF-α: glomerulo-podocytic communication in IgA nephropathy

Kar Neng Lai,[1] Joseph C. K. Leung,[1] Loretta Y. Y. Chan,[1] Moin A. Saleem,[2] Peter W. Mathieson,[2] Fernand M. Lai,[3] and Sydney C. W. Tang[1]



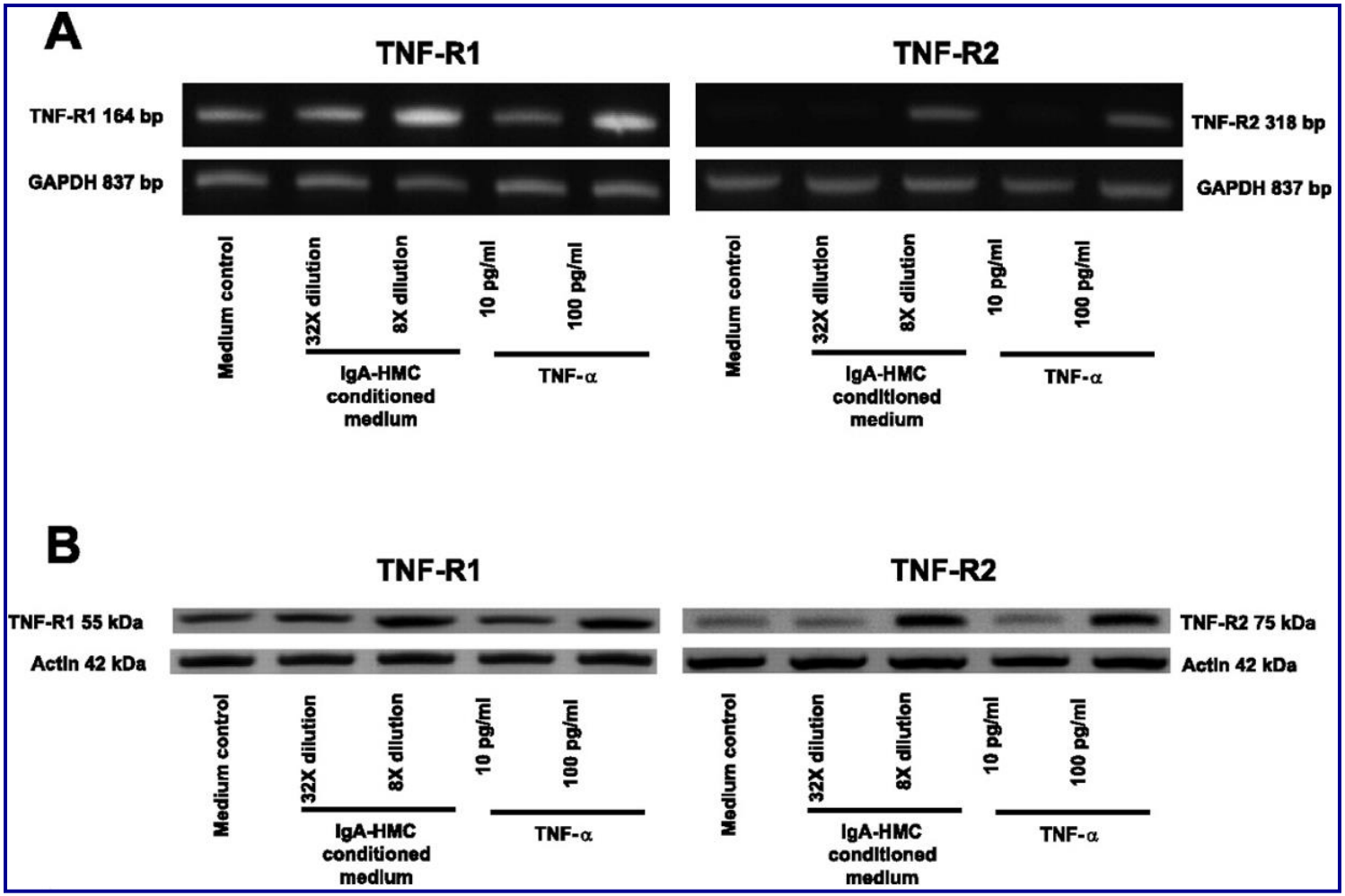
- TNF-α produced by mesangial cells following stimulation by polymeric IgA from IgAN patients led to increased synthesis of TNF-α by podocytes in an autocrine fashion.

Mesangial-podocytic communication through humoral factors modulates podocytic proteins expression



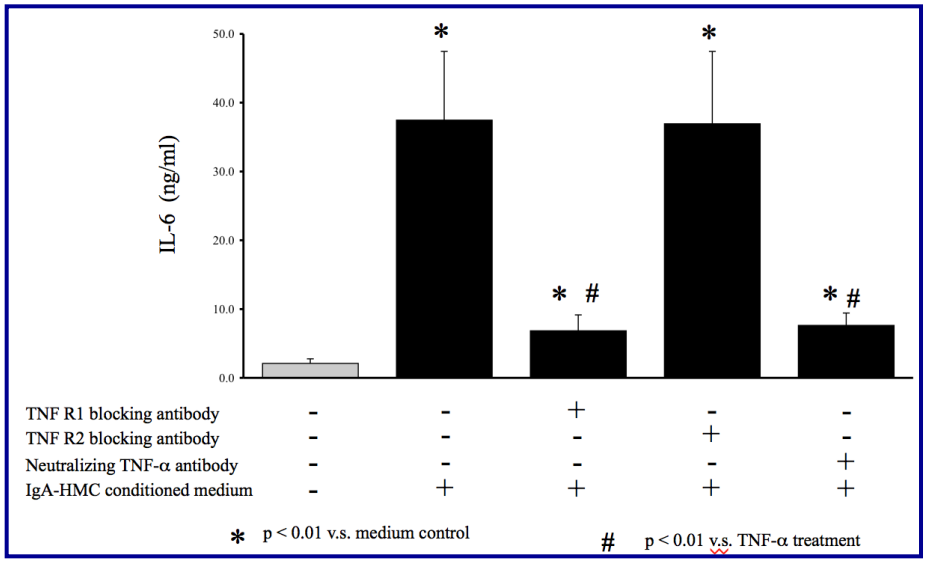
- The down-regulation was reproducible in podocytes cultured with of tumor necrosis factor-α (TNF-α) or transforming growth factor-β (TGF-β) at concentration comparable to that in the IgA–HMC conditioned medium. The expression of these podocyte proteins was restored partially with a neutralizing antibody against TNF-α or TGF-β and fully with combination of both antibodies.

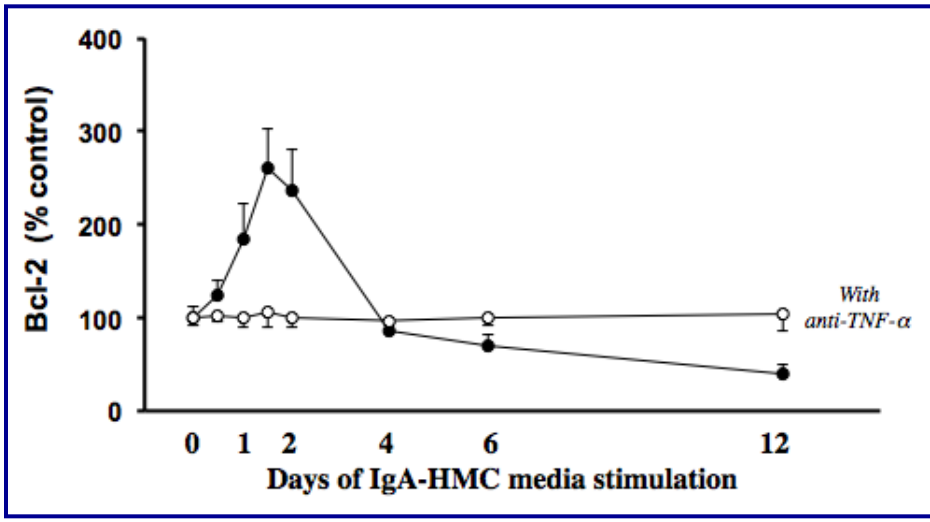
IgA-HMC media up-regulate TNF-α receptors expression in podocytes



- TNF-α receptor-1 (TNF-R1) and receptor-2 (TNF-R2) are constitutively present in normal podocytes. Expression of these receptors is readily up-regulated by IgA-mesangial cell conditioned medium from patients with IgAN or exogenous TNF-α.

Functional roles of podocytic TNF-R1 & TNF-R2

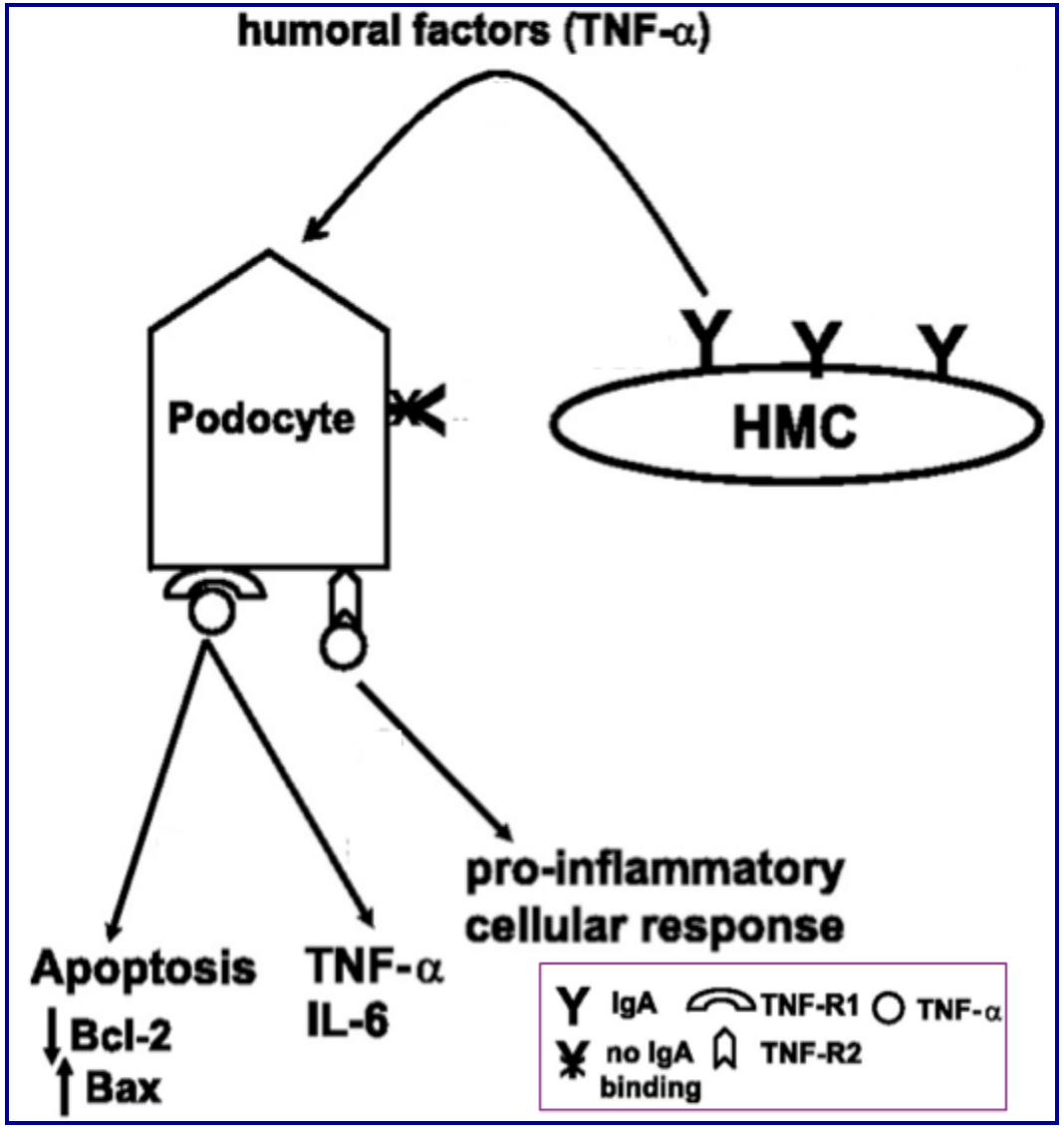




- Two functional roles of TNF-R1 in podocytes following stimulation by IgA-mesangial cell conditioned medium from patients with IgAN: IL-6 synthesis and favoring apoptosis. TNF-α released from MC after IgA deposition modulates the expression of Bcl-2 by podocyte.

- The up-regulation of TNF-R2 suggests podocytes are in a chronic pro-inflammatory state in IgAN.

A schema of mechanisms operating between the HMC and podocytes, following mesangial IgA deposition in IgAN progression.



TNF-α released from the glomerular mesangium after IgA deposition induced TNF-α synthesis by podocytes. Podocyte-derived TNF-α further upregulated the TNF-α production in an autocrine manner. TNF-α derived from mesangial cells and podocytes upregulated the expression of TNF receptors. The binding to TNFR1 leads to IL-6 synthesis and apoptosis, while binding to TNFR2 maintains proin-flammatory cellular responses.

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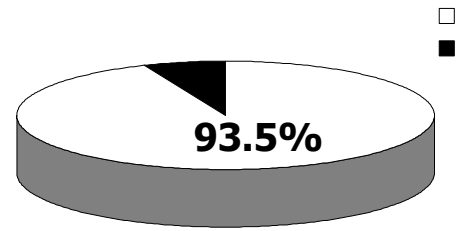
Percentage of Angiotensin II subtype receptor 1 (ATR1) and Angiotensin II subtype 2 (ATR2) in and saturation analysis of specific binding of Ang II to different renal cells



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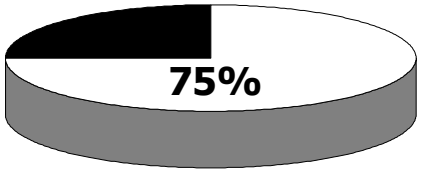
	Human mesangial cells (HMC)
Bmax (fmol/mg protein)	541.7
Kd (nM)	1.84



Podocyte

	Podocytes
Bmax (fmol/mg protein)	52.0
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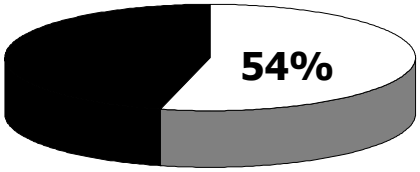


PTEC

	Proximal tubular epithelial cells (PTEC)
Bmax (fmol/mg protein)	452.2
Kd (nM)	2.63

[Data adopted from Wang L *et al.* (2003), Chan LY *et al.* (2005), and Lai KN *et al*, (2009)]

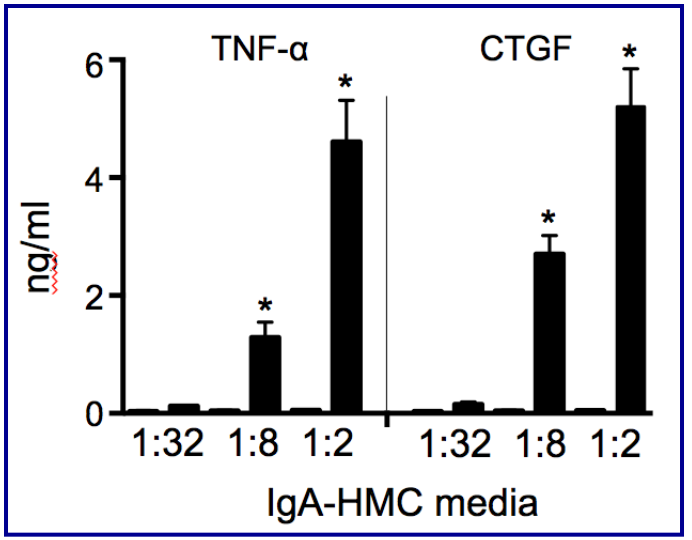
Roles of AT1R and PRR in podocytic apoptosis in IgAN

Apoptosis (2015) 20:907–920
DOI 10.1007/s10495-015-1117-1

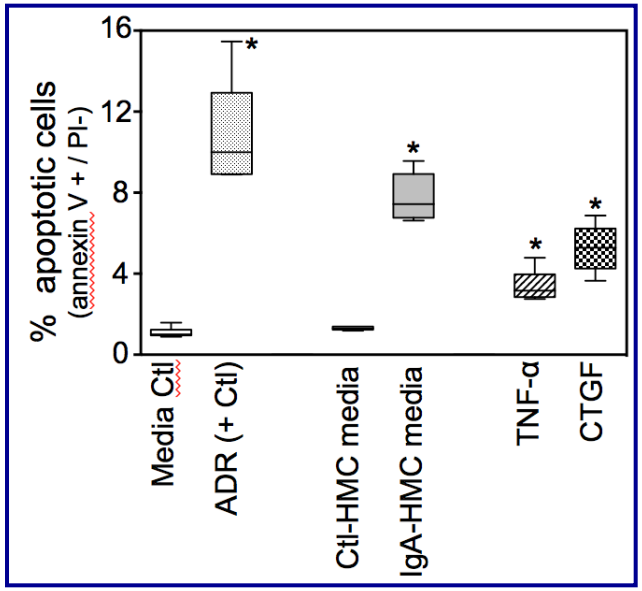
ORIGINAL PAPER

Combined blockade of angiotensin II and prorenin receptors ameliorates podocytic apoptosis induced by IgA-activated mesangial cells

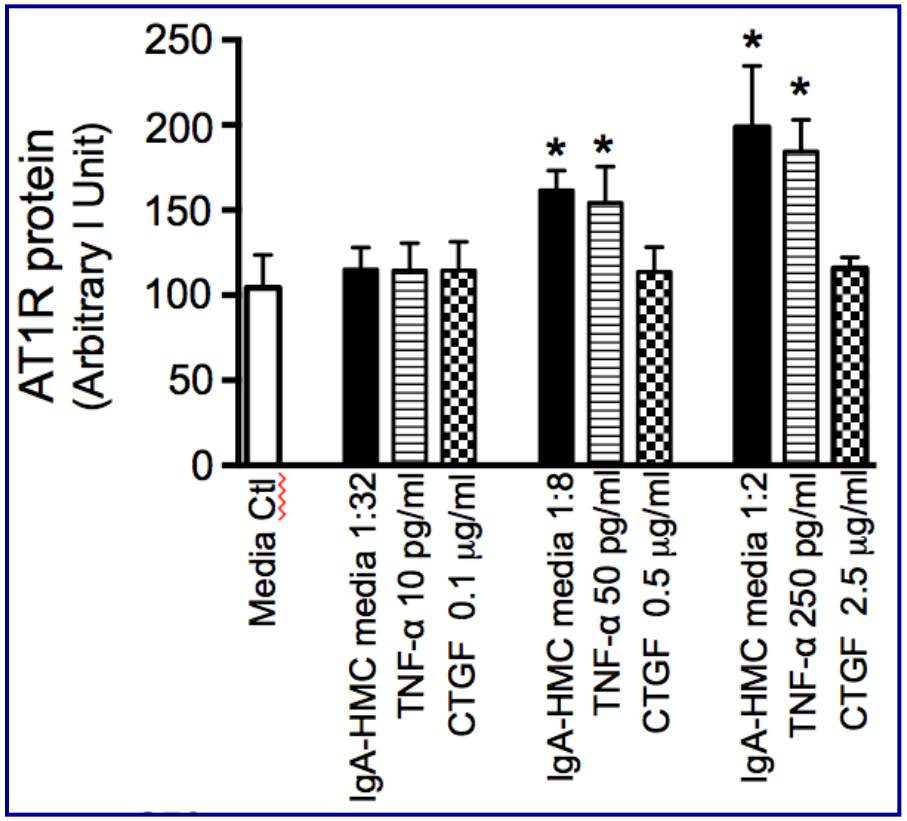
Joseph C. K. Leung[1] · Loretta Y. Y. Chan[1] · M. A. Saleem[2] · P. W. Mathieson[1] · Sydney C. W. Tang[1] · Kar Neng Lai[3]

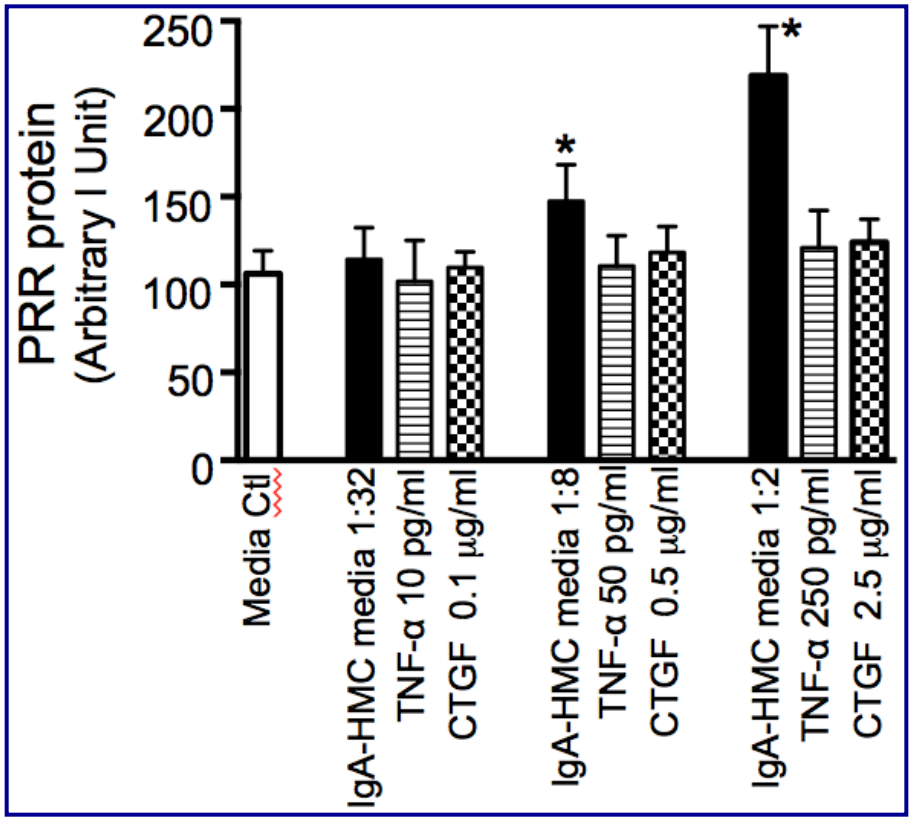


- IgA-HMC media up-regulate the protein expressions of TNF-α and connective tissue growth factor (CTGF) synthesis in podocytes.
- IgA-HMC-media, TNF-α and CTGF induce podocytic apoptosis.



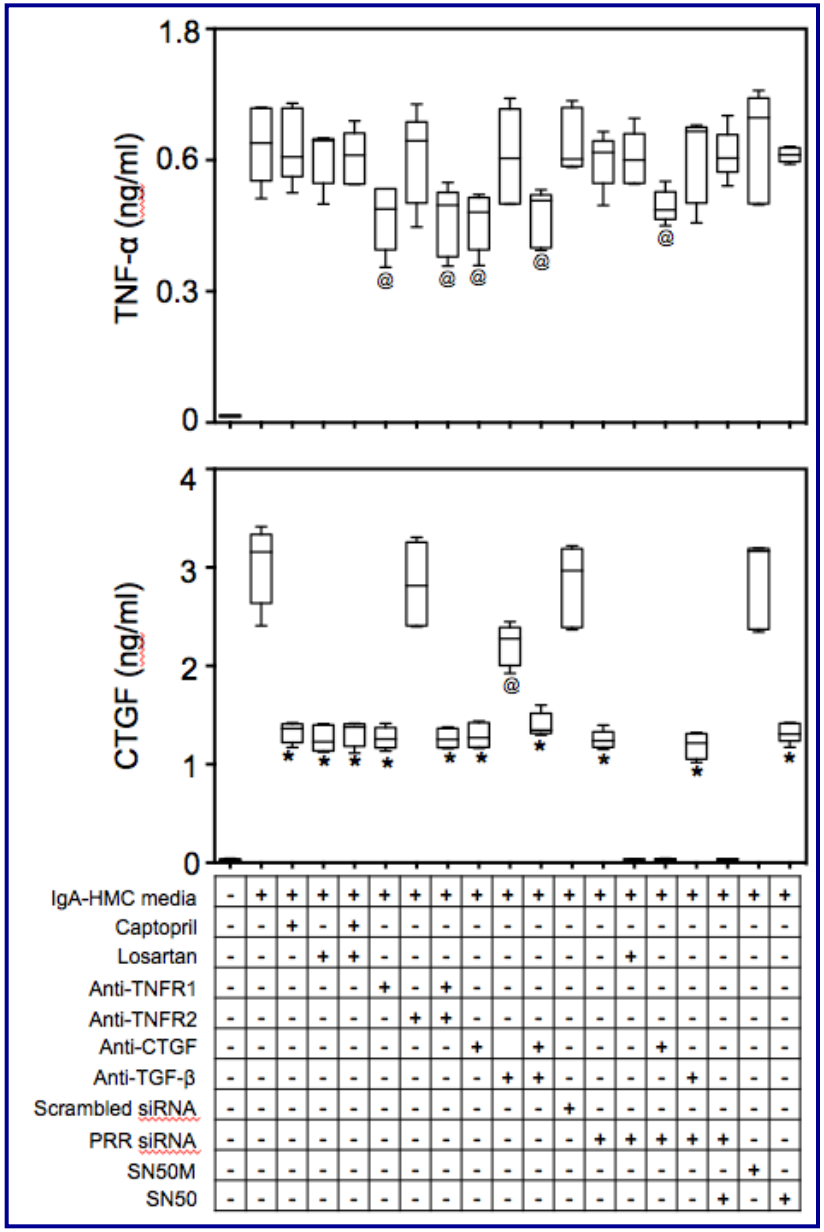
IgA-HMC media up-regulate AT1R and PRR expression in podocytes





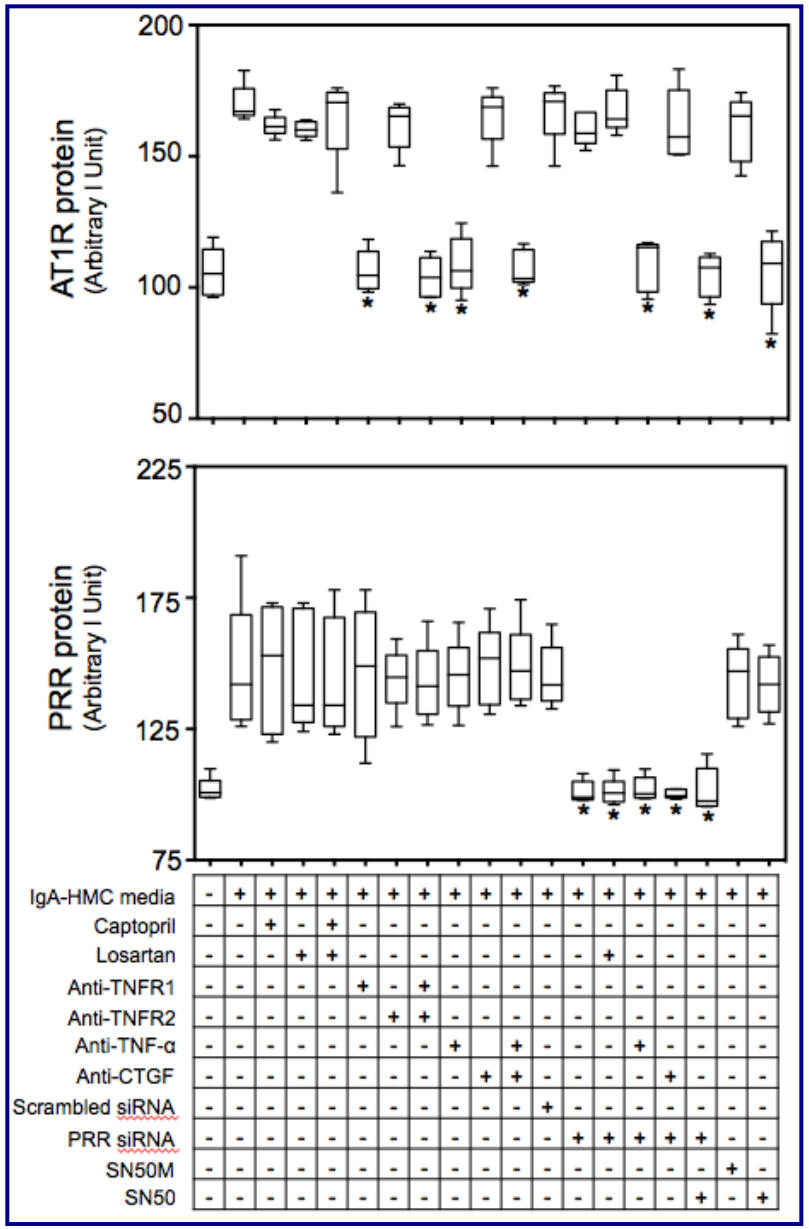
- Although there were no changes in AngII release or the expression of enzymes that controlling the formation or degradation of AngII, there were significant increases in the expression levels of angiotensin II receptor subtype 1 (AT1R) and prorenin receptor (PRR) in podocytes incubated with IgA-HMC media when compared with Ctl-HMC media. These upregulated expressions of RAS receptors by podocytes may play an essential role in the podocytic pathogenesis in IgAN.

Effect of blockade of RAS, NFκB, TNF-α receptors on TNF-α or CTGF release by podocytes cultured with IgA-HMC media



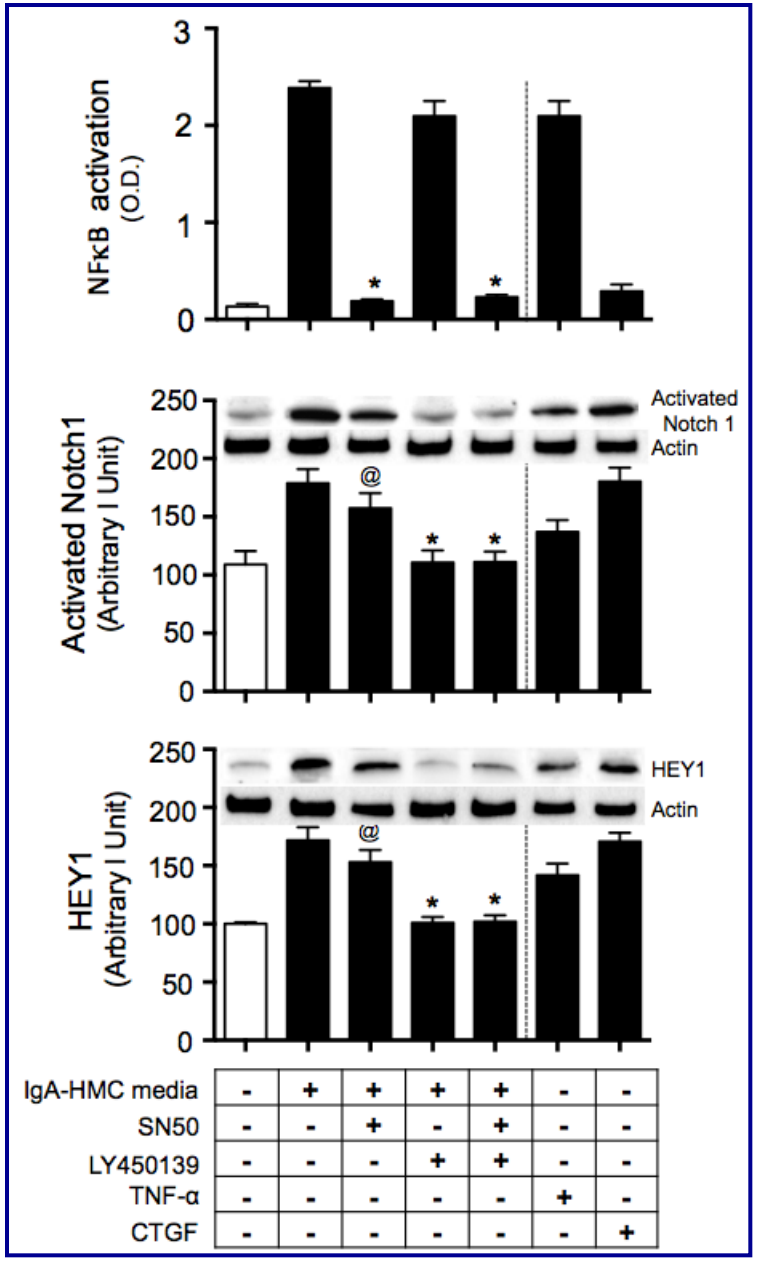
- Incubation with angiotensin-converting enzyme inhibitor (ACEi), blockade of AT1R, PRR, TNFR2, NFκB, and CTGF neutralization did not alter the TNF-α release by podocytes incubated with IgA-HMC media. Blockade of TNFR1 or TNF-α neutralization ameliorated but not abolished the TNF-α release by podocytes, suggesting the autocrine mechanism through TNFR1 contributed partly on the TNF-α release by podocytes incubated with IgA-HMC media. Suppression but not abolishment of CTGF release by podocytes incubated with IgA-HMC media was achieved by incubation with ACEi, blockade of AT1R, PRR, TNFR1, NFκB, CTGF, or TNF-α neutralization whereas TNFR2 blockade has no effect.

Effect of blockade of RAS, TNF-α receptors on AT1R or PRR expression by podocytes cultured with IgA-HMC media



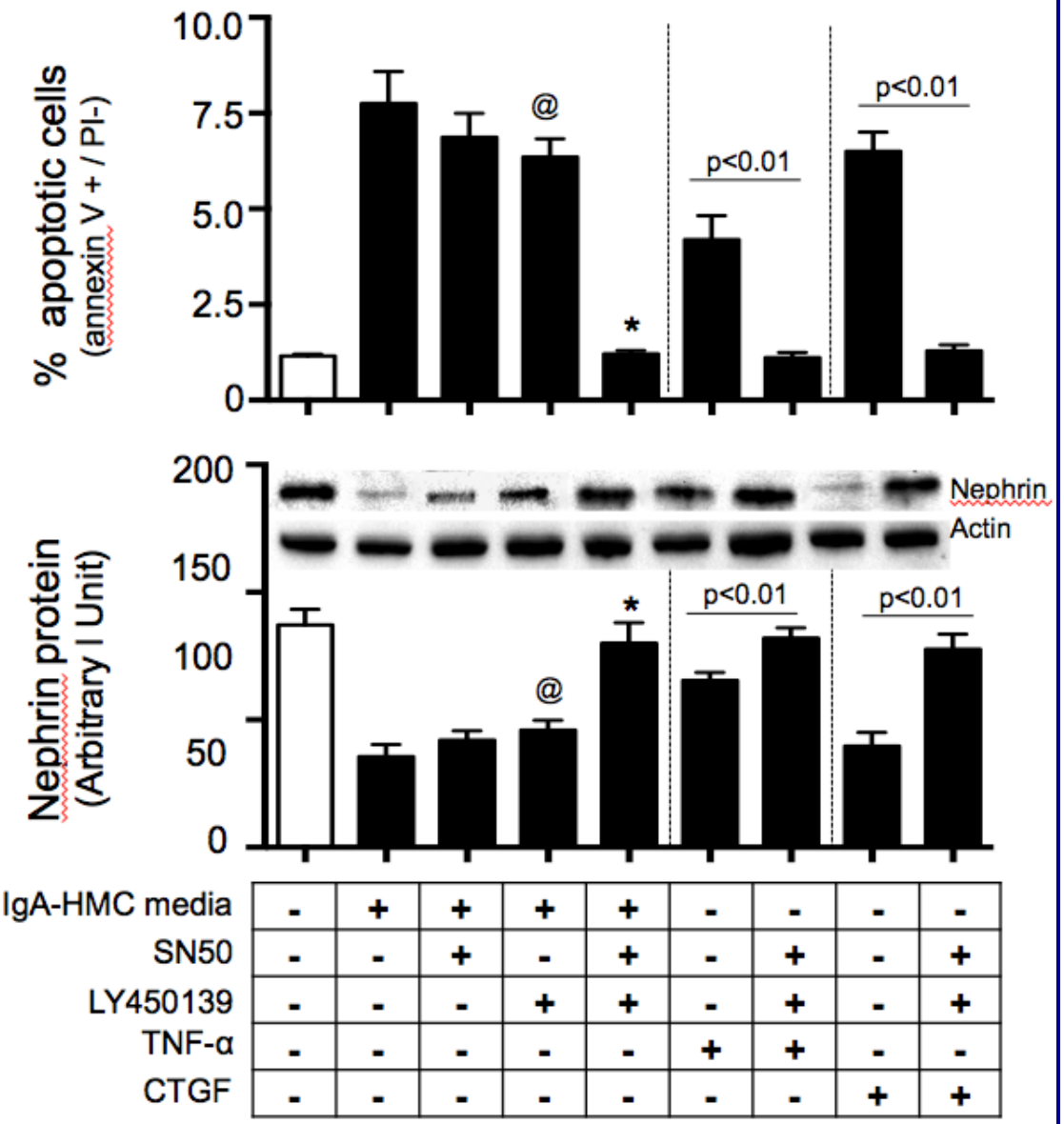
- ACEi incubation, blockade of AT1R, PRR, TNFR2, or CTGF neutralization did not affect the AT1R expression by podocytes incubated with IgA-HMC media. Blockade of TNFR1, TNF-α neutralization, or incubation with cell permeable NFκB inhibitor significantly abolished the up-regulated AT1R expression by podocytes. The up-regulated expression of podocytic PRR cultured with IgA-HMC media was only abolished by blockade with PRR siRNA. ACEi incubation, blockade of AT1R, PRR, TNFR1, TNFR2, TNF-α or CTGF neutralization have no effect on the PRR expression by podocytes incubated with IgA-HMC media.

Activation of the podocytic NFκB and notch1 signals by IgA-HMC media



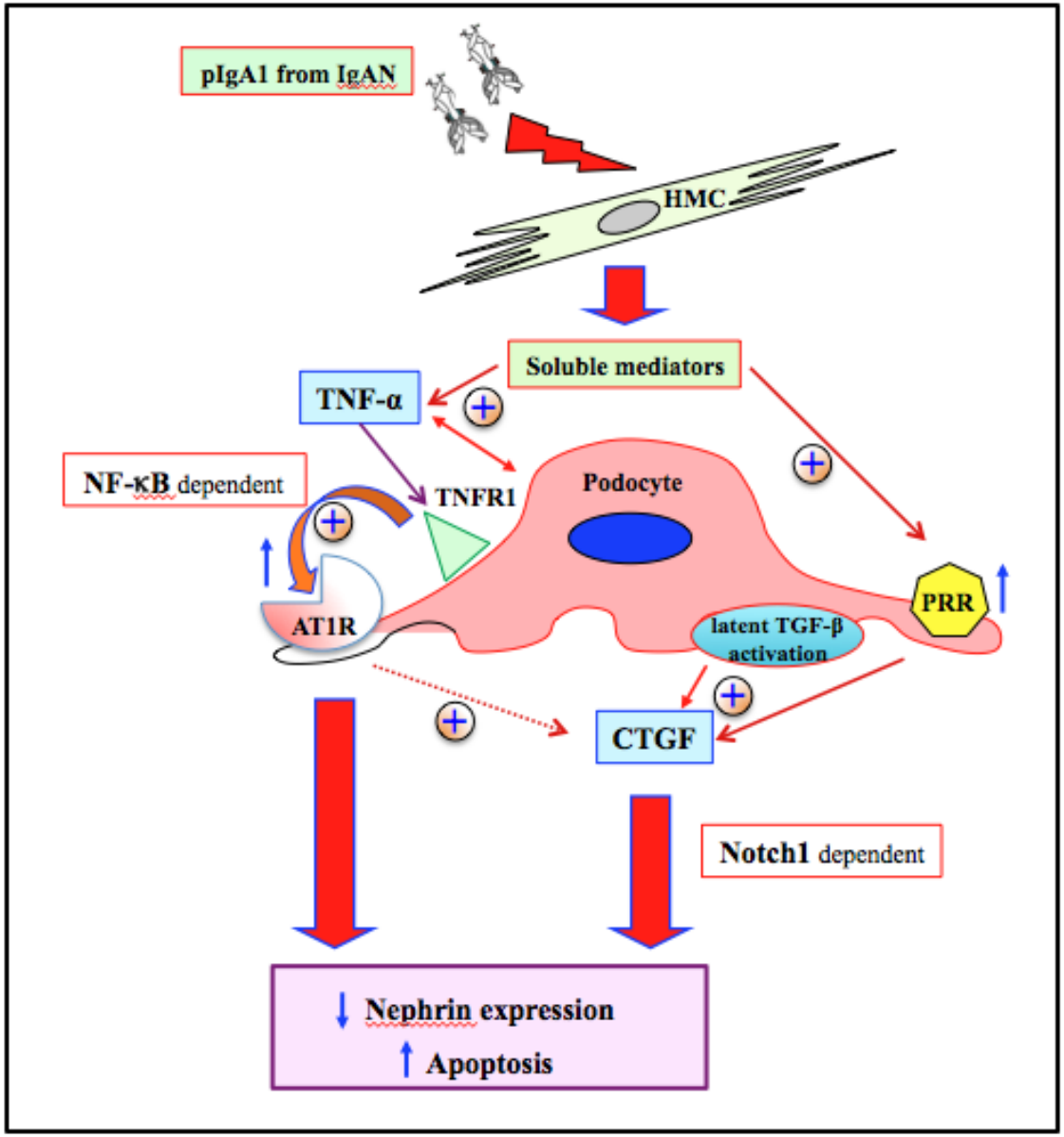
- IgA-HMC media induced the activation of NFκB, and up-regulated the expression of activated Notch1, or HEY1. Incubation with exogenous TNF-α or CTGF activated both the NFκB and notch signals. Incubation with SN50 (peptide inhibits the translocation of the active NF-κB complex into the nucleus) reduced NFkB, notch 1 and HEY1 activation. Incubation with LY450139 (a γ-Secretase or the notch receptor blocker) only eliminated the activation of podocytic notch1 and HEY1 by IgA-HMC media or CTGF.

Dual blockade of the podocytic NFκB and notch1 activation rescues nephrin expression and prevents apoptosis



- Blockade with either SN50 or LY450139 incompletely restored the reduced nephrin expression, partially ameliorated the increased percentage of apoptotic cell by podocyte incubated with IgA-HMC media. Simultaneous incubation with SN50 and LY450139 was essential to achieve complete prevention of these apoptotic events and restoration of the reduced podocytic nephrin expression induced by IgA-HMC media.

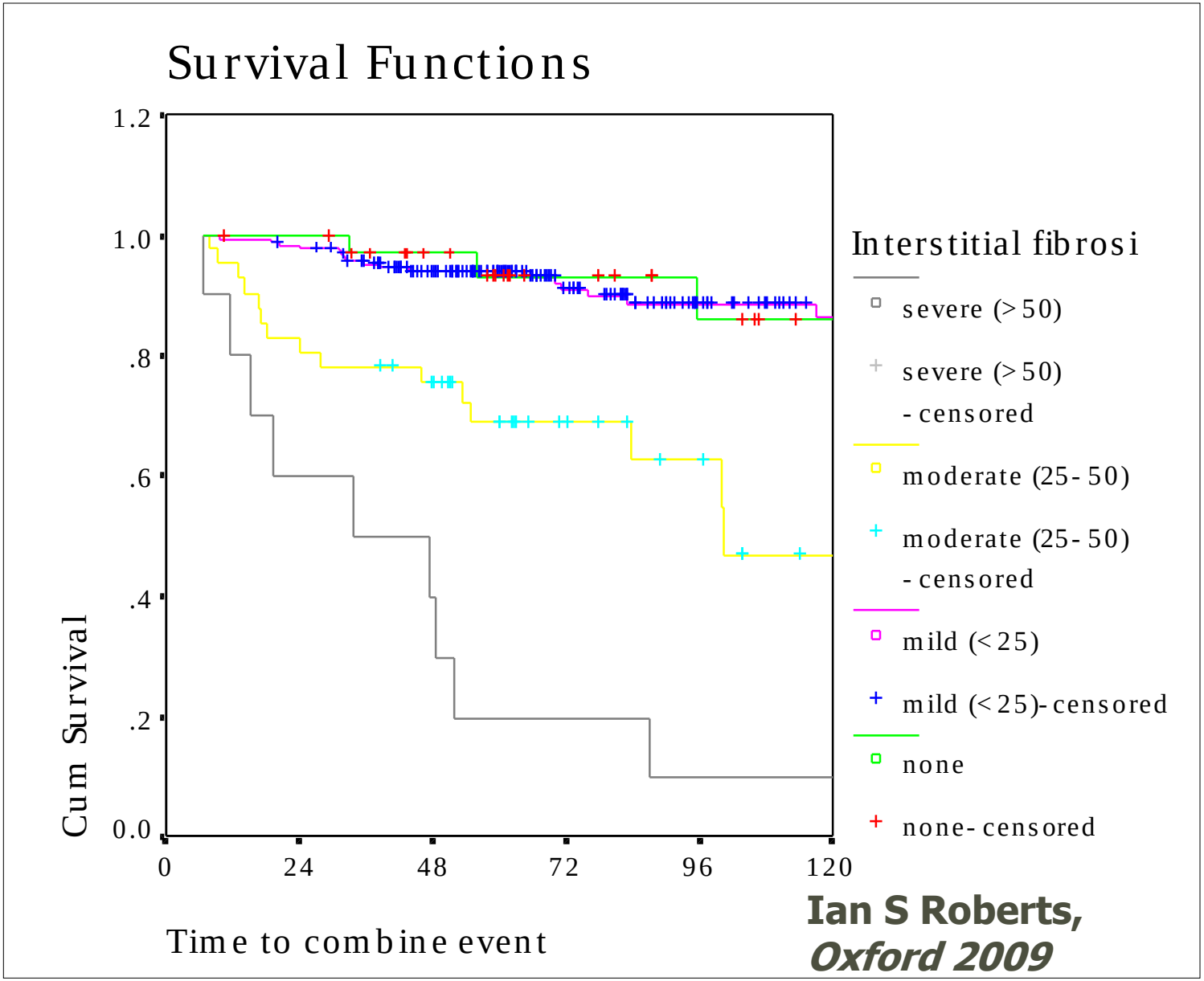
A schema on the role of mesangial-podocytic communication related to the development of podocytic injury in progressive IgAN



Mesangial-podocytic communication and the development of podocytic injury in progressive IgAN

- TNF-α released from the glomerular mesangium following IgA deposition up-regulate the expression of podocytic TNF receptors and AT1R through a NFκB dependent mechanism. This TNF-α/TNFR1/AT1R axis leads to further apoptosis and nephrin reduction in podocyte. Humoral mediators released from the glomerular mesangium following IgA deposition also lead to enhanced podocytic PRR expression and CTGF production. This PRR/CTGF axis leads to podocytic apoptosis and nephrin reduction through activation of notch1. Furthermore, AT1R activation also triggers the CTGF production by podocytes and forming a link between the two axes of podocytic apoptosis induction. Dual blockade of activation of NFκB and notch1 is essential to abolish the podocytic damage following mesangial pIgA deposition.

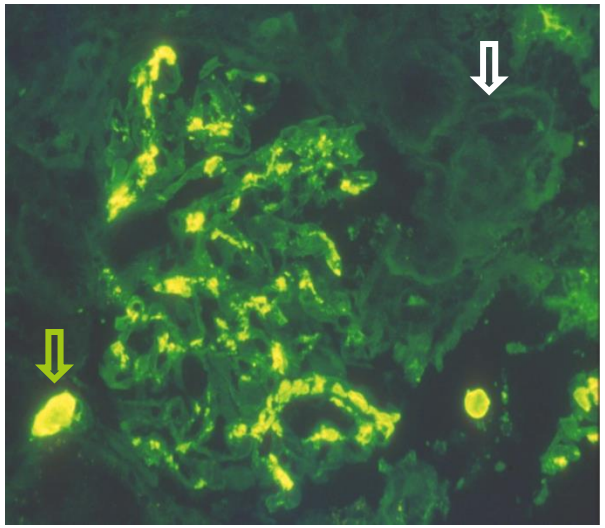
Interstitial changes as a determinant of renal survival in IgA nephropathy

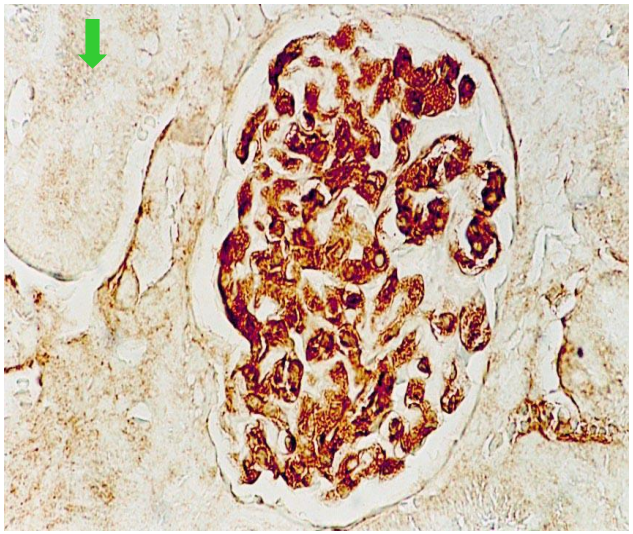


Ian S Roberts,
Oxford 2009

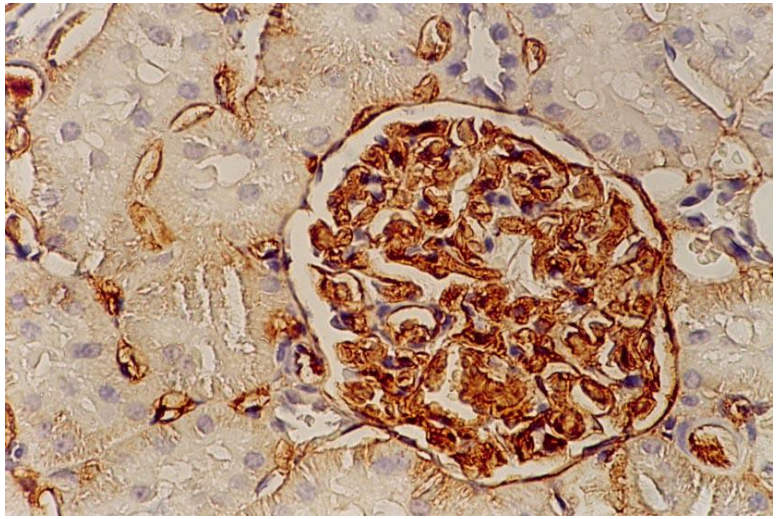
IgA deposits are present in the glomerular mesangium but rarely in the interstitium

IgA IF





IgA DEPS

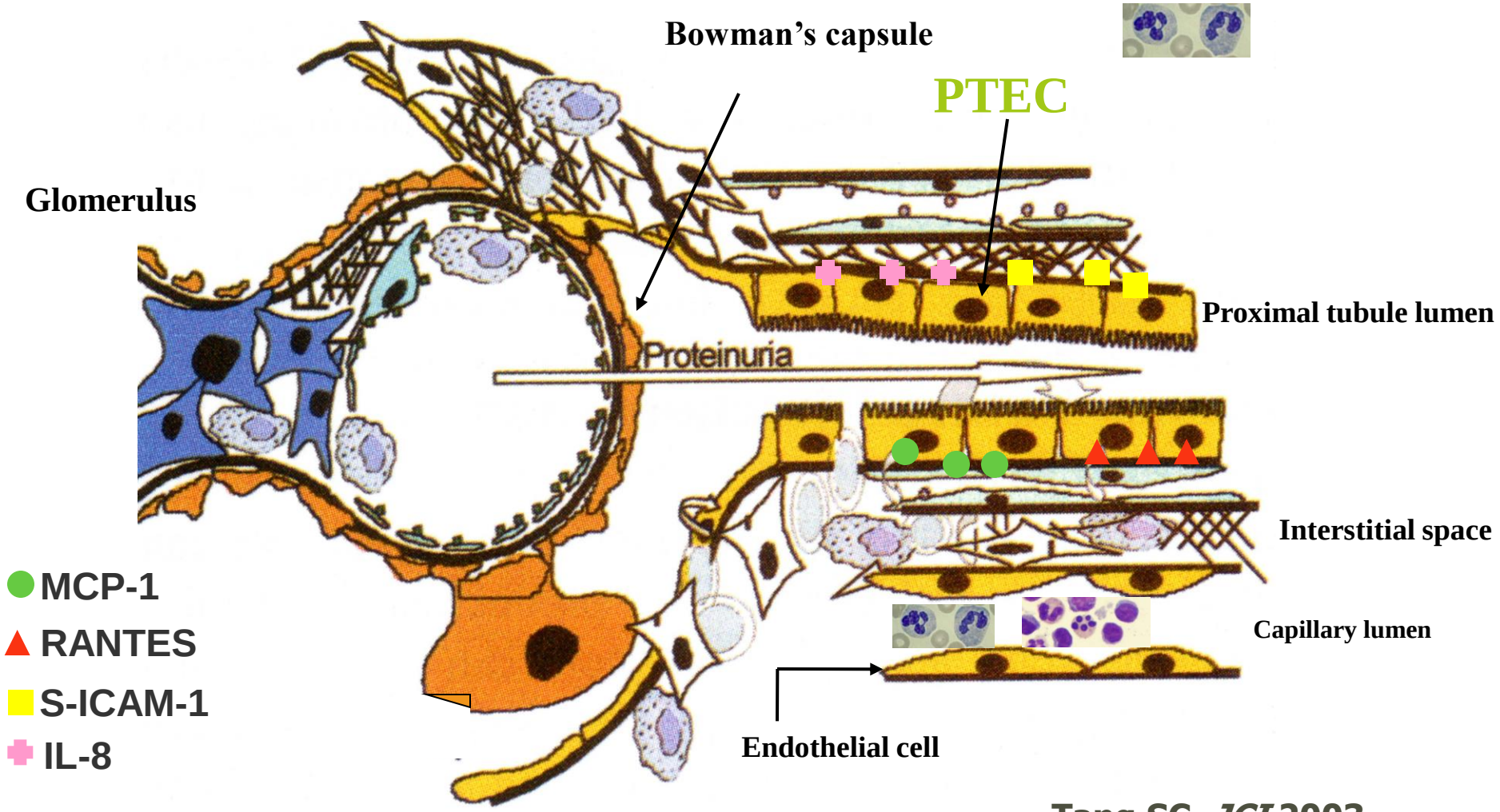


TGF-β DEPS

DEPS = Dako Envision Plus System

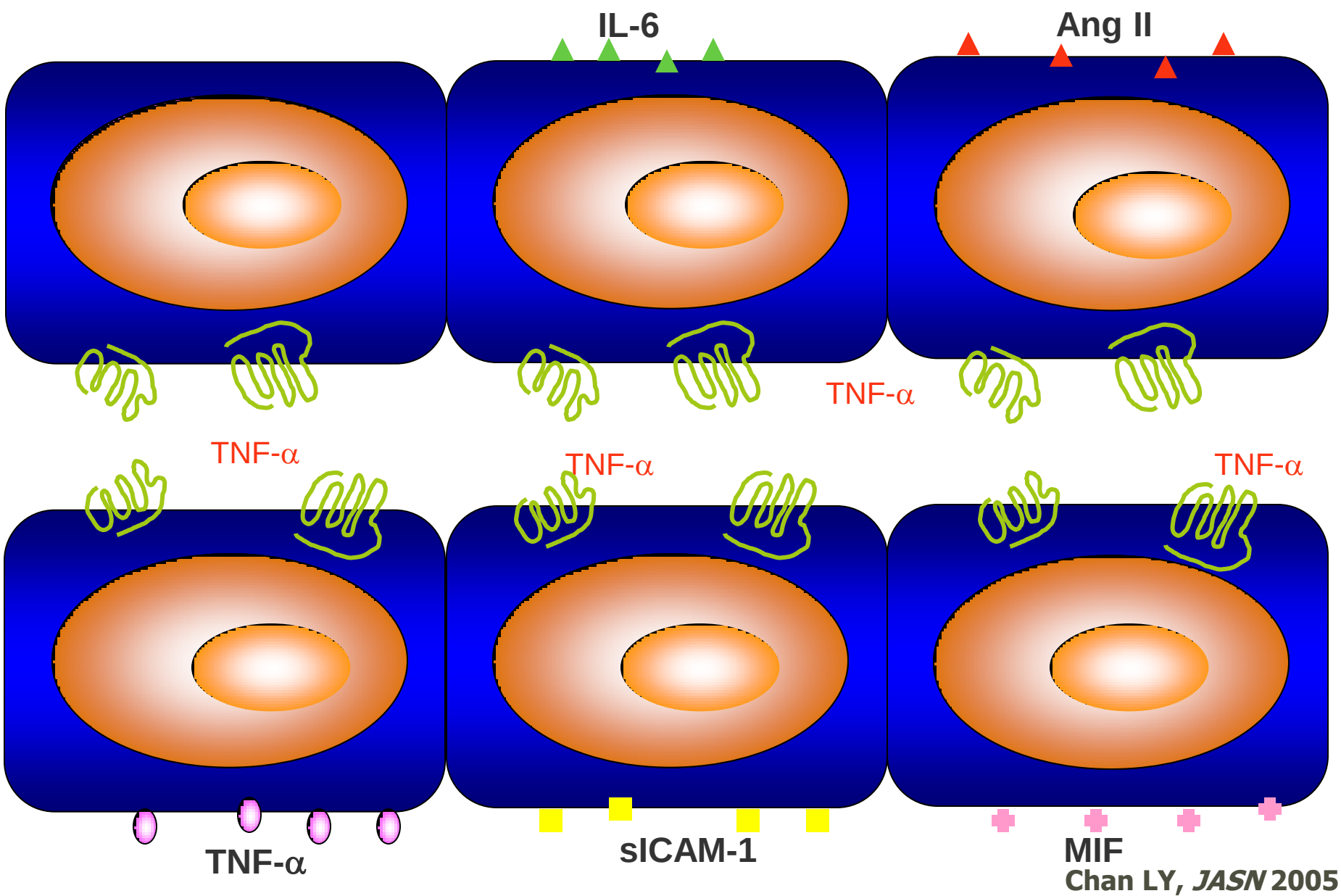
Lai KN, *KI* 2005

Proteinuria Exerts Profound Pathophysiological Effect on Proximal Renal Epithelial Cells



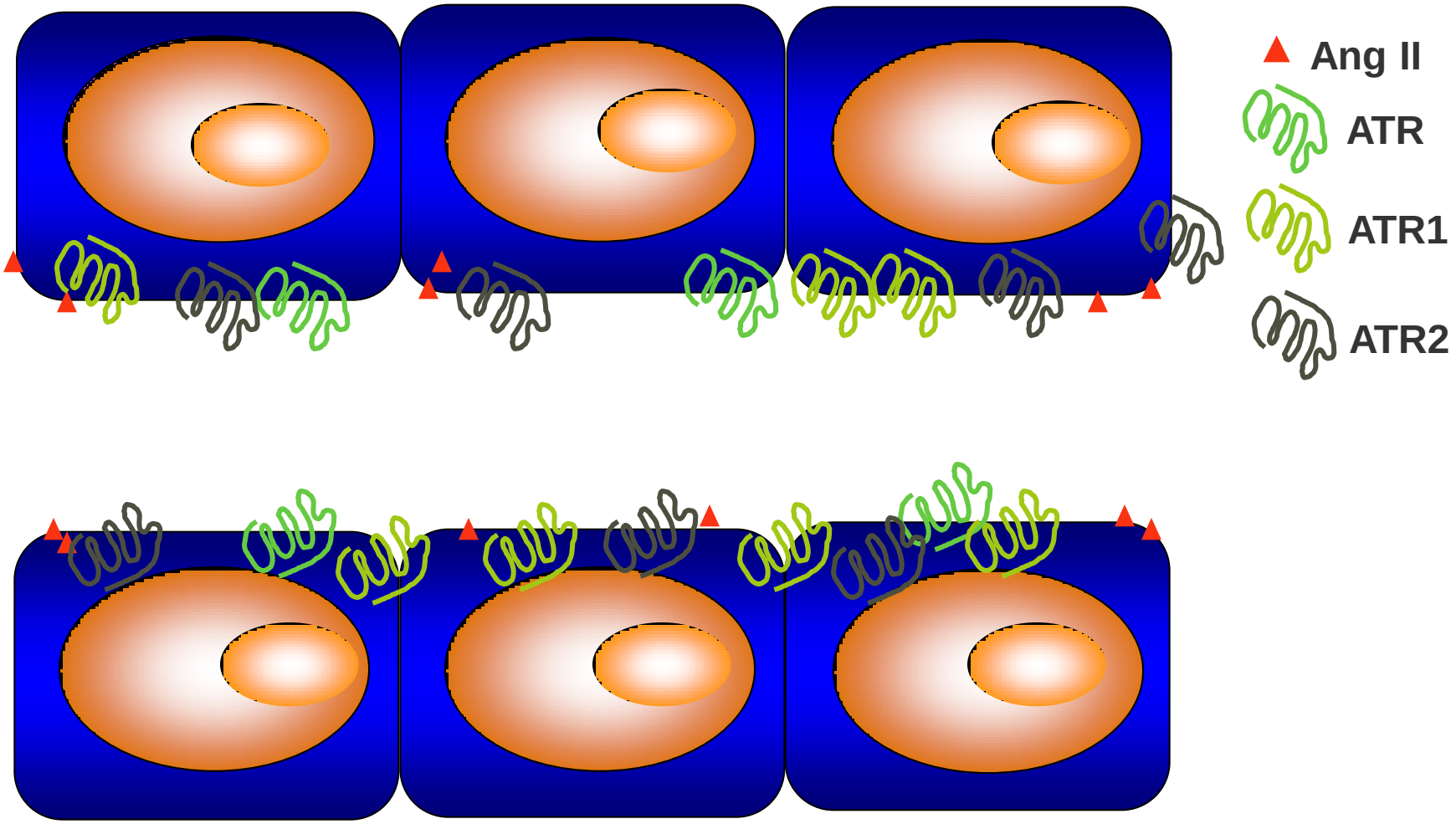
Tang SC, *JCI* 2003
Leung JC, *KI* 2006

Mesangial-derived TNF-α induces synthesis of growth factors, adhesion molecules and Ang II in renal tubular epithelial cells in IgAN



Chan LY, *JASN* 2005

Mesangial-derived Ang II induces synthesis of Ang II and expression of angiotensin receptors in renal tubular epithelial cells in IgAN



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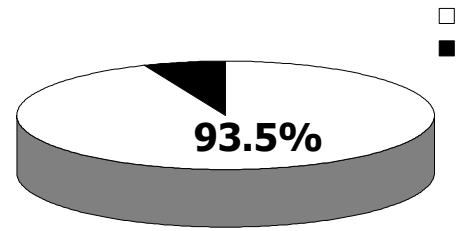

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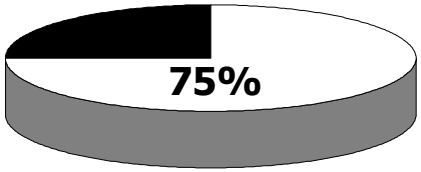

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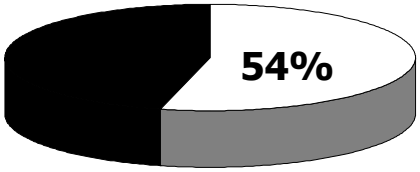

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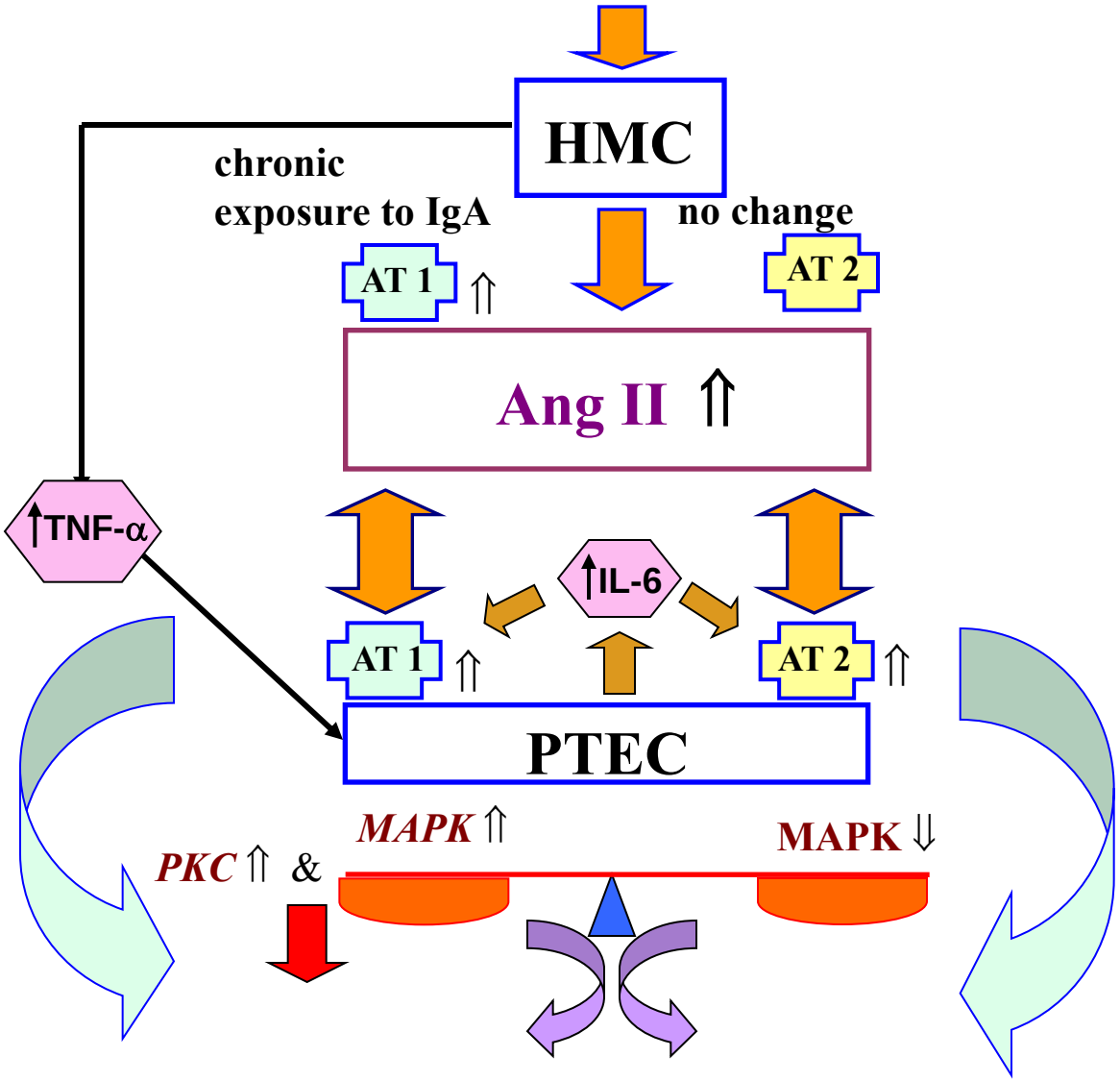
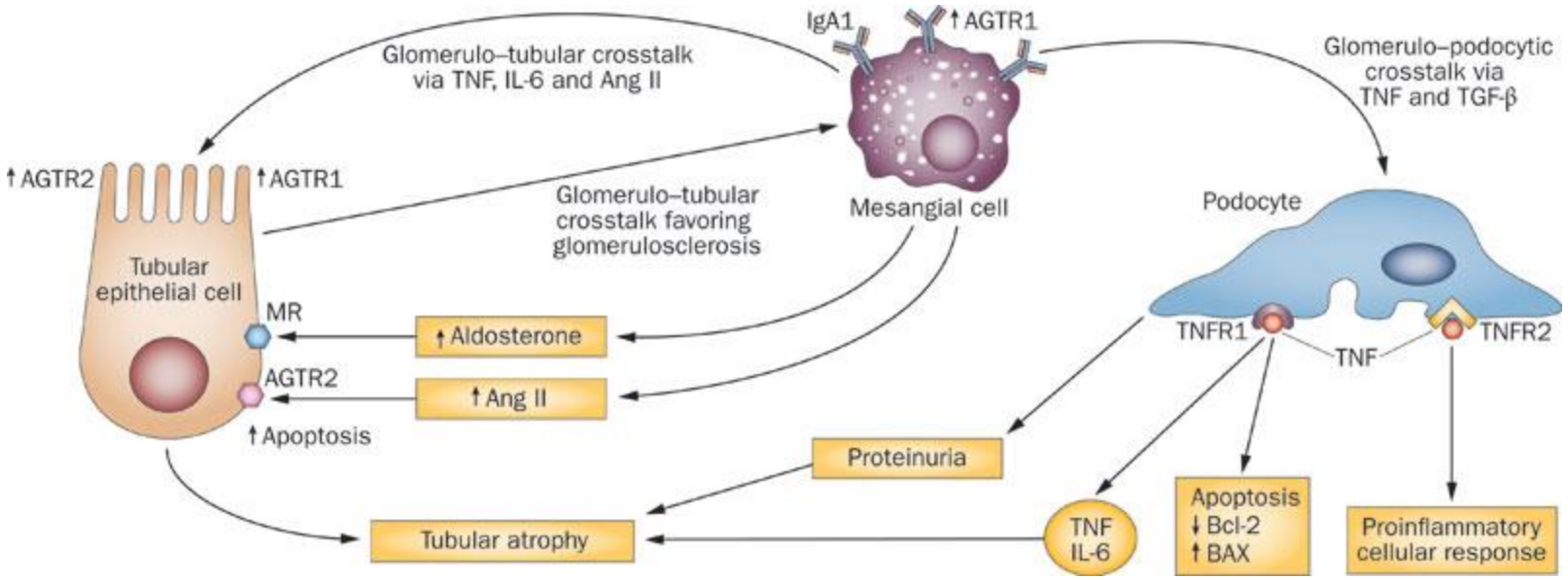
pathogenic pIgA



Chan L, JASN 2005

Mesangial-podocytic-tubular crosstalks in progressive IgAN



Lai, K. N. and Tang, S.C.W.: IgA nephropathy, Oxford Textbook of Clinical Nephrology, 4th edition, 2015

Summary

- Podocyte markers are reduced in IgAN. Our *in vitro* data implicate humoral factors (predominantly TNF-α) released from mesangial cells are likely to alter the glomerular permeability in the event of proteinuria and tubulointerstitial injury in IgAN. .

- The mesangial-podocytic crosstalk after mesangial pIgA deposition increases the synthesis of TNF-α and CTGF, up-regulates the expression of AT1R and PRR, and induces apoptosis by podocytes. These events are regulated by the NFκB and notch1 dependent signals and dual blockade of these pathways is essential to ameliorate the podocytic injury in IgAN.

- Mesangial-derived TNF-a mediates tubular injury with increased local release of cytokines and adhesion molecules leading to infiltration of macrophages and leukocytes. Induction of local Angiotensin II production also contributes to tubular injury and chronic interstitial fibrosis

Further development

- Progressive dysfunction in podocytes is a common phenomenon in acquired glomerular diseases, including IgAN, diabetes and focal segmental glomerulosclerosis. Recent studies have focused on dissecting the dynamic interaction between podocytes and other glomerular resident cells including mesangial and endothelial cells.

- Our present data address the podocytic apoptosis induced by glomerulo-podocytic communication in IgAN, nonetheless, the possible communication or crosstalk between tubular epithelial cells and podocytes, which is also an important determinant of the progression of chronic kidney disease (CKD), however, is less investigated. Bridging this gap will facilitate the invention of more mechanism-oriented and targeted therapeutics for proteinuric glomerular or tubular diseases.

Acknowledgment

- Dr. Sydney C.W. TANG
- Dr. Joseph C.K. LEUNG
- Dr. Loretta Y.Y. CHAN
- Dr. Hong GUO
- Dr. Jing XIAO
- Miss Kam Ying TAM
- Dr. Fernand M. LAI