PT-gliadin as a chemo-attractant factor for neutrophils

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Introduction: Gliadin triggers celiac disease (CD) in genetically predisposed individuals. Neutrophil influx is implicated in CD. We have shown that gliadin increases gut permeability through zonulin release, so gaining access to the lamina propria and initiating the host immune process.

Aim: to establish whether neutrophil recruitment is triggered by gliadin.

Methods: Ten C57BL/6 mice were given gliadin (1 mg/ml) or PBS by gavage. After 2h mice were sacrificed and duodenal tissue analyzed for (a) tight junction (TJ) disassembly by fluorescence microscopy and (b) immune cells (dendritic cells, T cells, granulocytes) by flow cytometry. Effects on neutrophil recruitment caused by gliadin was monitored *in vivo* after luminal exposure to gliadin (n=5) or PBS (n=5) for 2h by intravital microscopy technique using Lys-GFP mice that have green-fluorescent neutrophils. Furthermore, neutrophils were isolated from bone marrow of 8 C57BL/6 mice, and applied in the so-called taxi-scan assay, an *in vitro* model that allows monitoring neutrophil chemotaxis. Gliadin, and, as positive control N-Formyl-Methionyl-Leucyl-Phenylalanine (fMLP), were applied to this model.

Results: gliadin caused TJ disassembly in C57BL/6 mice as visualized by redistribution of both E-cadherin and ZO-1 tight junction proteins and increased number of GR1+ granulocytes (32% PBS, 47% PT-gliadin), but not dendritic cells or T cells. *In vivo* monitoring by intravital microscopy technique revealed a rapid and massive recruitment of neutrophils within 2h after luminal challenge with gliadin. Taxi-scan assay confirmed that gliadin and fMLP had similar chemo-attractant potential.

Conclusion: gliadin induced a massive neutrophil recruitment that occurred too soon to be induced by chemokine production. *In vivo* intravital microscopy and taxi-scan assay confirmed that PT-gliadin itself has chemo-attractant properties for neutrophils.