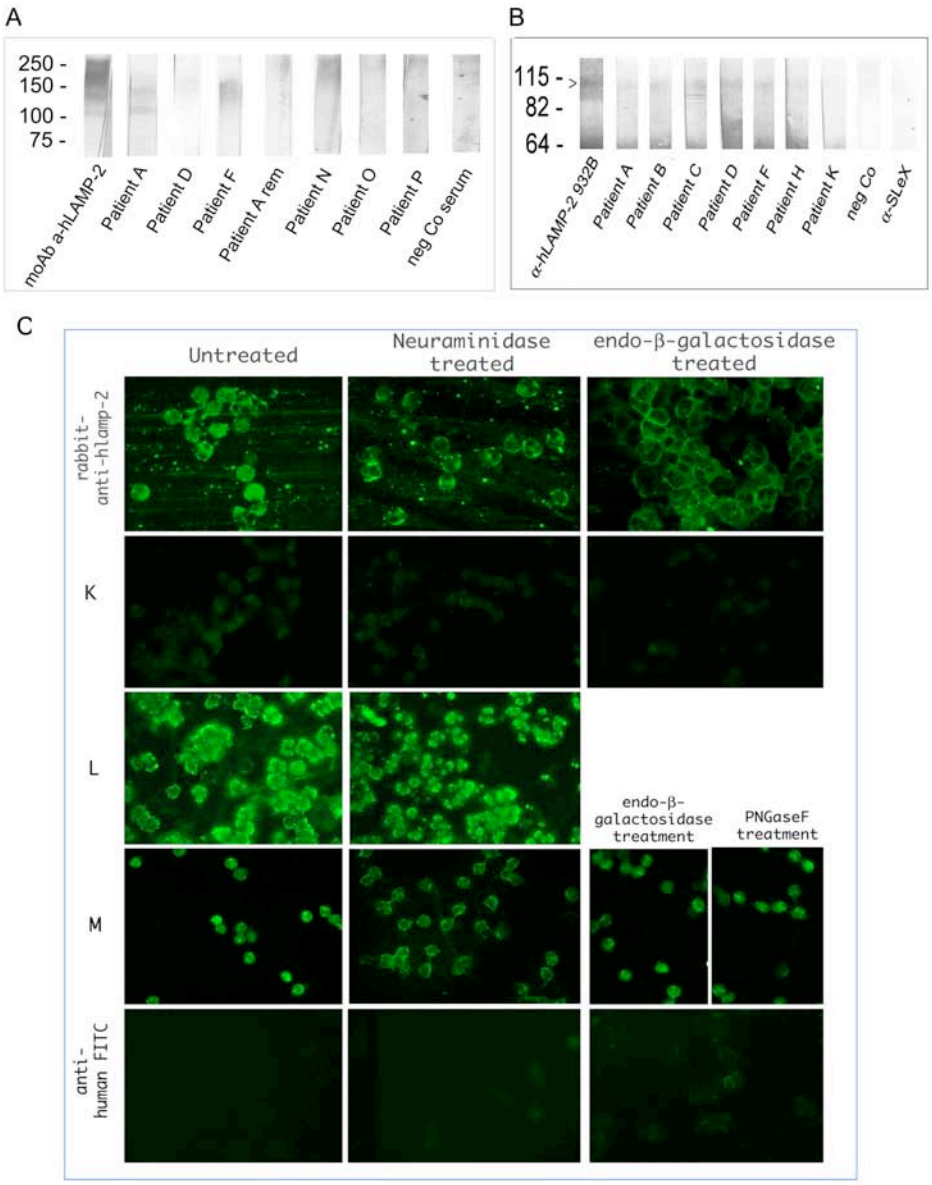
Autoantibodies to LAMP-2 in ANCA negative pauci-immune focal necrotizing glomerulonephritis

Supplementary material

Supplementary figure 1



Supplementary figure 1. Patients' autoantibodies binding to native human glomerular and neutrophil LAMP-2. Sera with antibodies to hLAMP-2 (patients A, D, and F) but not sera from patients in remission (patient A rem) or without antibodies to hLAMP-2 (patients N, O, and P) bound by immunoblot to a band recognized by a polyclonal antibody to hLAMP-2 (932B). This corresponds to native hLAMP-2 immunopurified from isolated human glomeruli (A).

Next we tested whether removal of glycans from neutrophil hLAMP-2 could restore binding of patients' IgG. hLAMP-2 from PMN lysates was immunopurified using monoclonal and polyclonal antibodies to hLAMP-2, treated for 1 hour with Neuraminidase (diluted in 50mM acetate pH 5.5, 154mN NaCl and 9mM $Cacl_2$), subjected to SDS-PAGE and subsequent transfer onto nitrocellulose.

Removal of sialic acid residues was confirmed with an antibody to Sialyl LewisX and yielded a dominant band of approx. 110 kDal identified by a polyclonal antibody to hLAMP-2 (932B) that was also recognized by sera that exhibited reactivity with glomerular hLAMP-2 (patients A, B. C, D, F, and H) but not hLAMP-2 negative (patient K) patients or controls (B).

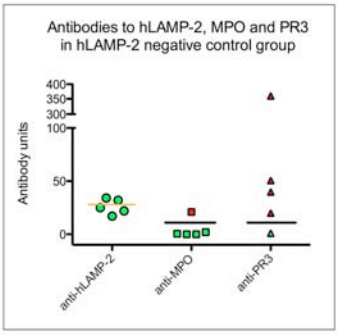
PMN preparations were used either untreated, treated with Neuraminidase, PNGaseF or endo-β-glactosidase to test whether removal glycans influenced the ANCA pattern obtained with either anti-PR3 or anti-MPO ANCA. Patients' sera were used as primary antibodies and binding was visualised using FITC conjugated anti-human IgG. As demonstrated for two of these sera, one with pANCA/anti-MPO (patient L) and one with cANCA/anti-PR3 (patient M) antibodies, reactivity with normal human granulocytes does not change after removal of either sialic acid residues with neuraminidase (patients L and M) or poly-lactosamines and *N*-glycans with endo-β-galctosidase and PNGaseF, respectively (patient M). Similarly, staining pattern of a polyclonal rabbit-anti-hLAMP-2 serum or human serum that does not contain antibodies to either ANCA or hLAMP-2 (patient K) does not change after treatment of PMN with de-glycosylating enzymes (C).

Supplementary figure 2

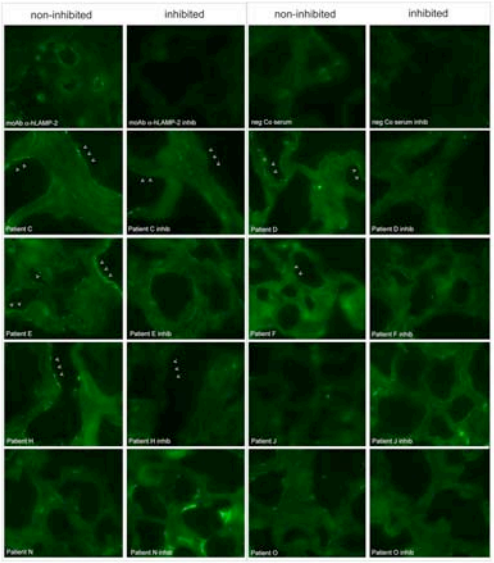
A

						Anti-hLAMP-2 assay results			Overall
No	Sex/Age	Diagnosis	Organ	ANCA	MPO/PR3	ELISA	Western	IIF ldID/hL-2	Summary
ANCA pos/hLAMP-2 neg									
L	M 78	FNGN	K	p	MPO 22	34.13	Neg	Neg	Neg
M	F 57	FNGN	K renal mass	c	PR3 20	31.98	Neg	Neg	Neg
N	M 58	FNGN	K/ENT	c	PR3 50.5	24.95	Low pos	Neg	Neg
O	F 76	FNGN	K	c	PR3 360	17.03	Low pos	Neg	Neg
P	M 45	FNGN	K	c	PR3 39.8	22.01	Neg	Neg	Neg

B



C



Supplementary Figure 2. Characteristics of ANCA positive/hLAMP-2 negative patients (A) and influence of glycan removal on sera from ANCA negative and ANCA positive control groups. Five sera from patients presenting with active piFNGN and ANCA specificity for either MPO (n = 1) or PR3 (n = 4) were chosen as control group (A). They had either limited renal disease (patient K) - one with a granulomatous renal mass (renal mass) - or systemic vasculitis with involvement of the upper respiratory tract (ENT). They did not have autoantibodies to hLAMP-2 that could confound the results obtained in experiments removing carbohydrate moieties from neutrophilic granulocyte proteins (A, B).

Binding to glomerular endothelial cells was assessed by incubating 1:40 dilutions of sera with 4 micron unfixed frozen sections of normal human kidney that had been depleted of non-specifically bound IgG by pre-incubation with high salt (1.8% NaCl) citrate buffer. Sera from ANCA-negative/hLAMP-2 positive (patients C, D, E, F, H) bound specifically to glomeruli with a predominantly endothelial pattern (B; non-inhibited). The slightly granular linear pattern seen after incubation with sera was identical to the endothelial staining obtained with the monoclonal antibody to hLAMP-2 (H4B4). In both cases binding was abrogated by pre-incubating the sera or monoclonal antibody with 8.5 ng/µl immunopurified native glomerular hLAMP-2 (B, inhibited). By contrast, sera from ANCA negative/hLAMP-2 negative (patient J), ANCA positive/hLAMP-2 negative (patients N, O) patients and serum from a healthy control did not bind to normal glomeruli.