EFFECTS OF GLIADIN AND THE GENETIC BACKGROUND IN THE TIGHT JUNCTION STRUCTURE INTEGRITY

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Background:

Tight junction structures (Tj) are crucial for intestinal epithelium homeostasis as they control the paracellular flux and maintain the apico-basal polarity of intestinal cells. In celiac disease (CD), disrupted barrier function and increased paracellular permeability are observed, and these could be due to Tj disassembly caused by gliadin-induced innate and adaptive immune responses. Moreover, association and gene expression studies suggest a genetic implication in the Tj disruption observed in disease.

The aim of this study is to determine the effect of gliadin and the genetic background on epithelial barrier integrity, using the Caco-2 subclone C2BBe1 as an intestinal epithelium model.

Main findings:

1. Effect of gliadin on intestinal cell monolayer integrity

Intestinal C2BBe1 cells were grown for 2 weeks and monolayer formation was monitored by measuring transepithelial electrical resistance (TEER) and paracellular permeability. Incubation with pepsin-trypsin digested gliadin (PT-G) from the very beginning (Fig. 1a) was able to inhibit the formation of new monolayers and enhanced permeability. On the contrary, there was no PT-G-induced disruption of the integrity of previously formed monolayers (Fig. 1b).

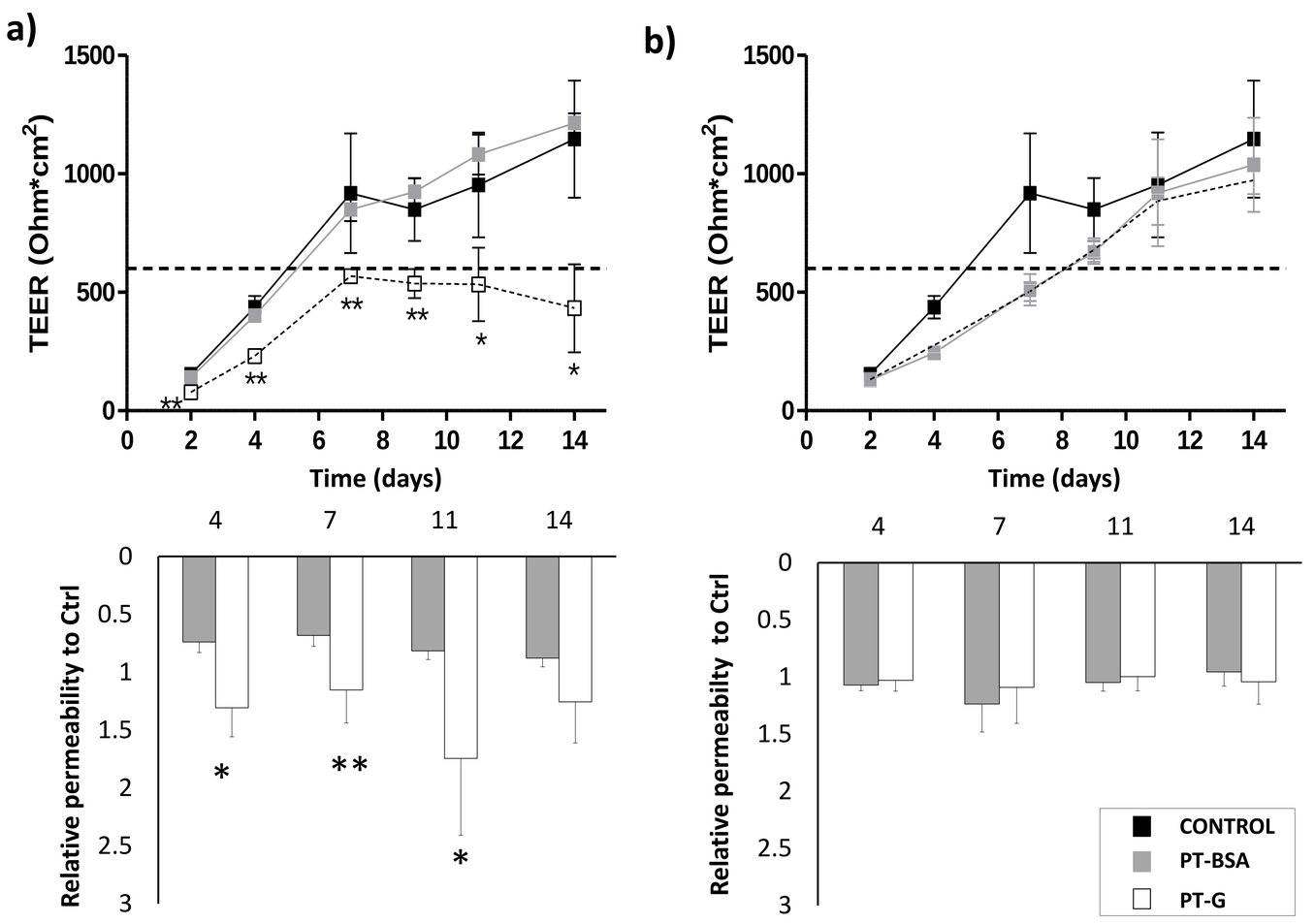

Figure 1. Measurement of transeptithelial resistance (TEER) and paracellular permeability to lucifer yellow in C2BBe1 monolayers during 2 weeks. *Cells were grown in normal conditions (Control) or stimulated with 1mg/ml pepsin-trypsin digested gliadin or BSA (negative control) from inoculation (a) or once the monolayer was formed (b), TEER > 600 ohm*cm².*

2. Effect of PT-G and gene silencing on Tj related gene panel

We analyzed the effect that PTG stimulation (1 mg/ml for 4 h) and crucial genes silencing by siRNA (48 h) has on the expression of a gene panel related to barrier integrity (Fig. 2). When genes that are crucial for Tj formation were silenced, PTG stimulation resulted in the alteration of the Tj pathway and other connected networks like Toll-like receptor signaling, as well as, increased expression of several chemokines (Fig. 3).

Figure 2. Effect of gliadin in C2BBe1 cells with a candidate gene silenced. *Columns show the silenced candidate gene and rows show analyzed genes. The key color is a gradual change from red (upregulation) to green (downregulation).*

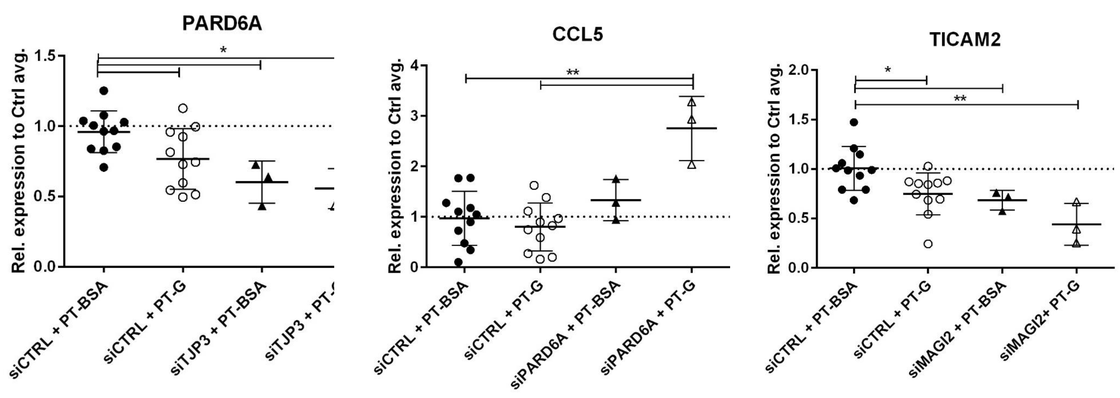

Figure 3. Three examples of significant gene expression alteration in C2BBe1 cells. *Each graphic shows sample conditions: Negative control , PTG stimulation, gene silencing or both PTG stimulation and gene silencing. (p value < 0,05).*

Conclusions:

- While gliadin does not affect intestinal epithelial barrier function in the normal gut, in an immature gut barrier may provoke increased intestinal permeability.
- Constitutively altered expression of Tj genes in CD might result in an immature gut barrier that could enhance CD-related gliadin toxicity leading to gut damage and activation of proinflammatory cytokine response.

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