of the population, are risk factors for reduced long-term survival, acute exacerbations of allergic inflammation associated with high pollen exposures may also precipitate death due to cardiovascular disease, COPD, or pneumonia in patients already suffering from these disorders. High concentrations of pollen allergens have also been shown to occur in thoracic particles (<10 μm in diameter) and respirable particles (<2·5 μm), and these correlated well in time with airborne pollen concentrations.[2] This finding means that airborne pollen results in exposure of the lower airways and lung to pollen allergens. The association between air pollution and the number of daily deaths may be related to the inflammatory potential of very small particles,[4] and our study suggests that particles of biological origin may have similar effects. Our findings require replication, but if substantiated, they suggest that high airborne pollen concentrations, which nowadays are mainly seen as triggers of allergic symptoms, may have far more serious effects than previously thought.

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Environmental and Occupational Health Group, Wageningen University, Wageningen (Prof B Brunekreef PhD, G Hoek PhD)**; Laboratory for Exposure Analysis, National Institute of Public Health and the Environment, Bilthoven** (P Fischer MSc)**; and Laboratory of Aerobiology, Department of Pulmonology, Leiden University Medical Centre, Leiden, Netherlands** (F Th M Spieksma PhD)

Correspondence to: Prof Bert Brunekreef, Environmental and Occupational Health Group, Utrecht University, PO Box 80176, 3508 TD Utrecht, Netherlands
(e-mail: Bert.Brunekreef@Staff.ech.wau.nl)

Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease

Alessio Fasano, Tarcisio Not, Wenle Wang, Sergio Uzzau, Irene Berti, Alberto Tommasini, Simeon E Goldblum

We identified zonulin, a novel human protein analogue to the *Vibrio cholerae* derived Zonula occludens toxin, which induces tight junction disassembly and a subsequent increase in intestinal permeability in non-human primate intestinal epithelia. Zonulin expression was raised in intestinal tissues during the acute phase of coeliac disease, a clinical condition in which tight junctions are opened and permeability is increased.

We have shown that zonula occludens toxin (ZOT), a protein elaborated by *Vibrio cholerae*, reversibly regulates the permeability of tight junctions.[1] ZOT interacts with a specific surface receptor[1] with subsequent protein kinase C α-dependent polymerisation of actin microfilaments strategically localised to regulate the paracellular pathway. On the basis of this observation, we investigated whether ZOT might mimic an endogenous modulator of tight junctions. We also postulated that ZOT and its putative eukaryotic analogue could be structurally and immunologically related.

Accordingly, specific anti-ZOT antibodies and an ex vivo intestinal permeability assay[1] were used in combination to screen for one or more human intestinal ZOT analogues. Non-primate intestinal tissues were used as an indicator system to identify and purify this analogue. Fetal and adult tissues were obtained from the brain and tissue bank for developmental disorders at the University of Maryland. A single protein (that we named zonulin) with a molecular weight of about 47 kDa was purified to homogeneity from both adult and fetal intestine (figure 1). To establish whether zonulin preparation was biologically active, it was tested on Rhesus monkey intestine with an *ex vivo* assay.[1] Intestinal tissues from the same animal with similar baseline tissue resistances were simultaneously exposed to either zonulin or media alone. Zonulin reversibly increased the monkey intestinal permeability compared with the media control in both jejunum (mean 35·0 [SE 1·8]% *vs* 3·0 [1·5]% permeability increment; p<0·0001) and ileum (26·0 [5·6] *vs* 4·9 [1·5] permeability increment), but not in the colon (1·3 [0·6] *vs* 1·1 [0·5] permeability increment, p=0·37, Student's *t* test). This increased permeability allowed the transepithelial passage of insulin, a macromolecule normally not absorbed when given orally.[2]

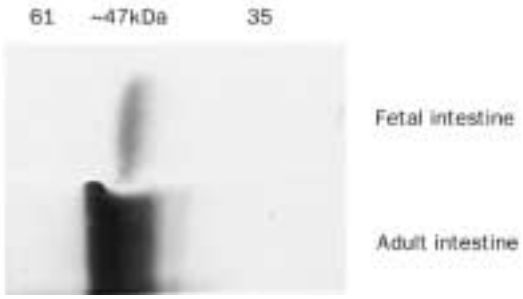

Figure 1: **Immunoscreening of human intestinal tissues with affinity-purified polyclonal anti-ZOT antibodies**
Proteins in tissue lysates of human fetal and adult intestine were subjected to sequential purification steps, resolved by sodium dodecylsulphate polyacrylamide gel electrophoresis, transferred onto polyvinylidene difluoride membranes, and probed with affinity-purified anti-ZOT antibodies. A single protein was purified that migrated with an apparent relative molecular mass of about 47 kDa and immunoreacted with anti-ZOT antibodies.

To establish whether zonulin is perturbed during coeliac disease, a condition in which tight junctions are opened through an as yet undefined mechanism,[3] intestinal tissues were obtained from seven patients with active coeliac disease and six controls and probed for zonulin with anti-ZOT antibodies. Immunofluorescence analysis of coeliac disease tissues showed enhanced zonulin expression within the intestinal submucosa with a characteristic reticular pattern that was consistently absent in control tissues. Quantitative immunoblotting of intestinal tissue lysates from patients with active coeliac disease confirmed higher zonulin protein concentrations than in control tissues (figure 2).

Since intestinal zonulin expression was increased during the acute phase of coeliac disease, when tight junctions are opened, this suggests a causal role of this endogenous mediator in

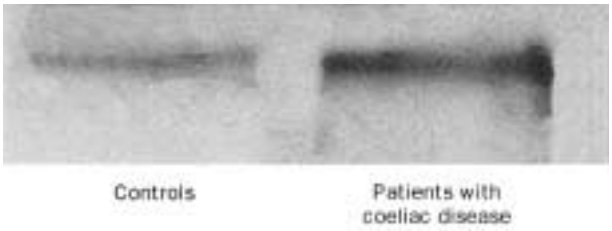

Figure 2: **Zonulin protein in intestinal tissues from coeliac disease patients and controls**
The increased expression of zonulin in intestinal tissues from coeliac patients was confirmed by western analysis. The amount of zonulin normalised to the total protein content in the tissues analysed was about 3-fold higher in intestinal specimens from patients with coeliac disease than in control tissues. These blots are representative of six specimens.

coeliac disease pathogenesis. Further, this increased expression of zonulin in the face of tight junctions disassembly might allow zonulin presentation to the submucosal gut immune system. Accordingly, we used a ZOT-based ELISA to detect antibodies to zonulin in the serum samples of patients with coeliac disease and controls. Anti-zonulin IgG was not higher in patients with coeliac disease than controls. By contrast, anti-zonulin IgA was raised in the serum samples of 25 of 117 (21%) patients with coeliac disease during the acute phase of the disease but in none of the 30 patients in remission ($p<0{\cdot}0001$). Only nine of 163 (6%) healthy controls had a minimally but significantly increased anti-zonulin IgA titre ($p<0{\cdot}0001$). The incidence of anti-zonulin antibodies during the acute phase of coeliac disease is consistent with the incidence of other auto-antibodies described in coeliac disease.[4] In seven patients with coeliac disease followed longitudinally, the raised anti-zonulin IgA returned to normal after 3–6 months symptomless remission on a gluten-free diet.

It has been recently reported that untreated coeliac disease predisposes to autoimmune disorders such as insulin-dependent diabetes mellitus, Hashimoto's thyroiditis, autoimmune hepatitis, and connective tissue diseases.[4] Perhaps zonulin opens small intestinal tight junctions during the early stage of coeliac disease and allows entry of putative allergens into the intestinal submucosa, in which an autoimmune response is elicited. In a spontaneous diabetic rat model, β-islet cell destruction and other autoimmune features develop only 3–4 weeks after the rise in gastrointestinal paracellular permeability.[5] Notably, these permeability changes always precede the autoimmune process.[5] Further, the barrier dysfunction is restricted to the small intestine,[5] paralleling the regional distribution of the zonulin regulatory system within the gastrointestinal tract.[1] Our findings that enhanced intestinal permeability in this diabetic rat model was associated with increased concentration of intraluminal zonulin (unpublished) further supports the pathogenic role for this protein at the onset of autoimmune disorders, such as diabetes mellitus and coeliac disease.

Our results support the idea that zonulin participates in the physiological regulation of intercellular tight junctions in the small intestine. Dysregulation of this conceptual zonulin model might contribute to the perturbation of the intestinal barrier functions, leading to the passage of environmental antigens involved in the pathogenesis of coeliac disease and related autoimmune disorders.

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Division of Paediatric Gastroenterology and Nutrition, and Gastrointestinal Pathophysiology Section, Center for Vaccine Development (A Fasano MD, W Wang MD, S Uzzau MD, I Berti MD), **Department of Physiology** (A Fasano), **and Division of Infectious Diseases** (S E Goldblum MD), **Department of Veterans Affairs Medical Center, University of Maryland, School of Medicine, Baltimore, Maryland 21201; and IRCCS Burlo Garofolo, Clinica Pediatrica Universita' di Trieste, Italy** (T Not MD, A Tommasini MD)

Correspondence to: Dr Alessio Fasano
(e-mail: afasano@umaryland.edu)

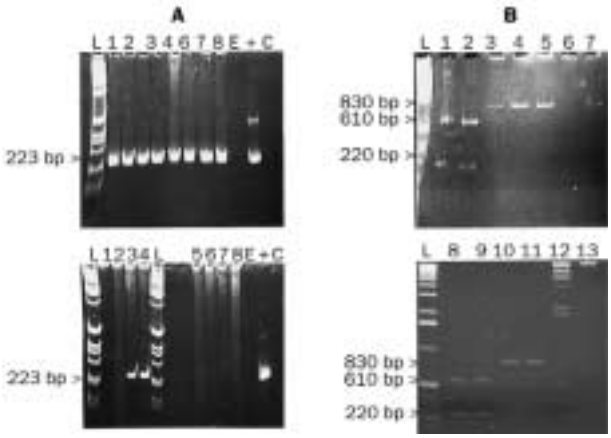
Detection of human herpes virus 6 DNA in fetal hydrops

Ahmed M Ashshi, Robert J Cooper, Paul E Klapper, Osama Al-Jiffri, Lynette Moore

Human herpes virus 6 (HHV6) DNA was detected in two of eight fetuses with hydrops and none of ten non-hydropic dead fetuses. Both cases with HHV6 DNA had chromosomal abnormalities. Positive results were confirmed with a second PCR specific for an alternate region of the HHV6 genome. Restriction endonuclease analysis confirmed that the viral DNA was representative of HHV6 type A.

Viruses including cytomegalovirus, herpes simplex virus, adenovirus, and more frequently, parvovirus B19, have been described in association with non-immune hydrops.[1] We describe the association of human herpes virus 6 with cases of fetal hydrops using PCR.

Paraffin-embedded tissue sections were obtained from the paediatric pathology archive. The eight cases of non-immune hydrops selected included two cases of trisomy 21 (females, 17 and 20 weeks gestation), one of Turner's syndrome (18 weeks), one with endocardial fibroblastosis (male, 34 weeks), one of fetal akinesia syndrome (female, 29 weeks) and three of unexplained hydrops (two males, 19, 32 weeks; female 29 weeks), one with unilateral pleural effusion and two with bilateral effusions. Non-hydropic control cases selected (ten) were four of extreme prematurity following pre-term delivery resulting from acute chorioamnionitis (three females, 18, 19, 20 weeks; 1 male, 24 weeks), one sepsis secondary to group B streptococcal infection (female, 41 weeks), one unexplained macerated stillbirth (female, 40 weeks), one trisomy 20 (female, 19 weeks), one *Pseudomonas* infection (male, 17 weeks) one hydrocephalus



A: Detection of HHV-6 DNA by uniplex PCR in tissue sections from fetal hydrops

Positive amplification is shown by a band at 223 bp. An extra (non-specific) band in the + lane is occasionally seen when HHV6 plasmid DNA is used as control. Lane L=1 Kb DNA markers; lane E=extraction negative control; lane+=PCR positive control; lane C=PCR negative control. Top: lanes 1 and 2=liver; lanes 3 and 4=kidney; lanes 5 and 6=heart; lanes 7 and 8=lung from case 1. Bottom: lanes 1–4=liver, kidney, heart and lung, respectively from case 2; lanes 5–8=liver, kidney, heart and lung, respectively from a HHV6-negative fetal hydrops case.

B: Typing of HHV-6 to A and B.

Lane L=1 Kb DNA ladder. Lanes 1, 2, 8, and 9=HHV-6 positive urine samples cut by *Hind* III (HHV-6 type B); lane 3=positive. Pleural fluid from case 1 uncut by *Hind* III (type A); lane 4, 5, 6, and 7=positive tissue sections from case 1 (liver, kidney, heart and lungs, respectively) uncut by *Hind* III (type A); lane 10 and 11=positive tissue sections from case 2 (heart and lung) uncut by *Hind* III (type A; lane 12=cut lambda DNA; lane 13=uncut lambda DNA.