We asked whether this effect is mediated via surface or lysosomal (phagosomal) receptors. IL-1ß promotes phagocytosis and clearance of bacteria and aids the gut in eliciting inflammation. Recent studies have shown that treatment with SCFAs (acetate, propionate or butyrate) stimulated release of IL-1ß and IL-18. SCFAs act on free fatty acid receptors FFAR2 and FFAR3, inhibition of histone deacetylases (HDAC) and as a source of energy. SCFAs are known to impact cytokine production by primary human immune cells. Treatment with SCFAs decreased TNFalpha (TNFa), Interleukin (IL)-10, IL-6, IL-18, IL-1ß).

We investigated the role of SCFAs in dextran sulfate sodium-induced colitis and their effect on the cytokine production by primary human immune cells. Treatment with SCFAs (acetate, propionate or butyrate) stimulated release of IL-1ß and IL-18 by buffy coat leukocytes or U937 cells without causing increased expression of corresponding genes. This raises the possibility of activation via the NLRP3 (NOD-like receptor family, pyrin domain-containing-3) inflammasome. NLRP3 is a multi-molecular inflammasome complex. Once activated, NLRP3 inflammasome releases caspase 1 leading to formation of mature IL-1ß and IL-1ß. Recent studies have shown that IL-1ß promotes phagocytosis and clearance of bacteria and aids the gut in eliciting an effective response in early stages of IBD.

We asked whether this effect is mediated via surface or lysosomal (phagosomal) receptors? To answer this question, we prepared a series (SYD010) of novel compounds which are able to accumulate in the phagolysosome of immune cells through their macrolide backbone and deliver SCFAs bound as esters to the lumen of the activated lysosome. In vitro, together with LPS stimulation, the substances modulated secretion of TNFalpha, IL-1ß, IL-6 and IL-8 at concentrations about 100x lower than free SCFAs (Figure 1). When tested in a DSS colitis mouse model, the SYD010 series caused a decrease in diarrhea scoring compared to the vehicle-treated control group at a concentration of 0.1 mg/kg (Figure 2) which corresponds to a total dose of ca. 100 nmol/kg (the compounds are systemically distributed). This is lower than the known luminal concentrations of SCFAs which is in the range of 40 mM. Our underlying hypothesis is that lysosomal reception of SCFAs leads to beneficial immune modulation in colitis in so far as stimulation of IL-1ß release promotes bacterial clearance. Furthermore, that concentrative uptake to the phagolysosome leads to enhanced stimulation of these receptors leading to responses at lower ambient concentrations or doses. We are assessing this substance class as potential IBD therapeutics.

Figure 1. Effect of sodium butyrate or CSY4286 (lysosomal butyrate donor) on cytokine production by U937 cells. Supernatants were harvested after 24 h (IL-6) and 48 h (IL-1ß, IL-6, IL-8) incubation with the test substances and cytokines determined by ELISA. The dotted line represents levels with LPS stimulation alone. SEM was applied for error bars.

Figure 2. Results from DSS-induced (2.5% in drinking water) IBD study in BALB/c mice (8 mice per group). Scoring of body weight and diarrhea score over 8 days (a and b show data from final day of study). SEM was applied for error bars.

Diagnoses in IBD

PO56

DISCRIMINATORY ROLE OF ANTI-MICROBIAL ANTIBODIES IN DIAGNOSIS OF CROHN’S DISEASE AGAINST NORMAL AND OTHER IMMIMICS

Peilin Zhang, Lawrence Minardi, J. Todd Kuenstner, Steve Zekan, Rusty Kruzelock

Anti-microbial antibodies have been found useful for diagnosis of Crohn’s disease (CD) and Sjogren’s syndrome (Sjo). These antibodies are also elevated in other autoimmune disease such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and multiple sclerosis (MS). The prevalence of these antibodies in the normal healthy population is unknown. We set to survey the normal population for these anti-microbial antibodies in comparison with Crohn’s disease and others. Totally 288 blood samples from the donor units of the leukocyte-reduced red blood cells from the American Red Cross were examined for the presence of anti-microbial antibodies using direct ELISA assays established in our laboratory using the recombinant microbial protein antigens. Our results showed that the prevalence of RPOB antibody in the normal blood donor population is 2.4% (7 positive of 288 samples). The prevalence of EF-G antibody is 4.2% (12 positive of 288 samples), ATP5a 5.2% (15 positive), Hsp65 2.8% (8 positive), EF-Tu 5.6% (16 positive), and NMPC 4.2% (12 positive). Meanwhile, in 109 patients with Crohn’s disease and 28 patients with Sjogren’s syndrome, these anti-bacterial antibodies were also elevated in ulcerative colitis (UC) and rheumatoid arthritis (RA). ROC curve analysis showed excellent sensitivity and specificity (Figure 2). These results indicate that the specific anti-microbial antibodies within the normal general population are uncommon, but frequent in chronic disease states. The presence of anti-microbial antibodies in patients but not in normal controls can serve as biomarkers for chronic diseases such as Crohn’s disease (CD) and Sjogren’s, and their presence indicates abnormal B-cell/plasma cell function in response to the commensal/pathogenic microbes. Since the antigens were derived from the common microbes associated with Crohn’s disease, we hypothesized that the specific anti-microbial antibodies in patients with diseases