IgA1-PROTEASE TREATMENT CLEARS MESANGIAL DEPOSITS AND AMELIORATES SYMPTOMS IN A HUMANIZED MOUSE MODEL OF IgA1 NEPHROPATHY

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Background and aims

IgA Nephropathy (IgAN) is the most common primary glomerulonephritis worldwide. This disease, is a major cause of end-stage renal disease (Wyatt and Julian, Nejm 2013), and affects both native and transplanted kidneys with recurrence after transplantation occurring in about one third of patients. Pathogenesis of IgAN involves hypo-galactosylated IgA1 complexed with soluble CD89, an IgA Fc receptor expressed on monocytes and neutrophils, which are deposited in the mesangium often with other proteins (IgG, IgM or C3) (Moura *et al.*, Sem Nephrol 2008). Despite all this knowledge, clinicians have only few tools to treat patients, with a lack of specificity and major secondary effects. Considering that mice do not express IgA1 nor CD89, we have developed a humanized mouse model of IgAN following expression by transgenesis of human IgA1 (Duchez *et al.*, PNAS 2010) and human CD89, the $\alpha1^{KI}$-$CD89^{Tg}$ mouse (Berthelot *et al.*, J Exp Med, 2012), which can be used as a powerfull tool for proof of concept experiments of new potential drugs targeting IgA1. These animals develop mesangial IgA1 deposits associated with proteinuria and hematuria at 12 weeks of age.

The aim of this study was to examine in vivo the effects of a recombinant IgA1 protease (IgA1P) in our humanized IgAN model, the $\alpha1^{KI}$-$CD89^{Tg}$ mouse. This IgA1P produced by human related bacteria such as *haemophilus Influenzae* has the capacity to cleave IgA1 in the hinge region but not IgA2. IgA1P was injected into $\alpha1^{KI}$-$CD89^{Tg}$ using different protocols to test the hypothesis that IgA1P will remove IgA1 mesangial deposition and ameliorate IgAN symptoms.

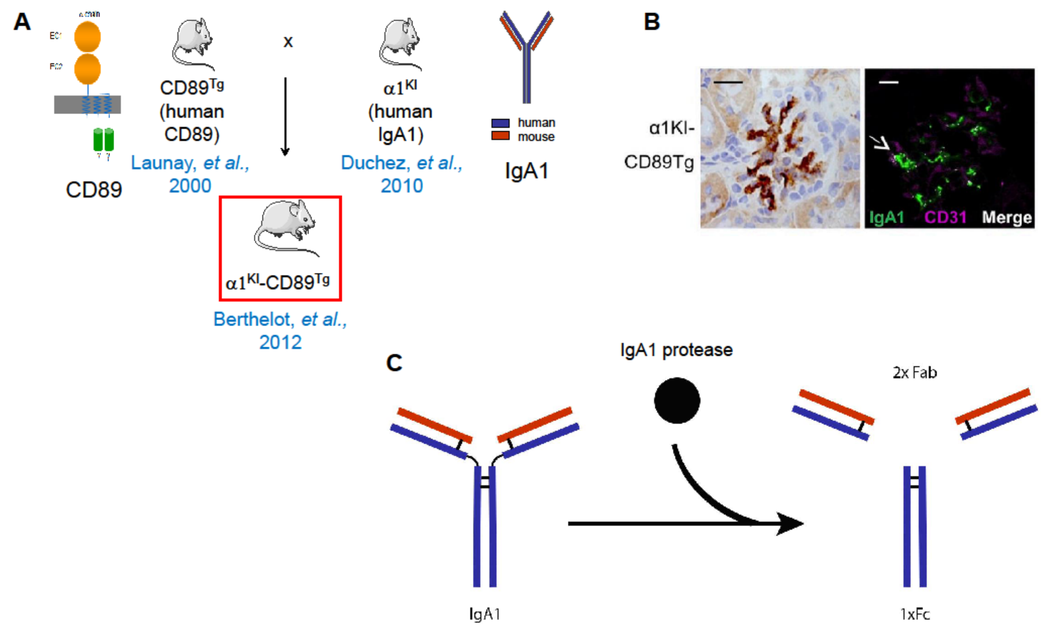

Figure 1: **A**: $\alpha1^{KI}$-$CD89^{Tg}$ mice: express human IgA1 and the human IgA Fc receptor CD89 on monocytes. **B**: Left panel: Immunohistochemistry against IgA and right panel: immunofluorescence against IgA and CD31 (endothelial cells) on frozen sections of kidney from $\alpha1^{KI}$-$CD89^{Tg}$ mice. **C**: Schematic representation of IgA1P activity on IgA1

IgA1P cleaves IgA1 in vitro and in vivo

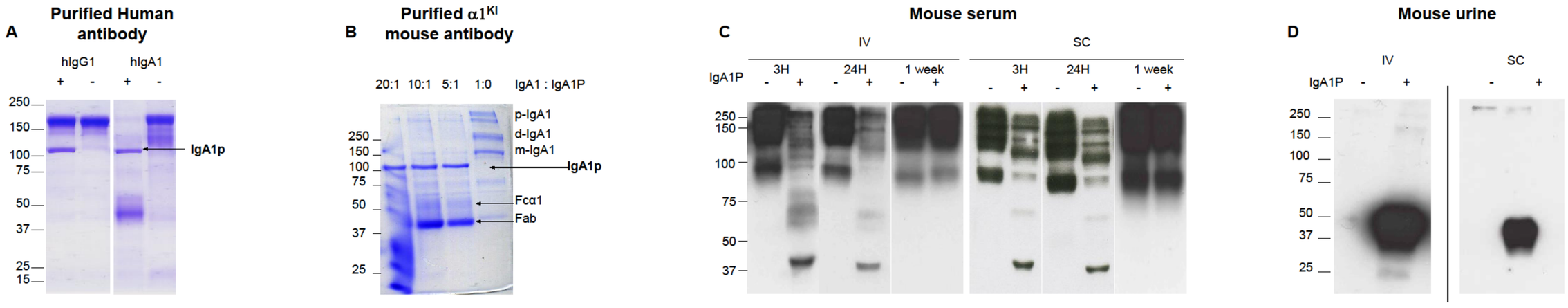

Figure 2: **A**: SDS Page of human IgG1 and human IgA1 with (+) or without (-) IgA1 protease. **B**: SDS Page of IgA1P with an increased concentration of chimeric IgA1 from $\alpha1^{Ki}$ mouse model. **C:** Western blot targeting IgA1 Fc fragments using sera of $\alpha1^{KI}$-$CD89^{Tg}$ mouse before and 3, 24 and 168h after IgA1P injection (subcutaneous or intravenous). **D**: Western blot targeting IgA1 Fc fragments using urines of mouse before and 24h after Iga1P injection (subcutaneous or intravenous).

IgA1 protease is efficient to cleave human and chimeric IgA1 specifically but not human IgG1. In vivo, the protease is able to cleave serum IgA1 up to 24h post injection (intravenously or subcutaneously) and fragments of cleaved IgA1 are found in urines up to 24H post injection.

Kinetic of IgA1 removal after treatment

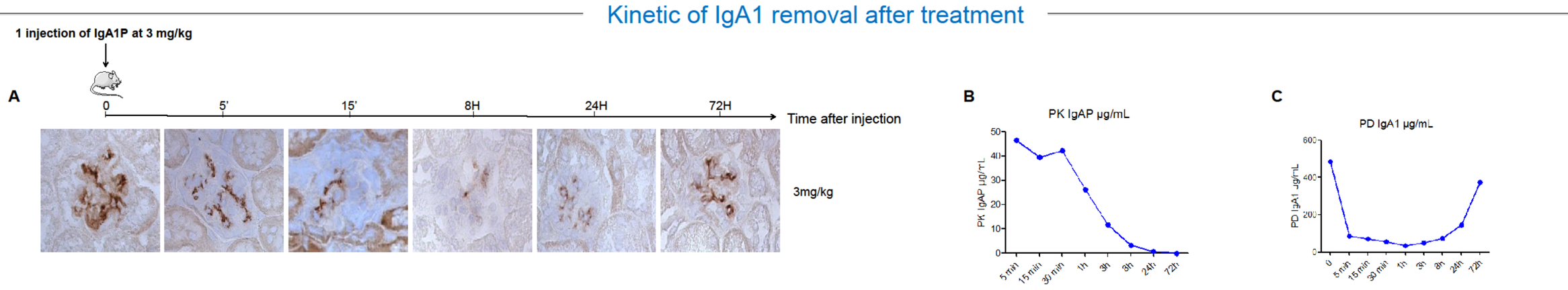

Figure 3: **A**: IgA1 immunohistochemistry on frozen kidney section from $\alpha1^{KI}$-$CD89^{Tg}$ mouse model at different time after IgA1P injection of IgA1P at 3mg/kg **B:** IgA1P dosage in the serum of our $\alpha1^{KI}$-$CD89^{Tg}$ mouse model at different time after IgA1P injection **C**: IgA1 dosage in the serum of our $\alpha1^{KI}$-$CD89^{Tg}$ mouse model at different time after IgA1P injection.

One IV IgA1P injection is sufficient to induce a decrease of IgA deposition as soon as 15 minutes after injection in $\alpha1^{KI}$-$CD89^{Tg}$ mouse glomerular and is maintained for at least 24 hours. This results are in correlation with IgA1P level which are not detectable after 24h and with the level of serum IgA increasing at the same time point.

Effects of IgA1P treatment

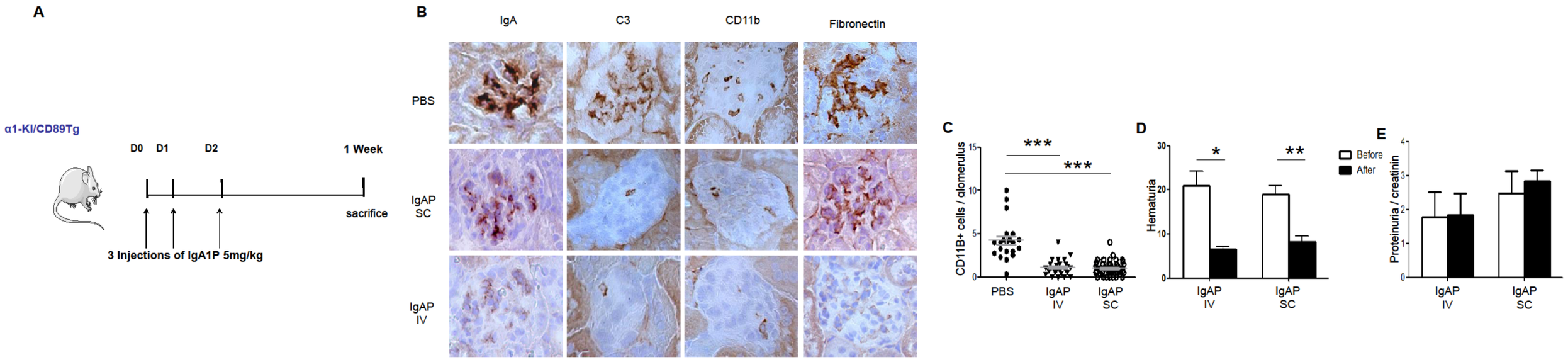

Figure 4: **A**: $\alpha1^{KI}$-$CD89^{Tg}$ mice were injected 3 times with IgA1P or PBS, intravenously (IV) or subcutaneously (SC) and sacrificed 1 week after **B**: IgA, C3 or Cd11b immunohistochemistry on frozen kidney section of $\alpha1^{KI}$-$CD89^{Tg}$ mouse model, injected 3 times with either PBS or IgA1P subcutaneously (SC) or intravenously (IV) **C:** CD11b+ cells per glomerulus counted after CD11b immunohistochemistry on frozen kidney section in mouse injected or not with IgA1P intravenously or subcutaneously. **D**: Hematuria on $\alpha1^{KI}$-$CD89^{Tg}$ mouse urines before and after IgA1P injection intravenously or subcutaneously.

Three injections of IgA1 protease in the $\alpha1^{KI}$-$CD89^{Tg}$ mouse model resulted in a strong decrease of IgA1 and C3 deposition, significantly less recruitment of macrophages, and important reduction of fibronectin expression in kidney. Moreover, a significant decrease of hematuria was observed after IgA1 protease injection. However no differences were observed in proteinuria.

In conclusion, these results reveal that recombinant IgA1 protease injected systemically markedly reduced mesangial IgA1 and C3 depositions, fibronectin production, inflammatory macrophage infiltrates and hematuria. The absence of modifications in the proteinuria after one week of treatment suggests that alterations in the glomerular basement membrane may require a longer time for resolution or additional reparative drugs. These experiments constitutes a proof of concept for recombinant IgA1P as an effective treatment for IgAN.

References: Wyatt and Julian, N. Engl. J. Med. 368: 2402–2414, 2013; Moura IC *et al*, Semin. Nephrol. 28: 88–95, 2008 Duchez *et al.*, Proc. Natl. Acad. Sci. 107: 3064–3069, 2010; Berthelot *et al.*, *J. Exp. Med.* 209: 793–806, 2012.

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