Proceedings of the 34th Meeting

WORKING GROUP on PROLAMIN ANALYSIS and TOXICITY

Edited by
Peter Koehler

15 - 16 October 2020
Postal, Italy

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Cover picture*
Dr. Schaer AG / SPA

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* Cover picture: View of the Dr. Schaer company building, location of the 34th PWG-meeting, 2020

Preface

The 34th meeting of the Working Group on Prolamin Analysis and Toxicity (PWG) was different from all previous PWG meetings. Planned as a face-to-face-meeting hosted by Dr. Schaer in Postal, Italy, the global Covid 19 pandemic made this type of meeting impossible. Due to the flexibility of the hosts Jacqueline Pante and Fabiana Saorin, it was possible to hold the meeting as an online event with a small core group of the Italian PWG-members present in Postal. Carlo Catassi was among them and spontaneously volunteered to be the on-site moderator of the meeting and except from the beginning and the end of the meeting, he accompanied the participants through the scientific programme. All other group members and the audience participated online. Apart from the group members, the audience comprised an invited speaker, guests from academia, industry, and international coeliac societies. Representatives from cereal starch producers, producers of gluten-free foods, as well as manufacturers of kits for gluten analysis participated from industry. In spite of the online format, the audience was very interested, and the presentations were lively discussed.

Analytical and clinical work in the field of CD, non-coeliac gluten/wheat sensitivity (NCGS/NCWS), gluten and amylase-trypsin inhibitors done by PWG members were presented in seven talks and discussed at the two meeting sessions. The symposium "Triggers and drivers of coeliac disease" comprised four presentations of PWG members and one invited speaker and highlighted the main drivers that are responsible for triggering CD. In addition, one presentation focussed on regulatory aspects of gluten analysis and labelling. In particular, the issue of quantitating gluten in fermented foods such as beer by competitive ELISA was an important part of this talk.

I would like to express my thanks to all participants of the meeting for their active contributions and the discussions that resulted thereof. I am in particular grateful to Fabiana Saorin and Jacqueline Pante for their flexibility and dedication, and Carlo Catassi for being the moderator in Postal. This made the meeting a success in spite of the general restrictions due to the corona pandemic. Finally, I express my gratitude to all friends, colleagues, sponsors and participants for their inspiration and ongoing support of the PWG and the meeting.

Esslingen, December 2020 Peter Koehler

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1 Executive summary

Twelve presentations covered aspects related to gluten, coeliac disease (CD) and other relevant hypersensitivities, amylase-trypsin inhibitors (ATI) as well as legal issues. Most authors have sent abstracts that are compiled in this proceedings book. Starting with analytical aspects of gluten, the programme included breeding for low occurrence of CD-active epitopes, and genetic modification to reduce or eliminate ATI from wheat. Furthermore, therapies of CD were reviewed, the pathomechanism of CD and the gluten-free diet were covered and reasons for the onset of CD from several perspectives were presented.

Analytical session

Four presentations were given in this session. One presentation was about the analysis of gluten to detect coeliac disease-relevant epitopes by LC-MS/MS. Linked to this topic was a report on activities for producing novel reference materials for gluten analysis. A main point in this presentation was, that the PWG would take the lead in preparing a new gliadin reference material, and that an isolated protein preparation is preferred over flour because of limited stability of flour. Another talk described ongoing research from the Wageningen group on the generation of wheat with low occurrence of CD-active epitopes and this included traditional breeding strategies as well as the CRISPR/Cas9 approach. Finally, RNAi and CRISPR/Cas9 approaches were reported to reduce or eliminate ATI from wheat.

Clinical session

This session included three presentations and started with a comprehensive overview on approaches for the therapy of CD. The second talk dealt with the immunophenotypic and functional changes occurring in the gut biopsies of pediatric patients with potential- or acute CD and showed a direct correlation between the number of TCRγδ+ cells and the serum levels of anti-TG2 in CD patients. The last presentation of this session was on the measurement of contaminating gluten in the daily diet of CD children following a gluten-free diet. It was found that in all investigated children the daily gluten intake was always well below the safety threshold of 10 mg/day.

Symposium: Triggers and drivers of coeliac disease”

Four presentations of the symposium covered the currently relevant triggers of CD starting with the relevance of specific gluten peptides and continuing with the role of viruses and bacteria. A very interesting finding is that specific bacteria produce peptides with amino acid sequences very similar to gluten peptides. The last presentation on T cell immunology in CD showed gluten-specific, HLA-DQ restricted CD4+ T cells as the critical checkpoint and effector cell in CD with a concurrent importance in the innate gluten-induced responses in CD.

Speakers at the 34th Meeting of the Working Group on Prolamin Analysis and Toxicity (PWG), held online in Postal, Italy, 15 – 16 October 2020.

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3 Programme

Thursday, 15 October 2020

14:00 Opening of the meeting
Carlo Catassi, Peter Koehler, Jacqueline Pante, Hansjoerg Prast

Analytical research reports

14:10 Analysis of gluten to detect coeliac disease-relevant epitopes
Marie Lay, Freising, Germany

14:30 Wheat lines with specific ATI genes silenced by RNAi and CRISPR-Cas9 for the understanding of their role in Non Celiac Wheat Sensitivity
Stefania Masci, Viterbo, Italy

15:00 Wheat modified for low occurrence of CD epitopes
René Smulders, Wageningen, The Netherlands

15:20 Looking beyond PWG-gliadin at future reference materials for gluten
Katharina Scherf, Karlsruhe, Germany

15:40 Break

Clinical research reports

16:30 Update on clinical studies for pharmacological treatment of coeliac disease
Detlef Schuppan, Mainz, Germany

16:50 Densities of IL4+ and TCRγδ+ T cell subsets as biomarkers of intestinal mucosa damage in coeliac disease
Carmen Gianfrani, Naples, Italy

17:30 Quantification of contaminating gluten into the GFD of Italian children
Carlo Catassi, Ancona, Italy

17:50 End of day 1

18:00 The Prolamin Working Group Executive Meeting (members only)

Friday, 16 October 2020

Symposium "Triggers and drivers of coeliac disease"

14:10 Gliadin and its peptide 31-43 as a proinflammatory molecule
Riccardo Troncone, Naples, Italy

14:30 The role of viruses as triggers of coeliac disease
Valentina Discepolo, Naples, Italy

15:00 The role of bacteria as triggers of coeliac disease
Frits Koning, Leiden, The Netherlands

15:20 The adaptive CD4+ T cell response; current understanding of immunobiology and implications for diagnosis and clinical trials
Knut Lundin, Oslo, Norway

15:40 Discussion of symposium talks

16:00 Break

Discussion of current developments concerning gluten analysis, clinical and legal aspects

16:30 Update on regulatory issues of gluten
Hertha Deutsch; Vienna, Austria

16:50 General discussion

17:10 Outline: Action plan 2021 of the Prolamin Working Group
Peter Koehler, Esslingen, Germany

17:30 End of PWG meeting
Peter Koehler, Esslingen, Germany

4 Analytical research reports

4.1 Analysis of gluten to detect coeliac disease relevant epitopes

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Abstract

Coeliac disease (CD) is a chronic immune-mediated inflammatory disease of the small intestine, triggered by the ingestion of gluten. So far, 38 T-cell epitopes consisting of nine amino acids each are known that provoke the adaptive immune reaction in CD patients.

The aim of the project is the development of a comprehensive liquid chromatography tandem mass spectrometry (LC-MS/MS) method to detect peptides with at least one CD-active epitope in different wheat flours and products.

Based on the T-cell epitopes, 27 epitopes occurring in wheat proteins were identified by reversing the transglutaminase-mediated deamidation of specific glutamine residues. In a second step, the wheat proteins containing these epitopes were identified.

Isolated wheat gluten samples and wheat flours were prepared according to a bottom-up proteomics workflow using chymotrypsin and trypsin for enzymatic digestion. The generated peptides were analysed with a tripleTOF-LC-MS/MS system. Based on the about 1900 identified peptides containing at least one CD-active epitope, marker peptides were selected. These marker peptides were analysed with a QTRAP-LC-MS/MS system in the flours of ten different common wheat cultivars and one commercial wheat flour.

The first results of the relative quantification of the marker peptides in the different common wheat flours showed differences between the samples. Especially the cultivar Winnetou showed higher amounts of peptides belonging to the ω-gliadin proteins compared to the other cultivars. The highest relative proportion of the peptides belonged to peptides of the α-gliadin fraction. To interpret these results, it should be taken into account that also the ratio of the α-gliadin fraction within gluten proteins and of the α-gliadin peptides within the marker peptides are the highest.

The next step of the project will be the absolute quantification of the marker peptides in a large sample set of different common wheat, emmer, einkorn, spelt and durum wheat cultivars.

4.2 Wheat lines with specific ATI genes silenced by RNAi and CRISPR-Cas9 for the understanding of their role in Non Celiac Wheat Sensitivity

Stefania Masci[1], Francesco Camerlengo[1], Stefano D'Amico[2], Sandra Denery-Papini[3], Angela Doherty[4], Arianna Frittelli[1], Shahidul Islam[5], Raviraj M. Kalunke[1], Domenico Lafiandra[1], Colette Larré[3], Roberta Lupi[3], Wujun Ma[5], Damiano Martignago[4], Francesco Sestili[1], Caroline Sparks[4], Silvio Tundo[1]

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Abstract

Non Celiac Wheat Sensitivity (NCWS) is gaining a great importance, due to its diffusion, but the real culprit has not been ascertained yet. Among the most likely candidates, there are alpha-amylase/trypsin inhibitors (ATI) that are involved also in some respiratory allergies. These latter polypeptides are a group of exogenous protease inhibitors, which are encoded by a multigene family dispersed over several chromosomes in durum and bread wheat. With the aim of obtaining wheat genotypes showing a lower amount of these proteins, we have pursued this goal by using two methods, RNAi and CRISPR-Cas9 silencing. Silencing by RNAi interference that is considered a totally transgenic technique has been carried out on the bread wheat cultivar Bobwhite on the three ATI genes *CM3*, *CM16* and *0.28*, whereas the durum wheat cultivar Svevo has been silenced by CRISPR/Cas9 by using a multiplexing strategy to edit the two ATI genes *CM3* and *CM16*, and a marker-free approach, that makes these latter lines potentially non-transgenic.

RNAi silenced lines do not show differences in terms of yield, have an effective decrease of the target genes, but also a range of pleiotropic effects, including a higher trypsin inhibition and a strong decrease in HMW-GS accumulation. They generate a lower reaction when tested with sera of patients allergic to wheat, accounting for the important role of the three target proteins in wheat allergies.

As regards the genome-edited lines, they also show absence of the target genes, but also the activation of the ATI 0.28 pseudogene present in durum wheat, as a pleiotropic effect.

The development of wheats accumulating a lower amount of ATI, not only allows to use them as a basis for the production of varieties with a lower impact on adverse reaction, but also to test if these proteins are actually implicated in those pathologies for which the triggering factor has not been established yet, as is the case for NCWS.

4.3 Wheat modified for low occurrence of CD epitopes

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Abstract

In wheat, coeliac disease (CD) epitopes occur mostly in gliadins, while the baking quality is determined predominantly by glutenins. Thus, removing and mutating gliadins can be used to lower the immunogenicity of wheat. Nevertheless, plant breeding cannot generate bread wheat that is safe for coeliac disease patients while retaining baking quality solely by combining natural or randomly induced mutations in gliadins and glutenins, due to the large number of genes in these gene families that have to be modified or removed, and because these genes are closely linked in clustered loci on the three genomes of bread wheat. Combining recently developed biotechnological approaches with classical breeding now offers the opportunity to change this situation.

A good starting point would be to use lines with deletions of some of the major gliadin loci. Deletion lines already exist, but lines with smaller, more focussed chromosomal deletions may be generated using γ-irradiation or fast neutrons, and these may have improved performance as a crop. CD-safe barley has been produced by combining such chromosomal deletions.

Next, various approaches may be used to lower the expression of the other gliadin genes. One interesting approach is to introduce the recessive, low-prolamin mutation lys3a. Other approached that may be used are RNAi of gliadin gene families, or RNAi of DEMETER, a gene necessary for activation of storage protein genes during wheat endosperm development. However, as the RNAi construct must remain present, this classifies as a GM approach.

Subsequently, the CD epitopes in the remaining gliadins that are expressed may be modified using gene editing with CRISPR/Cas, or by base editing. As the major epitopes are well known, the targets are clear. The challenge is to deal with the multiple loci that need to be modified, but recent improvements in the regeneration of wheat make this more realistic. Selection and screening must initially be done at DNA and protein level and confirmed with T cell tests, etc. However, once a clustered locus on a chromosome is devoid of major epitopes, it can be combined with other hypoallergenic loci through regular crossing and selecting, and these loci may be tracked in a breeding programme using linked molecular markers.

Thus, in the countries where targeted mutagenesis using gene editing is not considered as genetic modification, developing coeliac-safe wheat now appears doable. An intermediate product will be low-gluten wheat varieties.

4.4 Looking beyond PWG-gliadin at future reference materials for gluten

Katharina Scherf

Karlsruhe Institute of Technology (KIT), Institute of Applied Biosciences, Department of Bioactive and Functional Food Chemistry, Karlsruhe, Germany

Abstract

Reference materials are essential to validate analytical methods, calibrate instruments, verify laboratory performance, ensure quality control and estimate uncertainty. There is no certified reference material available for gluten, mainly because its protein composition is very complex and depends on genetic and environmental variability. Prolamin Working Group (PWG)-gliadin was isolated from a mixture of 28 representative European wheat varieties and the material is purified, homogeneous, completely soluble in 60% ethanol, stable, well-characterised and used to calibrate ELISA test kits and other methods for gluten detection. However, as the supply is limited, a new reference material for gluten needs to be developed.

The first discussions among the gluten reference material team concluded that a blend of five or more varieties of wheat, rye or barley from different continents appears to be most suitable to cover the genetic variability while evening out year-to-year variations of protein content and composition. The varieties should be widely used and milled to white flour for reasons of stability. Concerning long-term stability, the PWG continues to support the use of protein isolates, because PWG-gliadin has been stable for almost 20 years now when kept frozen at -80 °C. Lab-scale experiments using five varieties of wheat including Akteur (Germany), Carberry (Canada), Mv Magvas (Hungary), Yitpi (Australia) and Yumai-34 (China), as well as their blend, confirmed that gluten and gliadin isolates can be prepared while keeping the original protein composition of the flour. Further experiments based on ELISA, gel electrophoretic and chromatographic techniques are currently underway to select five suitable rye and barley varieties each from a collection of 123 barley and 57 rye varieties.

Based on its expertise and previous work with the PWG-gliadin reference material, the PWG will take the lead in the production of a new reference material for gluten and provide the new material to all stakeholders. The PWG agreed that a new gliadin isolate seems to be most appropriate for various applications such as clinical research and compliance monitoring of gluten-free products.

5 Clinical research reports

5.1 Update on clinical studies for the pharmacological treatment of coeliac disease

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Abstract

Coeliac disease (CD) is a small intestinal inflammatory condition that affects up to 2% of most populations worldwide (1). CD is triggered and maintained by the ingestion of gluten from wheat and related grains and occurs only in carriers of the human leukocyte antigens (HLA) DQ2 or DQ8, a necessary but not sufficient genetic precondition for the manifestation of CD. Classical symptoms of CD include diarrhea, weight loss, and malnutrition, but CD often manifests with nonspecific or atypical symptoms, or combined with autoimmune diseases that share the same major genetic predisposition as CD, HLA DQ2 or DQ8 (2-5).

The currently only treatment for CD is the life-long adherence to a strict gluten-free diet (GFD), i.e., complete avoidance of even traces of gluten in the diet, but maintaining the GFD poses significant practical and social challenges. Moreover, some patients are exquisitely sensitive to hidden traces of gluten, and up to 50% of celiacs do not show complete mucosal healing after one year of the GFD (6,7). Therefore, there is a need for an efficient (supportive) pharmacological therapy as add-on to the GFD to prevent complications, such as consequences of malabsorption and possibly autoimmunity and malignancy that can be due to continuous (minor) gluten ingestion (8).

Several pharmacological therapies to prevent gluten induced mucosal damage in patients with CD, and some made it towards phase 1b-3 clinical dose finding and efficacy studies, and the best study design for phase 2 is the gluten challenge in patients in remission and histological readout, to be complemented by patient related outcome measures (9). The most prominent or promising therapeutic approaches are shortly discussed:

1. Oral proteases that cleave immunogenic gluten peptides that otherwise escape intestinal digestion: While a phase 2a study showed promise for the combination of the barley germinating seed glutamine specific with a microbial proline specific endoprotease (Alvine), the *real life* phase 3 study failed (10,11). Currently, a phase 2 study with a synthetic glutenase (Kuma030, Takeda), with a more than 100fold

higher in vitro activity to degrade immunogenic gluten peptides, is ongoing (12). A major challenge for enzyme therapy remains: to secure rapid and complete enzymatic digestion of immunogenic gluten peptides that are embedded in a complex food matrix. This must occur within the stomach and proximal small intestine before these peptides reach the mucosal immune system of the small intestine (13).

2. Blocking antibody to IL-15: This based on a prominent role of epithelial and immune cell derived IL-15 as a driver of intestinal inflammation in CD. However, using the antibody AMG 714 (Amgen/Provention Bio) a phase 2 study showed no protection from mucosal damage, although patient symptoms improved (14). An IL-15 antibody that has a much higher potency to block the IL-15 receptor (Calypso) will be tested in a phase 1b study in 2021.
3. Tolerizing therapies: The aim is to induce tolerance to gluten in CD patients. While a long lasting development of a tolerizing vaccine, utilizing 3 major gluten antigenic epitopes finally failed (Nexvac (15)], the encapsulation of immunogenic gluten in PLGA nanoparticles that target myeloid cells to induce intestinal tolerance showed efficacy in a CD mouse model in vitro (16), and signs of efficacy in a phase 1b study in patients (TAK-101, Takeda). Another approach employs immunogenic gluten peptide-loaded and liver sinusoidal endothelial cell targeted nanoparticles that effectively dampened experimental multiple sclerosis. This has effectively suppressed the development of Multiple Sclerosis in Mice (17). A phase 1b study is planned for 2021.
4. Transglutaminase (TG) inhibitors: The aim is to block the conversion of gluten peptides that exhibit with low immunogenicity to deamidated gluten peptides that are optimally presented on HLA-DQ2 or -DQ8 to induce intestinal T cell activation and inflammation (18-20). This approach has shown proof-of-concept in *in vitro* systems and in an IL-15 transgenic celiac mouse model, where a general transglutaminase inhibitor attenuated gluten-induced T cell activation (21). A phase 2 clinical study using a highly specific oral inhibitor of TG2, the CD-specific transglutaminase, in 160 CD patients in remission and challenged with gluten for 6 weeks will be published soon.

In conclusion, several pharmacological studies aiming at protection of CD patients from gluten induced intestinal inflammation have been developed and are currently being assessed in clinical efficacy studies. CD therapeutics have become a “hot field” in view of, e.g., the saturated area of therapeutics for chronic inflammatory bowel disease. The aim is to abolish the detrimental effect of up to ~3-5 grams of daily gluten exposure, equivalent to 15-30% of normal gluten consumption. Drugs that mainly act in the intestinal mucosa, protecting it from immune activation, and tolerizing approaches that may provide longterm protection appear most attractive.

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5.2 Densities of IL4+ and TCRγδ+ T cell subsets as biomarkers of intestinal mucosa damage in coeliac disease

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Abstract

Coeliac disease (CD) comprises several clinical conditions, all characterised by the presence of HLA-risk alleles and specific antibodies. Furthermore, a large spectrum of intestinal lesions is reported that ranges from morphologically normal mucosa, condition known as potential-CD, to villous atrophy, typical of acute disease. It is envisaged that the disruption of the immunological balance is responsible of normal to atrophic mucosa transition. As the immune mechanisms underlying the CD natural history are not completely elucidated, we investigated the immunophenotypic and functional changes occurring in the gut biopsies of pediatric patients with potential- or acute-CD. Forty-seven young subjects underwent endoscopy for suspicion of CD disease. Nineteen children had a diagnosis of acute CD (CD, mean age 5.9 yrs), 16 had normal mucosa but were positive for anti-tissue transglutaminase (TG2) antibody, (potential-CD, mean age 8.7 yrs), and 12 non-coeliac control subjects (HC, mean age 6.3 yrs). Cell phenotype (CD3, CD4, CD8, and TCRγδ+ T cells) and cytokine production (INF-γ, IL4, IL21, IL17) were analysed by flow cytometry either in ex-vivo mucosal cells or in short-term T-cell lines.

T cells bearing the TCRγδ (TCRγδ+ cells) were markedly increased in children with overt-CD compared to potential-CD or HC ($p<0.05$). In contrast, T cell producing IL4 (IL4+ cells) were significantly increased in potential-CD and HC ($p<0.05$). An indirect correlation between the frequency of TCRγδ+ and IL4+ cells was observed in all children enrolled ($r=-0.5141$, $p=0.0013$). A direct correlation was found between the number of TCRγδ+ cells and the serum levels of anti-TG2 in CD patients (both overt- and potential-CD), ($r=0.4635$, $p=0.0086$). Conversely, IL4+ cells indirectly correlated with the anti-TG2 titers ($r=-0.5863$, $p=0.0013$). In conclusion, the transition to villous atrophy in CD patients is characterised by the expansion of TCRγδ+ cells concomitantly with the disappearance of IL4+ cells in gut biopsies. These findings, along with the indirect correlation between the anti-TG2 titers and IL4+ cell frequency, suggest that a shift from Th2 to Th1 phenotype of mucosa infiltrating T lymphocytes occurs in the transition from potential- to acute-CD.

Further studies are required to validate the IL4+ and TCRγδ+ cells as biomarkers of the different CD forms. If these pilot findings will be confirmed, the combined detection of these two cell subsets by flow cytometry could support the diagnosis of CD, mainly in case of histological pitfall or borderline value of CD-serology. Furthermore, the combined assessment of these cellular biomarkers could also provide a laboratory tool for the clinical management of potential-CD patients.

5.3 Contamination of gluten in the gluten-free diet: a quantitative study in children with coeliac disease

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Abstract

Background: A strict gluten-free diet (GFD) is notoriously difficult to maintain. Protracted ingestion of gluten traces (10-50 mg/day) is sufficient to cause significant damage in the architecture of the small intestinal mucosa in patients on treatment for coeliac disease (CD). Only few data are available on the daily intake of contaminating gluten in treated CD patients.

Objective: The aim of this study was to directly measure the level of contaminating gluten in the daily diet of CD children following a GFD.

Design: From April 2019 to December 2019, consecutive CD children (2-18 years old) on GFD for ≥ 6 months were offered to participate in the study. Patients and their caregivers were invited to provide a representative portion (about 10 g) of all meals consumed during a 24-hour period. The participants were requested to weight all ingested food and report items in a 24-hour food diary and to document brand, and ingredients. Gluten content of all food samples was quantified by R5 sandwich enzyme-linked immunosorbent assay method.

Results: Overall, 12/448 (2.73%) food samples contained detectable gluten contamination; of them, 11 (92%) contained 5-20 part per million (ppm) and 1 (8%) >20 ppm. The 12 contaminated food samples belonged to 5 of the 69 enrolled patients. In all investigated children the daily gluten intake was always well below the safety threshold of 10 mg/day.

Conclusions: The present findings suggest that in a country characterised by high CD awareness, the daily unintended exposure to gluten of treated CD children is very low; reassuringly, the presence of gluten traces did not lead to exceed the tolerable threshold of 10 mg/day of gluten intake in the GFD. These favourable results may be explained by several factors: (a) pediatric age of investigated subjects. The diet of children is more easily and fully controlled by the caregivers; (b) inclusion of highly compliant patients who are regularly seen at the Coeliac Clinic; (c) generalised conformity of GF products marketed in Italy with the International regulations for labelled gluten-free food; (d) high level of awareness of the requirement of the GFD by the general population in Italy, particularly due to the national Coeliac Protection law (n.123/2005) and the pro-active role of the Italian Coeliac Association.

6 Symposium: Triggers and drivers of coeliac disease

6.1 Gliadin and its peptide 31-43 as proinflammatory molecules

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Abstract

Coeliac disease (CD) is an immune-mediated enteropathy triggered in genetically susceptible individuals by a group of wheat proteins (commonly called gluten) and related prolamins from toxic cereals. The HLA-restricted gliadin-specific intestinal T cell response plays a central role in the pathogenesis of CD. A central question remains unanswered, why a pro-inflammatory T cell response is generated instead of a regulatory response, which normally promotes oral tolerance to dietary protein antigens. In an inflamed environment enriched in cytokines such as IL-15 or type I interferons, T cells tend to acquire a pro-inflammatory phenotype. Mice-based studies as well as epidemiological data have suggested viral infections to create such an environment. However, a number of other factors may contribute to the generation of a "sterile" inflammation. Most of the evidence point to gliadin itself and in particular, a peptide from the N-terminal portion of α-gliadin, named peptide p31-43.

The p31-43 peptide is part of the p31-55 peptide from α-gliadins that remains undigested for a long time, and can be present in the small intestine after ingestion of a gluten-containing diet. Different biophysical methods and molecular dynamic simulations have shown that p31-43 spontaneously forms oligomeric nanostructures. Experimental approaches using in vitro assays, mouse models, and human duodenal tissues have shown that p31-43 is able to induce different forms of cellular stress by driving multiple inflammatory pathways. Increased proliferative activity of the epithelial cells in the crypts, enterocyte stress, activation of TG2, induction of Ca2+, IL-15, and NFκB signalling, inhibition of CFTR and activation of the inflammasome platform are some of the biological effects of p31-43.

One possible mechanism candidate as responsible for this condition of inflammatory environment is the alteration of vesicular trafficking. Interestingly in fact, inducing a

delay of the endocytic trafficking by silencing the HRS protein, produces at cellular level the same alterations that p31-43 does. Another important question to be answered is why p31-43 affects particularly CD patients? The answer may reside in the constitutive alterations present in coeliac subjects. These involve several biological pathways, such as signalling/proliferation, stress/innate immune response and inflammation, ultimately due to the alterations of vesicular trafficking.

In conclusion, CD is the prototype of a chronic inflammatory disorder induced by dietary components. p31-43 comes into the spotlight as an important player in CD pathogenesis. Its particular conformation and its ability to induce different forms of cellular stress drive multiple inflammatory pathways, which, in the presence of appropriate susceptibility and environmental factors, may act together to drive the disease.

6.2 The role of viruses as triggers of coeliac disease

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No abstract provided.

6.3 The role of bacteria as triggers of coeliac disease

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No abstract provided.

6.4 T cell immunology in coeliac disease (the Oslo experience)

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Abstract

The role of the adaptive immune system, and particularly the recognition of gluten by CD4+ T cells from the gut, has been the focus of the Oslo group under the leadership of professor Ludvig M. Sollid for decades (1). This summary gives a short overview on some aspects. In 1989 Sollid reported that «all» coeliacs expressed certain HLA-DQ molecules, directly pointing to the possibility that gluten-reactive T cells were involved (2). The presence of such T cells was published some very few years later, a major break-through in this research field (3, 4). The next milestone was reached when the immunogenic peptides were defined and we and others in the late 1990's showed that they were modified by the enzyme Transglutaminase 2 (5). Similar observations were soon reported by others (6, 7). The anti-gluten T cell response is «strong», the frequency of gluten-specific T cells in the gut is in the range 0,5 – 1,8% and the same cell persist for decades (8, 9). Direct demonstration of such CD4+, gluten specific T cells by HLA-DQ:gluten tetramers in the blood may be used as a diagnostic test (10) and immunobiological studies have shown how they are key drivers of the immunopathology in this disease (11). Importantly, gluten challenge experiments show rapid activation of the gluten-specific T cells in all patients (12), and secretion of the T cell cytokine Interleukin-2 correlate very well with symptoms like nausea and vomiting after challenge (13). Taken together, these findings all points to the gluten-specific, HLA-DQ restricted CD4+ T cells as the critical checkpoint and effector cell in coeliac disease, and that they control much of the innate gluten-induced responses in coeliac disease as well.

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7 Statements on current developments concerning gluten analysis, clinical and legal aspects

7.1 Update on regulatory issues of gluten

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Abstract

AOECS, the Association Of European Coeliac Societies, has Observer status in the Codex Alimentarius Commission since 1992. In the past months, some items important for coeliacs regarding the gluten-free diet were discussed in the Codex Committee on Food Hygiene (CCFH) in November 2019 and in the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) in November 2019. Because of the Covid-19 situation, the Codex Alimentarius Commission (CAC) was hold in virtual sessions in several days in September, October and November 2020. The reports of the sessions are published on the Codex website www.fao.org. A short summary follows:

CCFH: The Committee agreed to forward the Draft Code of Practice on Food Allergen Management for Food Business Operators (COP) for adoption at Step 8 (Appendix II, page 24 - 44 of the report); to inform the Codex Committee on Food Labelling (CCFL) of the status of the work; and that the COP could be revised upon completion of the work on precautionary allergen labelling in CCFL and advice from FAO/WHO.

CCNFSDU: At the CCMAS session in May 2019, CCMAS agreed to refer the AACCI proposal to delete „Gluten-free Foods“ in the Commodity of the Codex Standard 234 - 199 and replace it with „Corn- and Rice-Based Gluten-Free Foods“ and „Oat-Based Gluten-Free Foods“ to CCNFSDU for consideration. CCNFSDU noted that it was premature to consider this proposal as research is still ongoing to determine the most appropriate method for determination of gluten and agreed to wait for the completion of ring trial tests and to consider this matter at a future date when more information became available. Furtheron, the Committee agreed to align section 5.2 of the Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten (CXS 118 - 1979) with the wording from the Procedural Manual. That means that any method of analysis will only be contained in the Codex Standard 234 - 1999 and not in individual Standards. The Committee agreed to submit this editorial amendment to CXS 118-1979 to CAC for adoption.

CAC: The Draft Code of Practice on Food Allergen Management for Food Business Operators and the editorial amendment to CXS 118-1979 were adopted.

In the USA, the FDA issued a final rule on the gluten-free labeling of fermented or hydrolyzed foods on August 12, 2020. The PWG statement to this rule is available.

8 Perspectives and action plan of the PWG

Peter Koehler

Biotask AG, Esslingen, Germany

The Prolamin Working Group executive meeting and joint discussion held on 15 October 2020, led to the decisions and statements outlined below.

Action plan

I. Analytical

- The PWG gliadin reference material is available from Arbeitsgemeinschaft Getreideforschung e.V. (Association of Cereal Research), Mr. Tobias Schumacher, Schuetzenberg 10, 32756 Detmold, Germany, E-mail: info@agf-detmold.de. The PWG has set the price for one batch (100 mg) to 150 Euro.
- The collaborative study *Gluten in a broad range of food ingredients and food products by Quantitative Enzyme Immunoassay R-Biopharm RIDASCREEN® Gliadin Test Kit* supervised by Katharina Scherf was carried out in 2020.
- The PWG will take the lead in preparing new gliadin reference material. An isolated protein preparation is preferred over flour because of limited stability of flour. Possible production facilities for the reproduction of PWG-gliadin reference material will be identified.

II. Clinical

- The PWG keeps considering becoming a working group under the umbrella of the International Society For The Study Of Celiac Disease

III. Members, Policy

- Stefania Masci, University of Tuscia, Viterbo, Italy is a new member of the group.
- Olivier Tranquet, INRAE, Nantes, France left the group and will be replaced by Sandra Denery from the same institute.
- Bob Anderson, Wesley Medical Research Ltd, Brisbane, Australia has been suggested as a new member of the group.
- A joint position paper *Recent Progress and Recommendations on Celiac Disease From the Working Group on Prolamin Analysis and Toxicity* has been published Open Access in March 2020 (doi: 10.3389/fnut.2020.00029).
- A joint opinion letter *Statement of the Prolamin Working Group on the Determination of Gluten in Fermented Foods Containing Partially Hydrolyzed Gluten* has been published Open Access in December 2020 (doi: 10.3389/fnut.2020.626712).

- An Open Access publication with the working title *Update on gluten analysis and considerations on the effect of low gluten doses on intestinal health* has been suggested by Carlo Catassi.
- Proceedings of this meeting will be available free of charge in electronic form from the PWG website (http://www.wgpat.com).

Next meeting: 2021

We are very pleased to announce the venue for our next meeting:

Wageningen, The Netherlands

Hosts:

René Smulders, Twan America, Ingrid van der Meer, Peter Weegels
Wageningen University & Research
E-mail: rene.smulders@wur.nl

Time: 28 - 30 October 2021 (preliminary)

Focus of the meeting:

- Gluten analysis and clinical effects of low gluten doses

The format of the meeting will depend on the global Corona pandemic situation. At the moment, no decision has been taken.

The invitation and registration deadline will be sent in summer 2021.

Very special thanks to the hosts for this kind invitation!