SEQUENTIAL ACTIVATION OF PROINFLAMMATORY GENES IN THE GLOMERULAR AND TUBULOINTERSTITIAL COMPARTMENTS IN TYPE II DIABETIC NEPHROPATHY (DN)

L. Cappuccino[1], G. Garibotto[1], E. D'Amato[1], B. Villaggio[1], F. Gianiorio[1], M. Mij[2], F.Viazzi[1], G. Salvidio[1], D. Verzola[1]
[1] Department of Internal Medicine, Nephrology Division, Genoa University and IRCCS AOU San Martino-IST Genoa, Italy
[2] Nephrology Division, Imperia Hospital, Italy.

INTRODUCTION AND OBJECTIVES

- ✓ An emergent hypothesis is that glucose-driven inflammatory changes are the trigger for subsequent oxidative stress and chronic kidney damage in type II DN (Liu, JASN 2010)
- ✓ The transcription factor NF-κβ helps to control the expression of numerous genes activated during inflammation, cell apoptosis and survival.
- ✓ Enhanced NF-κβ signaling has been observed in the tubulointerstitial compartment of patients with advanced diabetic disease (Mezzano, NDT 2004 and Schmid, Diabetes 2006).
- ✓ Whether NF-κβ signaling is activated at an early stage of human DN has never been studied.

➔ ***To verify early (microalbuminuric) activation of apoptotic as well as inflammatory processes mediated by NF-kB in type II DN***
➔ ***To study gene and protein expression of pro-inflammatory cytokines in renal tissue from microalbuminuric patients with type II DN***

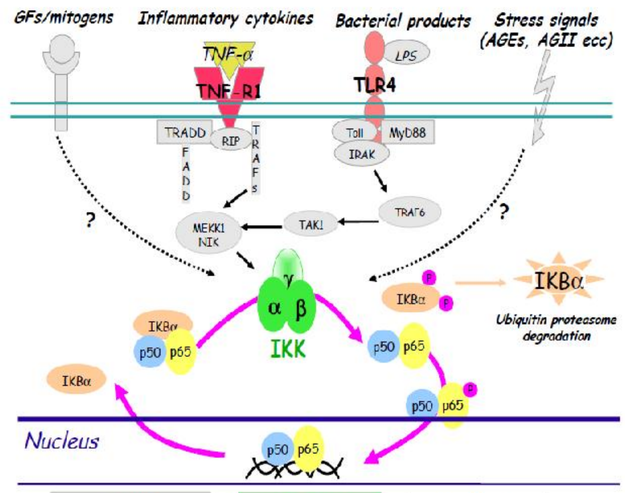


Fig. 1: Modified Santoro, Embo J, 2003.

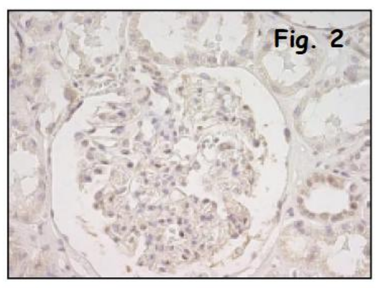
METHODS

- Renal biopsies from 22 patients with different stages of type DN (microalbuminuria or clinical proteinuria).
- Phospho-p65 (rabbit polyclonal Ab, Santa Cruz, USA) and p-IKB (mouse monoclonal Ab, Cell Signaling, USA) expression.
- Apoptosis: TUNEL, anti-ssDNA monoclonal Ab (Bender, Wien, Austria; Verzola et al. Kidney Int, 2008).
- From microdissected glomeruli and tubulointerstitial compartment: RNA extraction, cDNA-RT and quantitative RT-PCR (primers of proinflammatory genes: CCR2, CCL2, CCL5, CCR5,TLR-4, TNF-a, TNFR1) and β-Actin. Immunohistochemestry for cytokines protein detection.

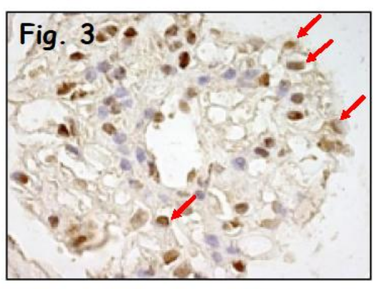
	Control (C)	Microalbuminuria	Proteinuria
Number of subjects	9	12	10
Age (yr)	60±4	60±12	63±3
Gender	5M/4F	6M/6F	4M/6F
AER (μg/min)		158±15	
Proteinuria (g/die)			4.7 ±1
eGFR (MDRD)	92±3	99±4	32±3

RESULTS

NF-kB /pp65 expression in C

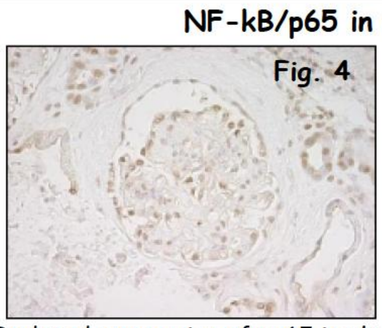


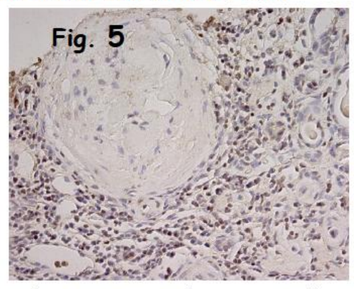
NF-kB/pp65 in pts with precocious DN



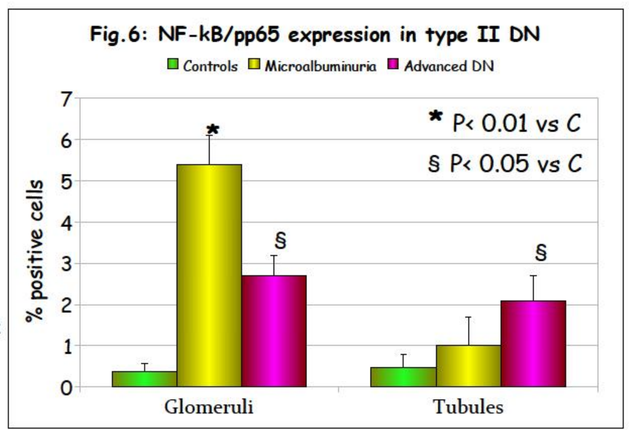
In microalbuminuric pts NF-kB signaling both pp65 (Fig. 3 and 6) and p-IKB (data not shown) were ~4 fold higher (p<0.01) in the glomeruli (podocytes and endothelial cells), but not in tubules as compared to C.

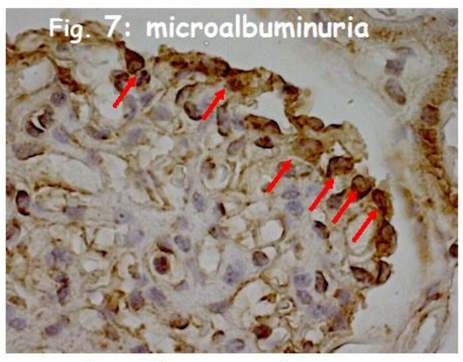
NF-kB/p65 in pts with advanced DN





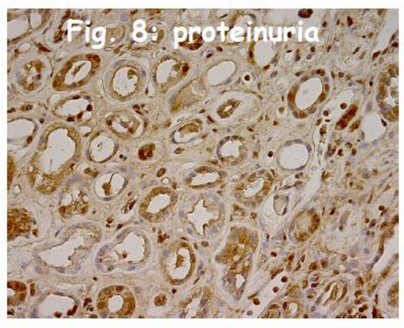
Reduced expression of pp65 in glomeruli as compared to microalbuminuric pts (Fig. 4 and 6), but still upregulated as compared to C (p<0.05). Increased expression of pp65 in tubulointerstitial cellular infiltrates (Fig. 5 and 6, p<0.05).



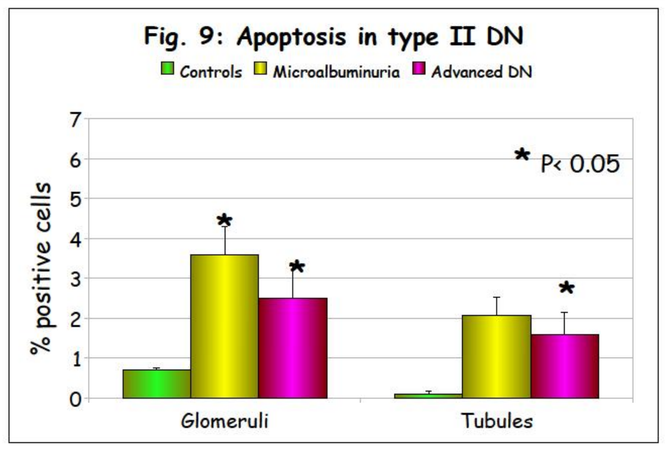


Microalbuminuric pts showed increased apoptotic processes (Fig. 7 and 9) in glomeruli (p<0.05), but not in tubules.

Apoptosis in type II DN



In proteinuric pts apoptosis increased in tubulointerstitial compartment (Fig. 8, p<0.05).



PRO-INFLAMMATORY TRANSCRIPTIONAL PROGRAMS

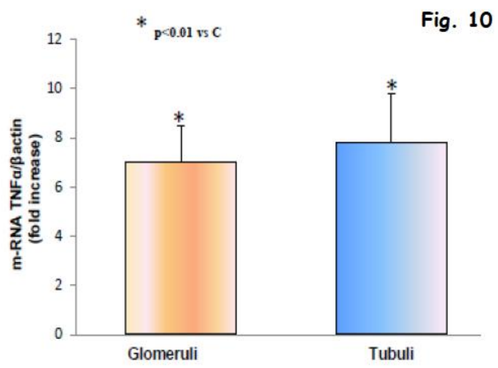
MICROALBUMINURIA

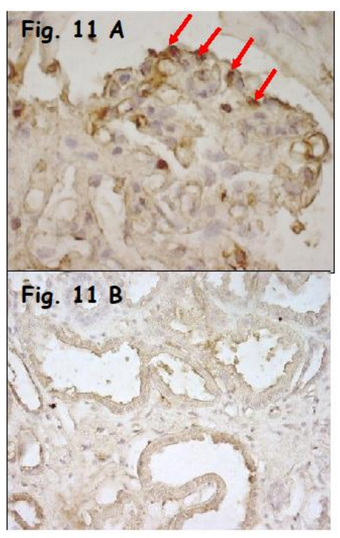
TLR4 (Fig. 10, 11A and 11B), TNFa, CCR-5, and CCL-5 mRNAs (data not shown) were markedly (p<0.01) upregulated in microdissected glomeruli.

Only TLR-4 (Fig. 10) was upregulated in the tubulointerstitial compartment (p<0.01).

Upregulated TNF-a was mainly expressed in endothelial cells, podocytes and proximal tubules (data not shown).

Fig. 10



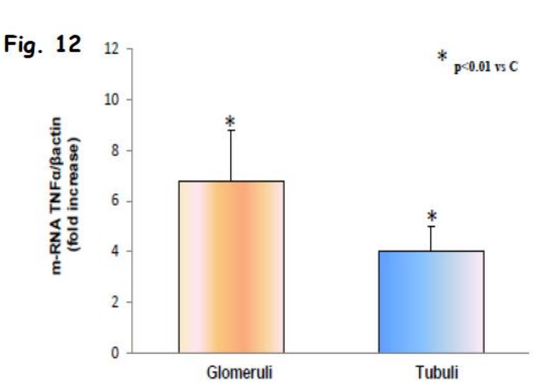


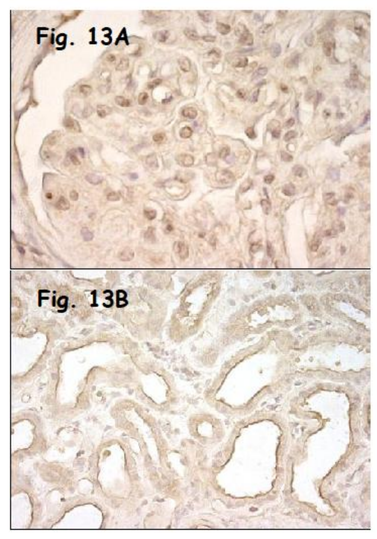
PROTEINURIA

TLR-4 (Fig. 12, 13A) and TNFa but not CCR-5 and CCL-5 persisted to be upregulated (microdissected glomeruli) (p<0.01).

In tubulointerstitial compartment TLR4 (Fig. 12 and 13B) and several genes (TNF-a,TNFRS and CCR-5) showed mRNA expression above background (p<0.04-0.02, data not shown).

Fig. 12





CONCLUSIONS

- ✓ Our data are consistent with the activation of NF-κB dependent pro-inflammatory transcriptional programs in the glomerular compartment, at an early stage of DN.
- ✓ At this stage, the acceleration of apoptotic processes in the glomeruli likely contributes to an early decrease in remodeling and albuminuria onset.
- ✓ In patients with more advanced DN NF-kB dependent signaling pathways are enhanced in the interstitial compartment, owing to inflammatory cell recruitment.

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Laura Cappuccino
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