ImmunoTools *special* Award 2014

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Dysbalance in BCL-2 family proteins may cause resistance to standard medical therapies in IBD patients and thus therapy failure

IBD is a prototypic chronic inflammatory disease with increasing incidence in the industrialized world (20000 Swiss people living with IBD) and is characterized by a chronic inflammation of the intestinal wall. One third of the patients cannot be sufficiently treated. IBD comprises two main disease conditions, ulcerative colitis (UC) and Crohn's disease (CD). In the pathogenesis of IBD, a chronic activation of the mucosal immune system leading to chronic inflammation occurs. It has been assumed that chronic inflammation is due to an imbalance between inflammatory and anti-inflammatory cytokine production (*1-3*). An exaggerated response of T lymphocytes to luminal antigens is suggested to increase intestinal inflammation. In CD, the reason for this exaggerated response is associated with a diminished turnover of mucosal T-cells.

Dysregulated apoptosis of lymphocytes has major impact on the pathogenesis of inflammatory bowel disease (IBD). T-lymphocytes escaping normal apoptosis and showing exaggerated response to luminal antigens trigger intestinal inflammation. Disturbed lymphocyte apoptosis during IBD is associated with an enhanced expression of the pro-survival protein B-cell lymphoma-2 (BCL-2) as determined in lamina propria T-cells of patients with CD compared to controls.

Sulfasalazine, glucocorticoids, azathioprine (AZA) and anti-TNF agents are standard medical therapies in IBD regulating both synthesis of pro-inflammatory cytokines and inducing apoptosis of activated lymphocytes by a decrease of anti-apoptotic BCL-x_L and BCL-2 or by triggering pro-apoptotic BAX accumulation.

Failure to respond to medical therapies is a common indication for surgery in IBD. As many as 80% of patients with CD and approximately 20% of patients with ulcerative colitis (UC) require surgery in their lifetime as a result of poor response to therapy. Though there are no known predictive factors for resistance to or relapse upon medical therapies. We propose to characterize the BCL-2 family-related risk of therapy resistance and its usefulness as parameter for the prediction of clinical relapse upon medical therapy.

In our previous work we showed that chronic experimental colitis was aggravated in *Bim*$^{-/-}$ mice. In line with our hypothesis on the role of Bim an impaired removal of autoreactive lymphocytes contributed to aggravated chronic mucosal inflammation. In contrast, increase in free available BIM (by pro-apoptotic ABT-737, a potent inhibitor of BCL-2) ameliorated intestinal inflammation. We propose to further study the pro-apoptotic mechanisms in the pathophysiology of colitis.

<u>Hypothesis:</u> Based on our findings we hypothesize that apoptosis mediated by BCL-2-inhibitors ameliorates colitis.

Inducing apoptosis of autoreactive lymphocytes may be a new promising therapeutic strategy for IBD patients. Treatment with BCL-2-inhibitors could improve the value of medical therapy by boosting the initiation of cell death in apoptosis-resistant lymphocytes. A long-term goal is the development of new therapeutic options for the treatment of IBD via the regulation of the turnover of lymphocytes.

BIM-mediated apoptosis by BCL-2-inhibitors adjusts the balance between activated and regulatory lymphocytes and ameliorates colitis

Pro–apoptotic protein BIM modulates lymphocyte killing by neutralizing it's opponent BCL-2 (as well as BCL-x_L, BCL-ω and MCL-1) which attenuates apoptosis. The balance of pro- and anti-apoptotic proteins determines the life span of lymphocytes. Upon ABT-737-treatment the amount of free available BIM in the cellular compartment could be increased followed by an ameliorated colitis. In this application we suggest the $CD4^+CD62L^+$ T-cell transfer model of CD to determine the effectiveness of ABT-737-treatment. The potential of ABT-737 to initiate apoptosis in

autoreactive lymphocytes from IBD patients with clinical relapse upon therapy will be determined *in vitro*.

ImmunoTools reagents will be used for flow cytometry as well as sorting of cells from murine spleen, peripheral blood and intestinal mucosa as well as human peripheral blood and intestinal mucosa.

ImmunoTools *special* AWARD for **Martin Hausmann**

includes 23 reagents

FITC - conjugated anti-human CD4, CD8, CD25, CD45RA, CD45RB, CD62L, Annexin V,

PE - conjugated anti-human CD25, CD62L,

PerCP - conjugated anti-human CD4, CD8,

APC -conjugated anti-human CD4, CD8, CD62L Annexin V,

FITC - conjugated anti-mouse CD4, CD8a, CD45R, CD62L,

PE - conjugated anti-mouse CD62L,

APC - conjugated anti-mouse CD4, CD8a, CD62L

DETAILS