Targeting the T cell: novel diagnostics and therapies for coeliac disease

Jason A. Tye-Din

Immunology Division, The Walter and Eliza Hall Institute, Parkville, Australia
Department of Medical Biology, University of Melbourne, Parkville, Victoria, Australia
Department of Gastroenterology, The Royal Melbourne Hospital, Parkville, Victoria, Australia

Coeliac disease (CD) is caused by a loss of immune tolerance to dietary gluten and is one of the most strongly HLA-linked illnesses afflicting humankind. Although it is similar to ‘traditional’ autoimmune diseases like type 1 diabetes where HLA-associated T-cell immunity plays a key role in pathogenesis, a unique feature of CD is the causative antigen, gluten, is exogenous (dietary) and well characterized. Clinically CD is often regarded as a malabsorptive disease and its diagnosis is based on demonstrating a characteristic enteropathy of villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis. However, the discovery that 99% of Caucasian CD patients express HLA-DQ2 and/or DQ8 and harbor pathogenic gluten-specific CD4+ T cells in the small intestine and blood indicate CD is best viewed as an aberrant T cell immune response to a common food protein with systemic features rather than a “food intolerance” affecting the bowel. Gluten is the viscoelastic protein that remains after washing dough and is composed of alcohol-soluble gliadins, and alcohol-insoluble glutenins. Modern wheat gluten is derived from a hexaploid genome making it genetically complex. Similar proteins rich in glutamine and proline are found in barley and rye prolams and generally considered safe for consumption in CD, although adverse immune and clinical effects have been reported and some cultivars may be more immunogenic than others. Further research into the toxicity of oats in CD has been recommended.

The prevalence of CD varies with sex, age, and geographic location with the frequency of predisposing HLA haplotypes in the general population and per capita wheat consumption the 2 major determinants of prevalence. In a systematic review and meta-analysis, the global seroprevalence and biopsy-confirmed prevalence of CD was estimated to be 1.4% and 0.7%, respectively. While CD is common around the globe and is rising in prevalence in many populations, it is frequently undetected in clinical practice. Concerningly, symptomatic and untreated disease is associated with elevated morbidity and mortality and impaired quality of life. Current issues of importance to clinicians and patients include expediting the detection and diagnosis of CD, improving quality of life and health outcomes for those diagnosed, and developing treatments that are more effective and less burdensome than a lifelong and strict gluten-free diet (GFD). Current issues with suboptimal diagnosis are compounded by the high uptake of the GFD in the general community, which means traditional serology and histology approaches to diagnose CD are not accurate. Approaches that avoid the need for prolonged gluten exposure and that can identify people with CD from those with non-coeliac gluten or wheat sensitivity are needed.

The emerging story of CD development is one where environmental factors increase the risk of CD in genetically predisposed individuals by shaping the immunologic context in which gluten is presented and shifting the balance from gluten tolerance to reactivity, and that this may be in part mediated through microbiome-host interactions. While environmental factors are important for CD development, a notable feature of CD is its high heritability and strong HLA association. This strong genetic association reflects the central role of CD4+ T cells as the HLA molecules associated with CD bind specific gluten peptides that activate T cells. Ninety percent of Caucasian CD patients possess the HLA-DQ2.5 haplotype (encoded by the DQA1*05:01 and DQB1*02:01 alleles) and the remaining carry either HLA-DQ8...
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(encoded by the DQA1*03:01 and DQB1*03:02 alleles), HLA-DQ2.2 alone (encoded by the DQB1*02:02 allele) or HLA-DQ7 alone (encoded by the DQA1*05:01 allele). Less than 1% of European and Australian CD patients lack these HLA haplotypes and their absence is usefully exploited in the clinical setting to assist in excluding a diagnosis of CD.

For any HLA-associated autoimmune illness, defining the repertoire of disease-specific T cells and how they respond to the driving antigen over time is crucial to understanding pathogenesis and providing insights that underpin development of new diagnostics and therapies. This knowledge has largely remained a mystery because the identity of the driving antigens in most HLA-associated diseases has not been well defined. Fortunately, CD affords the opportunity to explore this question in detail. The strong HLA association highlights a key role for pro-inflammatory CD4+ T cells responding to a restricted set of gluten peptides. These proline-rich peptides from wheat, rye and barley resist digestion and become highly immunogenic due to post-translational modification (deamidation) by transglutaminase. The structural restraint on HLA-peptide binding drives the selection of a biased, high affinity T cell receptor (TCR) repertoire. Uniquely, gluten can be removed from the diet to induce CD remission and can be reintroduced to trigger a recall immune response where gluten-specific T cells become expanded to levels detectable in blood and accessing disease-relevant tissue is readily achieved by gastroscopy and biopsy.

These unique features of CD means it has been possible to isolate and study gluten-specific T cells from the small intestine and blood of CD patients after gluten challenge to define the immunogenic gluten peptides (specifically, the T cell epitopes) pathogenic in CD. Most studies have focused on the 90% of CD patients who are HLA-DQ2.5 and here the most immunogenic peptides following wheat ingestion are those from α- and ω-gliadin. Much of the field has focused on the immunogenicity of T cell epitopes in α-gliadin, specifically those encompassed within a protease resistant 33mer. However, gluten challenge studies show that the most immunogenic peptides induced by gluten depends on whether wheat, rye or barley is ingested, and that a sequence from ω-gliadin (encompassing the T cell epitopes DQ2.5-glia-01 and DQ2.5-glia-02) is dominant irrespective of which grain is consumed. Despite the multitude of immunogenic peptides, just 3 peptides from wheat and barley appear to recapitulate most of the response to gluten in CD patients with HLA-DQ2.5 and this has been the basis for the development of an epitope-specific immune therapy, Nexvax2, discussed later. Interestingly, after oat ingestion, about 8% of CD patients have detectable T cells specific for avenin peptides that share close sequence homology with barley hordein, suggesting that cross-reactive T cells may mediate immune responses to oats in some CD patients.

Work in both children and adults with CD has shown that gluten-specific T cells in blood induced by oral wheat challenge, or expanded from the small intestine during active disease, share the same specificity for deamidated, immunodominant T cell epitopes. Indeed, the same gluten-specific T cell clonotypes persist in patients’ blood and intestinal tissue up to several decades and share the same TCR gene use motifs in CD patients from Norway, Finland and Austra-

lia. Their stability over such long periods of time may be maintained by ongoing gluten exposure as inadvertent gluten intake is common in CD even when a strict gluten-free diet is attempted. The findings support the concept that gluten-specific memory T cells are generated early in disease and their specificity remains constant thereafter. Collectively, these findings suggest that targeting the stable, long-lived population of gluten-specific T cells is a logical approach for novel diagnostics and therapies in CD.

Current diagnosis of CD is imperfect: while villous atrophy remains the cornerstone of CD diagnosis there is the growing realization that this “gold-standard” is only accurate if adequate mucosal samples are collected due to the patchy nature of disease, the biopsies are oriented correctly and a standardized approach to histopathology reporting adhered to. Ultra-short CD where villous atrophy is present only in the duodenal bulb and “mild enteropathy CD” where villous atrophy is absent in the setting of positive CD serology both present diagnostic challenges and highlight some of the limitations of relying on histology for diagnosis. The high rate of community adoption of the GFD means that, for those seeking a CD diagnosis, reintroduction of dietary gluten, generally for several weeks to months, is recommended prior to testing. Unfortunately, many patients are reluctant to undertake this and for those that do many fail to tolerate it. This issue is further complicated by the fact that the serologic and histologic response to gluten challenge is highly heterogeneous so the optimal duration of gluten challenge necessary to make a diagnosis is uncertain. Immune diagnostics that measure the gluten-specific immune response target a fundamental component of CD and may overcome the limitations of current diagnostics. The use of tetramers or cytokine release assays to identify gluten-specific T cells induced in blood after short-term oral gluten challenge is highly sensitive and specific for CD. Diagnostics that are accurate with limited or even no gluten exposure such as tetramer-based detection of gluten-specific T cells are particularly appealing as they may avoid the current need for prolonged gluten challenge. Large validation studies that confirm the accuracy of assessing disease-specific T cells as a CD diagnostic may force a re-think of how CD should be defined.

What about the treatment of CD? While strict adherence to a lifelong GFD remains the single proven treatment for CD it can be complicated, onerous and expensive. In several longitudinal studies, fewer than half of adults achieve small intestinal histological remission on a GFD. Persistent disease activity may be driven by low-level and potentially inadvertent gluten exposure, such as that through contaminated meals when eating out. These limitations of the GFD, and demand from patients, have driven research to find new therapeutic approaches. Insight into the molecular mechanisms underpinning CD pathogenesis provides several opportunities for novel therapeutics development and a range of pharmaceuticals are currently being assessed in pre-clinical and clinical trials. Eradication or suppression of the long-lived, pathogenic gluten-specific T cells presents a rational and attractive therapeutic approach. One way this might be achieved is by targeting these T cells and deleting or rendering them functionally unresponsive (anergic) and
inducing suppressive Tregs. As the target T cell population is stable in established CD, it is believed these approaches will apply similarly to children as they do in adults with CD. Phase 1 studies of Nexvax2, a therapeutic vaccine composed of 3 gluten peptides encompassing immunodominant HLA-DQ2.5‑restricted T cell epitopes, initially caused gastrointestinal symptoms similar to those triggered by gluten, however after later administration of Nexvax2 symptoms were no different from those after placebo.

The recall immune response to gluten was modified in people with CD receiving Nexvax2. The results of Phase 2 clinical trials currently underway are awaited to determine the efficacy of such an approach in addressing the adverse effects of gluten in HLA-DQ2.5 + CD patients. The findings that Nexvax2, and also oral gluten challenge, induce a strikingly similar cytokine response dominated by interleukin‑2 in the bloodstream 2‑4 hours after exposure in people with CD but not those without raises the possibility of a novel diagnostic approach that avoids the need for prolonged gluten exposure to be accurate. Preliminary work suggests this approach is highly accurate in distinguishing people with CD from those with gluten sensitivity however more work is required to validate this approach in the clinic.

It is an exciting time in CD research with an ever growing range of novel therapies entering pre‑clinical and clinical studies. However, the trial landscape in CD is still in its infancy and continues to evolve, with optimal endpoints and how these should be measured still being established. Emerging studies on the role of environmental factors and the microbiome and how they might impact gluten immunity and tolerance may one day shift the focus from CD diagnosis and treatment to disease prevention.

Disclosures

JT‑D has served as a consultant and scientific advisory board member for ImmusanT Inc., USA, and owns shares in Nexpep Pty Ltd. He is a co‑inventor of patents pertaining to the use of gluten peptides in CeD therapeutics, diagnostics and non‑toxic gluten. Nexpep Pty. Ltd. and ImmusanT Inc. were formed to develop novel diagnostics and treatments for CeD.

References


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