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Maria Laura Cupi

PhD Supervisor: Prof. Giovanni Monteleone

University of Tor Vergata, Department of Systems Medicine
Gastroenterology Unit, Via Montpellier 1, 00133 Rome, Italy

Immunologic characterization and role of cytotoxic B-lymphocytes in intestinal mucosa of IBD patients

Background

Inflammatory Bowel Diseases (IBDs) are chronic, relapsing inflammatory diseases of gastrointestinal tract. IBDs comprise two types of intestinal disorders: Crohn's disease (CD) and ulcerative colitis (UC), which are clinically distinguished by intestinal localization, local features of inflammation, a profile of complications and familial aggregation. Although the aetiology of CD and UC is still unknown, a large body of evidence suggests that IBDs result from an interaction between genetic and environmental factors, which eventually leads to an excessive and poorly controlled mucosal inflammatory response directed against components of the normal microflora. Both innate and adaptive immune responses have been implicated in the pathogenesis of IBD-related tissue damage. Indeed, in IBDs the inflammatory response often results in continued epithelial injury, which causes erosions, ulcerations, and a decrease in the production of defensin. The epithelial damage is often mediated by the activity of cytotoxic molecules mainly secreted by CD8 T lymphocytes and Natural Killer cells. This results in the increased exposure to intestinal microbiota and amplification of the inflammatory response.

B cells play an important role in human gastrointestinal health by producing elevated levels of immunoglobulins, which contribute to immune protection and epithelial barrier integrity. Various alterations in B cells number and function have been described in IBDs, raising the possibility that dysfunction in this cell type contribute to the amplification and maintenance of the IBD-associated-tissue damaging inflammation. In animal models of colitis, both anti-inflammatory and pro-inflammatory roles of B cells have been described. However, despite the large body of data, the role of B cells in IBDs is not yet clear. It was recently shown that CD19-positive cells are able to produce cytotoxic molecules in other systems.

Aim

To evaluate whether B cells have the ability to cause epithelial damage in the gut.

Specific aims:

1. To perform phenotypic analysis of B cells infiltrating the gut of patients with IBD.

We will isolate lamina propria mononuclear cells (LPMCs) from the colonic mucosa of CD, UC and from the non-inflamed mucosa of non-IBD patients (as control) with a well-established protocol. We will evaluate the percentage of CD19-positive cells that co-express CD20, CD5, CD27, CD38, CD21, CD80, CD86, CD11b, CD45, CD71, CD40 and CD72 by flow-cytometry. We will compare the percentage of the different B cell sub-populations among CD, UC and non-IBD controls.

2. To determine whether B cells produce cytotoxic molecules.
We will evaluate the percentage of CD19- and IgA-positive cells, which express perforin and granzyme B in CD, UC and non-IBD patients by flow cytometry. Data will be confirmed by performing Real-Time PCR in CD19-positive cells purified from colonic mucosa of IBD and non-IBD patients. Moreover, we will characterize which subset of B cells produces granzyme B and perforin.
To assess the ability of B cells to induce epithelial damage, we will evaluate the percentage of Annexin V- and/or propidium iodide (PI)-positive CFSE-labeled HCT-116 cells (colonic epithelial cells) cultured in the presence or absence of CD19-positive cells, purified from the intestine of IBD patients.

3. To evaluate the molecular mechanism that regulates the expression of cytotoxic molecules.
B cells express the IL-21 receptor. IL-21, a cytokine highly expressed in IBDs colonic mucosa, is known to regulate B cells development and functions (proliferation, differentiation into plasma cells and apoptosis). To evaluate if IL-21 positively regulates the expression of cytotoxic molecules of colonic CD19-positive B cells, purified from non-inflamed colonic mucosa of non-IBD patients, will be cultured in the presence or absence of increasing doses of IL-21 and the expression of granzyme B and perforin evaluated by flow-cytometry, Real-time PCR and ELISA. Moreover we will culture LPMC isolated from CD and UC patients in the presence or absence of a specific neutralizing antibody anti-human IL-21 and evaluate the percentage of CD19+ cells which express granzyme B and perforin by flow-cytometry.

We will use CD19, CD20, CD5, CD27, CD38, CD21, CD80, CD86, CD11b, CD45, CD71, CD40 and CD72 to characterize B cell populations. To Exclude T cells and APC we will also use CD3, CD4, CD8, HLA-DR, CD11b and CD11c. We will use Annexin V to evaluate the percentage of apoptotic CFSE-positive HCT-116. Finally, we will use Control-IgG isotypes to exclude non-specific signals.