Sulphate-Reducing Bacteria from Ulcerative Colitis Patients Induce Apoptosis of Gastrointestinal Epithelial Cells

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Abstract

The human microbiome consists of a multitude of bacterial genera and species which continuously interact with one another and their host establishing a metabolic equilibrium. The dysbiosis can lead to the development of pathology, such as inflammatory bowel diseases. Sulfide-producing prokaryotes, including sulphate-reducing bacteria (SRB) constituting different genera, including the *Desulfovibrio*, are commonly detected within the human microbiome recovered from fecal material or colonic biopsy samples. It has been proposed that SRB likely contribute to colonic pathology, for example ulcerative colitis (UC).

The interaction of SRB with the human colon and intestinal epithelial cell lines has been investigated using *Desulfovibrio indonesiensis* as a model mono-culture and in a co-culture with *E. coli* isolate, and with SRB consortia from human biopsy samples.

We find that *D. indonesiensis,* whether as a mono- or co-culture, was internalized and induced apoptosis in colon epithelial cells. This effect was enhanced in the presence of *E. coli*. The SRB combination obtained through enrichment of biopsies from UC patients induced apoptosis of a human intestinal epithelial cell line. This response was not observed in SRB enrichments from healthy (non-UC) controls. Importantly, apoptosis was detected in epithelial cells from UC patients and was not seen in epithelial cells of healthy donors. Furthermore, the antibody raised against exopolysaccharides (EPS) of *D. indonesiensis* cross reacted with the SRB population from UC patients but not with the SRB combination from non-UC controls. This study reinforces a correlation between the presence of sulphate-reducing bacteria and an inflammatory response in UC sufferers.

Keywords: SRB; ulcerative colitis; apoptosis; human epithelial cells

1. Introduction

Sulphate reducing bacteria (SRB) are a diverse group of anaerobic prokaryotes able to reduce sulphate to sulphide [1]. They are ubiquitous in aquatic and terrestrial environments, and in man-made systems [2], and are associated with plants, animals and humans [3]. In humans, SRB are known colonizers of the intestine and have been implicated in several clinical and inflammatory conditions such as periodontitis, Pouchitis, metabolic syndrome and obesity [4]; [5-8].

More than nine hundred bacterial species are known to colonize the human gut [9]. The delicate balance between pathogenicity and host-commensal bacterial mutualism is maintained with constant tolerance of bacterial antigens [10, 11],[12]. It is well accepted that an imbalance in the number or composition of gut microbiota (known as dysbiosis) is associated with a vary inflammatory diseases [13].. While some bacteria are used as probiotics in clinical studies [14], others can be harmful if they break across the epithelial barrier [13, 15, 16]. It has been proposed that induction of apoptosis of epithelial cells is one mechanism whereby the bacteria can cause pathology [17]. Some bacteria may also secrete virulence factors that can destroy the mucus barrier, allowing direct contact between bacteria and the epithelium [10, 18].

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) of multifactorial etiology, *i.e.*, susceptibility genes combine with environmental factors to produce the diseased phenotype [19, 20]. Bacterial infection could be one of the environmental factors. Although the involvement of SRB in the initiation and/or maintenance of UC in both humans and animals has been proposed [21, 22], the exact mechanism by which SRB could contribute to UC etiology remains unknown. The modificated environment may contribute to unproportional growth of SRB. Furthermore SRB are resistant to broad spectrum antibiotics [23], what can facilitates the burst of these bacteria in condition of repeated antibiotic use. The main product of metabolic the activity of SRB, sulphide, is toxic for human cells as it can destroy the sulphate-bridges in the mucus layer, thus neutralizing the ability of mucus to protect the colon epithelium [24, 25]. The mesophilic Gram-negative species representing the *Desulfovibrio* genus are of interest among the SRB. It has been demonstrated that members of *Desulfovibrio* colonize surfaces of

intestinal epithelial tissue of UC animals and are absent in healthy animals [26]. In human studies, it has been reported that, compared to healthy controls, the abundance of *Desulfovibrio* cells in UC patients are higher than in controls [21, 27-29]. Although the role of diet in the etiology of UC remains uncertain, evidence suggests that in UC patients, low-fat diets and insoluble oligosaccharides (prebiotics) could be beneficial [30]. A decrease in the level of anaerobic bacteria such as SRB has been observed in the microflora of the intestinal tract following the beneficial diet change [31-33]. It is also known that diets poor in sulphur containing-compounds are beneficial to UC patients [27]. While the numbers of SRB are similar for UC patients and healthy controls, differences were noted in the proliferative rates of bacterial colonies enriched from these samples [34].

Other studies confirmed that samples from UC patients and healthy controls harbor the same level of SRB, but there are significant differences in the structure of the SRB community between the two groups [35]. Here we report that SRB of the *Desulfovibrio* genus can interact with the surface of human intestinal epithelial cells and induce their apoptosis.

2. Material and Methods

2.1. Human intestinal tissue samples

Specimens of intestinal mucosa were taken during colonoscopy from the proximal colon of 29 patients with chronic ulcerative colitis and 37 control individuals with non-inflammatory conditions from the Department of Gastroenterology, Queen Alexandra Hospital. Portsmouth, UK. The biopsy procedure is described elsewhere [36]. Ulcerative colitis was diagnosed based on clinical, endoscopic and histological findings; the clinical data of patients and controls are shown in the Table 1. All human samples examined in this study were collected with the approval of local ethics committee (Portsmouth, Hampshire, UK) approval number 01/01/1106, and written informed consent was obtained from all participants.

Following sampling, specimens of mucosa were immediately transferred with a sterile needle from the forceps into an Eppendorf tube containing 0.2 ml of sterile physiological saline solution. The mucosal samples were maintained under an atmosphere of oxygen-free nitrogen gas from the time of the initial collection until use, and then vigorously aspirated into and out of a Pasteur pipette to ensure complete dissociation of bacterial cells from mucosa. The aliquots of mucosal samples were used for inoculating 10-ml vials with lactate- and sulfate-supplemented liquid VM medium I, as follow described.

2.2. Bacterial growth conditions

D. indonesiensis (NCIMB 13468) and *Escherichia coli* isolated from UC patients were cultivated at 37^{o}C. The VM medium I was composed of (g l^{-1} distilled water): KH_2PO_4, 0.5; NH_4Cl, 1.0; Na_2SO_4, 4.5; NaCl, 9.0; $CaCl_2{\cdot}2H_2O$, 0.04; $MgSO_4{\cdot}7H_2O$, 0.06; sodium lactate, 6.0; sodium citrate, 0.3; casamino acids, 2.0; tryptone, 2.0; thioglycolic acid, 0.1; $FeSO_4{\cdot}7H_2O$, 0.5; modified Wolfe's mineral elixir [37] and vitamin solution. The pH of the medium was adjusted to 7.5 prior to adding vitamin solution. The combination of vitamins was as following (mg l^{-1} distilled water): ascorbic acid, 100; nicotinic acid, 0.5; vitamin B_1, 0.6; vitamin B_{12}, 0.05; vitamin B_2, 0.2; vitamin B_5, 0.6; vitamin B_6, 0.6; vitamin H, 0.01. The vitamin solution and the modified Wolfe's mineral elixir were filter sterilized. Culture tubes were filled with the medium (without vitamins), purged with a

N_2 flux and autoclaved at 121 ^oC for 30 min. Due to the presence of Fe ions, VM medium I acts as an indicator medium for SRB. Upon the production of hydrogen sulfide by active SRB cells, the development of a black color, resulting from the presence of anaerobically formed iron sulfide compounds, indicates bacterial growth. The cultures were inspected for the change of color at regular time intervals over a period of 28 days.

2.3. Intestinal cryo-sections and Immunostaining assay

Dot blotting assay

The bacterial cells were pelleted from the culture by centrifugation for 15 min at 13,000 rpm. The cells were then suspended in 1 ml of distilled water, disrupted by eight freezing and thawing cycles (-180^{o}C and +37^{o}C, respectively) and spun at 13,000 rpm for 15 min. The supernatants were transferred into another set of tubes and the pellets were dissolved in 100 µl of distilled water. Nitro-cellulose membrane (Bio-Rad) was adjusted to a dot blot apparatus (Jencons - PLS) and the samples were applied under vacuum. After loading the samples, the membrane was dried, soaked in egg albumin 1 % solution for 1 h at room temperature and then washed three times using PBS-Tween 0.02 %, 10 min each time. After washing the membrane was incubated with normal goat serum 1:5 at room temperature for 3 h and then at 4^{o}C overnight with rabbit polyclonal antibody anti-exopolysaccharides (EPS) of *D. indonesiensis* diluted 1:50. The membrane was again washed three times with PBS-Tween and incubated with goat antibody anti-rabbit IgG conjugated to horseradish peroxidase (Sigma Co.) diluted 1:2000 for 2 h at 37^{o}C. The membrane was, then, washed five times with PBS-Tween, 10 min each time. The reaction was assessed by revealing peroxidase enzyme activity using 30 mg of 4-chloro-1-naphtol dissolved in 10 ml of cold ethanol, 50 ml of PBS and 30 µl of 30 % H_2O_2.The positive reaction is detected by dark bluish precipitate on the sample spots applied to the membrane.

2.4. SRB interaction with human epithelial cells assay.

The human epithelial cells line HCT8 cells from ATCC were distributed on 24 wells culture plates with and without slides on each well and left to grow in RPMI medium for

24h, achieving a monolayer culture. *D. indonesiensis* and *E. coli* 2R/BP were grown on VMNI medium and centrifuged for 1 min at 12,000 rpm on Eppendorf refrigerated centrifuge. The bacteria were then resuspended on RPMI medium. The bacteria ($2x10^5$ cells in 100 µl of medium) were added on wells containing $2x10^4$ cells in 300 µl of RPMI and let to interact for 1h at 37 ^oC. After that, the cells were washed, 2 ml of RPMI medium were added, and the cells were maintained in a CO_2 incubator at 37 ^oC for two additional hours. In some wells, epithelial cells were pre-incubated with 10 µg/ml of cytochalasin D, a phagocytosis inhibitor drug, for 30 min before the infection. At the end of the experiments, the cells were washed and the slides were prepared for immunostaining assay. The remaining cells which had grown directly on wells were released by treatment with PBS/EDTA 1mM for 10 min at 37 ^oC, collected on Falcon tube and processed for flow cytometry analysis.

2.5. Immunostaining assay

The cells attached to slides were processed for immunostaining using the same incubation steps with antibodies, but additionally incubated with DAPI for bacterial DNA and nucleus of epithelial cells staining. The cells were analyzed using Zeiss Axioplan (Zeiss, Germany) microscope coupled with Leica DC 200 image acquisition system (Leica, Cambridge, UK).

2.6. Flow cytometry analysis

For flow cytometry analysis, the cells were fixed on paraformaldehyde (PFA) 4% in PBS for 10 min at room temperature. The cells were then washed with PBS and incubated with 10% of calf serum in phosphate buffer saline PBS to block unspecific bindings, followed by sequential incubations for 30 min at room temperature with: 1) rabbit anti-*D. indonesiensis* extracellular polymeric substances (anti-*D. indo* EPS) polyclonal antibody diluted 1:50; 2) calf antibodies anti-rabbit IgG FITC-conjugated diluted at 1:50. All antibodies were diluted in PBS/BSA 1% saponin 0.1%. After each antibody incubation step, the cells were washed twice with PBS. Final 10.000 cells were acquired for flow cytometry FACScan Becton Dickson for fluorescence analysis.

2.7. Apoptosis assay using flow cytometry

The HCT8 human intestinal epithelial cells were infected or not with pure strain (*D. indonesiensis* or *E. coli* 2R/BP or *E. coli* K12) or with mixed culture bacteria (*D. indonesiensis* + *E. coli* 2R/BP or SRB combination from UC patient or from control individual) for 12 to 40 h. The infections were also performed with the bacteria but in the presence of Z-VAD, a drug that inhibit apoptosis mediated by caspases. The epithelial cells were also incubated with EPS from *D. indonesiensis*. The attached cells were then released from the wells with PBS/EDTA and recovered in Falcon tubes, washed and centrifuged for 7 min at 250g. The cells were finally resuspended in apoptosis buffer containing 0.1% sodium citrate, 0.1% Triton X-100 and 5 g/ml of propidium iodate. The DNA content of the acquired cells (10.000 cells per tube) was analyzed using a FACScan Becton Dickinson flow cytometry.

2.8. TUNEL labelling

Terminal deoxythymidine transferase-mediated dUTP nick end labelling (TUNEL) staining was performed using an *in situ* cell death detection kit (Boehringer Mannheim; Meylan, France) according to the manufacturer's recommendations. Briefly, harvested adhered and supernatant cells were fixed in 4 % paraformaldehyde (PFA; BDH Laboratory Supplies, Poole, UK) at room temperature for 30 min and permeabilized with a buffer containing 0.1% Triton X-100 and freshly-prepared 0.1% sodium citrate. Fixed cells were labeled with fluorescein isothiocyanate (FITC)-dUTP using terminal deoxythymidine transferase. The FITC-stained cells were visualized with a Zeiss Axioplan microscope (Jena, Germany) coupled to a Leica DC 200 image acquisition system (Cambridge, UK). The figures were prepared using the Adobe Photoshop 5.0 program.

2.9. Statistical analysis

Statistical analysis was performed using the unpaired Students't-test. Values of $P < 0.05$ were considered significant.

3. Results

3.1. The pure strain of SRB interacts with human intestinal epithelial cells in culture

The flow cytometry analysis showed that *D. indonesiensis* interacts with HCT8 human intestinal epithelial cells in culture (Fig. 1). The mean fluorescence intensity obtained from cells incubated/infected with *D. indonesiensis* was higher than for uninfected controls. There was no significant increase in staining when the cells were permeabilized with saponin during the incubation with antibodies, suggesting that the bacteria interacted mainly with the surface of the cells. However, the pre-treatment with cytochalasin D (a drug that inhibits phagocytosis) reduced the mean fluorescence intensity of HCT8 cells by 30%, suggesting that there was some internalization of SRB by the host cells (data not shown). These findings were confirmed by analysis of immunofluorescence staining, observed by fluorescence microscopy. The SRB interacted with the cell membrane of epithelial cells, usually creating aggregates (presumably biofilms) (Fig. 2), both when *D. indonesiensis* infected alone (Fig. 2e) or in combination with *E. coli* 2R/BP (Fig. 2g). The *E. coli* 2R/BP strain was not labelled with the antibody against EPS of *D. indonesiensis* (Fig. 2, d, h; shown by arrows).

3.2. The pure *D. indonesiensis* and *E. coli* 2R/BP strains induce apoptosis of human intestinal epithelial cells

The infected and uninfected HCT8 cells were analyzed for hypo-diploid cells by flow cytometer as described in Material and Methods. We found that *D. indonesiensis* and *E. coli* BP individually induced apoptosis in HCT8 cells and that the combination had a larger effect than either of them separately (Fig. 3a). Z-VAD treatment inhibited partially apoptosis induced by either strain of bacteria, suggesting that the apoptosis induced by either strain individually may require the activity of caspases, but treatment was not significantly effective on infection by the combination of bacterial strains. The TUNEL analysis confirmed the data obtained by flow cytometry, showing nuclear fragmentation only in cells treated with *D. indonesiensis*, *E. coli* BP or the combination of both strains (fig. 3b).

We then investigated whether the apoptosis of HCT8 cells induced by *D. indonesiensis, E. coli* BP or by the combination was specific or a general effect of other bacterial species. We observed that *D. indonesiensis* or *E. coli* BP separately or in combination induced apoptosis (5 ±1%, 29 ±2%, or 41 ±1% respectively), while another strain of *E. coli* (*E. coli* K12) also induced a low level of apoptosis (5 ±0.6%) in HCT8 cells. But the combination of bacteria enriched with SRB extracted from mucosal biopsies of healthy subjects did not induce apoptosis (Fig. 4). Conversely, the combination of bacteria enriched with SRB from patients with ulcerative colitis clearly induced apoptosis (13 ±1%) on HCT8 cells. We also investigated the effect of soluble bacterial products on the apoptosis obtained and observed that the extracellular polymeric substance extracted from *D. indonesiensis* did not induce apoptosis on intestinal epithelial cells HCT8. To better characterize the effect of *D. indonesiensis* on induction of death of HCT8 cells, we performed kinetic experiments growing the cells in the presence of the antibiotic penicillin. Under this experimental condition, the specific apoptosis induced by *D. indonesiensis* was 9.3 ±1.5% and 18 ±1 2.5% after 12h and 40h of infection, respectively (Fig. 5). The apoptosis induced by *D. indonesiensis* was also dependent on the bacterial concentration, since doubling the initial inoculum induced almost twice as much apoptosis after 24h.

3.3. Apoptosis in human intestinal epithelial biopsies from ulcerative colitis patients

We examined apoptosis in biopsies of epithelial tissue from patients (n=3) and healthy donors (n=3) by the TUNEL technique. We observed many apoptotic cells in samples from colitis patients (Fig. 6a and 6b). No apoptotic cells were observed on slides from healthy donor samples. Using antibodies against EPS of SRB, we also identified SRB on samples from ulcerative colitis patients, where they were found in association with the surface of intestinal epithelia (Fig. 6c). These results suggested that the epithelial barrier was broken in the patients and the colonic epithelium was infiltrated with SRB bacteria.

3.4. Antibodies against EPS of *D. indonesiensis* discriminate between SRB isolated from ulcerative colitis patients and control samples

The polyclonal antibody against EPS of *D. indonesiensis* was used to recognize SRB in bacteria consortium isolated in biopsies from the intestinal mucosa of ulcerative colitis patients and control groups by the dot blotting assay. As shown in table 1, the antibody could recognize 81% of samples from human colon biopsies from patients with ulcerative colitis and only 20.8% of samples from the control groups.

These results were confirmed by the immunofluorescence technique, which showed SRB staining in bacteria consortium isolated in biopsies from intestinal mucosa of ulcerative colitis patients (Fig.7a and b) but not control samples (Fig. 7c and d). These results suggest that the antibody against EPS of *D. indonesiensis* can be used as an additional marker for differential recognition of SRB present on human colon biopsies with and without ulcerative colitis.

4. **Discussion**

Intestinal bacteria are believed to play a role in the pathogenesis of inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease. Genetically engineered animal models have shown the importance of commensal bacteria in development of disease [38, 39], antibacterial treatment could improve symptoms of IBD [40], and some SRB are susceptible to drugs used in active treatment of UC patients [41]. Inhibition of BRS-sulfide production by 5-aminosalicylic acid (5-ASA)-containing drugs has been proposed for therapy of UC patients [42]. There are also circumstantial and sometimes conflicting reports regarding the differences in SRB populations (strains, growth rate) and metabolic activity (expressed as sulphide production) in healthy and diseased specimens. Furthermore, most of the reported data were obtained from fecal samples [43, 44], which do not necessarily provide a reliable readout for SRB infection in the colon.

We show here that SRB can interact with the human epithelial intestinal cell membrane and are cytotoxic for those cells. The apoptosis induced by SRB was significantly higher when the cells were co-infected with *E. coli* commensal bacteria, and these findings were confirmed when the SRB and *E. coli* were obtained from UC patients. The mechanism how SRB induces cytotoxity in human intestinal epithelial cells is not completed

understood. Interesting the Hydrogen sulfide (H_2S) that is produced by both SRB and *E. coli* [45], at higher concentrations is associated with inhibition of cellular bioenergetics and mitochondrial respiration, pro-oxidant effects, DNA damage, suppression of cell viability and promotion of cell necrosis and/or apoptosis [46]. Depending of cell type the H_2S treatment increases cell death in human lung fibroblast or inhibits cell cycle progression in rat smooth muscle, oral epithelial cells and human colon cancer cells inhibiting cyclin dependent kinase [47]. Furthermore, the H_2S at lower concentration can develop a beneficial effect protecting intestinal cancer Caco2 cells from TNF and IFN-γ induced cell death [48].

Previous studies have suggested that cell wall products derived from the luminal bacteria of the colon could cause colitis in immunodeficient mice [49], and that bacterial flagellin is a dominant antigen in IBD [50]. Besides, LPS of SRB was associated with microbiota inflammatory properties [51] while *E. coli* LPS induces increase in plasma H_2S levels in mice during inflammation [52], what prompted us to consider the LPS as an additional signalling molecule responsible for the SRB effects on intestinal epithelial cells. In addition, some probiotic bacteria can confer health benefits to the host by improving the microbial composition of the indigenous microflora in UC patients [53, 54]. In this direction the infection by *Yersinia enterocolitica* induces chronic bowel inflammation with dysbiosis favoring SRB proliferation and treatment using probiotics could prevent the microbiota alterations changes and inflammation showing the importance of dysbiosis of the gut microbiota for development of IBD [12].

Finally, recently we described that germ-free mice colonization with *Desulfovibrio indonesiensis* or with a human SRB consortium (from patients with colitis), induced changes in colonic architecture with increased cell infiltration in the lamina própria, and upregulation of IL-17 and Treg profiles of cytokine production/cell activation in cells from mesenteric lymph nodes [55]. What favors the hypothesis of SRB involvement in initiation of IBD.

5. **Conclusion**

We propose that SRB could contribute to initiation of IBD, by impairing the barrier function of the intestine and/or impairing the healing response to local inflammation.

Figure Legends

Fig. 1 *D. indonesiensis interacts in vitro with intestinal epithelial cells*. Labeling with anti-EPS antibodies with HCT8 cells was analyzed by flow cytometry. HCT8 cells infected with *D. indonesiensis* showed higher fluorescence intensity than control cells not infected or infected with *E. coli* 2R/BP (**p<0.001). Data are expressed as mean ± S.E.M. of 3 independent experiments. The asterisk represents a significant difference relative to the control value.

Fig. 2 *Immunostaining reaction showing the in vitro interaction of D. indonesiensis with human intestinal epithelial cells*. The left panel (**a**,**c**,**e**,**g** and **i**) shows in green SRB staining with anti-EPS antibodies, the right panel (**b**,**d**,**f**,**h** and **j**) shows the double staining of SRB (green) and the nucleus (blue) of eukaryotic cells and bacteria. Note that *D. indonesiensis* interacts with the surface of HCT8 cells (**e**,**g**,**i**, arrow), usually showing a cellular aggregate. In **h,** the wide arrow shows the absence of green staining in cells incubated with *E. coli* 2R/BP. Magnification (a-j) x 1000 gain.

Fig. 3 *Apoptosis of intestinal epithelial cells after bacterial infection*. The HCT8 cells were infected with *E. coli* 2R/BP (BP) and *D. indonesiensis* (D. ind), individually or in combination, analyzed by hypodiploid cells by flow cytometry (**a**) and by TUNEL (**b**), where the apoptotic nuclei are shown in green. *P<0.05, **P<0.001, ***<P<0.001 compared to control; #P<0.05 compared to Z-VAD untreated. The experiment is representative of three independent experiments in triplicate.

Fig. 4 *Apoptosis of epithelial cells induced by different bacteria*. Specific apoptosis is displayed as % of hypodiploid cells. The HCT8 cells were infected with bacteria or incubated in the presence of bacterial exopolysaccharides (EPS) for 12h (BP: *E. coli* 2R/BP; D. ind: *D. indonesiensis*; EPS D. ind: extracellular polymeric substance purified from *D. indonesiensis*; Cons (patient); sample bacteria consortium enriched in SRB isolated from intestinal mucosa biopsies from ulcerative colitis patient; Cons (control);

consortium enriched in SRB isolated from intestinal mucosal biopsies from healthy individuals. *P<0.05, **P<0.001, ***<P<0.001 compared to untreated control. Data are expressed as mean ± S.E.M. of 3 independent experiments performed in triplicate. The asterisk represents a significant difference relative to the Cons (control) value. (**b** 1000x gain)

Fig. 5 *Time and concentration dependence of BRS-induced apoptosis in epithelial cells.* HCT8 cells were infected with *D. indonesiensis* for 12h and 40h in RPMI medium without antibiotics. The percentage of apoptosis was dependent on the dose of inoculum and time of infection (*p<0.01). The experiment were performed in triplicate and repeated three times. The asterisk represents a significant difference relative to the 1x dose inoculum value.

Fig. 6 *Apoptosis and SRB interaction on intestinal epithelial cells from UC patients.* Apoptotic cells detected by TUNEL reaction (**a**-**b**) and immunostaining for SRB (**c**) on frozen samples from biopsies of intestinal mucosa from UC patients. Apoptotic cells were observed in the human colon epithelium (**a**- 400x; **b**-1000x gain; green staining). In (**c**) bacteria were stained with anti-EPS of *D. indonesiensis* (shown in green) associated with intestinal epithelial cells (nucleus stained in blue).

Fig. 7 *Selective staining of EPS of SRB from UC patients*. The presence of total bacteria is shown in blue for DAPI-stained nuclei (right panel). The staining of bacteria with anti-SRB antibody is shown in green (left panel). The bacteria enriched with SRB isolated from colon biopsies of UC patients (**a**, **b**) and healthy volunteers (**c**, **d**) shows positive staining in patients but not healthy volunteers. Note that on patient consortium there are few bacteria that are not recognized by antibody (**b**, arrow). (**a**-**d** 1000x gain)

Table 1

Dot blotting results showing the percentage of bacterial samples labeling using anti-EPS of *D. indonesiensis* antibody. The immune reactions were done against bacterial lysates from consortiums enriched with SRB isolated from patients with ulcerative colitis and or from control group.

Acknowledgments

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Compliance with ethical standards

Conflict of interest. All authors declare that they have no conflict of interest. Dr. Callum Pearce was working at Department of Gastroenterology, Queen Alexandra Hospital, Portsmouth, UK, where patient samples were collected. All experiments using patient samples were performed at University of Portsmouth, during the Post doc period of Dr. Claudia Coutinho, at laboratory headed by Dr. Iwona Beech. Dr. Beech and Dr. Pearce moved to other universities abroad after that. The *in vitro* experiments using epithelial cell line were performed later in Brazil.

Ethical approval: "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

Informed consent

Informed consent was obtained from all individual participants included in the study.

Highlights

- Sulphate -reducing bacteria interact with human intestinal epithelial cells.
- Sulphate-reducing bacteria induce apoptosis of human epithelial cells.
- Sulphate-reducing bacteria obtained through enrichment of biopsies from UC patients induce apoptosis of human intestinal epithelial cells.

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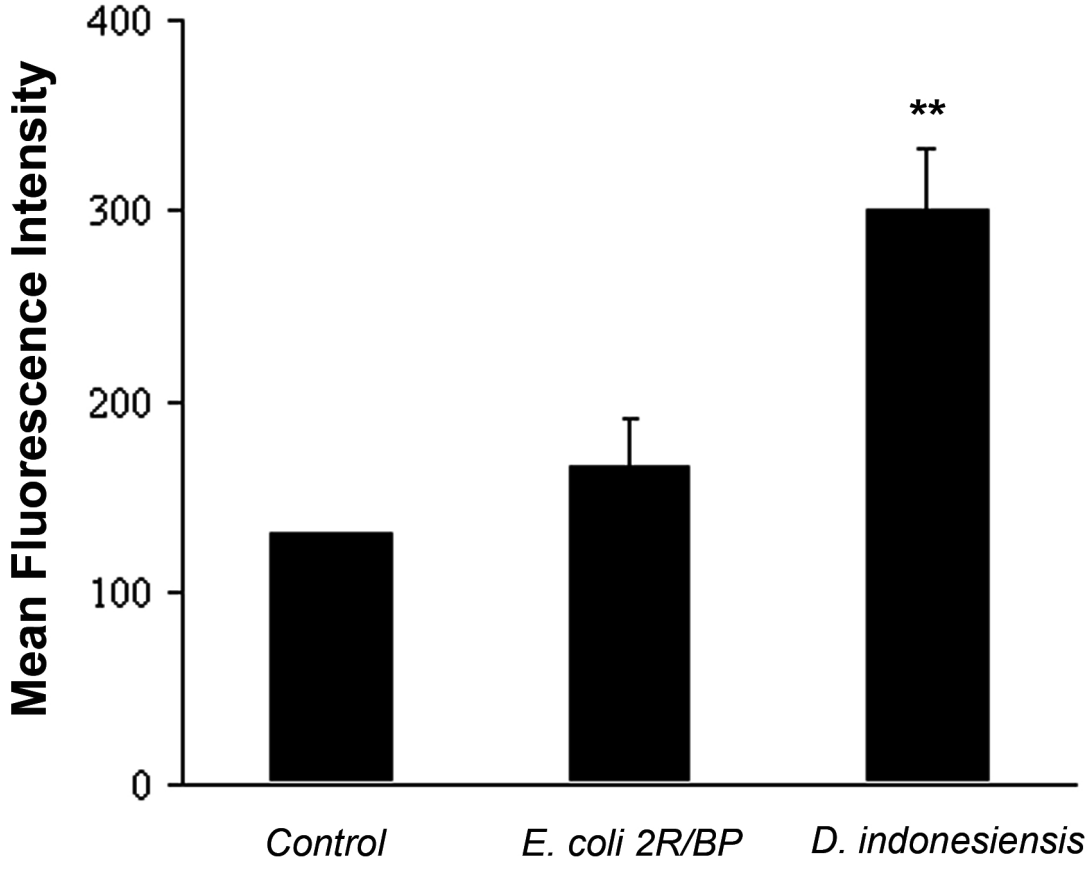
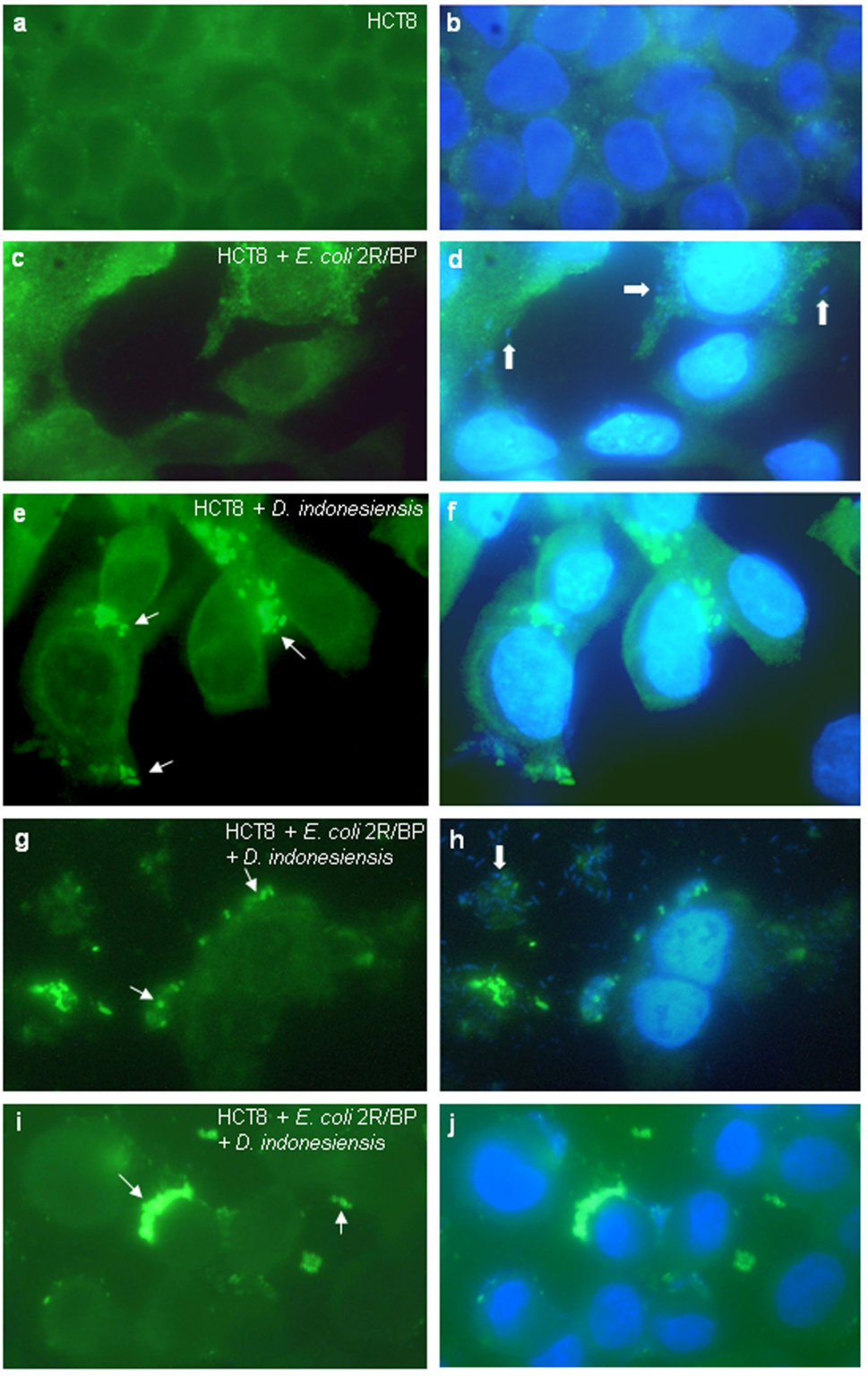
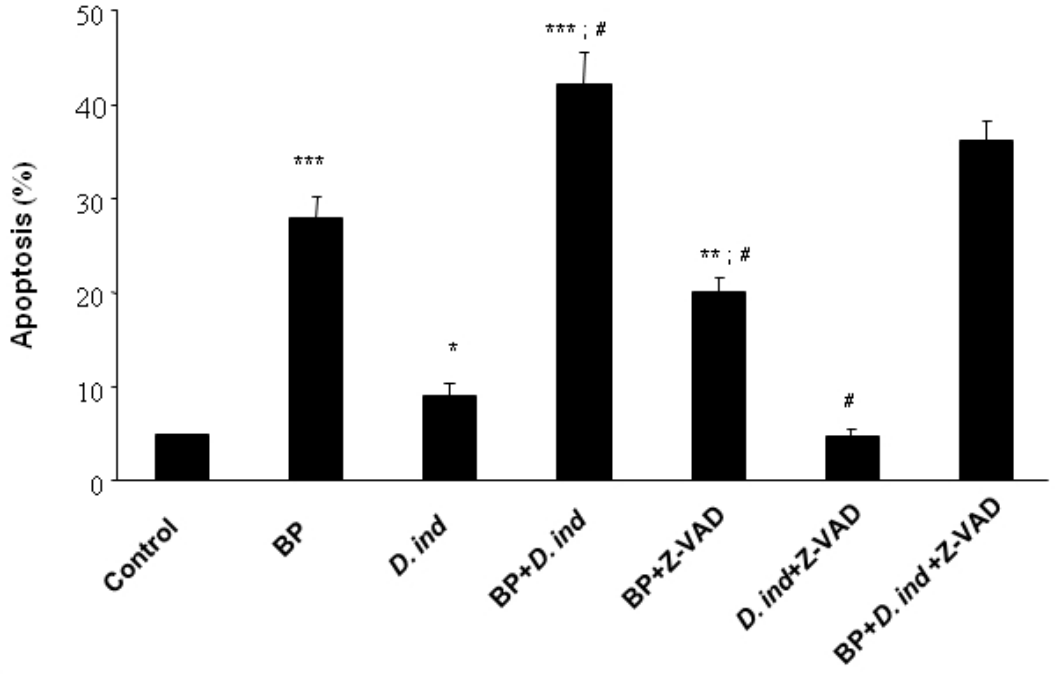
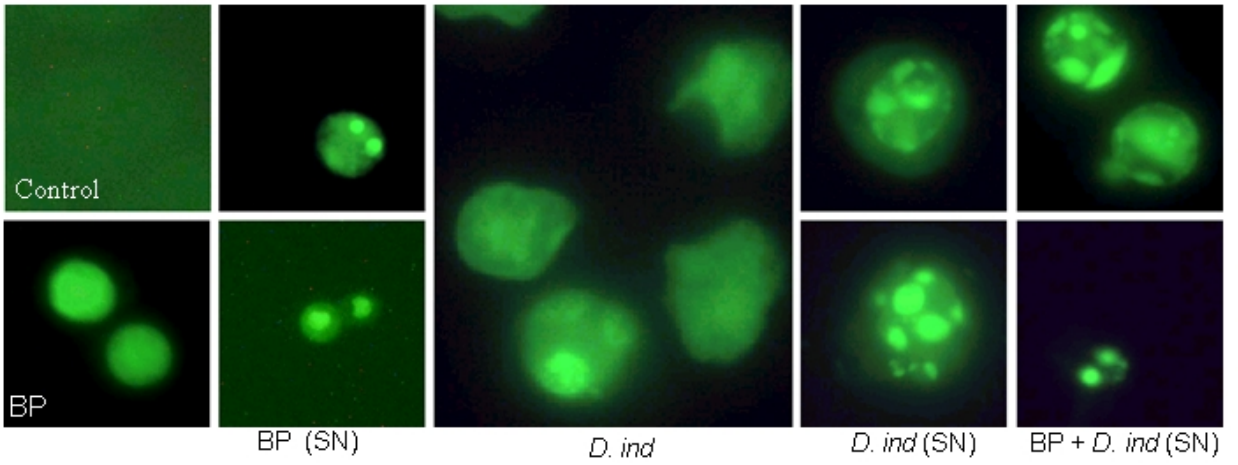

Mean Fluorescence Intensity
400
300
200
100
0
**
Control
E. coli 2R/BP
D. indonesiensis

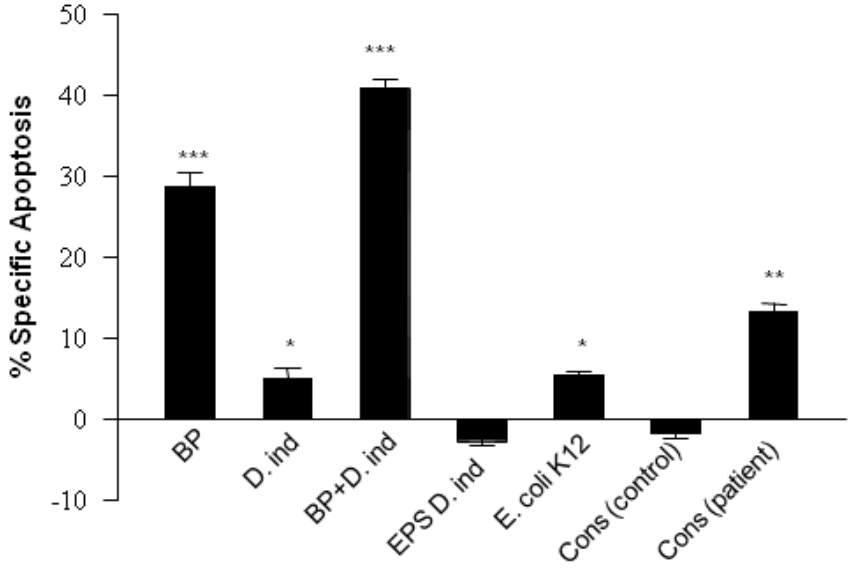

a
HCT8
b
c
HCT8 + E. coli 2R/BP
d
e
HCT8 + D. indonesiensis
f
g
HCT8 + E. coli 2R/BP
+ D. indonesiensis
h
i
HCT8 + E. coli 2R/BP
+ D. indonesiensis
j

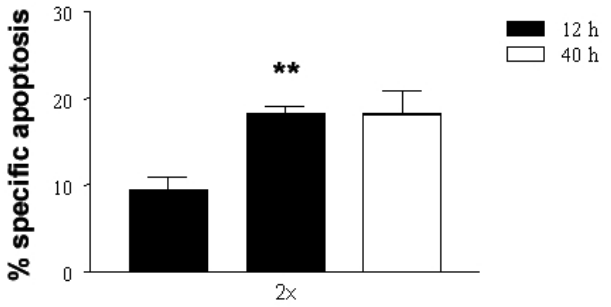
a

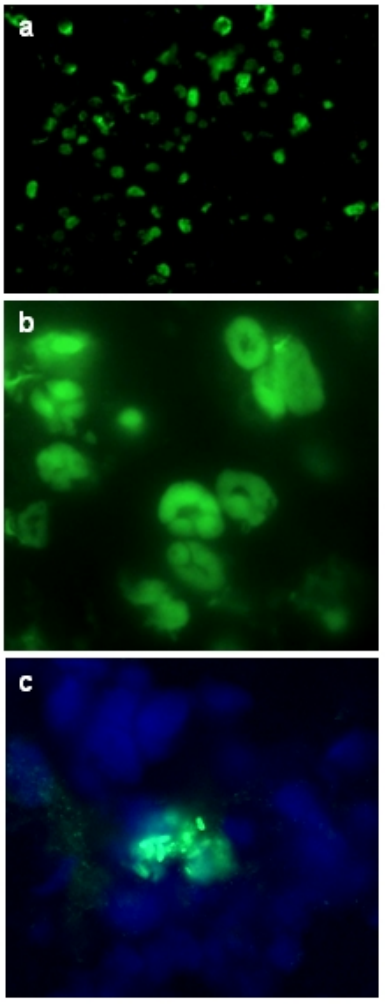

Apoptosis (%)
50
40
30
20
10
0
***
*
*** ; #
** ; #
#
Control
BP
D. ind
BP+D. ind
BP+Z-VAD
D. ind+Z-VAD
BP+D. ind+Z-VAD

b


Control
BP
BP (SN)
D. ind
D. ind (SN)
BP + D. ind (SN)


% Specific Apoptosis
50
40
30
20
10
0
-10
***
*
***
*
**
BP
D. ind
BP+D. ind
EPS D. ind
E. coli K12
Cons (control)
Cons (patient)


% specific apoptosis
30
20
10
0
**
2x
12 h
40 h


a
b
c

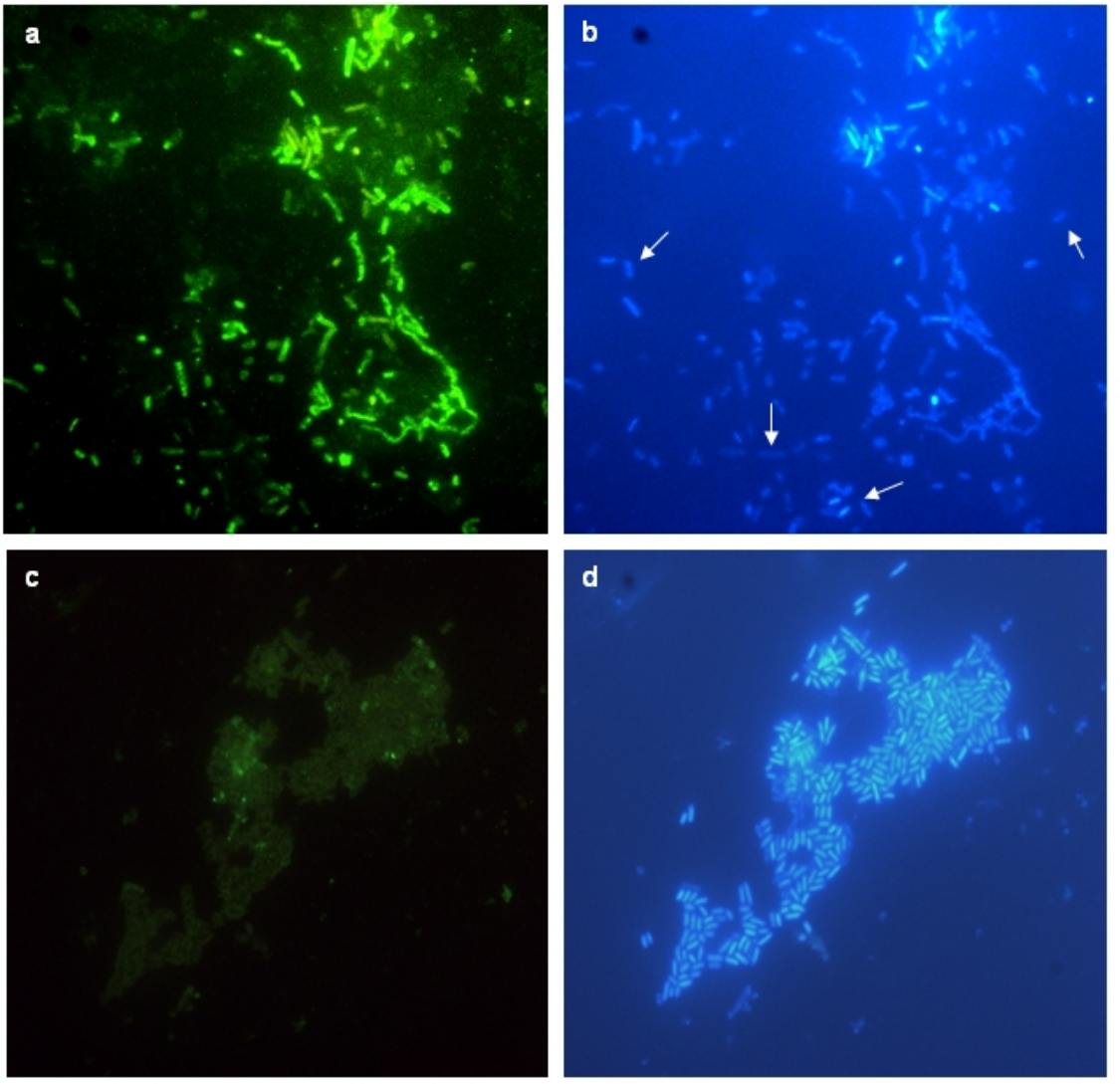

a
b
c
d

Table 1: Dot blotting of SRB enriched consortiums from patients or from control group using anti-EPS of *D. indonesiensis* antibody

Reaction	**Ulcerative colitis patients (n = 21)**	**Control group (n = 24)**
Positive	17 (81 %)	5 (20.8 %)
Negative	4 (19 %)	19 (79.2 %)