

Hypothesis

Is *Candida albicans* a trigger in the onset of coeliac disease?

W F Nieuwenhuizen, R H H Pieters, L M J Knippels, M C J F Jansen, S J Koppelman

Coeliac disease is a T-cell-mediated autoimmune disease of the small intestine that is induced by ingestion of gluten proteins from wheat, barley, or rye. We postulate that *Candida albicans* is a trigger in the onset of coeliac disease. The virulence factor of *C albicans*—hyphal wall protein 1 (HWP1)—contains aminoacid sequences that are identical or highly homologous to known coeliac disease-related α -gliadin and γ -gliadin T-cell epitopes. HWP1 is a transglutaminase substrate, and is used by *C albicans* to adhere to the intestinal epithelium. Furthermore, tissue transglutaminase and endomysium components could become covalently linked to the yeast. Subsequently, *C albicans* might function as an adjuvant that stimulates antibody formation against HWP1 and gluten, and formation of autoreactive antibodies against tissue transglutaminase and endomysium.

Coeliac disease is a T-cell-mediated autoimmune disease of the small intestine that is induced by ingestion of gluten proteins from wheat, barley, or rye. Exposure of the small intestine to gluten induces an inflammatory response, leading to destruction of the villous structure of the intestine. Coeliac disease affects 0.5–1.0% of the European and US population, and can arise in early childhood with severe symptoms, such as chronic diarrhoea, and signs, such as growth retardation. Later in life, the disease could manifest itself with symptoms such as fatigue or diarrhoea, neurological symptoms, weight loss, and nutritional deficiencies due to malabsorption. Moreover, patients with coeliac disease are at increased risk of development of specific types of cancer, of being infertile, or of having miscarriages. The only remedy, until now, for coeliac disease is a strict gluten-free diet.

A clear genetic predisposition exists for coeliac disease. The HLA-DQ2 genotype is most typical, whereas the HLA-DQ8 genotype is less frequent. The reason why only 20–50% of genetically predisposed individuals develop symptoms of the disease is not known.¹

Coeliac disease is diagnosed by assessment of the villous structure of biopsy samples from the small intestine. Less invasive serological testing of antiglutin antibodies and autoreactive antibodies against tissue transglutaminase and endomysium are also indicative for the disease,² but biopsy specimens are still used for final diagnosis.

Gluten is a complex mixture of storage proteins found in various food grains. The main, toxic, wheat-gluten components are a family of closely related proline-rich and glutamine-rich alcohol-soluble proteins called gliadins. The gliadins consist of protein subtypes A, α , β , γ , and ω . α gliadins contain several immunoreactive aminoacid motives (eg, epitopes), and seem to account for most

immunoreactivity in coeliac disease,³ but γ gliadins and ω gliadins also provoke immune responses.^{1,5}

The HLA DQ2/DQ8 peptide-binding motive preferentially binds negatively charged aminoacids. However, gliadins have few negative charges, but these can be enzymatically introduced by transglutaminases. These enzymes have a pivotal role in blood clotting and wound healing (ie, factor XIIIa), and usually form isopeptide bonds between glutamines and lysines in proteins, thus forming cross-linked protein networks. Expression of tissue transglutaminase is raised in patients with coeliac disease, and this enzyme could have a key role in pathogenesis of the disease. Tissue transglutaminase, present in the small intestine, can selectively convert glutamines in gliadins into glutamic acid. Indeed, deamidation of some clustered T-cell epitopes in α gliadin and γ gliadin has been noted,⁵ and deamidation of some synthetic gliadin peptides raises their T-cell stimulatory potency.^{3,6,7} In young patients who have just had onset of coeliac disease, however, T cells are stimulated to a similar extent by native and deamidated peptides.⁴ Furthermore, tissue transglutaminase is an autoantigen recognised by autoreactive antibodies.⁸

Proteins from different sources can contain identical or highly homologous aminoacid sequences (eg, epitopes), and hence immunological crossreactions might arise. Comparison of the aminoacid sequences of wheat gliadins with those of the cell-wall component hyphal wall protein 1 (HWP1) of *Candida albicans* (figure), in particular with HWP1 aminoacids 40–197, shows many identical and homologous sequences in the proteins. More important, however, is the observation that HWP1 contains sequences of known coeliac disease-related T-cell epitopes from α gliadins and γ gliadins (panel). HWP1 and γ gliadin have three and five identical PQQPQ repeats, respectively, that are also present in most T-cell stimulating epitopes. The sequence YPQQPQ is present in HWP1 and in the DQ2 γ -gliadin epitopes DQ2- γ -V, T-cell γ -III plus γ -IV, and an unspecified DQ2 T-cell epitope. The homologous sequence FPQQPQ is present in epitope DQ2- γ -III. Sequence PQQQ is present in HWP1 and in epitope DQ2- γ -IV. It is noteworthy that the immunodominant sequence PQPQLPY from α gliadin is selectively deamidated by tissue transglutaminase to give PQPELPY.⁷ This sequence is highly homologous to sequences PQPDPIC and PQQDVPC that both arise twice in HWP1.

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**Netherlands Organisation for Applied Scientific Research (TNO)
Nutrition and Food Research, PO Box 360, 3700 AJ Zeist,
Netherlands (W F Nieuwenhuizen PhD, L M J Knippels PhD,
M C J F Jansen PhD, S J Koppelman PhD); and Institute for Risk
Assessment Sciences, Department of Immunotoxicology, Utrecht
University, PO Box 80176, 3508 TD Utrecht, Netherlands
(R H H Pieters PhD)**

Correspondence to: Dr W F Nieuwenhuizen
(e-mail: nieuwenhuizen@voeding.tno.nl)

1 MRLSTAQLIA IAYYMLSIGA TVPQVDGQGE TEEALIQKRS

41 YDYYQEPCDD YPOOOOQOQEP CDYPOOOOQOE EPCDYPOOOQP QEPCDYPOOQP QEPCDYPOOQP

101 QEPCDYPOOQP QEPCDNPPQP DVPCDNPPQP DVPCDNPPQP DIPCDNPPQP DIPCDNPPQP

161 DQPDDNPPIP NIPTDWIPNI PTDWIPDIPE KPTTPAT

198 TPN IPATTTTSES SSSSSSSSSS TPKTSASTT PESSVPATTP

241 NTSVPTTSSE STTPATSPES SVPVTSGSSI LATTSESSSA PATTPNTSVP TTTTETKSSS

301 TPLTTTTTEHD TTVVTVTSCS NSVCTESEVT TGVIVITSKD TIYTTYCPLT ETTPVSTAPA

361 TETPTGTVST STEQSTTVIT VTSCSESSCT ESEVTTGVVV VTSEETVYTT FCPLTENTPG

421 TDSTPEASIP PMETIPAGSE SSMPAGETSP AVPKSDVPAT ESAPVPEMTP AGSQPSIPAG

481 ETSPA VKSD VPATESAPAP EMT PAGTETK PAAPKSSAPA TEPSPVAPGT ESAPAGPGAS

541 SSPKSSVLAS ETSPIAPGAE TAPAGSSGAI TIPESSAVVS TTEGAIPTTL ESVPLMQPSA

601 NYSSVAPIST FEGAGNNMRL TFGAAIIGIA AFLI

HWP1 aminoacid sequence of *C albicans*

The known transglutaminase substrate sequence is underlined, and identical or homologous sequences in T-cell epitopes, shown in the panel, are in bold.

C albicans is present in the intestinal flora of many people.⁹ Adherence of the yeast to intestinal epithelial cells is mediated by transglutaminase, and a peptide that represents aminoacids 40–197 of HWP1, which is a tissue transglutaminase substrate.^{10,12} In mice infected with *C albicans*, invasion and lysis of the villi in the intestine has been reported.¹³ Development of lethal oro-oesophageal candidiasis in immunodeficient mice depends on both epithelial transglutaminase activity and HWP1 in the cell wall of the yeast. HWP1-deficient *C albicans* were unable to colonise the intestine and the mice survived.¹⁴ The direct interaction between *C albicans* and transglutaminase was also noted in an immunological study. When the yeast has been in contact with human tissue (including skin) or human cell lines it binds a component on its cell wall that is immunoreactive with rabbit polyclonal antibody against human plasma transglutaminase factor XIIIa.¹¹ Factor XIIIa might, thus, be covalently linked to *C albicans*. Factor XIIIa and tissue transglutaminase have large stretches of identical aminoacid sequences, and antibody crossreactivity can arise. Since the gliadin T-cell epitopes and aminoacid sequences in HWP1 contain

identical and highly homologous sequences, and transglutaminase binds to *C albicans*, we propose a mechanism for *C albicans*-mediated onset of coeliac disease.

Hypothesis

Under certain conditions, such as antibiotic treatments, *C albicans* can damage the intestinal epithelial cell barrier, thus raised extracellular concentrations of tissue transglutaminase will become available in the intestine. This enzyme will link itself covalently to the cell surface of *C albicans* and, concomitantly, will covalently link the yeast to the intestinal epithelium and endomysium, with HWP1 as a bridge. Because of intestinal damage, *C albicans* and the conjugates *C albicans*-endomysium and *C albicans*-tissue transglutaminase will be exposed to the immune system. Moreover, *C albicans* will function as an adjuvant and activate antigen-presenting dendritic cells. These cells will engulf the yeast, including both protein-yeast conjugates, and present the peptides and isopeptides of tissue transglutaminase, endomysium, and HWP1 to T cells. People with the HLA DQ2/DQ8 genotype, who are predisposed to develop gluten sensitivity, will be sensitised to HWP1 and, at the same time, to the homologous α gliadins and γ gliadins. Similarly, autoreactive antibodies to tissue transglutaminase and endomysium develop, since peptides from these proteins have become part of a foreign immunoreactive adjuvant. Involvement of one A-gliadin sequence in the adenovirus in development of coeliac disease, and existence of gluten-tissue transglutaminase hybrids that provoke formation of autoreactive antibodies against tissue transglutaminase, has been postulated.^{7,15} The reported aminoacid sequence of the adenovirus is, however, not abundant in gluten, does not arise in known T-cell epitopes, and the role of the virus in the persistence of coeliac disease has been questioned.¹⁶ Hypothetical gluten-tissue transglutaminase hybrids have, by contrast to the *C albicans*-transglutaminase species, never been found.

Epidermal transglutaminase was identified as the autoantigen of dermatitis herpetiformis.¹⁷ This disease is

T-cell gliadin epitopes homologous to HWP1 from *C albicans*

T-cell gliadin epitopes	Sequence	Source	Reference
DQ2- γ -III	F PQQPQQPYPQQP	γ gliadin	5
DQ2- γ -IV	F SQPQQQFPQPQ	γ gliadin	5
DQ2- γ -V	L QPQQPFPPQQPYPQQPQ	γ gliadin	5
γ -I	Y QQLPQPQQPQQSFPQQRRPF	γ gliadin	3
γ -III plus γ -IV	L QPQQPFPPQQPYPQQPQ	γ gliadin	3
DQ2	Q QPYPQQPQQPFPPQ	γ gliadin	6
DQ2	Q PQQPQQSFPQQRRPF	γ gliadin	4
DQ2	P QQPYPQPQPQ	α gliadin	4
DQ2	Q LQPFPPQPQLPY	α gliadin	4
DQ2	P QPQLPYPQPQLPY	α gliadin	4

genetically related to coeliac disease, and patients also have a gluten-sensitive enteropathy.¹⁸ Since *C albicans* colonises the skin and might acquire epidermal transglutaminase, autoreactive antibodies against this protein can develop in a similar way as described above.

Testing the hypothesis

The hypothesis can be tested with a well-documented population to assess time of onset of coeliac disease and the time course of development of antigliuten, antiHWP1, and autoreactive antibodies to tissue transglutaminase and endomysium. With immunoblot and ELISA inhibition studies, crossreactivities and affinities for gliadins, synthetic gliadin peptides, HWP1, and synthetic HWP1 peptides of coeliac disease patients' antibodies and T cells can be assessed, and the primary sensitising antigen can be established. Furthermore, *C albicans* could cause symptoms of coeliac disease in patients who do not respond to a gluten-free diet, and anti-*C albicans* treatment might relieve these symptoms.

Models of coeliac disease in animals are being developed,^{19,20} but these will not yield conclusive results because these models have no DQ-restriction like human beings do. Proof of the mechanistic principle for adjuvant activity of *C albicans* and its tissue transglutaminase complexes, for induction of antibodies against tissue transglutaminase and crossreactive antibodies to gliadins and HWP1, could be obtained by characterisation of the specific affinities of mouse antibodies and T cells in mice exposed to *C albicans*, synthetic HWP1 peptides, tissue transglutaminase, gluten, and combinations of these antigens.

Conclusion

This hypothesis on the role of *C albicans* in the onset of coeliac disease offers an explanation for development of antigliuten and autoreactive antibodies against tissue transglutaminase and endomysium in patients with this disease.

Conflict of interest statement

None declared.

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References

- Sollid LM. Coeliac disease: dissecting a complex inflammatory disorder. *Nat Rev Immunol* 2002; **2**: 647–55.
- Stern M. Comparative evaluation of serologic tests for celiac disease: a European initiative toward standardization. *J Pediatr Gastroenterol Nutr* 2000; **31**: 513–19.
- Shan L, Molberg O, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. *Science* 2002; **297**: 2275–79.
- Vader W, Kooy Y, Van Veelen P, et al. The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. *Gastroenterology* 2002; **122**: 1729–37.
- Arentz-Hansen H, McAdam SN, Molberg O, et al. Celiac lesion T cells recognize epitopes that cluster in regions of gliadins rich in proline residues. *Gastroenterology* 2002; **123**: 803–09.
- Vader LW, de Ru A, van der Wal Y, et al. Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. *J Exp Med* 2002; **195**: 643–49.
- Piper JL, Gray GM, Khosla C. High selectivity of human tissue transglutaminase for immunoactive gliadin peptides: implications for celiac sprue. *Biochemistry* 2002; **41**: 386–93.
- Seissler J, Wohlrab U, Wuensche C, Scherbaum WA, Boehm BO. Autoantibodies from patients with coeliac disease recognize distinct functional domains of the autoantigen tissue transglutaminase. *Clin Exp Immunol* 2001; **125**: 216–21.
- Lacour M, Zunder T, Huber R, Sander A, Daschner F, Frank U. The pathogenetic significance of intestinal *Candida* colonization: a systematic review from an interdisciplinary and environmental medical point of view. *Int J Hyg Environ Health* 2002; **205**: 257–68.
- Staab JF, Bradway SD, Fidel PL, Sundstrom P. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science* 1999; **283**: 1535–38.
- Arrese JE, Pierard GE. Factor XIIIa-related antigen immunoreactivity of fungal cell wall: a biologically relevant feature? *Dermatology* 1995; **190**: 119–23.
- Bradway SD, Levine MJ. Do proline-rich proteins modulate a transglutaminase catalyzed mechanism of candidal adhesion? *Crit Rev Oral Biol Med* 1993; **4**: 293–99.
- de Repentigny L, Phaneuf M, Mathieu LG. Gastrointestinal colonization and systemic dissemination by *Candida albicans* and *Candida tropicalis* in intact and immunocompromised mice. *Infect Immun* 1992; **60**: 4907–14.
- Sundstrom P, Balish E, Allen CM. Essential role of the *Candida albicans* transglutaminase substrate, hyphal wall protein 1, in lethal oroesophageal candidiasis in immunodeficient mice. *J Infect Dis* 2002; **185**: 521–30.
- Kagnoff MF, Austin RK, Hubert JJ, Bernardin JE, Kasarda DD. Possible role for a human adenovirus in the pathogenesis of celiac disease. *J Exp Med* 1984; **160**: 1544–57.
- Vesy CJ, Greenson JK, Papp AC, Snyder PJ, Qualman SJ, Prior TW. Evaluation of celiac disease biopsies for adenovirus 12 DNA using a multiple polymerase chain reaction. *Mod Pathol* 1993; **6**: 61–64.
- Sardy M, Karpati S, Merkl B, Paulsson M, Smyth N. Epidermal transglutaminase (Tgase 3) is the autoantigen of dermatitis herpetiformis. *J Exp Med* 2002; **195**: 747–57.
- Hervonen K, Hakonen M, Kaukinen K, Collin P, Reunala T. First-degree relatives are frequently affected in coeliac disease and dermatitis herpetiformis. *Scand J Gastroenterol* 2002; **37**: 51–55.
- Tlaskalova-Hogenova H, Stepankova R, Farre M, et al. Autoimmune reactions induced by gliadin feeding in germ-free AVN rats and athymic nude mice: animal models for celiac disease. *Ann N Y Acad Sci* 1997; **815**: 503–05.
- Black KE, Murray JA, David CS. HLA-DQ determines the response to exogenous wheat proteins: a model of gluten sensitivity in transgenic knockout mice. *J Immunol* 2002; **169**: 5595–600.