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ELEVATED SERUM INTERLEUKIN-2 AFTER GLUTEN CORRELATES WITH SYMPTOMS AND IS A POTENTIAL DIAGNOSTIC BIOMARKER FOR COELIAC DISEASE

Short Title: Interleukin-2 and reactions to gluten in coeliac disease

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ABSTRACT

Background: Coeliac disease patients on gluten free diet experience reactions to gluten, but these are not well characterised or understood. Systemic cytokine release was recently linked to reactivation of gluten immunity in coeliac disease.

Aims: To define the nature and time-course of symptoms and interleukin-2 changes specific for coeliac disease patients.

Methods: Coeliac disease patients on gluten free diet (n=25) and healthy volunteers (n=25) consumed a standardised 6-gram gluten challenge. Coeliac Disease Patient Reported Outcome survey and global digestive symptom assessment were completed hourly up to 6 h after gluten. Adverse events over 48 h were recorded. Serum interleukin-2 was measured at baseline, and 2, 4, and 6 h.

Results: Serum interleukin-2 was always undetectable in healthy controls, whereas interleukin-2 was undetectable at baseline and elevated > 0.5 pg/ml at 4 h in 92% of coeliac disease patients. All patient reported outcome severity scores increased significantly after gluten in coeliac disease patients ($P < 0.001$ Wilcoxon signed rank test), but not controls. Symptoms began after 1 h, and peaked in the third. Nausea and vomiting characterised severe reactions, but mild reactions were limited to headache and tiredness. Peak interleukin-2 correlated with symptom severity, particularly for nausea, and vomiting.

Conclusions: Serum interleukin-2 elevations correlate with timing and severity of symptoms after gluten in coeliac disease. Standardised bolus gluten food challenge and interleukin-2 assessment could provide a valuable clinical test to monitor and diagnose coeliac disease in patients established on a gluten free diet.

Key words: Coeliac disease, cytokine release, diagnostic, gluten, gluten free diet, interleukin-2, patient reported outcome.

Abbreviations: Fermentable oligo-, di-, monosaccharides, and polyols (FODMAPs), human leukocyte antigen-DQ (HLA-DQ).

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INTRODUCTION

The use of acute single-dose oral food challenge is well established as an informative diagnostic maneuver and trial monitoring approach for food allergy, but has not previously been shown to be useful in coeliac disease.^{1,2} Coeliac disease is caused by ingestion of dietary gluten and activation of gluten-specific CD4⁺ T cells; chronic gluten exposure results in characteristic histological abnormalities in the duodenal mucosa and production of autoantibodies specific for transglutaminase.³ Symptoms caused by gluten in coeliac disease are generally considered non-specific, and delayed for days or weeks after gluten is reintroduced into the diet.^{4,5} Thus acute provocation testing has not played a role in the diagnosis of coeliac disease.⁶

There has been widespread adoption of gluten free diet by patients who have not been medically evaluated, but may have coeliac disease.^{7, 8} Diagnostic tests for coeliac disease, serology and duodenal histology, normalise with strict gluten exclusion.⁶ Genetic testing can rule out coeliac disease in patients on gluten free diet, but its clinical utility is limited because of the high prevalence of coeliac-associated HLA-DQ genotypes.^{9, 10} Consequently, gluten challenge for 2 to 6 weeks followed by gastroscopy with duodenal biopsy and serology is recommended to evaluate coeliac disease in patients on gluten free diet.⁶ There is no standardisation of diagnostic gluten challenges, and this approach is often impractical because troubling symptoms prevent continuation of gluten challenge for the period required to rule out coeliac disease.

Recently, we found that consuming gluten-containing food increases symptoms on the day it is consumed, and elevates serum interleukin-2 within hours in patients on gluten free diet who have coeliac disease.¹¹ Activated gluten-specific CD4+ T cells are implicated as the source of interleukin-2 because similar elevations of interleukin-2 follow administration of antigenic gluten peptides in patients with coeliac disease on gluten free diet. Furthermore, coeliac disease patients administered antigenic gluten peptides also experience acute digestive symptoms that mimic symptoms reported by coeliac disease patients after presumed gluten exposure.^{12, 13} These observations suggested that standardising a potent gluten challenge could enable a diagnostic oral challenge test that would also allow evaluation of cytokine responses and their relationship to acute symptoms caused by gluten exposure in coeliac disease patients on gluten free diet.

The aim of the present study was to define the nature and time-course of symptoms and interleukin-2 changes specific for coeliac disease patients on gluten free diet following an oral food challenge test that could serve as a diagnostic tool in coeliac disease.

METHODS

Participants and study design

The study design is outlined in Figure S1. Patients with coeliac disease on gluten free diet and healthy unaffected individuals consuming an unrestricted diet were separately studied to assess

the clinical and immunological effects of an unmasked gluten food challenge. The study in coeliac disease patients, which encompassed assessments and procedures on screening day 1 in the study “Nexvax2-1005” described in detail by Truitt et al ¹⁴, was approved by Bellberry Human Research Ethics Committee (Application No: 2017-12-962-A-2) and conducted at three sites in Australia from May to September 2018. The study was registered at ClinicalTrials.gov (NCT03543540). All patients gave written, informed consent prior to undergoing any trial-related procedures. Eligibility criteria for participants are listed in Table S1. Briefly, coeliac participants were aged from 18 to 70 y, with a medical diagnosis of coeliac disease based on assessment of duodenal biopsies, and had been on a gluten free diet for at least 1 y. The healthy unaffected, “true negative” cohort was recruited and studied separately from Nexvax2-1005 at a single Australian site from September to October 2018. Full eligibility criteria for the healthy volunteer study are listed in Table S1. Briefly, individuals who did not have coeliac disease and did not limit their dietary gluten intake gave written informed consent and underwent assessments and procedures aligned with those on the first day of screening in Nexvax2-1005. This study was approved by the Human Research Ethics Committees at the Walter and Eliza Hall Institute and Melbourne Health (identifiers 03/4 and 2003.009 respectively). Further details regarding the study protocols are available from the sponsor, ImmusanT, Inc. The sponsor designed studies and analysed the data.

In both studies, assessments performed on the same day, but before gluten challenge, included collection of demographics and medical history, and a review of prior/concomitant medications and procedures. Additional procedures in the coeliac study included review of medical reports supporting diagnosis of coeliac disease, and a coeliac disease characteristics survey (Figure S2).

Gluten challenge

Oral food challenge tests were performed using the contents of one custom-manufactured packet of vital wheat gluten flour that was thoroughly mixed in water, and consumed as a bolus by participants who had fasted overnight. Individual packets were filled with 11 g of a commercial-grade vital wheat gluten flour (Rutgers Food Innovation Center, Rutgers University, Bridgeton, New Jersey, USA). Eurofins Microbiology Laboratories Inc. (Lancaster, Pennsylvania, USA) tested food challenge material for protein and water content, and for microbes. Serial testing over

a 15-month storage period at room temperature confirmed the gluten challenge material, “vital wheat gluten flour”, contained 77-79% protein, 5.5-6.4% water, and was free of enteropathogens. Consistent with the approach adopted by others using the Osborne method to estimate gluten protein as 80% of total protein in wheat flour,^{15, 16} and assuming that approximately 1 g vital wheat gluten flour remained in the drinking vessel and was not consumed, participants would have ingested approximately 10 g vital wheat gluten flour containing 6 g gluten protein. Neogen Corp. (Lansing, Michigan, United States) performed the Veratox Gliadin R5-ELISA, which confirmed the presence of gluten in the test material. Content of fermentable oligo-, di-, monosaccharides, and polyols (FODMAPs) in food challenge material as well as plain wheat flour (“Strong Baker’s Flour”; Manildra Group, Auburn, New South Wales, Australia) were assessed at Monash University (Melbourne, Victoria, Australia). The gluten challenge material included approximately 0.1 g FODMAPs, mostly as fructan, which provided “low” exposure to FODMAPs (Table S2).¹⁷ FODMAPs content of baker’s wheat flour was similar to vital wheat gluten flour, but having approximately 8-times lower protein content would have required approximately 75 g to provide 6 g gluten protein and resulted in “high” FODMAPs exposure (0.9 g) that would potentially have contributed to irritable bowel symptoms after gluten challenge (Table S2).¹⁸

Symptom assessments and adverse event monitoring

Self-reported symptom severity was recorded using a modified version of the “Celiac Disease Patient Reported Outcome” tool (CeD PRO®).¹⁹ Within an hour before, and at 2, 3, 4, 5 and 6-hours after gluten challenge, patients responded to the prompt, “Thinking about your worst experience in the past *1 hour*, how severe was each of the following symptoms?” A whole number rating on a scale from 0 (no symptoms) to 10 (worst possible severity) was recorded individually for abdominal cramping, bloating, gas, pain, nausea, diarrhea, loose stool, headache and tiredness. A rating was not assessed at 1 h, as this early time-point was felt to be more subject to the influence of non-specific factors related to ingesting the flour slurry. In addition, a single-item patient “global impression of gastrointestinal symptom severity” (Figure S3) was recorded on the same 0 to 10 numeric scale as the modified CeD PRO, and in parallel a severity descriptor was selected from one of six options: “A. no symptoms”, “B. Very mild symptoms”, “C. Mild symptoms”, “D. Moderate symptoms”, “E. Severe symptoms”, or “F. very severe

symptoms". At 6 h, the Global Symptom Survey assessment also addressed what digestive symptom/s and what symptom/s overall had been the most troubling after gluten challenge. Adverse events were monitored continuously from the gluten challenge through to 14 days later in coeliac disease patients and up to 24 hours in healthy volunteers. All participants were contacted at 24 hours, and coeliac disease patients also at 14 days post-challenge to record adverse events. Adverse events were separately recorded and graded by investigators, according to Common Terminology Criteria for Adverse Events, Version 4.03.

Laboratory assessments

Laboratory pathology tests and coeliac serology (QUANTA Lite® R h-tTG IgA and Gliadin IgA II, INOVA Diagnostics, San Diego, California, USA) were performed by Dorevitch Pathology (Footscray, Victoria, Australia). *HLA-DQA* and *HLA-DQB* alleles were determined using leukocyte-derived DNA with a panel of sequence-specific primers (Australian Red Cross, Victorian Transplantation and Immunogenetics Service, Parkville, Victoria, Australia). Separate blood draws for serum interleukin-2 assessment were performed at baseline and at 2, 4, and 6 h. Blood was allowed to clot for 30 min and, within 3 h, aliquots of serum were frozen and stored at -60 to -80°C. Covance Laboratories, Inc. (Immunochemistry Services, Chantilly, Virginia USA) independently qualified a V-PLEX electrochemiluminescence assay for serum interleukin-2 performed according to the manufacturer's instructions using kits provided by Meso Scale Discovery (Rockville, Maryland, USA). Covance Laboratories, Inc. established that the lower level of quantitation for the interleukin-2 assay was 0.5 pg/ml before test samples were assessed. Sera from the two study cohorts were analysed in separate lots, but laboratory staff were not informed of the clinical diagnoses of participants.

Statistical analyses

No formal power calculation was undertaken for this exploratory study. Sensitivity and specificity for interleukin-2 in this exploratory study were based on the prospectively determined, optimal timing (4 h) for collection of sera after gluten challenge. Non-parametric tests were used to assess the significance (Wilcoxon signed rank test) and correlations (Spearman's coefficient) between changes in symptoms and serum interleukin-2 for individual participants. Differences in symptoms and serum interleukin-2 between the coeliac disease and

healthy cohorts were compared by Mann-Whitney test. All statistical tests were 2-tailed. Confidence interval ($\alpha = 0.05$) for response rate was computed using Clopper-Pearson Exact method for binomial proportions. Benjamini-Hochberg's false discovery rate method was used to adjust P-values for multiple comparisons. Summary and significance statistics were computed using a combination of Graphpad Prism V7.0d and Mathworks Matlab Ver 9.5 (R2018b). All figures were plotted using Graphpad Prism V7.0d.

RESULTS

Patient characteristics and recall of gluten-related symptoms

Altogether, 25 of 32 coeliac disease patients and 25 of 26 healthy volunteer screenees met all relevant eligibility criteria and completed the gluten challenge. Disposition of patients and reasons for failing screening are shown in Figure S4. Demographics of coeliac disease patients and healthy volunteers are shown in Table 1. Details collected in the "Characteristics of coeliac disease" survey are summarised in Table 1 and Table S3. Most coeliac disease participants anticipated symptoms after they consumed gluten (18 of 25, 72%), which appeared to contrast with only 9 (36%) having originally been diagnosed with coeliac disease because of digestive symptoms (Table 1, Table S3), and suggested gluten-related symptoms may be influenced by a placebo effect or that they worsen after adopting a gluten free diet.

Symptoms and adverse events after standardised gluten challenge

All participants completed the gluten challenge and 6 h-duration observation period per protocol. The gluten challenge was ingested at a similar time in the morning in the coeliac disease cohort (median: 10:18h, range: 9:26-13:33h) and in the healthy volunteers (9:15h, 7:58-10:35h). Peak symptom severity was in the 3rd hour after gluten challenge for coeliac disease patients (Figure 1). Peak severity of each of the symptoms assessed by participants after gluten challenge was significantly higher than baseline in the coeliac disease cohort, but not in the healthy volunteers (Table 2). Tiredness, headache, bloating and nausea, in that order, increased most from baseline in the coeliac disease cohort according to modified Coeliac Patient Reported Outcome ratings (Figure 1). The most troubling symptoms after gluten challenge for coeliac patients were, in order of frequency, headache ($n=12$, 48%), tiredness (10, 40%), nausea (10, 40%), bloating (7,

28%), vomiting (7, 24%), and gas (flatulence) in (5, 20%) according to the Global Symptom Survey at 6 h. There was a significant correlation between coeliac disease patients' peak individual Coeliac Patient Reported Outcome digestive symptom ratings and their highest hourly Global Symptom Survey score over the 6 h observation period ($n=25$, Spearman $r=0.857$, $p<0.001$). The median value for the worst rating given by coeliac disease patient for the Global Symptom Survey was 4 (range: 1-8) (Figure 1). Peak Global Symptom Survey ratings after gluten challenge were significantly higher than baseline for coeliac disease patients ($p<0.001$, Wilcoxon signed rank test), but not in healthy volunteers (median peak: 0, range: 0-0; $p=0.5$ by Wilcoxon signed rank test). Change in Global Symptom Survey score from baseline to peak for coeliac disease patients was significantly higher than for healthy volunteers ($p<0.001$, Mann-Whitney test). Table S5 lists the adverse events after gluten challenge. The only adverse event commonly experienced after gluten challenge that was not specifically queried in the modified Coeliac Patient Reported Outcome was vomiting, which affected 6 (24%) coeliac participants from 1 to 3 h after gluten challenge and lasted up to 2 h.

Symptom groupings and severity of gluten reactions

In addition to a numerical rating of overall digestive symptoms (from 0 to 10), the Global Symptom Survey prompted participants to select an accompanying severity assessment using one of five descriptors (no symptoms, very mild, mild, moderate, severe, or very severe symptoms) (Table S4). Accordingly, 4 (16%) coeliac disease patients reported peak overall gastrointestinal symptom severity as severe, 7 (28%) as moderate, 8 (32%) as mild, and 6 (24%) as very mild, which compared to 2 (8%) healthy volunteers reporting their most severe overall digestive symptoms as mild, 1 (4%) as very mild, and 22 (88%) as having no symptoms. Coeliac disease patients with a "severe" Global Symptom Survey rating had diverse digestive symptoms usually with nausea rated worst among them, and all had vomiting (Figure 1). In contrast, dominant symptoms for coeliac disease patients with maximal Global Symptom Survey ratings of "mild" or "very mild" were typically extra-intestinal, headache and tiredness, and peak symptom severity was often delayed until the 4th hour (Figure 2).

Serum cytokine assessments after gluten challenge

All coeliac disease patients and healthy volunteers had baseline serum levels of interleukin-2 below the lower level of quantitation (<0.5 pg/ml), but in coeliac disease patients serum interleukin-2 levels were significantly increased at 2, 4, and 6 h (Figure 3A). Median serum concentrations of interleukin-2 in coeliac disease patients were 1.8 pg/ml (range: <0.5 - 173) at 2 h, 3.5 (<0.5 - 33) at 4 h, and 1.7 (<0.5 - 14) at 6 h. Altogether, 23 (92%; CI: 74%-99%) coeliac disease patients had serum levels of interleukin-2 at or above 0.5 pg/ml at 4 h, which compared to none of the healthy volunteers (sensitivity: 92%, specificity: 100%).

When grouped according to maximal overall severity of digestive symptoms, serum interleukin-2 levels increased least and peaked late (at 4 h) in coeliac disease patients with very mild symptoms, whereas serum interleukin-2 increased most and peaked early (at 2 h) in coeliac disease patients with severe symptoms (Figure 3B). Peak serum interleukin-2 levels were significantly correlated with peak overall severity of digestive symptoms numerical score (Spearman $r=0.57$, $p=0.003$ false discovery rate-corrected for multiple comparisons) and with peak nausea score ($r=0.65$, $p=0.001$) (Figure 3C-D). For the 6 coeliac disease patients who experienced vomiting, their peak serum interleukin-2 was significantly higher (median: 24 pg/ml, 95% CI: 4.3-174) than coeliac disease patients who did not vomit (3.0, 1.5-5.4; $p<0.0005$, Mann-Whitney test) and peak interleukin-2 levels occurred earlier in those who vomited (median: 2 h, 95% CI: 2-4) compared to those who did not (4, 4-4; $p<0.005$, Mann-Whitney test) (Figure 3E-F). All 6 coeliac disease patients with vomiting had elevated interleukin-2 serum levels at 2, 4, and 6 h after gluten challenge.

DISCUSSION

This study defines a standardised gluten food challenge test that causes highly consistent elevations in serum interleukin-2 in coeliac disease patients. To our knowledge, and including in parallel studies we have conducted in patients with self-reported gluten sensitivity that have been reported in abstract form, elevations in circulating interleukin-2 after consuming gluten appears to be limited to patients with coeliac disease. Here we also showed that digestive symptoms as well as headache and tiredness increased significantly after unmasked gluten challenge in coeliac disease patients. Reactions to gluten were frequently associated with prominent nausea, and also

vomiting in the most severe cases, which began after about one hour and peaked in the third hour after gluten. To our knowledge, patient reported outcome symptom severity scores have not previously been correlated with cytokine elevations or any other contemporaneous disease-relevant biomarker in coeliac disease or any other condition. As such, interleukin-2 elevation triggered by standardised gluten challenge appears to be the first biomarker identified in coeliac disease that correlates with symptoms.⁵

The symptoms triggered by gluten in coeliac disease patients, their timing and association with elevations in interleukin-2 as a marker of systemic cytokine release, mimic our experience in coeliac disease patients on gluten free diet after receiving an injection of gluten peptides corresponding to immuno-dominant epitopes for gluten-specific CD4+ T cells. These observations are not altogether unexpected as the cytokine release syndrome following the first dose of biologics that activate T cells is also associated with elevation of interleukin-2 over a similar time course.²⁰ Elevation in plasma interleukin-2 has also been reported after oral food challenge in infants with food protein-induced enterocolitis syndrome.²¹ Interestingly, high dose interleukin-2 therapy and cytokine release syndromes are often associated with digestive symptoms, in particular nausea and vomiting.^{22, 23}

In this study, half the coeliac disease patients had only mild reactions to gluten that were often dominated by extraintestinal symptoms of headache and tiredness. Consequently, a diagnostic provocation test for coeliac disease relying on digestive symptoms alone, similar to the oral food challenge test for food protein-induced enterocolitis syndrome,^{2, 24} is unlikely to be robust or sensitive. In contrast, blood collection and assessment of serum interleukin-2 after gluten challenge was found in 92% of coeliac disease patients on gluten free diet and none of the healthy volunteers on unrestricted diets, who served as a “true negative” cohort. Optimal timing for interleukin-2 assessment after gluten challenge was at 4 h, but coeliac disease patients with more severe reactions including vomiting consistently showed elevated serum interleukin-2 from 2 to 6 h after gluten challenge.

Combining this standardised gluten challenge and paired serum collections at baseline and at 4 hours with a high-sensitivity laboratory assay for interleukin-2 now allows for this novel test to

be investigated in larger, more diverse cohorts of patients that will define its diagnostic utility. The bolus gluten challenge employed here lends itself to widespread, scalable implementation. A key aspect for understanding the sensitivity and specificity of this test will be selection of a “true positive” cohort to compare with a “true negative” cohort. Ideally, patients in the “true positive” cohort with a negative interleukin-2 response and minimal symptoms to gluten would be re-evaluated by re-introducing dietary gluten. Similarly, volunteers in a “true negative” cohort with a positive IL-2 response to gluten would also be carefully re-evaluated including re-introducing dietary gluten in those on a gluten free diet. Whether interleukin-2 release is provoked by gluten challenge in patients with untreated coeliac disease should be assessed in future studies. Ultimately, this diagnostic approach may be particularly useful for patients on gluten free diet who are unwilling or unable to maintain regular gluten consumption for the weeks or months necessary to cause unequivocal histological features of coeliac disease or for seroconversion.¹⁵

Current knowledge of acute clinical reactions to gluten is mostly based on recall of symptoms after presumed, but unproven, gluten exposure.^{13, 25} It is quite likely that non-gluten food constituents, in particular FODMAPs, contribute to symptoms reported by coeliac disease patients in retrospective studies, and also in clinical trials that either do not include gluten challenge, or utilise a gluten challenge high in FODMAPs.²⁶ Wheat flour is rich in FODMAPs,¹⁷ which is known to cause irritable bowel syndrome.¹⁸ Similarly, the potential for FODMAPs in gluten-containing food to cause symptoms in coeliac disease patients during food challenge studies has seldom been considered. Most studies have utilised gluten challenges consisting of one or more slices of bread daily, or the equivalent gluten load in wheat flour,^{15, 17} which we now show provides substantially higher exposure to FODMAPs than in vital wheat gluten flour with an equivalent amount of gluten protein. By utilising vital wheat gluten flour in the present study, FODMAPs exposure was low while also providing a defined 6 g amount of gluten protein in a simple format suited to standardisation, extended periods of storage at room temperature, and widespread clinical use.

Findings from this, and our other recent studies using various gluten challenge formats and injection of antigenic gluten peptides,^{11, 12} indicates the timing and magnitude of cytokine release after “antigen challenge” in coeliac disease patients is linked to digestive symptoms. In the

present study, demonstrating that interleukin-2 correlates with symptom severity addresses the criticism that symptoms in this unmasked food challenge were solely due to anticipation of a reaction to gluten.²⁷ Future double-blind placebo-controlled studies will be important to control for potential nocebo effects of gluten that may occur with “open-label” gluten challenge.

The poor correlation of histology and serology with symptoms has highlighted the need for suitable tests to monitor relevant clinical responses to gluten in trials of novel therapies intended for coeliac disease patients on gluten free diet.^{4, 5, 28} Symptoms measured by patient reported outcome instruments are increasingly used as efficacy endpoints in clinical trials.²⁹ Here we show that standardised oral gluten challenge, combined with cytokine assessment and patient reported outcomes adapted for hourly rating (as described for the first time in this study), is well suited to monitoring the effects of gluten in coeliac disease patients. Until now, clinical trials have not utilised one-off gluten challenge.^{4, 5} The validity of the coeliac patient reported outcome instrument as a measure of symptoms caused by gluten-mediated immune activation was strongly supported by showing significant increases in gastrointestinal symptoms after gluten challenge, and by finding that symptom severity correlated with elevations of serum interleukin-2, a cytokine derived from antigen-activated T cells.³⁰ Vomiting and severity of nausea stand out as strongly correlated with elevations in serum interleukin-2 after gluten challenge, but significant correlations between interleukin-2 and other symptoms would likely have been revealed if the coeliac cohort had been larger.

In conclusion, serum interleukin-2 measurement pre- and post- a standardised oral gluten challenge has the potential to improve diagnostic testing for coeliac disease in patients on gluten free diet, and to augment monitoring of clinical and immunological effects of gluten in coeliac clinical trials. Laboratory assessment of interleukin-2 after gluten challenge provides an objective biomarker for coeliac disease regardless of the specifics of associated symptoms. Further studies are warranted to establish the clinical role for this oral food challenge test in well-defined patient groups, and in patients with an uncertain diagnosis who have adopted a gluten free diet without medical oversight.

Statement of Interests: HE has no conflicts to disclose. GG, KEG, JLD, KMN, KET and RPA are employees of ImmusanT, Inc. JM and SA are consultants to ImmusanT, Inc. JAT-D and AJMD have served as advisors to ImmusanT, Inc. RPA is inventor of Patents, owned or licensed by ImmusanT, Inc, relating to the diagnostic application of gluten challenge.

REFERENCES

1. Ballmer-Weber BK, Beyer K. Food challenges. *J Allergy Clin Immunol* 2018; 141:69-71 e2.
2. Nowak-Wegrzyn A, Assa'ad AH, Bahna SL, Bock SA, Sicherer SH, Teuber SS, et al. Work Group report: oral food challenge testing. *J Allergy Clin Immunol* 2009; 123:S365-83.
3. Jabri B, Sollid LM. T Cells in Celiac Disease. *J Immunol* 2017; 198:3005-14.
4. Hindryckx P, Levesque BG, Holvoet T, Durand S, Tang CM, Parker C, et al. Disease activity indices in coeliac disease: systematic review and recommendations for clinical trials. *Gut* 2018; 67:61-9.
5. Ludvigsson JF, Ciacci C, Green PH, Kaukinen K, Korponay-Szabo IR, Kurppa K, et al. Outcome measures in coeliac disease trials: the Tampere recommendations. *Gut* 2018; 67:1410-24.
6. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA, American College of G. ACG clinical guidelines: diagnosis and management of celiac disease. *Am J Gastroenterol* 2013; 108:656-76; quiz 77.
7. Choung RS, Unalp-Arida A, Ruhl CE, Brantner TL, Everhart JE, Murray JA. Less Hidden Celiac Disease But Increased Gluten Avoidance Without a Diagnosis in the United States: Findings From the National Health and Nutrition Examination Surveys From 2009 to 2014. *Mayo Clin Proc* 2016.
8. Golley S, Corsini N, Topping D, Morell M, Mohr P. Motivations for avoiding wheat consumption in Australia: results from a population survey. *Public Health Nutr* 2015; 18:490-9.
9. Anderson RP, Henry MJ, Taylor R, Duncan EL, Danoy P, Costa MJ, et al. A novel serogenetic approach determines the community prevalence of celiac disease and informs improved diagnostic pathways. *BMC Med* 2013; 11:188.

10. Koskinen L, Romanos J, Kaukinen K, Mustalahti K, Korponay-Szabo I, Barisani D, et al. Cost-effective HLA typing with tagging SNPs predicts celiac disease risk haplotypes in the Finnish, Hungarian, and Italian populations. *Immunogenetics* 2009; 61:247-56.
11. Goel G, Tye-Din JA, Qiao S-W, Russell AK, Mayassi T, Ciszewski C, et al. Cytokine release and gastrointestinal symptoms after gluten challenge in celiac disease. *Science Advances* 2019 (In Press).
12. Goel G, King T, Daveson AJ, Andrews JM, Krishnarajah J, Krause R, et al. Epitope-specific immunotherapy targeting CD4-positive T cells in coeliac disease: two randomised, double-blind, placebo-controlled phase 1 studies. *Lancet Gastroenterol Hepatol* 2017; 2:479-93.
13. Silvester JA, Graff LA, Rigaux L, Walker JR, Duerksen DR. Symptomatic suspected gluten exposure is common among patients with coeliac disease on a gluten-free diet. *Aliment Pharmacol Ther* 2016; 44:612-9.
14. Truitt KE, Daveson AJM, Ee HC, Goel G, MacDougall J, Neff K, et al. A randomized, placebo-controlled clinical trial of subcutaneous or intradermal Nexvax2, an investigational immunomodulatory peptide therapy for coeliac disease. *Alimentary Pharmacology and Therapeutics* 2019 (In Press).
15. Bruins MJ. The clinical response to gluten challenge: a review of the literature. *Nutrients* 2013; 5:4614-41.
16. van Overbeek FM, Uil-Dieterman IG, Mol IW, Kohler-Brands L, Heymans HS, Mulder CJ. The daily gluten intake in relatives of patients with coeliac disease compared with that of the general Dutch population. *Eur J Gastroenterol Hepatol* 1997; 9:1097-9.
17. Varney J, Barrett J, Scarlata K, Catsos P, Gibson PR, Muir JG. FODMAPs: food composition, defining cutoff values and international application. *J Gastroenterol Hepatol* 2017; 32 Suppl 1:53-61.
18. Halmos EP, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; 146:67-75 e5.
19. Leffler DA, Kelly CP, Green PH, Fedorak RN, DiMarino A, Perrow W, et al. Larazotide acetate for persistent symptoms of celiac disease despite a gluten-free diet: a randomized controlled trial. *Gastroenterology* 2015; 148:1311-9 e6.
20. Winkler U, Jensen M, Manzke O, Schulz H, Diehl V, Engert A. Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8). *Blood* 1999; 94:2217-24.

21. Kimura M, Ito Y, Shimomura M, Morishita H, Meguro T, Adachi Y, et al. Cytokine profile after oral food challenge in infants with food protein-induced enterocolitis syndrome. *Allergol Int* 2017; 66:452-7.
22. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant* 2019; 25:625-38.
23. Rosenberg SA, Lotze MT, Yang JC, Aebbersold PM, Linehan WM, Seipp CA, et al. Experience with the use of high-dose interleukin-2 in the treatment of 652 cancer patients. *Ann Surg* 1989; 210:474-84; discussion 84-5.
24. Nowak-Wegrzyn A, Chehade M, Groetch ME, Spergel JM, Wood RA, Allen K, et al. International consensus guidelines for the diagnosis and management of food protein-induced enterocolitis syndrome: Executive summary-Workgroup Report of the Adverse Reactions to Foods Committee, American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol* 2017; 139:1111-26 e4.
25. Barratt SM, Leeds JS, Sanders DS. Factors influencing the type, timing and severity of symptomatic responses to dietary gluten in patients with biopsy-proven coeliac disease. *J Gastrointest Liver Dis* 2013; 22:391-6.
26. Barrett JS, Gearry RB, Muir JG, Irving PM, Rose R, Rosella O, et al. Dietary poorly absorbed, short-chain carbohydrates increase delivery of water and fermentable substrates to the proximal colon. *Aliment Pharmacol Ther* 2010; 31:874-82.
27. Molina-Infante J, Carroccio A. Suspected Nonceliac Gluten Sensitivity Confirmed in Few Patients After Gluten Challenge in Double-Blind, Placebo-Controlled Trials. *Clin Gastroenterol Hepatol* 2017; 15:339-48.
28. Canestaro WJ, Edwards TC, Patrick DL. Systematic review: patient-reported outcome measures in coeliac disease for regulatory submissions. *Aliment Pharmacol Ther* 2016; 44:313-31.
29. U. S. Department of Health Human Services FCfDER, U. S. Department of Health Human Services FCfBER, U. S. Department of Health Human Services FCfDRH. Guidance for industry: patient-reported outcome measures: use in medical product development to support labeling claims: draft guidance. *Health Qual Life Outcomes* 2006; 4:79.
30. Spolski R, Li P, Leonard WJ. Biology and regulation of IL-2: from molecular mechanisms to human therapy. *Nat Rev Immunol* 2018; 18:648-59.

Figure legends

Figure 1. Heatmap of symptoms after gluten challenge. Maximal severity of symptoms, and time profile of global assessments of digestive symptom severity (Global Symptom Survey) after gluten challenge for individual participants; 0 (no symptoms) to 10 (worst possible severity). Hourly nominal Global Symptom Survey descriptors were also recorded. Peak nominal Global Symptom Survey rating for participants are shown at far right. Whether participants vomited is shown on the left. BSL = Baseline

Figure 2. Timing and severity of individual symptoms according overall severity of gluten reaction. Coeliac disease patients were grouped by their peak nominal Global Symptom Survey rating after gluten challenge, and compared to healthy volunteers. Modified Celiac Disease Patient Reported Outcome score (0: no symptoms, to 10: worst possible severity) was used to assess individual symptoms for the previous hour. Mean and standard deviation are shown.

Figure 3. Serum interleukin-2 elevations after gluten challenge and their relation to symptom severity. Baseline serum interleukin-2 levels were always below 0.5 pg/ml at baseline for coeliac disease and healthy volunteers, and for healthy volunteers they remained below 0.5 pg/ml at all later time points. For each time-point, interleukin-2 serum concentrations for individual participants are shown in (A) with median and interquartile range. P-values were determined by Wilcoxon signed rank test. Responder rates, n (%) are shown. Serum interleukin-2 (mean and standard deviation) for coeliac disease patients grouped by the peak severity of symptoms (nominal Global Symptom Score) after gluten challenge is shown in (B). Peak numerical severity scores for g Global Symptom Score (C), and nausea (D) are compared to peak serum interleukin-2 after gluten challenge in coeliac patient (Spearman correlation r and associated P-values shown); circled symbols indicate individuals who vomited after gluten challenge. Profiles of serum interleukin-2 in coeliac participants who did (E), or did not vomit (F) after gluten challenge. The lower limit of quantitation for serum estimation of interleukin-2 was 0.5 pg/ml, indicated by dashed line.

Table 1. Demographics and characteristics of participants

	Coeliac patients (n=25)	Healthy volunteers (n=25)
Mean (SD) age in years	39 (16)	47 (11)
Number (%) females	22 (88)	17 (68)
Mean (SD) height in centimeters	169 (7)	167 (7)
Mean (SD) body mass in kilograms	72 (13)	77 (20)
Mean (SD) body mass index	25 (5)	27 (7)
Number (%) Caucasian	24 (96)	23 (92)
Number (%) positive for anti-TG2 IgA or DGP IgG †	4 (20)	0 (0)
Number (%) positive for HLA-DQ2.5	25 (100)	8 (32)
Number (%) positive for HLA-DQ2.5, 2.2, 8 or 7	25 (100)	20 (80)
Median (range) age/years at diagnosis of coeliac disease	29 (2-59)	NA
Median (range) years from coeliac diagnosis	4 (1-25)	NA
Median (range) years following gluten free diet	4 (1-20)	NA
Median (range) number of gluten exposures in last year	2 (0-12)	NA
Number (%) ingesting oats in last year	1 (4)	NA
Number (%) expecting symptoms after gluten ‡	18 (72)	NA

NA, not applicable. † DGP IgG was low positive in one, and TG2 IgA was low positive in 3 other coeliac participants. ‡ 4 of 25 coeliac participants did not know and one did not expect symptoms after gluten challenge

Table 2. Increase in hourly symptom scores after gluten from baseline to peak

Coeliac disease (n=25)	Healthy volunteers (n=25)
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Symptom	Median (25 th – 75 th %)	P value	Median (25 th – 75 th %)	P value
Global	3 (2 - 6)	<0.001	0 (0 - 0)	1
Tiredness	4 (1 - 6)	<0.001	0 (0 - 0)	1
Headache	3 (1 - 6)	<0.001	0 (0 - 0)	1
Nausea	2 (1 - 7)	<0.001	0 (0 - 0)	1
Bloating	3 (1 - 4)	<0.001	0 (0 - 0)	1
Gas	1 (0 - 3)	<0.001	0 (0 - 0)	1
Pain	1 (1 - 2)	<0.001	0 (0 - 0)	1
Cramping	1 (0 - 2)	<0.001	0 (0 - 0)	1
Loose Stool	0 (0 - 1)	0.009	0 (0 - 0)	1
Diarrhea	0 (0 - 1)	0.016	0 (0 - 0)	1

P value estimated by Wilcoxon signed rank test between baseline and peak symptom score. False discovery rate-adjusted P value estimated by Benjamini-Hochberg method

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Figure 1.

Figure 2.

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Figure 3.

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