FOOD AND INFLAMMATION

EXCLUSIVE ENTERAL THERAPY IN CROHN'S DISEASE: IMPACT ON IMMUNOMETABOLIC ASSET AND T REG FUNCTION

Caterina Strisciuglio

Principal investigator
Caterina Strisciuglio

Other components of the research group
Alessandra Vitale
Massimo Martinelli
Elena Scarpato

Background

The inflammatory bowel diseases (IBD) are chronic inflammatory disorders of gastrointestinal tissues that include Crohn's disease (CD) and ulcerative colitis (UC). IBD are characterized by a chronic course requiring a lifelong treatment. It has been reported that an overall 25% of cases are diagnosed in the childhood (1). In the pathogenesis of IBD, the adaptive immune system, antigen specific and conferring long lasting and immunological memory, plays a key role. In contrast, the innate immune system, that is the first line of defense with an immediate protective response against infections, likely contributes to the inflammation initiation (2). Regulatory T cells (Tregs) are able to prevent excessive inflammation that can lead to the rupture of intestinal homeostasis, by modulating cells of both the innate and adaptive immune systems. Indeed, it has been demonstrated that an altered balance between regulatory T cells and effector T cells in the intestinal microenvironment is involved in the pathogenesis of IBD (3). Although there are many different subpopulations of Tregs, the most investigated are the type 1 regulatory cells (Tr1) producing IL-10 and TGF-β, the conventional Forkhead box protein 3 (FOXP3) positive regulatory T cells, and the TGF-β-producing Th3 cells, these latters with a role in oral tolerance (4). While, to our knowledge, Tr1 cells have not yet been investigated in IBD, studies on conventional Tregs in human IBD are more numerous, although with contrasting

results, in particular on their frequency and suppressive function (5,6). Furthermore, the majority of studies on Tregs refers to adult IBD, and very few pediatric studies have been reported (7-9). However, it has been postulated that pediatric IBD represent a separate entity with specific clinical peculiarities requiring a specialized medical care (10). Indeed, pediatric IBD cohort represents a patient population ideal for the study of pathogenic mechanisms because of the different pathogenic origin, natural history and outcome compared to adult. For this reason, the aim of our study was to evaluate the frequency of peripheral blood Tr1 and FOXP3+ Tregs in the same patients at the onset of disease and during remission after pharmacological therapy. Moreover, we evaluated how the density of Tr1 and FOXP3+ Tregs correlated with disease activity.

Main achievements

We observed, in peripheral blood of both types of IBD, that the percentage of Tr1 cells at the diagnosis was increased compared to HC group, and this cell population critically decreased during remission under pharmacological treatment reaching the same frequencies as in HC subjects. Instead, we assessed that the frequency of FOXP3+ cells at diagnosis of IBD was comparable to that of controls and decreased after therapy. A more pronounced thymopoietic activity in children may explain the maintenance of a higher frequency of circulating Tregs during active IBD, which is different from what occurs in adult patients, in whom the recruitment of Tregs at mucosal sites correlates with a depletion of the systemic compartment. Moreover, our data suggest that the immunoregulatory potential of IBD may change with the duration of disease. Indeed, it has been demonstrated that there are clear differences in the production and in the response to cytokines by T cells in early and late Crohn's disease (11). During the initial manifestation of the disease, mucosal T cells are susceptible to the modulatory influence of Th1-driving cytokines, but once the disease progresses this immunoregulation changes (11). Modifications in the composition of tissue T cells thus appear more likely to be dependent on the basic biology of the disease process. We have, on the other side, also analyzed the activation status of FOXP3+ Tregs. We have examined some specific markers, such as CCR7, CTLA-4 and PD-1, involved in mediating the suppressive function and migration of FOXP3+ Tregs. CCR7 has been studied as a marker of homing to secondary lymphoid tissue (12), while CTLA-4 is a key inhibitory receptor, which might be required to restrain immune responses of conventional T cells (13). In summary, we found that reduced Tregs frequency at the follow-up was associated with an increased activation status of these cells compared to the Tregs of the same patients at the diagnosis. At the onset of disease, the increase percentage of Tregs, although not associated with an activated status, could be a natural process to counteract ongoing inflammatory responses.

Despite the fact that Tregs percentage decreases at follow-up, the pharmacological treatment could stimulate the fractions of Tregs to apply their suppressive functions through expression of these specific markers, for example CTLA-4, contrasting a destructive inflammatory response, as demonstrated for this and other diseases (14). Interestingly, we observed in IBD young patients during acute disease at diagnosis a direct correlation between FOXP3+ Tregs frequency and CRP, and an indirect correlation of Tregs and fecal calprotectin. The reason for this discrepancy could be due to the fact that we were analyzing Tregs derived from peripheral blood that well correlate with CRP, a marker of systemic inflammation measured in the blood, whereas calprotectin is a marker of intestinal inflammation, which could better and directly correlate to Tregs derived from the intestinal mucosa.
Our study demonstrates that, although children with IBD at diagnosis show a high number of Tregs, most likely this regulatory cells are not enough in terms of amount or functionality to counteract the inflammation. Moreover, once the patients are in remission under pharmacological therapy, the Tregs decrease in the number but there is an improvement of their activation status, most probably due a selective enhancement of functional Tregs. Recently, several studies considered the possibility to treat CD patients through a cell therapy based on Tregs(15-17). Despite the presence of unknowns and theoretical risks, the use of Tregs as medical strategy in IBD may help to reset the adverse intestinal immunity. Current approaches for IBD depend on the use of non-specific immunosuppressive agents such as steroids and anti-cytokine; these treatments are not effective in all patients, are nonspecific, and never provide a complete disease resolution. By contrast, Tregs therapy would offer an antigen-specific and potent cure by targeting peculiar antigens at the site of inflammation. In conclusion, since evidence to date suggests that Tregs could contrast the inflammatory immune response in IBD pediatric patients, the in vitro expansion of Treg cells (18) may be an appealing and feasible approach for future autologous cell therapy for IBD.

Future perspectives

Epidemiological studies have shown an increase in the incidence of the IBD in the more westernized countries, such as Europe and North America (19), demonstrating how a diet rich in fats and proteins, but low in fruit and vegetables, can favor the development of these inflammatory diseases (20). Among the environmental risk factors, a fundamental role seems to be played by the diet. To confirm this hypothesis, methods of treating the disease have been developed, specifically for Crohn's disease (MC), which offer an alternative to steroid therapy, one of which is enteral nutrition (Exclusive Enteral Nutrition, EEN), this last provides the exclusive administration of a polymer formula for eight weeks. The mucosal healing rate has

been shown to be significantly higher in patients treated with EEN compared to those with steroid-induced remission (21). Moreover, ENN is able to induce a more favorable sustained remission than steroids, for a maximum of 3 years (22). Studies conducted on the adult population also suggest that this type of treatment could reduce the risk of relapse and improve the biological response to drugs (23). But, pediatric patients have specific characteristics, not only because of the severity and extent of the disease, but also because the disease affects individuals in the growth phase, which could face possible irreversible consequences in adult life. Weight loss, growth retardation, delayed puberty and low bone mineral density are frequent signs in children with IBD, particularly in CD, due to malabsorption and malnutrition. In fact, children experience a reduced dietary intake, and since inflammation leads to greater loss of nutrients, there is an increase in energy demands and an altered metabolism (24,25). Furthermore, the linear growth and the correct nutritional status of children affected by IBD are also compromised by immunosuppressive drugs, in particular corticosteroids, used more frequently for the treatment of the disease (26). All these reasons justify the particular importance of nutritional therapy in pediatric age groups as a therapeutic alternative. Therefore, the aim of our future study is to analyze the immunological bases of EEN in a pediatric population, to better understand the pathogenetic mechanisms underlying the disease and to possibly identify alternative pathways on which to intervene with new therapeutic approaches. We will evaluate the impact of ENN on immunometabolic asset and T regulatory cells (Tregs) function. Moreover, we will stratify our patients in two groups, after ENN therapy, patients that reintroduce either a gluten free diet or regular diet containing gluten, to understand the role of gluten on immunometabolic asset of Tregs and to evaluate if this diet component can lead to a relapse of the disease. Moreover, using this type of approach it would be interesting to study if there are also diet elements that can play a role in reactivation of this pathology.

References

1. *Loftus EV Jr. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. Gastroenterology 2004; 126:1504-17.*
2. *Wallace KL, Zheng L, Kanazawa Y, et al. Immunopathology of inflammatory bowel disease. World. J. Gastroenterol. 2014; 20:6-21.*
3. *Mayne CG, Williams CB. Induced and natural regulatory T cells in the development of inflammatory bowel disease. Inflamm Bowel Dis. 2013; 19:1772-1788.*
4. *Shevach EM. From vanilla to 28 flavors: multiple varieties of T regulatory cells. Immunity 2006; 25:195-201.*
5. *Kelsen J, Agnholt J, Hoffmann HJ, et al. FOXP3(+)CD4(+)CD25(+) T cells with regulatory properties can be cultured from colonic mucosa of patients with Crohn's disease. Clin Exp Immunol 2005; 141:549-557.*

6. *Maul J, Loddenkemper C, Mundt P, et al. Peripheral and intestinal regulatory CD4+ CD25(high) T cells in inflammatory bowel disease. Gastroenterology 2005; 128:1868-78.*

7. *Sznurkowska K, Żawrocki A, Sznurkowski J, et al. Peripheral and Intestinal T-regulatory Cells are Upregulated in Children with Inflammatory Bowel Disease at Onset of Disease. Immunol Invest 2016; 19:1-10.*

8. *Reikvam DH, Perminow G, Lyckander LG, et al. Increase of regulatory T cells in ileal mucosa of untreated pediatric Crohn's disease patients. Scand J Gastroenterol 2011; 46:550-560.*

9. *La Scaleia R, Morrone S, Stoppacciaro A, et al. Peripheral and intestinal CD4+ T cells with a regulatory phenotype in pediatric patients with inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2010; 51:563-72.22.*

10. *Baecher-Allan C, Brown JA, Freeman GJ, et al. CD4+CD25high regulatory cells in human peripheral blood. J Immunol 2011; 167:1245-1253.*

11. *Kugathasan S, Saubermann LJ, Smith L, et al. Mucosal T-cell immunoregulation varies in early and late inflammatory bowel disease. Gut 2007; 56:1696-1705.*

12. *Procaccini C, Matarese G. Regulatory T cells, mTOR kinase, and metabolic activity. Cell Mol Life Sci 2012; 69:3975-87.*

13. *Walker LS, Sansom DM. The emerging role of CTLA-4 as a cell-extrinsic regulator of T cell responses. Nat. Rev. Immunol 2011; 11:852-63.*

14. *Boschetti G, Nancey S, Sardi F, et al. Therapy with anti-TNFα antibody enhances number and function of FOXP3(+) regulatory T cells in inflammatory bowel diseases. Inflamm Bowel Dis 2011; 17(1):160-70.*

15. *Desreumaux P, Foussat A, Allez M, et al. Safety and efficacy of antigen specific regulatory T-cell therapy for patients with refractory Crohn's disease. Gastroenterology 2012; 143:1207-1217.*

16. *Kumar P, Saini S, Khan S, et al. Restoring self-tolerance in autoimmune diseases by enhancing regulatory T-cells. Cell Immunol 2018; pii: S0008-8749(18)30318-6.*

17. *Jia X, Zhai T, Wang B, et al. Decreased number and impaired function of type 1 regulatory T cells in autoimmune diseases. J Cell Physiol 2019; doi: 10.1002/jcp.28092.*

18. *Himmel ME, Yao Y, Orban PC, et al. Regulatory T-cell therapy for inflammatory bowel disease: more questions than answers. Immunology 2012; 136(2):115-22.*

19. *Thia KT, Loftus EV, Jr Sandborn WJ, Yang SK. An update on the epidemiology of inflammatory boweldisease in Asia. Am. J. Gastroenterol. 2008; 103, 3167-3182.*

20. *Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: A systematic review of the literature. Am. J. Gastroenterol. 2011; 106, 563-573.*

21. *Borrelli O, Cordischi L, Cirulli M, Paganelli et al. Polymeric diet alone versus corticosteroids in the treatment of active pediatric Crohn's disease: arandomized controlled open-label trial. Clin Gastroenterol Hepatol 2006; 4: 744-753.*

22. *Grover Z, Burgess C, Muir R, et al. Early mucosal healing with exclusive enteral nutrition is associated with improvement outcomes in newly diagnosed children with luminal Crohn's disease. J Crohn's Colitis 2016; 1-6.*

23. *Yamamoto T, Nakahigashi M, Umegae S et al. Prospective clinical trial: Enteral nutritionduring maintenance infliximab in Crohn's disease. J. Gastroenterol. 2010; 45, 24-29.*

24. *Azcue M, Rashid M, Griffiths A et al. Energy expenditure and body composition in children with Crohn's disease: Effect of enteral nutrition and treatment with prednisolone. Gut 1997; 41, 203-208.*

25. Bannerman E; Davidson I; Conway C et al. Altered subjective appetiteparameters in Crohn's disease patients. Clin. Nutr. 2001; 20, 399-405.

26. Gerasimidis K, McGrogan P, Edwards CA. The aetiology and impact of malnutrition in paediatric inflammatory bowel disease. J. Hum. Nutr. Diet. 2011; 24, 313-326.

Publications

1. Strisciuglio C, Cenni S, Giugliano FP, Miele E, Cirillo G, Martinelli M,Vitale A, Tolone C, Staiano A, Miraglia Del Giudice E, Perrone L. The Role of Inflammation on Vitamin D Levels in a Cohort of Pediatric Patients With InflammatoryBowelDisease. J Pediatr Gastroenterol Nutr. 2018; 67(4):501-506.

2. Tolone C, Bellini G, Punzo F, Papparella A, Miele E, Vitale A, Nobili B, Strisciuglio C, Rossi F. The DMT1 IVS4+44C>A polymorphism and the risk of irondeficiency anemia in children with celiac disease. PLoS One. 2017; 12;12(10):e0185822.

3. Vitale S, Strisciuglio C, Pisapia L, Miele E, Barba P, Vitale A, Cenni S, Bassi V, Maglio M, Del Pozzo G, Troncone R, Staiano A, Gianfrani C. Cytokine production profile in intestinal mucosa of paediatric inflammatory bowel disease. PLoS One. 2017; 12(8):e0182313.

4. Strisciuglio C, Bellini G, Miele E, Martinelli M, Cenni S, Tortora C, Tolone C, Miraglia Del Giudice E, Rossi F. Cannabinoid Receptor 2 Functional Variant Contributes to the Risk for Pediatric Inflammatory Bowel Disease. J ClinGastroenterol. 2018; 52(5):e37-e43.

5. Strisciuglio C, Giugliano F, Martinelli M, Cenni S, Greco L, Staiano A, Miele E. Impact of Environmental and Familial Factors in a Cohort of Pediatric Patients With Inflammatory Bowel Disease. J Pediatr Gastroenterol Nutr. 2017; 64(4):569-574.

6. Strisciuglio C, van Deventer S. Regulatory T cells as potential targets for immunotherapy in inflammatory bowel disease. Immunotherapy. 2010; 2(6):749-52.

7. Giugliano FP, Strisciuglio C, Martinelli M, Andreozzi M, Cenni S, Campione S, D'Armiento M, Staiano A, Miele E. DoesAzathioprine induce endoscopic and histologic healing in pediatric inflammatory bowel disease? A prospective, observational study. Dig Liver Dis. 2018; 50(3):240-246.

8. Galatola M, Miele E, Strisciuglio C, Paparo L, Rega D, Delrio P, Duraturo F, Martinelli M, Rossi GB, Staiano A, Izzo P, De Rosa M. Synergistic effect of interleukin-10-receptor variants in a case of early-onset ulcerative colitis. World J Gastroenterol. 2013; 19(46):8659-70.

9. Galatola M, Izzo V, Cielo D, Morelli M, Gambino G, Zanzi D, Strisciuglio C, Sperandeo MP, Greco L, Auricchio R. Gene expression profile of peripheral blood monocytes: a step towards the molecular diagnosis of celiac disease? PLoS One. 2013; 8(9):e74747.

External collaborations

- Giuseppe M, Department of Molecular Medicine and Medical Biotechnology, University Federico II, Naples, Italy and Laboratory of Immunology, Endocrinology and Experimental Oncology Institute, National Research Council (IEOS-CNR), Naples, Italy