IMMUNOMODULATORY FUNCTION OF γδ+ INTRAEPITHELIAL LYMPHOCYTES

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Background

Although the expansion of γδ+ IELs is a hallmark of celiac disease (CD), to date we know little about the mechanism underlying their role in CD pathogenesis. An increased proportion of γδ+ IELs secreting TGF-β has been reported in intestinal biopsies from patients on gluten-free diet compared to patients with active CD, pointing to a regulatory role of small intestinal γδ+ IELs (Bhagat et al., 2008). More recently it was shown that γδ+ T cells isolated from the celiac gut display an activated, effector memory phenotype, and retain the ability to rapidly respond to in vitro stimulation by gliadin (Dunne et al., 2013). Despite the increased frequency in vivo and suppressive activity in vitro, γδ+ IELs do not control the development of inflammation in the small intestinal mucosa with active CD, suggesting a defect in the activation of regulatory mechanisms.
Cerf-Bensussan et al showed that IL-15, overexpressed in active CD, was involved in the local downregulation of TGF-β signaling (Benahmed et al., 2007, Hmida et al., 2012). It is known that the upregulation of IL-15 in CD correlates with the degree of mucosal damage (Di Sabatino et al. 2015). In addition, our group showed that IL-15 impairs the functions of Treg cells in CD (Zanzi et al., 2011). Most studies analyzed cytokine mRNA expression in IELs of CD patients, using techniques that fail to provide an isolation of highly pure cell populations, that is important to identify and define biological processes in CD pathogenesis. The development in the last decade of laser capture microdissection (LCM) has allowed this goal to be achieved (Iacomino et al., 2016). Furthermore, organ culture of the small intestine is a valuable model to study the immunological events occurring in the celiac intestinal mucosa following contact with gliadin peptides (Mazzarella et al., 2003, Mazzarella et al. 2008, Mazzarella et al., 2015).

Main achievements

In the last few years we obtained important results regarding the CD pathogenisis by using the organ culture model and the LCM technology. In particular, we have shown that the surface epithelium and lamina propria compartments play a prominent role in determining innate and adaptive mucosal immunity, respectively. We also showed that surface epithelium and lamina propria produce cytokines with dominant suppressive activity, suggesting that immune cells of both compartments are actively trying to downregulate ongoing inflammation.

Future perspectives

Our aim is to investigate if IL-15, could impair, in vivo or in vitro, the immunomodulatory function of γδ+ IELs, downregulating the production of anti-inflammatory cytokines. In particular, the study will focus on γδ+ IELs by investigating:

- Immunomodulatory function of γδ+ IELs, infiltrating the small intestinal mucosa of subjects in different CD histological stages (Marsh 0 to Marsh III), isolated by immuno-LCM.
- Immunomodulatory function of TCRγδ+ IELs isolated from small intestinal mucosa of treated CD patients, by immuno-LCM, after gliadin in vitro stimulation with or without IL-15.

The study will aim to assess whether IL-15 may have a role on the immunomodulatory function of γδ+ IELs, downregulating the production of anti-inflammatory cytokines, as we have demonstrated for the Treg cells in CD (Zanzi et al. 2011).

Immunomodulatory function of γδ+ IELs will be evaluated in small intestinal biopsies of CD subjects that were adequately adhering to a strict gluten-free diet (histological stages Marsh 0-I), in small intestinal biopsies of CD subjects that were on diet containing gluten (histological stages Marsh II-III) and in small intestinal biopsies obtained from treated CD patients cultured in vitro for 24 h with gliadin, in presence or absence of IL-15. Anti-inflammatory IL-10 and TGF-β cytokines production, will be assessed on highly pure population of γδ+ IELs, isolated by immuno-LCM. Moreover, also the eventual production of pro-inflammatory (INF-ϒ, TNF-α, IL-17) cytokines will be analyzed. Cytokines can have a pivotal role in many different immunomediated pathologies and sometimes they might represent an ideal therapeutic target as clearly shown for TNFα in rheumatoid arthritis (Elliot et al., 1994) and Crohn's disease (van Dullemen et al., 1995). Moreover, cytokines, such as IL-15, have been reported to be involved in the progression of different types of lymphomas (Dobbeling et al., 1998). If IL-15 interferes with the regulatory activity of γδ+ IELs, we might expect that in active CD patients, where IL-15 is overexpressed, we can find a downregulation of

anti-inflammatory cytokines production. Our study will provides insights into the immunomodulatory function of human small intestinal γδ+ IELs, which might help in the identification of potential therapeutic strategies for patients with CD. Besides to the role of human small intestinal γδ+ IELs, we could use immuno-LCM technique to identify other cell populations of the intestinal mucosa in its different compartments, to evaluate the different gene expression profile of each single cell population. In this way, we could improve the knowledge concerning the mechanisms that support the innate and adaptive immune response in CD.

Publications

1. *Gianfrani C, Maglio M, Rotondi Aufiero V, Camarca A, Vocca I, Iaquinto G, Giardullo N, Pogna N, Troncone R, Auricchio S, Mazzarella G. Immunogenicity of monococcum wheat in celiac patients. Am J Clin Nutr. 2012; 96(6):1339-45. doi:10.3945/ajcn.112.040485. Epub 2012 Nov 7.*
2. *Gianfrani C, Camarca A, Mazzarella G, Di Stasio L, Giardullo N, Ferranti P, Picariello G, Rotondi Aufiero V, Picascia S, Troncone R, Pogna N, Auricchio S, Mamone G. Extensive in vitro gastrointestinal digestion markedly reduces the immune-toxicity of Triticum monococcum wheat: implication for celiac disease. Mol Nutr Food Res. 2015; 59(9):1844-54. doi:10.1002/mnfr.201500126. Epub 2015 Jun 26.*
3. *Iacomino G, Marano A, Stillitano I, Aufiero VR, Iaquinto G, Schettino M, Masucci A, Troncone R, Auricchio S, Mazzarella G. Celiac disease: role of intestinal compartments in the mucosal immune response. Mol Cell Biochem. 2016; 411(1-2):341-9. doi:10.1007/s11010-015-2596-7. Epub 2015 Nov 5.*
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External collaborations

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