

Franka Kahlenberg · Daniel Sanchez ·  
Ingolf Lachmann · Ludmila Tuckova ·  
Helena Tlaskalova · Enrique Méndez ·  
Thomas Mothes

## Monoclonal antibody R5 for detection of putatively coeliac-toxic gliadin peptides

Received: 13 May 2005 / Revised: 17 June 2005 / Accepted: 21 June 2005  
© Springer-Verlag 2005

**Abstract** Monoclonal antibody R5 against rye secalin was recently suggested to be useful in analysis of gluten in food. The epitope specificity of R5 was characterized and compared with those of eight other monoclonal antibodies (mabs) against gliadins (gli) and secalins. Mabs were tested for binding to synthetic peptides spanning in overlapping manner sequences of gli. In a luminescence assay R5 bound to all peptides from the N-terminal part of  $\alpha$ -type gli hitherto known to induce in sensitive patients with coeliac disease after in vivo instillation. Thus, R5 proves to be very useful for gluten analysis. Sequences QQFPF, QQQFP, LQFPF, and QLFPF were bound most strongly. Substitution of glutamine by glutamic acid in the epitope may decrease binding of R5 dependent on surrounding amino acids. One of the positions of the substitutions diminishing antibody binding was a typical site of attack of tissue transglutaminase, the enzyme converting by deamidation cereal prolamins into their disease active form. Investigation of eight other mabs against gli and secalins showed binding properties very similar to R5. We speculate the sequence QQQ/FPF seems to represent an immunodominant structure in prolamins.

**Keywords** Coeliac disease · Epitope · Gliadin · Gluten-free · Monoclonal antibody · Synthetic peptides

### Introduction

Patients with coeliac disease (CD) have to keep a lifelong gluten-free diet. For control of gluten-free diet reliable and sensitive methods of gluten analysis are necessary. Recently, an ELISA was established based on monoclonal antibody R5 [1] the use of which for food analysis was suggested by Codex Alimentarius [2]. Antibody R5 was raised against ethanolic rye extracts [1]. This antibody detects, to the same extent, prolamins from wheat (gliadins), rye (secalins), and barley (hordeins). The core epitope of R5 was determined by means of phage display technique and pepscan studies to be QQFPF [3].

The main requirement for an antibody to be applied for food analysis is that it recognizes the peptides in prolamins toxic for CD patients. In order to assess toxicity of gliadin peptides different in vitro tests were used including organ culture and T-cell stimulation. However, in vivo gluten challenge and subsequent investigation of bioptically obtained small intestinal tissue still remains the gold standard for toxicity testing. Due to the invasive nature of jejunal biopsy the number of peptides tested by in vivo instillation is small and therefore, information on gliadin toxicity remains limited. From the N-terminal part of  $\alpha$ -type gliadin, the peptides 31–49 of A-gliadin [4], peptides 31–43 and 44–55 of  $\alpha$ -gliadins [5], and peptide G8 comprising amino acids 56–75 of  $\alpha_2$ -gliadin [6] were shown to be toxic in vivo. Another peptide from the C-terminal domain of  $\alpha$ -gliadin (206–217) was also shown to be toxic in vivo [7].

Native gliadin from wheat is subjected to different modifications until it reaches the intestinal mucosa where it induces CD in sensitive patients. One of the reactions is deamidation of glutamine residues a process which can be elicited by food manufacturing [8] but might also be induced by gastric acid [9] and is finally catalyzed by the enzyme tissue transglutaminase (tTG) in the small intestinal mucosa [9, 10]. Partially deamidated short peptides in

F. Kahlenberg · T. Mothes (✉)  
Institute of Laboratory Medicine, Clinical Chemistry, and  
Molecular Diagnostics, University Hospital Leipzig,  
Leipzig, Germany  
e-mail: mothes@medizin.uni-leipzig.de  
Tel.: +49-341-9722251  
Fax: +49-341-9722209

D. Sanchez · L. Tuckova · H. Tlaskalova  
Department of Immunology, Institute of Microbiology, Czech  
Academy of Sciences,  
Prague, Czech Republic

I. Lachmann  
Roboscreen GmbH Leipzig,  
Leipzig, Germany

E. Méndez  
Unidad de Gluten, Centro Nacional de Biotecnología, CSIC,  
Campus Universidad Autónoma,  
Cantoblanco, Madrid, Spain

the N-terminal region of  $\alpha$ -type gliadin were identified as potent stimulators of intestinal T-cell response [11, 12]. These peptides in their native, amidated form are contained in a 33-mer  $\alpha$ -type gliadin peptide which was shown to be resistant to gastric and pancreatic hydrolysis [13]. They are also part of peptide G8 of the N-terminal region of  $\alpha$ -type gliadin shown to be toxic for CD patients in vivo [6].

The aim of the present work was to define the epitope of R5 more precisely, to examine if R5 detects the putatively CD-toxic gliadin peptides in the N-terminal domain of  $\alpha$ -type gliadin, to investigate if deamidation of glutamine affects its reactivity, and to compare epitope specificity of R5 with a panel of other monoclonal antibodies directed against gliadins.

## Materials and methods

### Monoclonal antibodies

Mouse monoclonal antibodies R1, R4, R5 (against ethanolic rye extract isotype IgG2b [1]), 4D6 (against gliadin from wheat [14] isotype IgG1), 5B10, 1C6 (against gliadin from wheat [14] isotype IgM), 6H5, 8D4, and 5G7 (against crude gliadin from Sigma-Aldrich, Taufkirchen, Germany, isotype IgG1) were investigated.

### Synthetic peptides

The following complete amino acid sequences of prolamins (excluding the propeptide regions) were scanned for antibody binding:  $\alpha$ -type gliadin ( $\alpha/\beta$ -gliadin precursor, accession number C22364, 299 amino acids) [15],  $\gamma$ -type gliadin ( $\gamma$ -gliadin precursor, accession number P21292, 283 amino acids) [16],  $\omega$ -secalin ( $\omega$ -secalin precursor, accession number S18235, 339 amino acids) [17], peptides B3142 (53 amino acids), B3143 (53 amino acids), and B3144 (54 amino acids) extending from amino acid 3 to 55 or 56 of  $\alpha$ -gliadin, respectively [18], the corresponding sequence of A-gliadin (54 amino acids) [19], and the 33-mer peptide remaining after gastric/pancreatic digestion [13] of N-terminal region  $\alpha_2$ -recombinant gliadin [20]. Peptides were synthesized as octapeptides (overlapping by six amino acids) or as decapeptides (overlapping by eight or nine amino acids). The peptides on cellulose membranes were prepared by automated spot synthesis [21] as previously described in detail [22]. The peptides were covalently bound to cellulose membrane (Abimed, Langenfeld, Germany) via their C-termini. The peptides were N-terminally acetylated. Several of the peptides carried Q-E substitutions at defined positions. The single letter code for amino acids was used throughout.

### Pepscan

Binding of monoclonal antibodies to cellulose-bound peptides was measured as described [3]. In brief, after

washing in methanol and Tris-buffered saline (TBS: NaCl 137 mmol/l; KCl 2.7 mmol/l; Tris 50 mmol/l, pH 8.0) with 0.05% Tween 20 (TBST) the membranes were blocked (blocking buffer: TBS-T with 5% sucrose and 2.5% skimmed milk). After blocking the membranes were incubated with monoclonal antibody (1  $\mu$ g/ml). After washing the membranes were incubated with antimouse immunoglobulin G (IgG) conjugated with peroxidase (1:4000 in blocking buffer, Dianova GmbH, Hamburg, Germany). After further washing, luminescence was measured using the Supersignal CL-HRP kit (Pierce, Rockford, USA) and the ChemiImager (Alpha Innotech Corp., San Leandro, USA). Binding score was assessed qualitatively on a scale from 0 to 3 and results are given as mean luminescence score (MLS).

### Sequence alignment

All peptides recognized by antibodies with a score of at least 1 were considered. A set of successive peptides overlapping in sequence was aligned and the common amino acids were defined as epitope. Due to the repetitive nature, up to 38 epitope regions in the sequences tested could be identified for a single antibody. These epitopes were aligned again and the positions occupied by the most common amino acid expressed as a fraction of the number of epitopes. A frequency of 1 means that the respective position is always part of the epitope. If one and the same epitope sequence was recognised several times in different regions of the prolamins sequences, this epitope was included multiply into the calculation.

### Database searching

The database of the U.S. National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) was searched for short nearly exact matches with sequence QQPFP and with other, homologous sequences recognized by R5 using BLASTP 2.2.10 (March 3, 2005; 793,074,205 letters or 2,340,000 sequences in database, respectively). At this time, the database contained 9236 entries for proteins from Triticeae.

### Transamidation assay

Binding of biotin-cadaverine (Molecular Probes, Invitrogen GmbH, Karlsruhe, Germany) to gliadin-homologous decapeptides synthesized onto cellulose membranes was studied in a transamidation assay. For this, membranes were washed in methanol and TBST, blocked in TBS with 2% Tween-20, and incubated for 2.5 h at 37 °C in buffer (Tris 100 mmol/l, CaCl<sub>2</sub> 10 mmol/l, dithiothreitol 20 mmol/l, pH 8.5) with biotin-cadaverine (7.2  $\mu$ Mol/l) and human recombinant tTG (3.5  $\mu$ g/ml) [23]. After incubation with tTG and biotin-cadaverine, the membranes were washed in TBST, incubated with streptavidin-peroxidase

**Table 1** Epitopes of R5 in gliadin and effects of Q→E substitutions on antibody binding

Motif	MLS <sup>a</sup>	Decapeptides tested
QQPFP	2.7	VPLVQQQQFP; PLVQQQQFPG; QQQQFPGQQ; QFPGQQQQFP; PGQQQQFPPQ; GQQQQFPPQQ; QQQQFPPQQP; PQQPQQPFPQ; QQPQQPFPQP; QPQQPFPQPQ; PQQPFPQPQQ, QQPFPQPQQP
QQQFP	1.7	QFXGQQQQFPF; FLGQQQQFPPF; XGQQQQFPPFQ; GQQQFPPFQQ; QQQPFPFQQP
LQPFP	1.9	LQLQFPFPQPQ; QLQFPFPQQL; LQFPFPQQLP
QLPFP	3.0	PQQPQLPFPQ; QPQLPFPQPQ; QLPFPQPQQP
QQSFP	0.8	EQIISQQPFP; IISQQPFPQ; SQQPFPPLQP; PLQPQQSFPQ; QPQQSFPQPQ; QQSFPQPQHP
QLPYP	0.1	QFPFPQQLPY; PFPQPQLPYP; FPQPQLPYPQ; PQPQPYPQP; QPQLPYPQPQ; PQLPYPQQL; QLPYPQQLP
QRPFA	1.0	QPQRPFAQQP
QEFPFP	3.0	XGQEFPFPFPQ; QEFPFPFPQQP
PEFPFP	0.1	PQQPEFPFPQ; QQPEFPFPQP; QPEFPFPQPQ; PEFPPFPQQQ
QQEFPFP	0.5	XGQQEFPFPFPQ; QQEFPFPFPQQP

X=P or L

<sup>a</sup>Mean luminescence score of the decapeptides indicated in the right column

(Roche Diagnostics, Mannheim, Germany, 1:5000), and washed again in TBST. Luminescence was measured using Supersignal CL-HRP kit in the ChemiImager. Specificity of tTG was investigated by comparison of differently Q→E substituted peptides.

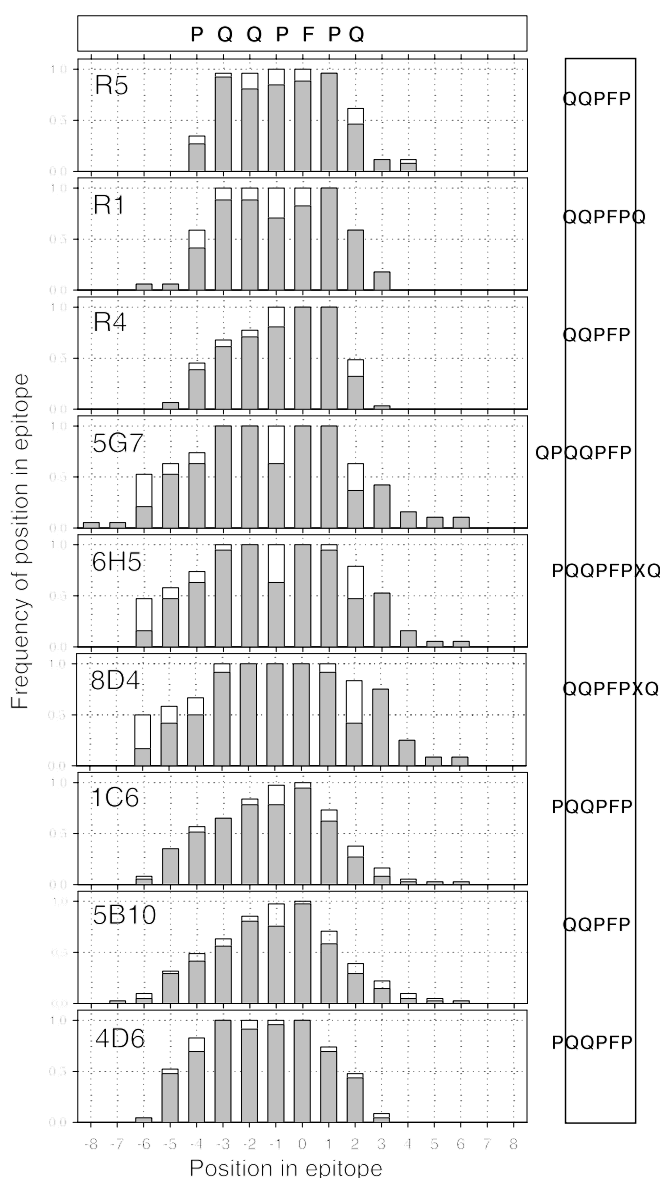
## Results

The N-terminal part of  $\alpha_2$ -gliadin comprises peptide B3142 (amino acid 3–55) and the 33-mer peptide (amino acid 56–88). Monoclonal antibody R5 strongly detects two regions in the N-terminal part of  $\alpha_2$ -gliadin. In peptide B3142, the sequences QQQFP (27–31) and in close proximity QQPFP (34–38) are recognized. In the 33-mer peptide, R5 reacts with LQPFP (58–62). The core epitope of R5 comprises not more than five amino acids. Our results confirm the essential role of the dipeptide FP for antibody binding as stressed earlier [3]. If F is replaced by Y, antibody binding is strongly diminished, however, a weak reactivity remains with PQLPYPQ occurring three times in the 33-mer (positions 64–70, 71–77, 78–84). Peptide PQQPFPSSQQ (44–53, although possessing a PFP unit) is not recognized by R5 showing that a P residue three positions upstream of F deletes binding.

The sequence of B3142 differs in positions 31 (P→L substitution) and 36 (P→Q substitution) from peptides B3143 and 3144 as well as from A-gliadin. Both substitutions were tested but did not affect antibody binding.

Scanning of the complete sequence of  $\omega$ -secalin confirmed prominent binding of R5 to the motif QQPFP. The sequence QLPFP was also found to be a strong binder. The motifs QRPFA and QQSFP are recognized more weakly (Table 1).

In the N-terminal region of  $\alpha_2$ -gliadin and in the fragment of  $\gamma$ -gliadin (amino acid 89–106), the effects of substitution of Q by E on binding of R5 were investigated (Table 1). Substitutions outside of the epitope were without effect. Introduction of an E residue directly in front of the PFP motif decreased antibody binding strongly. Substitution two positions in front of the PFP motif were only inhibitory if

**Fig. 1** Epitopes of nine different monoclonal antibodies against wheat gliadins and rye secalins

preceded by proline but not if preceded by a glutamine (see Table 1). This shows that further amino acids outside of the core pentapeptide structure influence antibody binding.

tTG is able to bind biotin-cadaverine to the second glutamine residue in the PQQFPF motif but not to other residues (results not shown). This sequence specificity is in accordance with previously published results [24] and shows that tTG can modify the sequence of gliadin peptides such that binding of R5 may be decreased.

Epitope specificity was investigated of eight further mouse monoclonal antibodies raised against gliadins and secalins. To our surprise, binding properties of all antibodies were very similar to that of R5 (Fig. 1). The motif QQPF is common for all antibodies investigated. The epitope specificity differs slightly according to the length of the sequence (range 5–8 amino acids). Furthermore, there are slight differences if the respective position can also be occupied by other amino acids. The amino acid phenylalanine constitutes the main common requirement for antibody binding and in most cases substitution of this aromatic amino acid by tyrosine is not tolerated.

The database of the U.S. National Center for Biotechnology Information was searched for exact matches with the QQPF motif and with other homologous sequences recognized by R5. For QQPF, 791 entries were found. From these, 492 entries referred to proteins from Triticeae (*Triticum*, *Hordeum*, *Secale*, *Aegilops*). Proteins from *Avena* did not contain this sequence. Further, 10 hits were found in *Oryza sativa* and 4 hits in *Sorghum bicolor*. The sequence QQPF was not found in proteins from maize. There were 14 entries for vertebrate proteins, 12 of them from man. The other sequences recognized by R5 were less specific for coeliac toxic cereals. Only in Triticeae, the epitopes were found to occur repetitively.

---

## Discussion

Detailed knowledge of the epitope recognized by an antibody used to detect harmful components in the diet is important to enhance the safety of food control. For CD patients gluten peptides have to be excluded from the diet. Ideally, an antibody applied for detection of gluten should recognize the partial sequence toxic for patients with CD [25]. Our results show that monoclonal antibody R5 is able to detect peptides, which are known to exert a toxic effect after instillation in CD patients in vivo. The number of gliadin sequences with known in vivo toxicity is still small. Gluten peptides remaining after gastrointestinal digestion and after modification by tissue transglutaminase and binding to MHC molecules on antigen presenting cells stimulate gliadin-specific T cells, which elicit tissue damage. Test systems assessing the stimulatory effect of gluten peptides on human T-cell clones have revealed a large number of T-cell epitopes. Additionally, innate immune mechanisms might be triggered by gliadin peptides of unknown sequence [26, 27]. In this investigation we have concentrated our search on gliadin sequences with established in vivo toxicity in the N-terminal part of  $\alpha$ -type gliadin.

The sequence QQPF detected by R5 is very specific for the cereal species toxic for CD patients. Five percent of all cereal proteins in the database contained the QQPF sequence, but only 0.01% of the noncereal proteins. Moreover, in the noncereal proteins, the epitopes of R5 do not occur repetitively thus not allowing detection in the sandwich ELISA system described [1] in which at least two epitopes within one molecule are required. Other cereal proteins not belonging to Triticeae (*Avena*, *Oryza*, *Zea*, *Sorghum*) are nearly completely devoid of QQPF. This corresponds well with immunoblot data [1] showing a strong reaction of the antibody with prolamins from wheat, rye, and barley but insensitivity to oats, maize, and rice prolamins [1, 25].

Although oats are well tolerated by most CD patients, some concerns remain [28]. Some patients have avenin-reactive mucosal T-cells that can cause mucosal inflammation [29]. Therefore, to detect oats in the diet, monoclonal antibodies with other epitope specificity should be applied. Another point to take into account is that gluten response can be directed not only to gliadins but also to glutenins [30]. The main epitopes recognized by R5, QQPF and QQPF are also contained in LMW glutenins, however, not repetitively. Actually R5 recognizes glutenins only very weakly, probably because the number of epitopes in glutenins necessary to react with the R5 antibody is very small as compared with gliadins. This was also recently confirmed for HMW glutenin subunits by ELISA analysis of a pure HMW subunit preparation from wheat flour [31]. In immunoblot, R5 recognizes the HMW glutenins but with less intensity than gliadins, probably due to the existence of a few epitopes in the HMW glutenins.

The reactivity of R5 with its epitope is decreased but not abolished by deamidation processes. Such processes do not only take place in vivo by the action of tTG but may also occur during food processing [7], e.g., by acid treatment or bacterial transglutaminases. Our investigations show that deamidations actually may affect antigenicity of gliadin and that investigation of processed food generally has to be interpreted with caution. Nevertheless, we can show that there is still some reactivity remaining.

The finding that a panel of different monoclonal antibodies raised in three different laboratories against gliadins and secalins more or less recognizes the same epitope is amazing. The epitope of another monoclonal antibody against gliadin PN3 [32] comprises the QQPF pentapeptide as well, but differs from R5 epitope by an additional N-terminal glutamine residue. In accordance with this, PN3 recognizes  $\alpha$ -type gliadin better than  $\gamma$ -type gliadin (which does not contain QQPF but only QQPF or QQPF), and  $\omega$ -gliadins were detected only very weakly as it was also the case with prolamins from rye, barley, and oats. Further three monoclonal antibodies were described against cereal proteins two of them detecting as core peptide the epitope QQPF, one of them the homologous sequence QQSF/Y [33], which is also recognized by R5. On the contrary, in another study, epitopes of monoclonal antibodies against gluten were described with very variable epitopes [34]. The authors, however, used for selection of antibodies not only gliadins but also glutenin subunits.

Human antibodies of the IgE class with the QQPFP and PQQPF specificity are also common in wheat allergic patients [35, 36]. Furthermore, the sequences QXQPFP (X for Q, P, or L) and PQQPF are also major epitopes for human gliadin antibodies of patients with CD [37] the binding of which to gliadin is considerably improved after conversion by tTG into QEQPFP [38].

Thus, the sequence motif QQQ/PFP seems to represent an immunodominant structure. Reasons for that may be that the proline and glutamine-rich sequences of gliadins and of other cereals form hydrophilic  $\beta$ -turns [39–41], which are exposed at the surface of the protein. Another reason may be the repetitive character. The sequence QQPFP occurs in prolamins up to 15 times within one molecule. Repetitive epitopes could enhance the immunological response of the B cells [42]. In an ELISA system for detection of gliadin it was recently demonstrated that R5 is able to act as capture as well as detection antibody [1] suggesting that at least two epitopes within the same molecule can be bound at the same time. Such mechanism might contribute to stimulation of B-cells even if only small amounts of polyvalent gliadin molecules remain after digestion. Cross linking of patients' IgE-antibodies via these epitopes was recently speculated to trigger subsequent allergic reactions [35].

To summarize, we have shown that a monoclonal antibody raised against secalin, R5, specifically detects as core epitope the sequence QQQ/PFP that is contained in gliadin peptides with known toxicity for CD patients. Therefore, this antibody proves to be very useful for analysis of gluten-free food for CD patients. The QQQ/PFP sequence seems to represent an immunodominant structure in prolamins the possible pathogenetic importance of which remains to be investigated.

**Acknowledgements** This work was supported by grants of Interdisciplinary Centre of Clinical Research (IZKF) of the University of Leipzig, 01KS9504, Project A5 (TM), of the German Academic Exchange Service, D/03/44431 and D/03/40308 (FK, TM) and AVCR D23-CZ/04-05 (DS, LT, HT), of the Saxon Ministry of Science and Art, 915-0504-WE (FK), of the Grant Agency of the Czech Republic, 310/04/P242 (DS), and of Plan Nacional AGL2004-02721/ALI, BIO2000-0403-P4-03, and PETRI 95-0778.OP (EM). Dr. J. Plicka (Immunotech, Prague) is thanked for help in preparing monoclonal antibodies 6H5, 8D4, and 5G7.

## References

- Valdés I, Garcia E, Llorente M, Méndez E (2003) *Eur J Gastroenterol Hepatol* 15:465–474
- Codex Alimentarius Committee, Report of the 28th Session, Budapest 4–8 April 2005, ALINORM 05/28/23, p 7
- Osman AA, Uhlig HH, Valdes I, Amin M, Méndez E, Mothes T (2001) *Eur J Gastroenterol Hepatol* 13:1189–1193
- Sturgess R, Day P, Ellis HJ, Lundin KE, Gjertsen HA, Kontakou M, Ciclitira PJ (1994) *Lancet* 26(343):758–761
- Marsh MN, Morgan S, Ensary A, Wardle T, Lobley R, Mills C, Auricchio S (1995) *Gastroenterology* 108:A871
- Fraser JS, Engel W, Ellis HJ, Moodie SJ, Pollock EL, Wieser H, Ciclitira PJ (2003) *Gut* 52:1698–1702
- Mantzaris G, Jewell DP (1991) *Scand J Gastroenterol* 26:392–398
- Riha WE, Izzo HV, Zhang J, Ho CT (1996) *Crit Rev Food Sci Nutr* 36:225–255
- Sjöström H, Lundin KEA, Molberg O, Körner R, McAdam SN, Anthonsen D, Quarsten H, Noren O, Roepstorff P, Thorsby E, Sollid LM (1998) *Scand J Immunol* 48:111–115
- Molberg O, Mcadam SN, Korner R, Quarsten H, Kristiansen C, Madsen L, Fugger L, Scott H, Noren O, Roepstorff P, Lundin KE, Sjöstrom H, Sollid LM (1998) *Nat Med* 4:713–717
- Anderson RP, Degano P, Godkin AJ, Jewell DP, Hill AVS (2000) *Nat Med* 6:337–342
- Arentz-Hansen H, Korner R, Molberg O, Quarsten H, Vader W, Kooy YM, Lundin KE, Koning F, Roepstorff P, Sollid LM, McAdam SN (2000) *J Exp Med* 191:603–612
- Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM, Sollid LM, Khoshla C (2002) *Science* 297:2275–2279
- Osman AA, Richter T, Stern M, Mothes T (1996) *Clin Chim Acta* 255:145–152
- Okita TW, Cheesbrough V, Reeves CD (1985) *J Biol Chem* 260:8203–8213
- Scheets K, Hedgcoth C (1988) *Plant Sci* 57:41–50
- Hull GA, Halford NG, Kreis M, Shewry PR (1991) *Plant Mol Biol* 17:1111–1115
- Wieser H, Belitz Z (1992) *Z Lebensm Unters Forsch* 194:229–234
- Kasarda DD, Okita TW, Bernardin JE, Baecker PA, Nimmo CC, Lew EJ-L, Dietler MD, Greene FC (1984) *Proc Natl Acad Sci USA* 81:4712–4716
- Arentz-Hansen EH, McAdam SN, Molberg O, Kristiansen C, Sollid LM (2000) *Gut* 46:46–51
- Frank R (1992) *Tetrahedron* 48:9217–9232
- Kramer A, Schneider-Mergener J (1998) *J Methods Mol Biol* 87:25–39
- Osman AA, Richter T, Stern M, Conrad K, Henker J, Brandsch C, Zimmer K-P, Mothes T (2002) *Eur J Gastroenterol Hepatol* 14:1217–1223
- Vader LW, de Ru A, van der Wal Y, Kooy YM, Benckhuijsen W, Mearin ML, Drijfhout JW, van Veelen P, Koning F (2002) *Exp Med* 195:643–649
- Méndez E, Vela C, Immer U, Janssen FW (2005) *Eur J Gastroenterol Hepatol* (in press)
- Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A, Goldblum SE. (2000) *Lancet* 355:1518–1519
- Londai M, Maiuri L (2004) *J Gastroenterol Hepatol Nutr* 39:S729
- Lundin KE, Nilsen EM, Scott HG, Loberg EM, Gjoen A, Bratlie J, Skar V, Mendez E, Lovik A, Kett K (2003) *Gut* 52:1649–1652
- Arentz-Hansen H, Fleckenstein B, Molberg O, Scott H, Koning F, Jung G, Roepstorff P, Lundin KE, Sollid LM (2004) *PLoS Med* 1:e1
- Vader W, Kooy Y, Van Veelen P, De Ru A, Harris D, Benckhuijsen W, Pena S, Mearin L, Drijfhout JW, Koning F (2002) *Gastroenterology* 122:1729–1737
- García E, Llorente M, Hernandez A, Kieffer K, Wieser H, Méndez E (2005) *Eur J Gastroenterol Hepatol* 17:529–539
- Ellis HJ, Rosen-Bronson S, O'Reilly N, Ciclitira PJ (1998) *Gut* 43:190–195
- Brett GM, Mills EN, Bacon J, Wellner N, Husain RD, Tatham AS, Shewry PR, Morgan MR (2002) *Biochim Biophys Acta* 1594:17–26
- Skerritt JH, Hill AS, Andrews JL (2000) *J Cereal Sci* 32:259–279
- Tanabe S (2004) *J Nutr Sci Vitaminol (Tokyo)* 50:367–370
- Battais F, Mothes T, Kanny G, Guérin L, Popineau Y, Bodinier M, Moneret-Vautrin DA, Denery-Papini S (2005) *Allergy* 60:815–821
- Osman AA, Günnel T, Dietl A, Uhlig HH, Amin M, Fleckenstein B, Richter T, Mothes T (2000) *Clin Exp Immunol* 121:248–254
- Schwartz E, Kahlenberg F, Sack U, Richter T, Stern M, Conrad K, Zimmer K-P, Mothes T (2004) *Clin Chem* 50:2370–2375
- Tatham AS, Shewry PR, Belton PS (1985) *Biochem J* 232:617–620
- Tatham AS, Marsh MN, Wieser H, Shewry PR (1990) *Biochem J* 270:313–318
- Tatham AS, Shewry PR (1995) *J Cereal Sci* 22:1–16
- Clark MR, Massenburg D, Zhang M, Siemasko K, Ann NY (2003) *Acad Sci* 987:26–37