Efgartigimod, the neonatal Fc receptor antagonist, protects human keratinocytes from loss of cell adhesion induced by pathogenic anti-Dsg3 antibodies

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INTRODUCTION

IgG recycling via FcRn can be blocked by efgartigimod

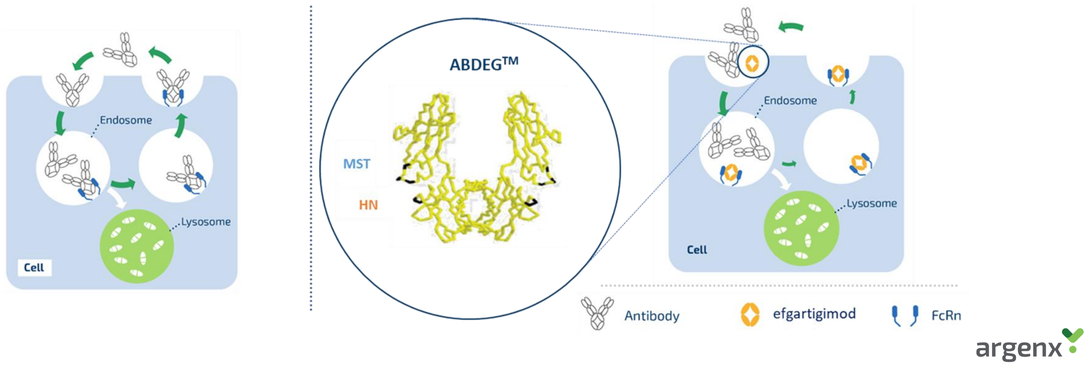

Figure 1: Schematic representation of the effect of FcRn inhibition by efgartigimod on IgG recycling. The neonatal Fc receptor (FcRn) has has been shown to bind IgGs and rescue them from lysosomal degradation, extending IgG half-life (left panel). Efgartigimod, an IgG1 Fc fragment, was designed for increased affinity for FcRn. It competes with IgG to occupy FcRn and reduce overall IgG recycling (right panel).

Novel antibody tools in pemphigus research

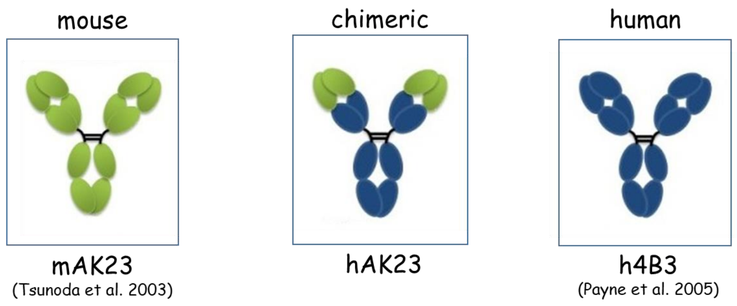

Figure 2: Schematic representation of the anti-Dsg3 antibody formats used in this study. Mouse IgG exhibit only negligible binding capacity to human FcRn. To investigate a potential role of FcRn in the pathogenicity of anti-Dsg3 antibodies novel antibody tools were developed. The well characterized mouse AK23 (**mAK23**) antibody was reformatted as a chimeric human/mouse IgG4 (**hAK23**). In addition, previously described, **h4B3** antibody isolated from a pemphigus vulgaris patient was produced as a recombinant human IgG1.

RESULTS

Pathogenicity of recombinant anti-Dsg3 antibodies

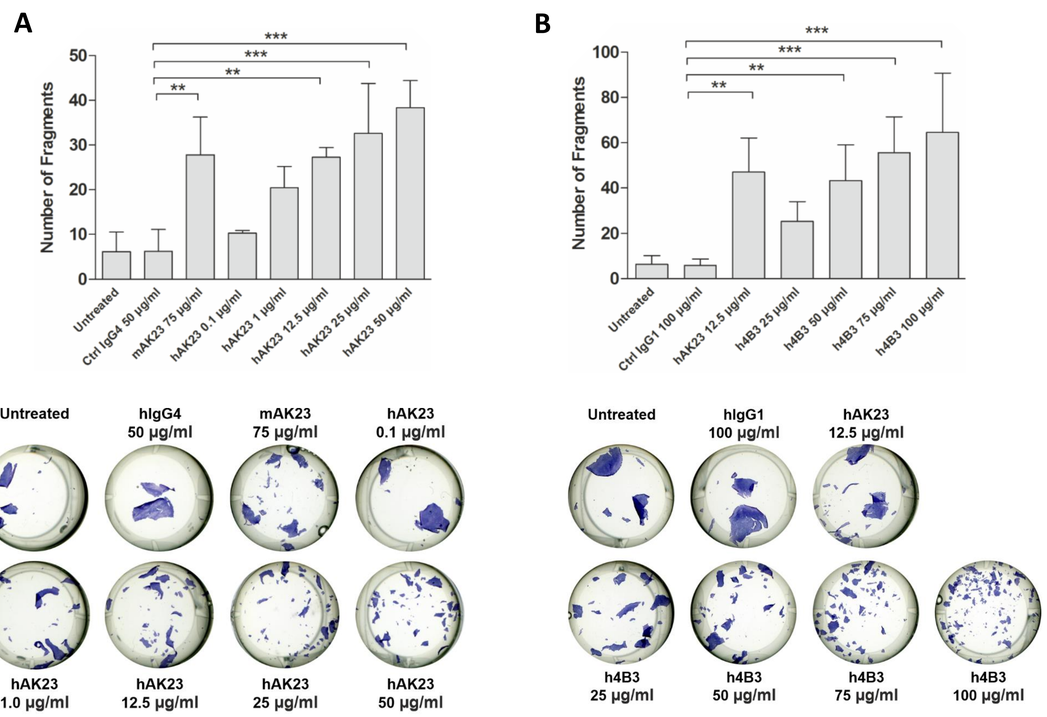

Figure 3: Recombinant anti-Dsg3 antibodies induce loss of cell adhesion and Dsg3 depletion in human keratinocytes. Dose-dependent effect of hAK23 **(A)** and h4B3 **(B)** antibodies on monolayer integrity, analysed in a dispase based dissociation assay in hTert cells. **(C)** Western blot analysis of Dsg3, Dsg1 and FcRn expression after treatment with hAK23, h4B3 and mAK23 antibodies. GAPDH serves as a loading control. **(D)** Densitometric analysis of western blot images performed with ImageJ software. Statistical analysis was done using one-way analysis of variance (ANOVA) with Dunnett´s post-test. Statistically significant differences are indicated by **, p≤ 0.01; ***, p≤ 0.001. The error bar represents SD of values obtained in at least four independent experiments.

Protective effect of efgartigimod on monolayer integrity

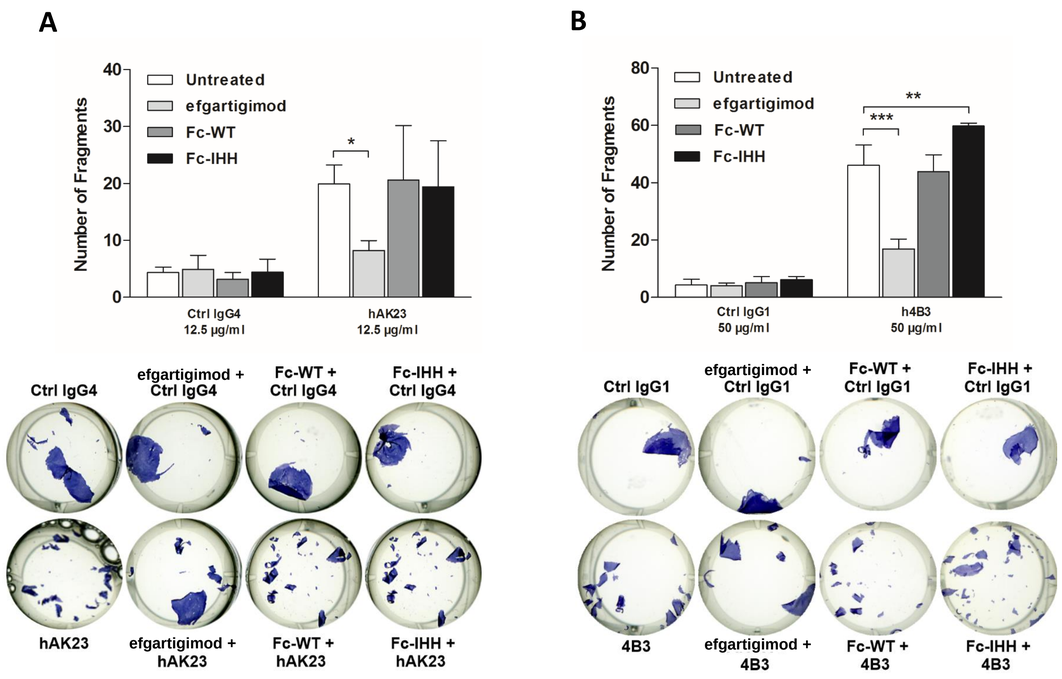

Figure 4: Efgartigimod treatment prevents the loss of monolayer integrity induced by pathogenic anti-Dsg3 antibodies. Effect of efgartigimod applied before treatment with pathogenic antibodies hAK23 **(A)** or h4B3 **(B)** was evaluated using a dispase based dissociation assay. Treatment with Fc-WT and Fc-IHH was included as a control. Statistical analysis was performed using two-way analysis of variance (ANOVA) with Bonferroni's post-test. Statistically significant differences are indicated in the figures by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The error bar represents SD of values obtained in at least four independent experiments.

Efgartigimod prevents h4B3 induced FcRn degradation

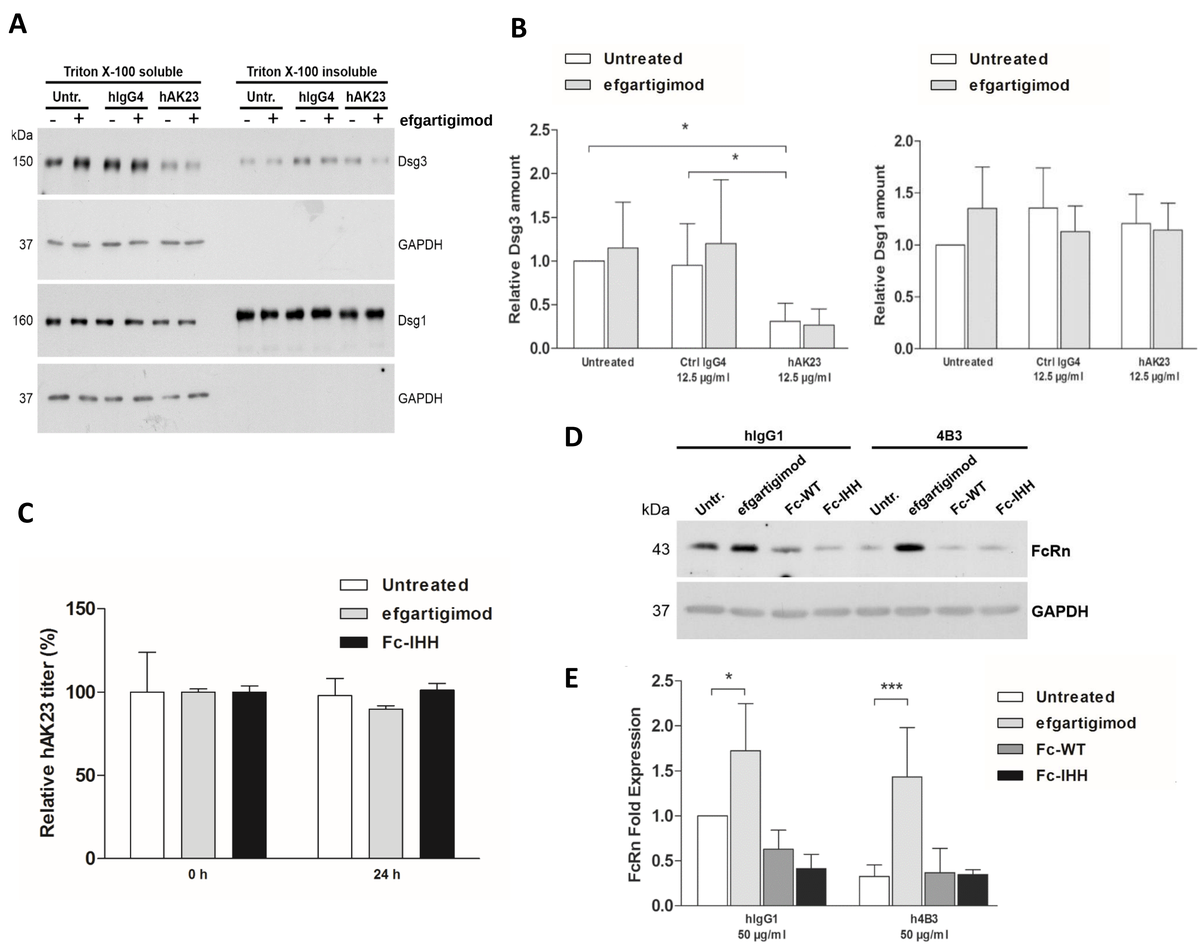

Figure 5: Efgartigimod does not rescue antibody mediated Dsg3 depletion but prevents h4B3 induced FcRn degradation without affecting anti-Dsg3 antibody levels. (A) Effect of efgartigimod on the non-desmosomal (Triton-X 100 soluble) and desmosomal (Triton-X 100 insoluble) pool of Dsg3 and Dsg1 was assesed by western blot. **(B)** Western blot signal in non desmosomal pool was quantified by densitometric analysis. **(C)** The amount of pathogenic antibodies was quantified with ELISA. **(D)** FcRn level was evaluated by western blot and quantified by densitometric analysis **(E).** Statistical analysis was done using two-way analysis of variance (ANOVA) with Bonferroni's post-test. Statistically significant differences are indicated in the figures by * $p < 0.05$, *** $p < 0.001$. The error bar represents SD of values obtained in at least four independent experiments.

CONCLUSIONS

- **Novel anti-Dsg3 Abs containing human Fc fragment induce loss of cell adhesion and depletion of Dsg3 in hTert cells and enable investigation of the role of FcRn in the pathogenesis of pemphigus in human keratinocytes**
- **The pathogenic effects on keratinocyte adhesion can be inhibited by treatment with efgartigimod**
- **FcRn may play a further role in the pathogenesis of pemphigus, beyond its known contribution to IgG recycling**