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Decoding the immunophenotype – New perspectives in understanding celiac disease

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CONTEXT AND DEFINITION OF CELIAC DISEASE

Each individual with celiac disease (CeD) develops their own immune response to gluten, conditioned by their genetic makeup and modulated by intercurrent environmental factors. CeD can occur at any age and has increased in prevalence in recent decades (1). The greatest contribution to an individual's genetic risk comes from the expression, in antigen-presenting cells (APCs), of histocompatibility molecules encoded by the HLA-DQ2 and DQ8 alleles, which bind gluten peptides deamidated by the tissue transglutaminase type 2 (TG2) enzyme and present them to helper CD4+ T lymphocytes. The resulting immune-mediated enteropathy is a dynamic process that involves a gluten-specific inflammatory response mediated by CD4+ T lymphocytes, culminating in the innate destruction of enterocytes by intraepithelial T lymphocytes (IELs), giving rise to a wide spectrum of clinical forms that are sometimes difficult to identify (2). As a consequence of the resulting inflammation, a malabsorptive enteropathy is induced, characterized by intraepithelial lymphocytosis accompanied by varying degrees of villous atrophy and crypt hypertrophy in the duodenal mucosa,

which is reversible after gluten withdrawal (3).

DIAGNOSIS

It is based on the detection of specific anti-TG2 antibodies and the demonstration of the lesion, although both diagnostic pillars have their weaknesses. Thus, 2-15 % of adult patients with CeD are primarily seronegative (4), or there may be a reduction in antibodies secondary to immunodeficiencies and/or immune dysregulation. In addition, characteristic histological lesions are not entirely specific (5), allowing for multiple histopathological mimics. All of this, together with the wide spectrum of digestive and extra-digestive clinical manifestations, or the voluntary reduction of gluten intake without a definitive clinical diagnosis, means that identifying this pathology remains a clinical challenge today and justifies the incorporation of new diagnostic tools.

IMMUNOPATHOGENESIS OF CELIAC DISEASE: TWO IMMUNE RESPONSE LEVELS

1. Specific immune response to gluten:

- Key players:
  - APCs with DQ2 and/or DQ8 restriction.
  - Gluten-specific CD4+ T cells.
- Location: lamina propria and enteric lymphoid follicles.

Expansion of deamidated gluten peptide-recognizing CD4+ T cells, in a setting of restricted presentation of HLA-DQ2 and/or DQ8 antigens, represents the unequivocal immune trait that initiates and directs the celiac inflammatory response. These effector lymphocytes produce pro-inflammatory cytokines IL-2, IL-21 and IFNγ, and are necessary though not sufficient to induce atrophic lesions. Following gluten discontinuation they persist for decades as resident memory lymphocytes in the lamina propria, and may be reactivated and mobilized to peripheral blood with each new re-exposure to gluten.

2. Epithelial damage and non-gluten-specific intraepithelial lymphocytes:

- Key players:
  - Cytotoxic effector intraepithelial T lymphocytes (T-IELs).
  - Interleukin 15 (IL-15).
- Location: duodenal epithelium.

The intraepithelial lymphoid (IEL) compartment is highly heterogeneous and includes subpopulations of diverse ontogeny and function, capable of integrating defensive immune responses with innate regulatory and trophic functions (6). It comprises a majority T-line (CD3+) fraction, which includes T-IEL subtypes TαβCD8$^+$ (50-75 %) and Tγδ (<10 %), and a remnant fraction (15-45 %) of IELs that fail to express the CD3 marker on their surface (CD3$_s^-$), whose function and origin are poorly understood, and that include both innate lymphoid cells (ILCs) and lymphoid precursors.

In active celiac disease T-IELs express non-gluten-specific, innate-type, activating "natural killer" receptors (NKRs) (NKG2D and NKG2G), and a highly cytotoxic, inflammatory effector phenotype with production of IFNγ, perforins and granzymes. Upon gluten withdrawal, both NKR expression and T-IEL function are reconfigured, and an intraepithelial resident memory phenotype persists (1,7).

IL-15 is a key cytokine that is overexpressed in active celiac mucosa. It decisively contributes to T-IEL activation and expansion, acting synergistically with the IL-21 and IFNγ cytokines released in the lamina propria by gluten-activated CD4+ T lymphocytes. It also induces the expression of receptors NKG2G and NKG2D in T-IELs, and of their ligands, stress antigens MICA/B-HLAE in enterocytes, enabling their cytotoxic functions through this innate signaling pathway, which ultimately results in direct enterocyte destruction (8).

DUODENAL LYMPHOGRAM: EXPERIENCE AT HOSPITAL UNIVERSITARIO RAMÓN Y CAJAL, MADRID (SPAIN)

A key immune finding in active CeD is the expansion of T-IELs, both Tαβ and Tγδ. However, while Tαβ levels regress after gluten withdrawal, Tγδ levels persist for a long time, the latter being considered a quasi-pathognomonic finding in celiac disease

(characteristic marker). On the other hand, the $CD3_s^-$ subpopulation virtually disappears during active phases, and gradually returns following gluten discontinuation (mucosal integrity marker). An assessment of this intraepithelial lymphoid compartment using flow cytometry, a so-called "duodenal lymphogram," allows identification of various lymphoid subset profiles, which reflect the dynamics of the underlying celiac process. Our team established cutoffs for $T\gamma\delta$ and $CD3_s^-$ using the values providing maximal diagnostic effectiveness according to ROC curves, and defined 4 lymphogram profiles:

- Complete celiac lymphogram ($\uparrow T\gamma\delta > 14$ % and $\downarrow CD3_s^- < 4$ %).
- Partial lymphogram (isolated $\uparrow T\gamma\delta > 14$ % or isolated $\downarrow CD3_s^- < 4$ %).
- Non-celiac lymphogram ($\downarrow T\gamma\delta < 14$ % and $\uparrow CD3_s^- > 4$ %).

An active celiac lymphogram is characterized by $T\gamma\delta/CD3_s^-$ imbalance. In our experience, jointly assessing both parameters ($T\gamma\delta/CD3_s^- \geq 5$) renders this diagnostic test maximally effective (Fig. 1). Its study in large pediatric and adult cohorts (9,10) confirms its usefulness as a diagnostic tool, even for the less conventional forms of the condition.

Over 80 % of active CeD forms have a complete celiac lymphogram, which offers specificity near 100 % and sensitivities around 80-90 %. The remaining patients have partial lymphograms, associated with less diagnostic certainty (9,10).

After gluten is removed from the diet the inflammation trigger is canceled and a mucosal recovery process begins, with changes in IEL subset distribution, phenotype and function. Under these circumstances, the majority group show partial lymphograms that, while preserving the characteristic $\uparrow T\gamma\delta$, also include an increased proportion of $CD3_s^-$ IELs. In such cases diagnostic certainty is significantly reduced. This scenario is shared by potential celiacs without histological lesion. Therefore, a lymphogram is a powerful tool not only for diagnosing but also for monitoring the natural course of the disease (9).

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CURRENT STATUS OF DUODENAL LYMPHOGRAM: FUTURE PROSPECTS AND REFLECTIONS

This technique was included in the Protocol for the Early Diagnosis of Celiac Disease, released by the Health Ministry in 2018 (11), and has been implemented in multiple national hospitals and by multiple international teams (12-19), demonstrating high reproducibility of results and diagnostic efficiency, although unanimous agreement regarding the interpretation of the data collected is still lacking.

It is a simple, rapid, precise technique that reveals a "nearly pathognomonic" imprint of the immunopathogenic process involving the duodenal epithelium in CeD. The celiac lesion is modulated by gluten and directly produced by effector T-IELs. However, to date no T-IEL subtype has demonstrated gluten-specific recognition—T-IELs perform an innate inflammatory-cytotoxic activity that parallels the gluten-specific response mediated by CD4+ T cells in the lamina propria.

Advances in the analysis of unicellular molecular signatures (omics) and the adoption of high-power, high-resolution cytometric approaches (spectral and mass/CyTOF) allow now to identify and describe different T-IEL subtypes, with distinct effector and/or memory functions (7,20), which may become more specific biomarkers for the underlying process. In addition, the migratory potential of these lymphocytes has been demonstrated to peripheral blood, where they may be identified by their expression of gut-homing molecules (21), and quantified using cytometry techniques (22,23). Gaining insight on the function and phenotype of these cells opens up a promising approach to the biopsy-free diagnosis of celiac disease.

Our experience with lymphograms results in the following *reflections:*

- A duodenal lymphogram provides specificity to histological findings and increases effectiveness throughout the diagnostic process, minimizing diagnostic errors and/or delays.
- Diagnostic certainty is maximal (LR+ 36.2) for complete celiac lymphogram (↑Tγδ > 14 % and ↓$CD3_s^-$ > 4 %), and decreases when partial lymphograms are used.

- Knowing whether a patient is taking gluten is key when interpreting a partial lymphogram: an isolated ↑Tγδ ≥ 14 % in a gluten-eating patient virtually excludes any form of active CeD, or introduces the option of potential CeD, not ruling out other conditions. However, in an established CeD case under treatment, it indicates good dietary compliance.

- Tγδ and $CD3_s^-$ IEL ranges remain undefined. They may be more or less stringent depending on whether sensitivity or specificity is prioritized.

- Increased Tγδ IEL levels represent the characteristic, though nonspecific, immune biomarker of most celiac enteropathy variants.

- The ratio of $CD3_s^-$ IELs is a sensor of celiac mucosal integrity.

- A non-celiac lymphogram almost excludes active CeD.

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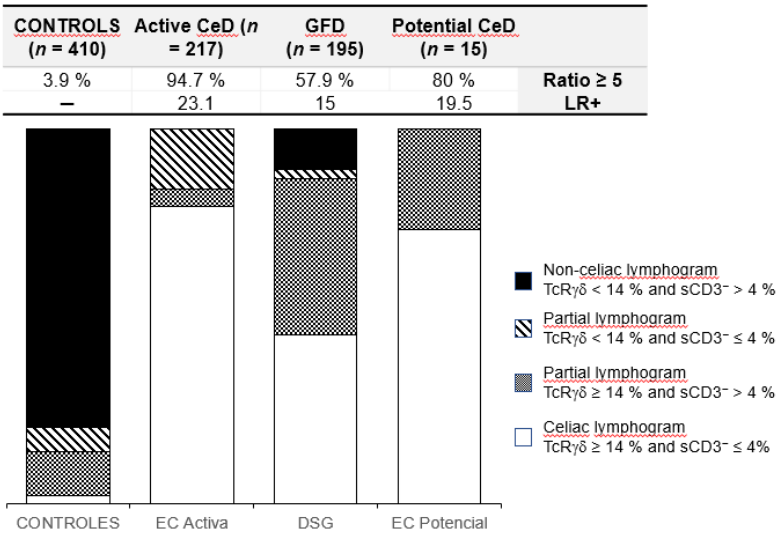

Fig. 1. Distribution of the four different lymphogram profiles in an adult celiac cohort (adapted from reference 10). The data table at the top of the figure shows the percentage of patients in each group that meet the Tγδ/CD3s⁻ ≥ 5 ratio requirement, and the calculated diagnostic accuracy (LR+) for each one (ACD, active celiac disease; CD, celiac disease; GFD, gluten free diet; LR+, positive likelihood ratio).