

## IMMUNOLOGICAL ASPECTS OF CROHN'S DISEASE: A REGULATORY FUNCTION OF TIM-3/ GALECTIN-9 IN LYTH1.

Francesco Carini <sup>1\*</sup>, Carola Gagliardo <sup>2</sup>, Margherita Mazzola <sup>1,3</sup>, Elena Lo Presti <sup>4,5</sup>, Marco Scaglione <sup>2</sup>, Abdo Jurjus <sup>6</sup>, Alice Gerges Geagea <sup>1</sup>, Raymond Zerbe <sup>6</sup>, Angelo Leone <sup>7</sup>, Francesca Rappa <sup>1</sup>, Sabrina David <sup>1</sup>, Giovanni Tomasello <sup>1\*</sup>

\* These authors contributed equally to the present work.

1. Department of Experimental Biomedicine and Clinical Neuroscience, Section of Anatomy, (BIONEC), University of Palermo, Italy
2. Student of School of Medicine and Surgery, Palermo University, Italy.
3. Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy.
4. Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR), University of Palermo, Italy.
5. Department of Biopathology and Medical Biotechnologies (DIBIMED), University of Palermo, Italy.
6. Department of Anatomy, Cell Biology and Physiological Sciences, American University of Beirut. Beirut-Lebanon.
7. Department of Experimental Biomedicine and Clinical Neuroscience, Section of Histology, (BIONEC), University of Palermo, Italy.

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### ABSTRACT

Crohn's disease (CD) is a type of inflammatory bowel disease (IBD) and its etiology is multifactorial and involves a combination of genetic and environmental factors. The interaction of these factors causes an imbalance in the microbiota, leading to the activation of several immunological and inflammatory mechanisms. From an immunological point of view, there seems to be an involvement of the TIM-3/galectin-9 pathway and of the autoregulation of LyTh1. The studies show that in patients with CD the autoregulation of LyTh1 is lost due to a reduced concentration of galectin-9 and a reduced TIM-3 expression in LyTh1. This could be one of the reasons for the state of perpetual activation in LyTh1, resulting in the chronic inflammatory process.

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## 1. Introduction

Crohn's disease (CD) is a type of inflammatory bowel disease (IBD), like ulcerative colitis (UC) and indeterminate colitis (IC). This disease is characterized by a chronic inflammatory process that can involve every segment of the digestive tract from the mouth to the anus; the last ileal loop is particularly susceptible to its effects. The chronic inflammatory process that occurs in CD leads to profound anatomical and functional changes of the mucosa, ranging from simple superficial ulcerations to perforations. The disease can cause various symptoms, such as abdominal pain, often bloody diarrhea, dehydration, intermittent and prolonged fever,

malabsorption of nutrients, weight loss, and sometimes anemia. Further common complications include stenosis, fissures and perianal fistulas (1-4).

The etiology of CD is multifactorial and involves a combination of genetic and environmental factors. A current key point of interest is intestinal dysbiosis (5). Indeed, the intestinal microbiota of subjects with CD show a dysbiosis characterized by changes in *Firmicutes* and *Proteobacteria* phyla (6).

Dietary habits, such as a diet rich in sugars and fats (2,3), smoking (2,7), and use of antibiotics (8) are some of the environmental factors that can influence the composition of the intestinal microbiota. The interaction of these factors causes an imbalance in the microbiota, leading to the activation of several immunological and inflammatory mechanisms. Such

\* Corresponding author: Francesco Carini, francesco.carini@unipa.it

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mechanisms include the alteration of tight junctions in the intestinal epithelial barrier (9) and the establishment of a chronic inflammatory state, characterized by the release of various chemical mediators of inflammation, such as heat shock proteins (HSP), interleukins and COX-2 causing the release of arachidonic acid metabolites (10-14).

From an immunological point of view, there seems to be an involvement of the TIM-3/galectin-9 pathway, which will be discussed in detail below.

## 2. TIM-3/galectin-9 pathway and CD

The enzymes of enteric bacteria induce tight junction alterations in the epithelium of the intestinal mucosa, promoting the passage of bacteria to the lamina propria, where the gut-associated lymphoid tissue (GALT) is located (15). GALT contains different cell types that produce cytokines, which activate cells and determine the differentiation of naive T lymphocytes (LyT) into LyTh1, believed to be the main protagonists of the immune response in CD (16-18). LyTh1 produce interferon gamma (IFN- $\gamma$ ) and matrix metallo proteinases (MMPs), which cause an amplified permeabilization of the epithelial barrier, thus intensifying the tissue damage and causing the inflammatory state to become chronic (15). The switch-off mechanism of LyTh1 is regulated through the interaction between two molecules produced by the lymphocytes themselves: a membrane protein called T-cell immunoglobulin and mucin domain, or TIM-3 and its ligand, a glycoprotein called galectin-9 (19). It has been shown that the interaction between TIM-3 and galectin-9 in LyTh1 downregulates the production of inflammatory cytokines, such as IFN- $\gamma$ , IL-17, IL-2, and IL-6 (12,13,20), by inducing peripheral tolerance (19) and mediating apoptosis in human and murine Th1 (21). However, the mechanisms through which TIM-3/galectin-9 interaction causes an increase in the transcription of cytokines and apoptosis of T-cells are still somewhat uncertain. Currently, it is thought that this interaction causes the entry of calcium into the cells, thus inducing apoptosis (22). The TIM-3/galectin-9 pathway appears to be an important factor in the pathogenesis of CD. TIM-3 is a surface molecules electively expressed in LyTh1 of the intestinal mucosa. Galectin-9 binding regulates these lymphocytes in the healthy mucosa. It has been observed that in patients with CD, the autoregulation of LyTh1 is lost due to a reduced concentration of galectin-9 and a reduced TIM-3 expression in LyTh1 (19). This could be one of the reasons for the state of perpetual activation in LyTh1, resulting in the chronic inflammatory process. This hypothesis opens up a possible pharmacological solution for disorders characterized by TIM-3/galectin-9 alterations, with the development of drugs targeted at increasing the amount of galectin-9 in patients with CD (23).

## 3. Conclusions

These data suggest that TIM-3 plays a pro-apoptotic role in immunosuppression and production of pro-inflammatory cytokines, dependent on the concentration of galectin-9. Based on these observations, drugs that induce the interaction between TIM-3 and galectin-9 could be created, aimed at reducing the inflammatory processes characteristic of CD, and the consequent chronicization. Further studies are needed to better determine the clinical and therapeutic aspects of TIM-3/galectin-9 binding.

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