



BECLIN-1 AND LC3 EXPRESSION IN AN EXPERIMENTAL LUPUS-LIKE NEPHRITIS MODEL



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INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex and common multisystem autoimmune disease, presenting with lupus nephritis (LN) in 30-60% of patients. Macroautophagy can be defined as an intracellular degradation system, it involves the fusion of a double-membrane autophagosome with a lysosome and has been associated with both cell survival and death. Among the various proteins synthesized from the expression of autophagy-related genes (ATG), there are Beclin-1 and LC3. Sato et al described that autophagy in podocytes occurred in 50% of children with evolved IgA nephropathy, correlating with a more aggressive histopathological diagnosis and the presence of LC3-positive autophagosomes was demonstrated in patients with membranous nephropathy compared to pre-transplant renal biopsies. Although it has been shown that autophagy is involved in the pathogenesis of SLE, to what extent it is present in the target organs remains uncertain, so we set out to analyze and describe the presence of macroautophagy in an LN-like experimental model.

METHODS



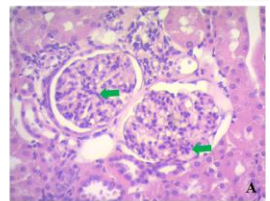
8-month-old Galectin-3 knockout mice (n:6 for each sex)



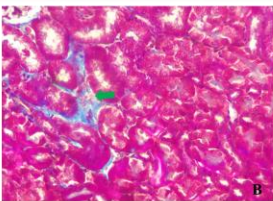
B6 strain mice (n:6 per sex)

Male and female Galectin-3 knockout mice of 8 months of age were used (n:6 for each sex), in which ANAs and renal functional compromise (proteinuria and increased creatinine) have been described, being the controls (C) mice of both sexes of the B6 strain (n:6 per sex). Methodologies used for the evaluation of macroautophagy were: transmission electron microscopy (4% Karnovsky fixative, inclusion in epoxy resins, contrast with heavy metals, observation in a Zeiss Leo 906-E electron microscope) and immunohistochemistry in paraffin for Beclina-1 and LC3, determining the number of immunopositive cells per tubule, counting between 710 and 790 tubules per control and experimental group. Statistics: mean and SD, Mann-Whitney test (two quantitative variable groups, unpaired, without normal distribution). $p<0.05$. Protocol approved by CICUAL-Universidad de San Martín.

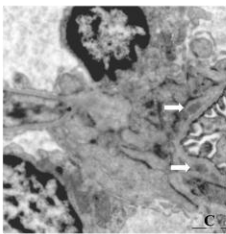
RESULTS



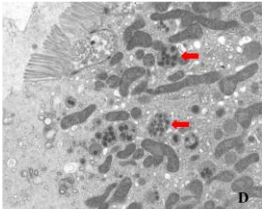
A- Optical Microscopy (H/E): Glomeruli with moderate mesangial hypercellularity and increased matrix (green arrows). Original magnification 400x.



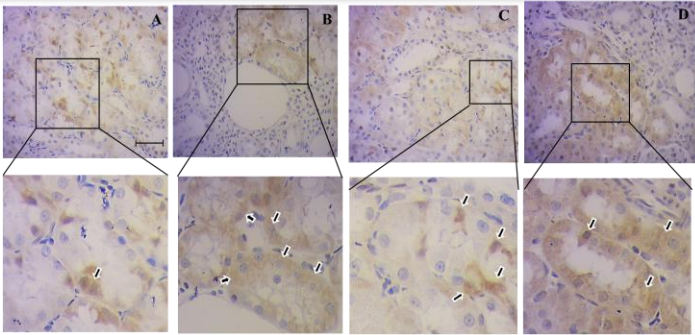
B- Optical Microscopy (Masson): Small focus of interstitial fibrosis (green arrow). Original magnification 400x.



C- Electron micrograph with electron-dense deposits of immune complex type in mesangial regions (white arrows).



D- Electron micrograph of tubular epithelial cell in which vacuoles with cytosolic content are observed, compatible with autophagic vacuoles (red arrows).



Beclin-1	Control male	Gal-3 male	p
Tubules (n)	780	790	
Cel+/tubules (x+-SD)	5±3	20±7	<0,05

LC3	Control male	Gal-3 male	p
Tubules (n)	710	730	
Cel+/tubules (x+-DS)	7±2	24±7	<0,05

Beclina-1	Control female	Gal-3 female	p
Tubules (n)	750	740	
Cel+/tubules (x+-DS)	7±2	25±4	<0,05

LC3	Control female	Gal-3 female	p
Tubules (n)	740	720	
Cel+/tubules (x+-DS)	8±2	22±7	<0,05

Immunohistochemistry photomicrograph: A- Cytoplasmic labeling for Beclin-1 in renal tubular cells from control females. B- Cytoplasmic labeling for Beclin-1 in renal tubular cells from Gal-3 KO females. C- Cytoplasmic labeling for LC3 in renal tubular cells from control females. D- Cytoplasmic labeling for LC3 in renal tubular cells from Gal-3 KO females. Original magnification 400x.

CONCLUSION

In the NL-like model the macroautophagy mechanism is increased compared to control animals, possibly linked to the proteinuric state in Gal-3 KO strain, and may also contribute to the chronic interstitial damage described.