Is there a primary increase in epithelial cell permeability in coeliac disease?

Alessio Fasano, M.D.

Despite the progress made in understanding the immunological aspects of coeliac disease (CD) pathogenesis, the early steps that allow gliadin to cross the intestinal barrier are still largely unknown. We have recently reported that zonulin, the eukaryotic analogue of the *Vibrio cholerae*-produced zonula occludens toxin (Zot), modulates the permeability of intestinal tight junctions (tj) and is up-regulated in CD. Both zonulin and Zot engage to an intestinal receptor with subsequent activation of an intracellular signaling leading to the disassembly of intercellular tj. To establish the direct effect of gliadin on the activation of the zonulin system, both mammalian intestines (rat, rabbit, and human tissues) and a non-tumoral murine cell line (IEC6 cells) were used. Incubation of IEC6 cells with gliadin led to a reversible, PKC-mediated actin polymerization temporarily coincident with luminal (but not serosal) zonulin release. A significant reduction in tissue resistance (TEER) was observed after mucosal addition of gliadin on either rabbit or rat small intestines. This reduction was paralleled by decreased mRNA expression (evaluated using PCR Real Time with the TaqMan probes technique) of occludin, the main tj protein. Pre-treatment with the zonulin inhibitor FZI/0 abolished the gliadin-induced actin polymerization and tj disassembly but not zonulin release. No significant changes were observed when tissue was treated with similar concentrations of bovine

serum albumin used as control. Similar results were obtained when duodenal biopsies from CD treated patients were mounted in the polarized microsnapwell system and exposed to gliadin added to the luminal side of the tissue. Gliadin induced a time-dependent decrement in both TEER and occludin mRNA expression that was blocked by pre-treatment with FZI/0. No significant changes were observed in untreated tissues. Interestingly, intestinal tissues obtained from healthy subjects and exposed to gliadin showed a decrease of the mRNA occluding and TEER similar to that observed in CD-derived tissues. In conclusion, gliadin induces zonulin-dependent actin polymerization, followed by decrement of occludin expression and tj disassembly both in healthy and CD intestinal tissues. These changes lead to an increased intestinal permeability, suggesting that gliadin may play a pivotal role in facilitating its own passage to the submucosal compartment and, therefore, initiating the immune activation typical of CD in genetically-susceptible individuals.