FOOD & FUNCTION

# Active hexose correlated compound exerts therapeutic effects in lymphocyte driven colitis

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Active hexose correlated compound (AHCC) is a commercial extract of *Basidiomycetes* fungi enriched in oligosaccharides that is used as a human nutritional supplement for various purposes in humans. Our aim was to study the anti-inflammatory effect of AHCC in the CD4+ $CD62L^+$ T cell transfer model of colitis, considered one of the closest to the human disease. Colitis was induced by transfer of $CD4^+$ $CD62L^+$ T cells to recombination activating gene $1^{-/-}$ mice. AHCC (75 mg/d) was administered by gavage as a post-treatment. Three groups were established: noncolitic, colitic ($CD4^+$ $CD62L^+$ transferred mice treated with vehicle), and AHCC (colitic treated with AHCC). AHCC improved colitis, as evidenced by a 24% lower colonic myeloperoxidase and a 21% lower alkaline phosphatase activity. In addition, a decreased secretion of proinflammatory genes assessed by RT-qPCR was observed, particularly TNF-α and IL-1β. Ex vivo mesenteric lymph node cells obtained from AHCC treated mice exhibited a fully normalized production of IL-6, IL-17, and IL-10 ($p < 0.05$). Also, AHCC treated mice exhibited decreased STAT4 and IκB-α phosphorylation in splenic $CD4^+$ cells. Our data provide validation of AHCC colonic anti-inflammatory activity in a chronic, T cell driven model of inflammatory bowel disease.

**Keywords:**
AHCC / $CD4^+$ $CD62L^+$ T cell / Inflammatory bowel disease / Nutraceutical / Prebiotic

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Active hexose correlated compound (AHCC) is a commercial extract obtained from *Basidiomycetes* under controlled conditions, yielding a 74% content in oligosaccharides, specially α-glucans, plus aminoacids, lipids, and minerals [1]. AHCC is currently used as a human nutritional supplement in the management of cancer [2–11], and has preclinical antidiabetic and hepatoprotective activity [12, 13], with excellent human tolerance [14, 15]. AHCC has immunomodulatory activities [5, 16, 17]. AHCC engages TLR4 in intestinal epithelial cells and macrophages, eliciting cytokine production and secretion, actions that may result in enhanced mucosal barrier function in vivo [18]. It is possible that TLR2 is similarly modulated by AHCC [19]. AHCC may be beneficial in inflammatory bowel disease (IBD), a condition characterized by chronic intestinal inflammation with ill characterized etiology and difficult management. Current preclinical evidence obtained in a chemically induced IBD model (trinitrobenzenesulfonic acid colitis) indicates that AHCC downregulates colitis either alone [20] or in association with *Bifidobacterium longum* BB536 [21], acting at least partly as a prebiotic.

However, these models are not strictly chronic (i.e. they heal with time) and they are not lymphocyte driven as is the

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**Abbreviations**: **AHCC**, active hexose correlated compound; **IBD**, inflammatory bowel disease; **IκB**, inhibitor of kappa B; **NFκB**, nuclear factor of kappa light polypeptide gene enhancer in B-cells; **REG3-γ**, regenerating islet-derived 3 gamma; **STAT4**, signal transducer and activator of transcription 4; **TNF-α**, tumor necrosis factor alpha

 

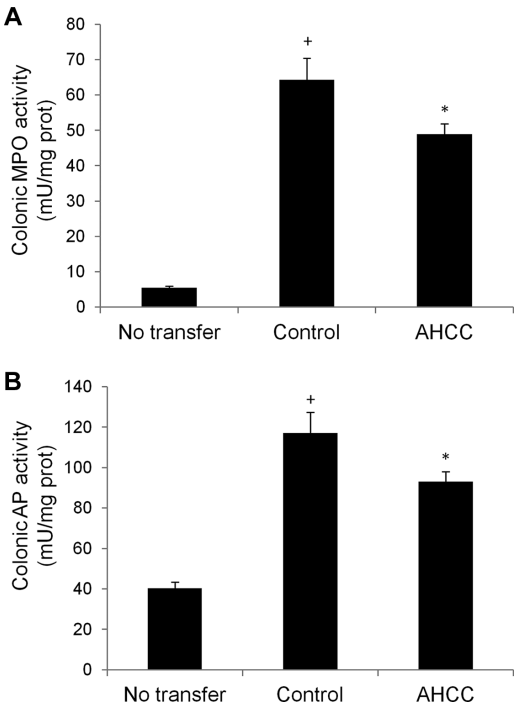


**Figure 1.** Colonic MPO and AP activity in mice with $CD4^+$ $CD62L^+$ transfer colitis. (A) MPO activity. (B) AP activity. Data are means ± SEM. $^+p < 0.05$ versus no transfer; $^*p < 0.05$ versus control.

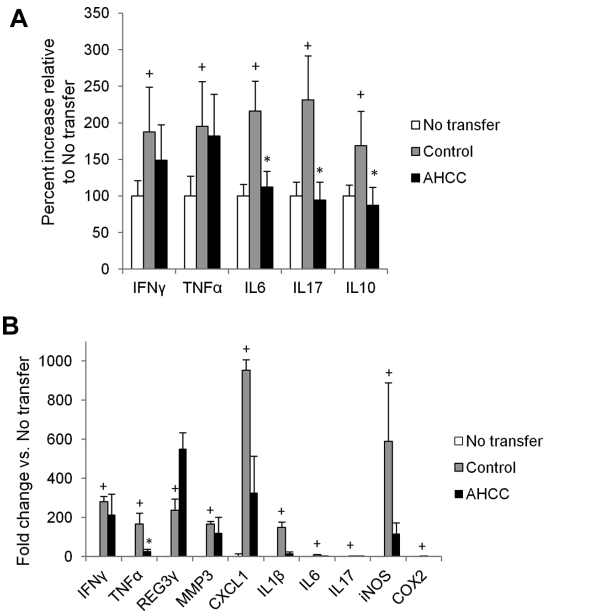


**Figure 2.** Expression of colonic inflammatory markers in mice with $CD4^+$ $CD62L^+$ transfer colitis. (A) Ex vivo cytokine production by MLN cells from mice with $CD4^+$ $CD62L^+$ transfer colitis. Cells were cultured for 48 h and cytokines were measured by ELISA and expressed as means ± SEM. (B) Colonic mRNA levels measured by RT-qPCR and expressed as means ± SEM. $^+p < 0.05$ versus no transfer; $^*p < 0.05$ versus control.

human disease. We set out to validate the use of AHCC in IBD in a more clinically relevant model, namely, $CD4^+$ $CD62L^+$ T cell transfer colitis. Briefly, immunodeficient mice are repopulated with naïve T lymphocytes for a period of 8 weeks, and which then react against the intestinal microbiota, producing colitis. Mice with established disease were randomized to receive AHCC (AHCC group, 75 mg/d, $n = 10$) by gavage or vehicle (control group, $n = 15$). In addition there was a non colitic control group (no transfer group, $n = 6$). Detailed materials and methods are contained in the Supporting Information.

AHCC treatment resulted in a positive response, based on lower disease activity index (2.8 ± 0.6 versus 4.6 ± 0.3, $p < 0.05$), colonic myeloperoxidase and alkaline phosphatase activities (Fig. 1), with a lower sensitivity of the latter to levamisole in vitro (not shown), and diminished ex vivo production of IL-6, IL-17, and IL-10 by mesenteric lymph node cells (Fig. 2A). Colonic weight:length ratio was doubled in the control animals (48.4 ± 5.5 versus 21.6 ± 1.4 mg/cm, $p < 0.05$) while it was not significantly increased in AHCC treated mice (36.5 ± 3.7 mg/cm). Since recombination activating gene $1^{-/-}$ mice have no lymphocytes, they have an underdeveloped mucosal immune system, which is restored by administration of exogenous T cells. Thus bowel wall thickening results both from mucosal immune maturation and inflammation. Consistent with this notion, the small intestine was thickened despite the apparent absence of inflammation, and AHCC had no effect at this level (not shown). Therefore, bowel wall thickening must be considered a less sensitive marker of inflammation than in other models.

Histological analysis revealed thickening of the mucosa and occasionally the submucosa, with a significant inflammatory infiltrate in control transfer animals, features that were mostly absent in AHCC treated animals (Supporting Information Fig. 1). Histological score (criterion modified from [22]) was 4.8 ± 1.1 for the control group and 1.9 ± 1.1 for the AHCC group, $p = 0.09$).

$CD4^+$ $CD62L^+$ transferred animals showed an increased colonic expression of a variety of inflammatory markers, including IFN-γ, tumor necrosis factor alpha (TNF-α), regenerating islet-derived 3-γ (REG3-γ; a bactericidal peptide produced by epithelial cells), matrix metalloproteinase-3, chemokine (C-X-C motif) ligand 1, IL-1β, IL-6, IL-17, inducible nitric oxide synthase, and cyclooxygenase 2 (Fig. 2B). Treatment with AHCC had a dramatic effect on most of them, particularly TNF-α and IL-1β, and generally tended to normalize the mRNA level increased by colitis. In contrast, REG3-γ exhibited the opposite trend i.e. it was further upregulated by AHCC rather than decreased. We confirmed the effect of AHCC at the protein level in the case of cyclooxygenase 2, finding a 20% decrease by AHCC (Fig. 3A).

Interestingly, AHCC treated mice exhibited a substantial inhibition of STAT4 phosphorylation in $CD4^+$ spleen cells (~50%, Fig. 3B). IκB-α phosphorylation was also reduced

 

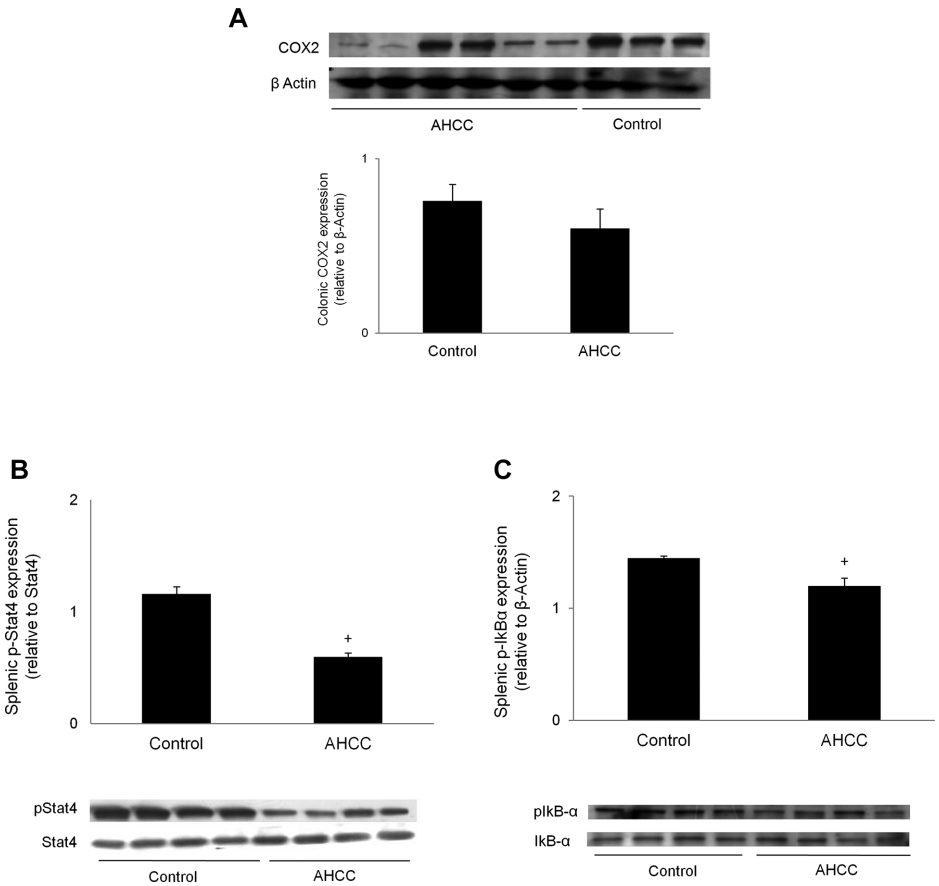


**Figure 3.** Expression of inflammatory markers in mice with $CD4^+$ $CD62L^+$ transfer colitis assessed by Western blot. (A) Colonic cyclooxygenase 2. (B and C) Activation of the STAT4 (phospho-STAT4) and NFκB (phospho-IκB-α) pathways in splenic $CD4^+$ cells of mice with $CD4^+$ $CD62L^+$ transfer colitis. Cells were isolated from the spleen of mice by positive magnetic bead separation and nuclear extracts were analyzed. Densitometry is expressed as means ± SEM. $^+p < 0.05$ versus no transfer; $^*p < 0.05$ versus control.

but to a lower extent, approximately 10% (Fig. 3C), indicating a minor role of NFκB signaling in this cell population. However, there was no significant change in the mRNA levels of IFN-γ, TNF-α, IL-1β in these cells (data not shown). These data suggest that AHCC exerts systemic immunomodulatory effects, possibly reducing the number of IFN-γ producing Th1 cells.

Although not tested in this study, the mechanism of AHCC probably involves its known prebiotic properties [20]. In addition, AHCC activates cytokine secretion in intestinal epithelial cells and in macrophages by a mechanism involving TLR4 [18], and it may also engage TLR2 [19]. These actions may paradoxically contribute to the anti-inflammatory effect, because a defective mucosal barrier function has been involved in IBD, suggesting that disturbances in the efficient control of mucosa invading microorganisms may ultimately evoke an inflammatory response. To cite some examples, Nenci et al. observed that conditional suppression of intestinal epithelial expression of IκB-α kinase-γ, resulting in reduced activation of the NFκB pathway, produced a severe inflammatory response [23]. Similarly, lack of expression of chemokine (C-X-C motif) ligand 1, considered the main chemokine responsible for neutrophil recruitment in the colon, is associated with augmented colitis [24]. In line with these findings, neutrophil depletion itself aggravates colitis [25]. Thus AHCC may limit colonic inflammation also by enhancing mucosal barrier function.

Our data offer evidence that AHCC is capable of dampening colonic inflammation in a chronic, T-cell driven model of IBD and complement our prior observations in the rat trinitrobenzenesulfonic acid model, strengthening its translational potential.

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