Anti-inflammatory effects of *Saccharomyces boulardii* mediated by myeloid dendritic cells from patients with Crohn’s disease and ulcerative colitis

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Thomas S, Metzke D, Schmitz J, Dörffel Y, Baumgart DC. Anti-inflammatory effects of *Saccharomyces boulardii* mediated by myeloid dendritic cells from patients with Crohn’s disease and ulcerative colitis. *Am J Physiol Gastrointest Liver Physiol* 301: G1083–G1092, 2011. First published September 8, 2011; doi:10.1152/ajpgi.00217.2011.— *Saccharomyces boulardii* (Sb) is a probiotic yeast that has demonstrated efficacy in pilot studies in patients with inflammatory bowel disease (IBD). Microbial antigen handling by dendritic cells (DC) is believed to be of critical importance for immunity and tolerance in IBD. The aim was to characterize the effects of Sb on DC from IBD patients. Highly purified (>95%), lipopolysaccharide-stimulated CD11c+CD123+CD123− myeloid DC (mDC) from patients with ulcerative colitis (UC; n = 36), Crohn’s disease (CD; n = 26), or infectious controls (IC; n = 4) were cultured in the presence or absence of fungal supernatant from Sb (SbS). Phenotype and cytokine production and/or secretion of IBD mDC were measured by flow cytometry and cytokometric bead arrays, respectively. T cell phenotype and proliferation were assessed in a mixed lymphocyte reaction (MLR) with allogenic CD4+CD45RA− naïve T cells from healthy donors. Mucosal healing was investigated in epithelial wounding and migration assays with IEC-6 cells. SbS significantly decreased the frequency of CD40−, CD80−, and CD197 (CCR7; chemokine receptor-7)–expressing IBD mDC and reduced their secretion of tumor necrosis factor (TNF-α) and interleukin (IL)-6 while increasing IL-8. In the MLR, SbS significantly inhibited T cell proliferation induced by IBD mDC. Moreover, SbS inhibited T cell (TNF-α and interferon-γ) polarization induced by UC mDC and promoted IL-8 and transforming growth factor-β-dependent mucosal healing. In summary, we provide novel evidence of synergistic mechanisms how Sb controls inflammation (inhibition of T cell costimulation and inflammation-associated migration and mobilization of DC) and promotes epithelial restitution relevant in IBD.

Inflammatory bowel disease; dendritic cells; Crohn’s disease; ulcerative colitis; yeast; fungus; probiotics; microbial antigen recognition

LYOPHILIZED *Saccharomyces boulardii* (Sb) is a probiotic yeast preparation. Preliminary studies have evaluated the effect of Sb in patients with inflammatory bowel disease (IBD). One study of patients suffering from Crohn’s disease (CD) with moderate activity found that the addition of Sb to conventional treatment significantly reduced stool frequency (30). A beneficial effect in the maintenance of remission in CD has been reported as well (44). In another pilot study, Sb significantly improved the clinical activity index of patients with left-sided ulcerative colitis (UC) when added to their maintenance regimen with mesalazine (29). While well studied in infectious diarrhea, little is known about the mechanisms how Sb may control inflammation in CD and UC.

Dendritic cells (DC) are thought to be critically involved in inflammatory T cell polarization and activation observed in human IBD (3, 62). Our group has recently demonstrated a striking correlation between DC subsets and disease activity and aberrant response of myeloid dendritic cells (mDC) to lipopolysaccharide (LPS) (4). Here, we report novel anti-inflammatory effects of Sb on primary human mDC from patients with IBD.

**MATERIALS AND METHODS**

**Patients and Controls**

Peripheral blood was obtained from patients with CD, UC, and infectious controls (patients with infectious diarrhea) (IC) seen at our IBD Center; CD and UC patients were characterized according to the Montreal Classification (51). Two validated scores, the modified Truelove Witts Severity Index (MTWSI) for UC and the Harvey Bradshaw Severity Index (HBSI) for CD, were used to assess disease activity (20). UC patients who scored ≥10 on the MTWSI and CD patients who scored ≥7 on the HBSI were classified to have active disease (flare up) (Table 1). Samples were exclusively analyzed from carefully selected patients off steroids, biologics, immunomodulators, and immunosuppressants. The study protocol was approved by Charité’s institutional review board. All patients gave informed written consent to the study.

**Purification of mDC**

Peripheral blood mononuclear cells (PBMC) were isolated from blood samples using a protocol published previously (4). In brief, mDC were isolated by magnetic cell separation from PBMC using CD1c (BDCA-1) antibodies and MicroBeads (Milenyi Biotec, Bergisch Gladbach, Germany) (24). In blood, the CD1c (BDCA-1) antigen is expressed specifically on DC, which are CD11c+CD123−. Additionally, a subset of B cells also expresses this antigen. Thus, depletion of B cells with CD19 MicroBeads was required before the enrichment of CD1c (BDCA-1) mDC. The purity of isolated mDC populations was checked by fluorescence-activated cell sorter (FACS) analysis, and samples with <95% mDC were discarded.

**Purification of naïve T cells**

Naive T cells were isolated by magnetic cell separation from PBMC using the CD4 MultiSort Kit and CD45RA MicroBeads (Milenyi Biotec) (24). In brief, CD4+ cells were positive selected with CD4 MultiSort MicroBeads. Afterward, magnetic particles were enzymatically released, which allows a second magnetic labeling and separation of the cells using CD45RA MicroBeads. CD4+CD45RA+ cells were positively selected from the preselected CD4+ cells. The purity of the isolated naïve T cell population was checked with FACS, and samples with <95% CD4+CD45RA+ T cells were discarded.
Preparation of Sb Supernatant

Lyophilized Sb was provided by Laboratories Biocodex (Montouge, France). Sb supernatant (SbS) was prepared as described previously (16, 59). In brief, Sb was cultured in RPMI 1640 cell culture medium for 24 h at 37°C in an initial concentration of 100 ng/ml. To separate the yeast from the supernatant, a 0.2-μm sterile filter (Fisher Scientific, Schwerte, Germany) was used. Previous work from our group (including serial dilutions) indicates that any dilution above 1:2 is not toxic (the viability does not differ between 1:8 and 1:32), and a dilution of 1:8 is most effective and was thus used throughout all experiments (59).

Culture and Stimulation of mDC

Highly purified mDC were cultured for 21 h in RPMI with 0.2% l-glutamine (GIBCO, New York, NY) + 10% human serum type AB (Cambrex, Charles City, IA), + 1% penicillin-streptomycin (GIBCO). To mimic bacterial microbial activation, mDC were stimulated with LPS, a prototypical microbial antigen they may encounter in the gut (35), in the presence or absence of SbS. Ultrapure LPS from Escherichia coli 055:B5 (Calbiochem, Darmstadt, Germany) was added at a concentration of 100 ng/ml (31). Finally, mDC were harvested and stained with the appropriate antibodies to measure the expression of costimulatory molecules by FACS. Supernatants were collected to measure cytokine secretion. Because of the limited cell numbers that can be obtained from one patient, not all experiments were performed in each individual patient.

Mixed Lymphocyte Reaction of mDC and Naïve T Cells

For mixed lymphocyte reaction (MLR) experiments, 1 x 10⁴ highly purified mDC were preincubated with LPS (12.5 ng/ml) in the presence or absence of SbS (see SbS fractionation above) for 3 h at 37°C and 5% CO₂ in RPMI with 0.2% l-glutamine (GIBCO) + 10% human serum type AB (Cambrex) + 1% penicillin-streptomycin (GIBCO). Naïve, allogenic T cells (1 x 10⁵) from healthy volunteers were added and cocultured for 5 days at 37°C and 5% CO₂. To rule out T cell unresponsiveness, staphylococcal enterotoxin B was added to naïve T cells at a concentration of 6.7 μg/ml (Sigma, St. Louis, MO). T cells were labeled with 5 μM carboxyfluorescein-diacetate-succinimidylester to identify and enumerate proliferating cells (39). For the detection of intracellular cytokine production and secretion in supernatants, T cells were restimulated with 1 μg/ml ionomycin, 10 ng/ml phorbol myristate acetate, and Golgi Stop Solution (BD Biosciences, Heidelberg, Germany) 5 h before harvesting as described elsewhere.

For intracellular staining, cells were harvested, washed in FACS buffer, and fixed in cytofix/cytoperm buffer (BD Biosciences). Cells were washed two times with BD Perm/Wash buffer. Finally, intracellular staining for tumor necrosis factor (TNF-α) and interferon (IFN)-γ (all antibodies were obtained from BD Biosciences) was performed in a total volume of 100 μl for 30 min at 4°C. As an isotype control, mouse IgG1 was used. Unbound antibody was removed by washing two times with Perm/Wash Buffer; cells were resuspended in FACS buffer and analyzed by flow cytometry.

Antibodies

Monoclonal antibodies against CD4, CD11c, CD14, CD19, CD40, CD45RA, CD80, CD83, CD86, and CD197 [chemokine receptor-7 (CCR7)] were obtained from BD Biosciences against CD1c (BDCA-1) from Miltenyi Biotec as fluorochrome conjugates. To rule out unspecific binding, mouse IgG1 and IgG2b (BD Biosciences) and mouse IgG2a (Caltag, San Diego, CA) isotype control antibodies were used.

Flow Cytometry

Three- or four-color flow cytometric analysis (FACS) was used to identify and enumerate blood mDC and T cells as described previously (4). Data were analyzed using Cell Quest (BD Biosciences) software (38, 54).

Cytometric Bead Array Analysis of Cytokine Secretion

Cytokine secretion of TNF-α, interleukin (IL)-6, IL-8, IL-10, IL-12/23p40, IL-17, and transforming growth factor (TGF)-β into culture supernatants was measured by cytometric bead array (CBA) analysis (BD Biosciences) according to the manufacturer’s guidelines. Briefly, bead populations with distinct fluorescence intensities coated with capture antibody proteins were first mixed with phycoerythrin-conjugated detection antibodies and recombinant standards or test samples and then incubated to form sandwich complexes. Cytokine concentrations were calculated using the proprietary FCAP (Softflow, New Brighton, MN) analysis software (13).

Migration Assay (In Vitro Mucosal Healing Model)

Wounding-healing assays were essentially performed as described previously using a modified method first described elsewhere (6, 49). Confluent monolayers of IEC-6 cells were incubated for 6 h in serum-deprived medium (DMEM + 0.1% FCS). Afterward, cells were scraped with a razor blade to produce wounds of 20–25 mm width and then washed two times with PBS to remove residual cell debris. Wounded monolayers were cultured for 24 h in DMEM (GIBCO), supplemented as described above in the presence if absence of IL-8 (10 ng/ml), TGF-β (10 ng/ml) (both from R&D, Wiesbaden-Nordenstadt, Germany), and SbS. The concentration of 10 ng/ml was chosen from a dilution series (1, 10, and 100 ng/ml; data not shown) and based on previous work that has demonstrated effects on IEC-6 with as little as 1 ng/ml TGF-β (7, 60) or IL-8 (56).

Migration of IEC-6 cells was assessed by counting the number of cells across the wound border in a blinded fashion. The number of cells was expressed as the mean number of cells across the wound border in a standardized wound area. Several wound areas per plate were used to quantitate the migration. Wound areas were standardized by taking pictures at 100-fold magnification using an inverted microscope (Zeiss Telaval 31) with a Nikon F-601 camera.

Statistical Analysis

For all studies, data are expressed as means ± SEM. Comparisons are by a two-tailed Mann-Whitney U-test with statistical significance accepted for P < 0.05.
RESULTS

*SbS Decreases the Number of CD40- and CD80-Expressing LPS-Stimulated mDC*

First, we studied the expression of CD40 and CD80, two costimulatory molecules that are known to be upregulated on activated human mDC and critical for the activation of naïve T cells (15, 41, 63). After stimulation with LPS and incubation for 21 h in the presence or absence of *SbS*, mDC were stained for CD40 and CD80 and eventually analyzed by flow cytometry (Fig. 1).

As expected, LPS induced a substantial expression of CD40-positive cells in remission (CD 93.61 ± 0.5%) (UC 87.58 ± 2.05%) and acute flare ups (CD 89.69 ± 5.0%) (UC 92.33%). However, addition of *SbS* to these cultures significantly (except for UC flare up) decreased the percentage of CD40-expressing mDC in remission (CD 47.58 ± 4.38%, *P* < 0.01) (UC 56.48 ± 3.91%, *P* < 0.001) and acute flare ups (CD 49.47 ± 8.01%, *P* < 0.05) (UC 40.20 ± 10.93%). (Fig. 1, A and C).

The same trend was observed for CD80, where LPS also induced a substantial expression of CD80-positive cells in remission (CD 92.35 ± 0.1%) (UC 92.57 ± 1.4%) and acute flare ups (CD 92.77 ± 2.3%) (UC 95.93%). As for CD40, addition of *SbS* to these cultures significantly decreased the fraction of CD80-expressing mDC and remission (CD 59.09 ± 7.2%, *P* < 0.05) (UC 65.70 ± 5.0%, *P* < 0.01) and acute flare ups (58.35 ± 5.8%, *P* < 0.01) (UC 43.52 ± 10.0%) (Fig. 1B).

The *SbS* effects on mDC isolated from IC (IC CD40 93.64% vs. 88.07% *P* < 0.01) (UC CD80 96.95% vs. 92.57% *P* < 0.001) (UC CD80 92.33%) (98.71%) (Fig. 2). Overall, the inhibitory effect of *SbS* was comparable in mDC isolated from UC and CD patients, as well as in IC.

*SbS Decreases the Number of CD197 (CCR7)-Expressing LPS-Stimulated mDC*

CCR7 is not only another maturation marker for DC, but also involved in the steady-state and inflammation-associated migration and mobilization of DC and T cells (25). LPS induced an expression of CCR7 by virtually all mDC-positive cells in remission (CD 96.09 ± 1.4%) (UC 95.68 ± 1.0%) and flare ups (CD flare up 95.14 ± 2.7%) (98.71%) (Fig. 2).

However, addition of *SbS* to these cultures decreased the percentage of CCR7-expressing mDC in remission (CD 73.47 ± 4.2%, *P* < 0.05) (UC 75.68 ± 2.7%, *P* < 0.001) and acute CD flare ups (CD 80.74 ± 3.5%, *P* < 0.05). The trend for flare ups (UC 86.12 ± 3.1%) did not reach statistical significance.

The *SbS* effects on mDC isolated from IC (IC 98.62 ± 0.2% vs. 88.07 ± 3.5%, *P* < 0.05) were comparable (Fig. 2).

There were no significant differences between the numbers of CD83- and CD86-expressing mDC (data not shown).

*SbS Impact on Cytokine Secretion by LPS-Stimulated mDC*

To further investigate the cytokine secretion by LPS-stimulated mDC from UC patients in remission (UC remission 315.9 ± 2.0 pg/ml vs. UC acute flare up 42.3 ± 2.05 pg/ml *P* < 0.01) were significantly reduced when *SbS* was added to the culture. The secretion of TNF-α by
LPS-stimulated mDC from flaring (UC flare up 355.5 ± 80 vs. 255.6 ± 99.7 pg/ml) and CD (CD flare up 197.6 ± 60 vs. 102.5 ± 25 pg/ml) patients as well as IC (IC 323.5 ± 72.2 vs. 296.5 ± 50 pg/ml) was decreased, but these trends did not reach statistical significance (probably because of low patient numbers). There was no difference seen in CD patients (CD remission 193.1 ± 32.6 vs. 191 ± 55 pg/ml) in remission (Fig. 3A).

IL-6.

The secretion of IL-6 by LPS-stimulated mDC from UC patients (UC remission 2,022 ± 578 vs. 1,198 ± 337 pg/ml) and CD patients (CD remission 1,355 ± 225.3 vs. 1,037 ± 302 pg/ml) in remission as well as flaring (UC flare up 3,084 ± 766 vs. 2,109 ± 696.4 pg/ml) and CD (CD flare up 2,637 ± 539.1 vs. 2,102 ± 509 pg/ml) patients decreased when SbS was added to the culture but did not reach statistical significance. A similar trend was seen with IC (IC 3,175 ± 40.3 vs. 2,355 ± 626 pg/ml) (Fig. 3B).

SbS Augments the Secretion of IL-8

The secretion of IL-8 by LPS-stimulated mDC from IBD patients in remission (CD remission 70,377 ± 5,699 pg/ml, UC remission 76,914 ± 14,503 pg/ml) and flare up (CD flare up 63,574 ± 8,081 pg/ml, UC flare up 73,473 ± 11,201 pg/ml) increased significantly (CD remission 174,392 ± 13,780 pg/ml, \( P < 0.001 \); UC remission 163,460 ± 11,578 pg/ml, \( P < 0.001 \); UC flare up 165,301 ± 15,770 pg/ml, \( P < 0.001 \); UC flare up 164,783 ± 23,685 pg/ml, \( P < 0.01 \)) when SbS was added to the culture. A similar secretion pattern was seen in IC (IC 84,031 ± 2,970 vs. 192,890 ± 18,237 pg/ml) but could not be statistically evaluated because of low patient numbers (see MATERIALS AND METHODS). (Fig. 3C).

SbS Inhibits mDC-Induced T Cell Proliferation in an Allogenic MLR

Because the first set of experiments suggested an effect of SbS on costimulatory molecules and DC maturation markers highly relevant for T cell activation, we decided to investigate its direct effect on T cell proliferation and phenotype (see below). We studied the effect in an allogenic MLR of naïve T cells and mDC.

Naïve T cells showed almost no autoproliferation after stimulation with LPS alone (2.47 ± 1.21%). However, in an allogenic MLR with LPS, stimulated mDC induced a robust proliferation in IBD (CD remission 45.6 ± 1.1%, CD flare up 44.24 ± 5.3%) and UC (UC remission 38.64 ± 5.2%, UC flare up 23.11 ± 1.9%). Addition of SbS significantly reduced T cell proliferation in patients with IBD (CD remission 27.77 ± 1.0%, \( P < 0.01 \); CD flare up 30.02 ± 7.7%; UC remission 18.03 ± 5.3%, \( P < 0.05 \); UC flare up 5.2 ± 1.8%, \( P < 0.001 \)). The effects of SbS were most striking in UC (Fig. 4).

SbS Induces an Anti-Inflammatory T Cell Phenotype in MLR With UC mDC

To further characterize the impact of SbS-exposed mDC on T cells, the cells from the previous MLR experiments were restimulated and phenotyped. Moreover, culture supernatants were analyzed for key proinflammatory cytokines TNF-\( \alpha \), IL-12/23p40, and IL-17, and the epithelial cell restitution and wound healing promoting cytokine TGF-\( \beta \) were measured by CBA.
Intracellular TNF-α production (Fig. 5A) in proliferating T cells decreased significantly in MLR experiments with LPS-stimulated mDC isolated from UC patients (62.13 ± 2.4 vs. 34.05 ± 10.2%, *P* < 0.05) in acute flare ups when SbS was added to the approach, but not during remission (data not shown). A similar trend was seen for IFN-γ (Fig. 5B). Its production in proliferating T cells decreased in MLR experiments with mDC isolated from UC patients (31.14 ± 3.5% vs. 19.71 ± 6.7%) in acute flare ups but not remission (data not shown). These effects were restricted to UC and were not seen with mDC from CD patients.

TGF-β. The secretion of TGF-β in MLR culture supernatants by LPS-stimulated mDC from UC patients in remission (31.14 ± 3.5% vs. 19.71 ± 6.7%) in acute flare ups but not remission (data not shown). These effects were restricted to UC and were not seen with mDC from CD patients.

TGF-β. The secretion of TGF-β in MLR culture supernatants by LPS-stimulated mDC from UC patients in remission (31.14 ± 3.5% vs. 19.71 ± 6.7%) in acute flare ups but not remission (data not shown). These effects were restricted to UC and were not seen with mDC from CD patients.

SbS-Induced Cytokines Promote Wound Healing in an Epithelial Cell Injury Model

To verify the functional relevance of the increased IL-8 and TGF-β concentrations in SbS-exposed cultures or cocultures, we studied their effect in an epithelial cell injury model.

The number of migrating epithelial cells in IL-8 (335 ± 16 cells)- or TGF-β (296 ± 5 cells)-treated cultures was significantly (*P* < 0.001 vs. *P* < 0.05, respectively) higher compared with medium (255 ± 14 cells). While SbS may have an (statistically not significant) intrinsic effect on migration, the data suggest it is predominantly mediated through interaction with mDC and/or T cells and the resulting cytokines IL-8 and TGF-β (Fig. 6).

DISCUSSION

The underlying mechanisms of Sb’s potential therapeutic efficacy in IBD compared with other diarrheal illnesses have not been systematically investigated in humans (17). Because of the long-standing use of Sb in infectious diarrhea, most experimental research from in vitro studies with organoid or...
cell cultures and animal models have focused on the prevention of microbial pathogen adherence (46, 58), translocation of the commensal microbial flora (8, 26), investigation of neutralization of bacterial toxins [i.e., Clostridium difficile toxin A (14, 16, 45) or Cholera toxin (9)], toxin-related signaling (9, 18, 19), maintenance of normal intestinal permeability and barrier function (45), as well as control of mucosal electrolyte transport and luminal secretion (16, 27, 28, 45, 50).

However, a positive outcome in preliminary clinical studies in IBD (29, 30, 44) and beneficial effects of Sb in immunological (adoptive transfer model in SCID mice) and chemical (DSS colitis) (21, 36) animal models of IBD imply additional efficacy and anti-inflammatory mechanisms in CD and UC.

Our study demonstrates for the first time that Sb controls DC maturation, T cell activation, production and secretion of cytokines critically relevant for T cell phenotype, T cell polarization, and epithelial cell restitution (mucosal healing) in patients with CD and UC and also infectious colitis.

An important prerequisite for T cell activation is the expression of costimulatory molecules on antigen-presenting cells such as DC (43). Their upregulation on the cell surface marks not only a phenotypic but substantial functional change from being inducers of peripheral tolerance to potent activators of immune effector cells, such as T cells (52, 53). We first investigated the expression of CD40 and CD80 on mDC from IC and IBD patients with or without the presence of SbS. Here, we decided to use the microbial antigen surrogate motif and model TLR ligand LPS to prime mDC, since this mimics the situation these cells may encounter in the gut. Consistent with the available literature, freshly isolated, i.e., immature and inactive, mDC expressed practically no detectable numbers of the two activation markers (data not shown) (40). Exposure to LPS induced the expected maturation of DC as indicated by their increased expression of costimulators. However, when cultured in the presence of SbS, substantially fewer mDC express CD40 and CD80.

CD197 (CCR7)-mediated signals control the migration of immune cells to secondary lymphoid organs and subsequently their positioning within defined functional compartments (25). The SbS-dependent reduced expression of CD197 in mDC observed by us may prevent their migration from the peripheral circulation to the sites of inflammation, i.e., the gut in IBD (4, 5).

The effects of SbS on mDC were not restricted to phenotypic changes but paralleled by functional consequences, since we were able to demonstrate that SbS suppresses LPS-stimulated mDC to secrete less TNF-α and IL-6. An increased secretion of these prototypical inflammatory cytokines upon contact with microbial antigens encountered in the gut, such as LPS, polarizes T cells toward a proinflammatory phenotype (Th1) and contributes to the perpetuation of inflammation known to occur in IBD (3, 62). Approved and investigational drugs individually targeting these important IBD cytokines are available and clinically effective (34, 57). We show that Sb can block the secretion of both. Moreover, SbS suppresses LPS-stimulated mDC-mediated T cell proliferation in an allogenic MLR and polarizes naïve T cells from flaring UC patients to acquire an anti-inflammatory phenotype. These data are in line with previous research reporting that Sb blocks TNF-α gene expression and secretion by lymphoid and nonlymphoid cells (1, 22, 23, 23).

In addition to these direct anti-inflammatory effects, Sb increased the secretion of TGF-β and IL-8. TGF-β has been not only attributed to the polarization of naïve T cells to acquire a regulatory phenotype but also in epithelial restitution and would healing (2, 7). While IL-8 is mostly known as a chemotactic agent for innate immune cells, very recent research indicates that IL-8 is a critical regulator of angiogenesis, cell migration, and other wound-closure mechanisms, ultimately resulting in mucosal healing (33, 42, 48, 64). Interestingly, similar effects have been recently demonstrated with supernatants of a bacterial species, where the intestinal wound-healing effects were also IL-8-dependent and even facilitated recovery of experimental colitis as shown by increased body weight and reduced rectal bleeding and histological severity (33). Thus, the perhaps initially paradoxically seeming increased IL-8 secretion may be in fact a synergistic wound-healing effect induced by Sb.
Previous work of our group suggests that the active component has a molecular weight smaller than <3 kDa. This implies that wall compounds of Sb such as glycoproteins or glycolipids may be responsible for the observed effects (37). Our current study was restricted to the investigation of primarily humoral factors of Sb. The anti-inflammatory effects of Sb are probably not limited to secreted compound(s) found in the supernatant but may also involve cellular wall compounds and/or their synergistic (inter)action. To better understand the cellular mechanisms of Sb’s interaction with mDC, the three principal fungal wall compound groups β-glucans, mannoproteins, and chitin and their respective receptors (TLR2, TLR4, Dectin-1, DC-SIGN, MR, CR, and others) and signaling pathways as well as comparison with related and unrelated species such as *Saccharomyces cerevisiae* and *Candida albicans*, respectively, is necessary and subject of ongoing research in our laboratory. Recent research in mice (61) and humans (32) indicates that myeloid-derived cells may have a role in desensitizing immune responses, since long-term NOD2 stimulation of macrophages significantly decreased production of proinflammatory cytokines, and this phenomenon is disturbed in mutant macrophages from CD patients (32) (discussed in Ref. 55). It remains to be studied whether this phenomenon may also relate to the observed effect of Sb on human mDC.

Our study has additional limitations. To the best of our knowledge, it is unknown (unstudied) what the actual distri-

Fig. 5. SbS induces an anti-inflammatory phenotype in MLR of ulcerative colitis mDC and T cells. MLR of ulcerative colitis mDC and naïve T cells. Intracellular TNF-α (A) and interferon (IFN)-γ (B) decreased significantly in acute ulcerative colitis flare ups when SbS was added. FACS plots depict representative experiments. C: the secretion of transforming growth factor (TGF)-β into MLR culture supernatants by LPS stimulated mDC from UC patients in remission and IBD patient flare ups increased when SbS was added. Bars represent means ± SE.
bution of the lyophilized pharmacological yeast product and more specifically their humoral components throughout the human intestine is or their concentration at the individual epithelial cell level. We based our supernatant concentrations and dilutions on the work of other investigators (14, 16) and our own dilution series (59) and know that they are at least not toxic (see above).

We cannot rule out that SbS-induced downregulation of co-stimulatory molecules on LPS-stimulated mDC is partially mediated through direct effects of SbS on LPS itself. One study in an animal model identified in Sb a protein phosphatase that had a greater ability to dephosphorylate LPS of E. coli, which when injected in rats produced substantially less TNF-α and no organic lesions compared with the non-Sb-exposed LPS (11, 12). However, this observation would not explain the other novel immunological and epithelial restitution effects demonstrated by us.

Additional evidence for a modulation of the immune response by Sb to explain its clinical benefit in inflammatory and...
infectious conditions comes from in vitro and animal studies. Orally administered Sb was shown to increase the production of secretory IgA and the secretory component of immunoglobulins in growing rats and monoassociated germ-free mice, thereby augmenting the host’s first line of defense of the innate immune system in the gut (10, 47).

In summary, we provide novel evidence of synergistic mechanisms of Sb to control inflammation and promote epithelial restitution relevant to IBD, but also infectious colitis (Fig. 7). Because the effects of Sb were most prominent in UC, it may be worthwhile to study its potential efficacy in patients with further well-designed clinical trials and additional translational research studies.

DISCLOSURES
None of the funders had any influence on the conduct of the experiments, analysis of the data or interpretation of the results.

REFERENCES