

ARTICLES

A microbial symbiosis factor prevents intestinal inflammatory disease

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Humans are colonized by multitudes of commensal organisms representing members of five of the six kingdoms of life; however, our gastrointestinal tract provides residence to both beneficial and potentially pathogenic microorganisms. Imbalances in the composition of the bacterial microbiota, known as dysbiosis, are postulated to be a major factor in human disorders such as inflammatory bowel disease. We report here that the prominent human symbiont *Bacteroides fragilis* protects animals from experimental colitis induced by *Helicobacter hepaticus*, a commensal bacterium with pathogenic potential. This beneficial activity requires a single microbial molecule (polysaccharide A, PSA). In animals harbouring *B. fragilis* not expressing PSA, *H. hepaticus* colonization leads to disease and pro-inflammatory cytokine production in colonic tissues. Purified PSA administered to animals is required to suppress pro-inflammatory interleukin-17 production by intestinal immune cells and also inhibits *in vitro* reactions in cell cultures. Furthermore, PSA protects from inflammatory disease through a functional requirement for interleukin-10-producing CD4⁺ T cells. These results show that molecules of the bacterial microbiota can mediate the critical balance between health and disease. Harnessing the immunomodulatory capacity of symbiosis factors such as PSA might potentially provide therapeutics for human inflammatory disorders on the basis of entirely novel biological principles.

Many human disorders seem to require a critical—and often unknown—environmental component. Inflammatory bowel diseases (IBDs) such as Crohn's disease and ulcerative colitis represent aberrant immune responses of the human gastrointestinal tract with adverse clinical outcomes¹. Abundant clinical and laboratory research has shown that, in IBD, commensal bacteria harboured within mammalian intestines are the targets of inflammatory responses^{1–4}. Antibiotic treatment alleviates intestinal inflammation in humans and experimental animals⁵. Germ-free re-derivation of animals genetically susceptible to colitis prevents development of intestinal inflammation⁶. Transfer of CD4⁺ T-cell clones specific for bacterial antigens induces disease in recipient animals⁷. In humans and other animals, inflammatory responses are apparently directed towards specific subsets of commensal organisms that have pathogenic potential but are not typically infectious pathogenic agents. All mammals harbour these species; why inflammation ensues only in those affected by IBD is unknown. Some investigators have predicted that, in addition to genetic factors, an imbalance in the normal microbiota without acquisition of an infectious organism is at least partially responsible for IBD⁸. Metagenomic studies have shown that entire classes of bacteria are lost or over-represented as part of the IBD process⁹. Perhaps in certain disorders where environmental factors are implicated, an imbalance between commensal bacteria with pathogenic potential (which we term pathobionts) and symbionts (commensal bacteria with beneficial potential) in the microbiota has a role in pathogenesis.

Humans maintain a lifelong association with innumerable commensal microorganisms that inhabit almost every environmentally exposed surface of the body. The gastrointestinal tract harbours > 10¹⁴ microorganisms of ~1,000 species¹⁰. Collectively, the intestinal microbiota represents a 'forgotten organ' that can execute many physiological functions and thus may profoundly influence human

biology. Germ-free animals, born and raised under sterile conditions, have defects in the development of intestinal tissues, show reduced vascular, nutritional and endocrine function, and are more susceptible to infection than conventionally colonized animals^{11,12}. Both gastrointestinal and systemic immune responses are deficient in the absence of commensal microorganisms¹³. Thus, mammals seem to depend on the microbiota to promote development and differentiation of host tissue¹⁴. Because of the complexity of the interactions of this diverse consortium of microorganisms with the mammalian host, the molecules responsible for host–microbe communication remain almost entirely unknown. As the microbiota has been implicated in disease, an understanding of these molecules may benefit human health¹⁵.

We have demonstrated that germ-free animals have defects in CD4⁺ T-cell development and that the human commensal bacterium *Bacteroides fragilis* corrects these deficiencies through the expression of PSA¹³. The precise immune-cell subset affected by PSA has not yet been identified. CD4⁺ T cells of the mammalian immune system can be generally divided into a naive ('uneducated') CD4⁺CD45Rb^{high} population and an antigen-experienced ('educated') CD4⁺CD45Rb^{low} population¹⁶. We found that splenic cells from germ-free animals included a smaller proportion of CD4⁺CD45Rb^{low} T cells than those from age-matched conventional mice with a complete bacterial microbiota (Fig. 1a). We examined the ability of *B. fragilis* to correct deficiencies in the CD4⁺CD45Rb^{low} T-cell population. Mono-colonization of germ-free mice with wild-type *B. fragilis* alone restores the CD4⁺CD45Rb profile to that found in animals with a complete bacterial microbiota (Fig. 1a; left panels). Notably, colonization with a mutant strain defective in the ability to produce PSA (*B. fragilis* ΔPSA) did not generate an expansion of the CD4⁺CD45Rb^{low} T-cell population (Fig. 1a; bottom right panel). It is well established that the latter population possesses potent

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anti-inflammatory properties and confers protection in animal models of inflammation¹⁷. These results suggest that *B. fragilis* may have evolved a molecular strategy to mediate protection from inflammation during host–bacterial mutualism.

Protection from colitis by *B. fragilis*

We used the well-established CD4⁺CD45Rb transfer model of experimental colitis¹⁸ to investigate whether *B. fragilis* colonization protects animals from inflammatory disease. In this model, pathogenic CD4⁺CD45Rb^{high} T cells are separated from protective CD4⁺CD45Rb^{low} cells and transferred into specific pathogen-free *Rag*^{-/-} mice. On cell transfer, mice were colonized with *Helicobacter hepaticus*^{8,19}, a pathobiont that is a benign commensal in wild-type animals but an opportunistic pathogen causing colitis in immunocompromised mice. After 8 weeks, animals were killed and colitis was assessed using a standard scoring system²⁰. The pathology scores show that *H. hepaticus* colonization and CD4⁺CD45Rb^{high} T-cell transfer are sufficient to induce severe colitis in *Rag*^{-/-} mice (Fig. 1b, first column), as previously reported^{19,21}. Co-colonization with wild-type *B. fragilis* resulted in significant protection from disease (second column); conversely, co-colonization with *B. fragilis* ΔPSA does not offer protection (third column).

Tissue damage in colitis is widely believed to result from production of inflammatory cytokines in response to commensal bacteria²².

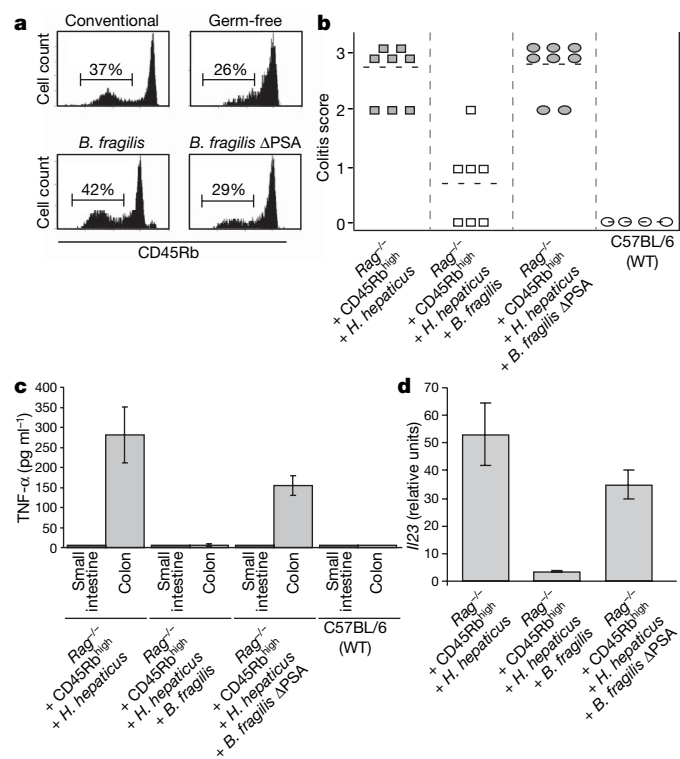


Figure 1 | *B. fragilis* colonization requires PSA for protection from experimental colitis. **a**, Mono-association of germ-free mice with wild-type *B. fragilis* expands the proportions of CD4⁺CD45Rb^{low} T cells in a PSA-dependent manner (mean ± s.d. for 3 experiments: conventional, 38.4% ± 2.2; germ-free, 26.7% ± 1.3; *B. fragilis*, 40.8% ± 3.1; *B. fragilis* ΔPSA, 28.8% ± 2.6). All cells were gated on CD4⁺ splenocytes. **b**, Co-colonization with *B. fragilis* ameliorates disease onset, whereas co-colonization with *B. fragilis* ΔPSA is not protective ($P = 0.004$, Mann–Whitney U -test). Combined data from two independent experiments are shown. **c**, ELISA of colon organ cultures demonstrates increased expression of pro-inflammatory cytokine TNF- α in diseased colons, with significant reductions in animals co-colonized with wild-type *B. fragilis* but not with *B. fragilis* ΔPSA. **d**, qPCR for *Il23p19* was performed on splenocytes and normalized to *L32* expression. Error bars represent s.d. for triplicate samples in all cases. WT, wild type.

The pro-inflammatory cytokines tumour-necrosis factor- α (TNF- α), interleukin (IL)-1 β and IL-23 are central to disease initiation and progression in this experimental model of colitis²³. Furthermore, amounts of these cytokines are elevated in patients with IBD²⁴, and therapies neutralizing TNF- α have yielded promising results in clinical trials in patients with Crohn's disease²⁵. We examined inflammatory cytokine concentrations during disease by directly culturing intestinal tissues of T-cell recipient colonized animals²⁶. Amounts of the pro-inflammatory cytokines TNF- α (Fig. 1c), IL-12p40 and IL-1 β (Supplementary Fig. 1) are elevated in the colons of *Rag*^{-/-} recipient mice colonized with *H. hepaticus*, but not in sections of small intestine—a site that is not affected in this model. Consistent with the protection observed by pathophysiological analysis of experimental colitis, TNF- α production was not elevated when these animals were co-colonized with wild-type *B. fragilis*. T-cell transfer plus co-colonization with *H. hepaticus* and *B. fragilis* ΔPSA results in increased colonic cytokine production similar to that seen in *Rag*^{-/-} animals colonized with *H. hepaticus* alone. Moreover, purified splenic CD4⁺ T cells from *H. hepaticus*-colonized animals demonstrate increased TNF- α production; this condition is corrected by colonization with wild-type *B. fragilis* but not with the PSA deletion strain (Supplementary Fig. 2). Expression of IL-23 is critical in the cascade of events leading to experimental colitis^{27,28}. We found that increases in *Il23* expression by splenocytes after disease induction are completely suppressed by intestinal colonization with PSA-producing *B. fragilis* (Fig. 1d). Over the course of the experiments, amounts of *H. hepaticus* and *B. fragilis* colonization did not differ between groups; thus, protection is not the result of bacterial clearance (Supplementary Figs 3 and 4). Instead, *B. fragilis* has evolved a specific immunomodulatory molecule that orchestrates beneficial immune responses to prevent its host from developing colitis.

Purified PSA prevents gut pathology

To determine whether PSA is sufficient for protection from disease in the absence of the intact organism, we purified PSA to homogeneity²⁹ and administered it by gavage (orally) to *Rag*^{-/-} mice. We measured disease progression by various pathological and histological criteria. Colitis scores after CD4⁺CD45Rb^{high} T-cell transfer in the absence of *H. hepaticus* colonization indicate the development of very mild colitis due to inflammation elicited by the animals' specific pathogen-free microbiota (Fig. 2a; first column). However, *H. hepaticus*-colonized *Rag*^{-/-} animals that received CD4⁺CD45Rb^{high} T-cell transfers developed severe colitis (Fig. 2a; second column). Oral PSA administration almost completely protected animals against *H. hepaticus*-induced colitis (Fig. 2a; third column), reducing disease to levels of control animals which do not develop colitis (Fig. 2a; fourth column).

The inability to gain weight is a hallmark of colitis in this experimental setting⁴. Wasting disease occurred in *Rag*^{-/-} animals after transfer of CD4⁺CD45Rb^{high} cells and colonization with *H. hepaticus* (Fig. 2b; PBS + *Hh*). These animals also developed intestinal pathology and expressed pro-inflammatory cytokines (as described above). Oral administration of PSA from the outset completely protected animals against *H. hepaticus*-mediated wasting disease (Fig. 2b; PSA + *Hh*). Demonstrating that *Helicobacter hepaticus* provides the necessary antigens for inflammation induction, no pathology was observed in uncolonized animals (Fig. 2b; PBS – *Hh*) or in animals without cell transfer.

We examined histological sections of colons for inflammation resulting in experimental colitis. Transfer of CD4⁺CD45Rb^{high} T cells into *H. hepaticus*-colonized *Rag*^{-/-} mice resulted in onset of severe colitis, as evidenced by massive epithelial cell hyperplasia and gross thickening of the gut wall (Fig. 2c; second panel). Consistent with previous studies, the combination of CD4⁺CD45Rb^{high} T-cell transfer and *H. hepaticus* colonization resulted in leukocyte infiltration of affected tissues—a hallmark of inflammation and disease

(Fig. 2c; second panel, bottom)^{19,21}. Oral administration of PSA into *H. hepaticus*-colonized cell transfer recipients conferred complete protection against experimentally induced colonic hyperplasia (Fig. 2c; third panel). Furthermore, PSA-treated animals showed no leukocyte infiltration in colonic tissues (Fig. 2c; third panel, bottom), indicating protection against inflammation. Taken together, these results suggest that PSA prevents colitis and protects mice against the associated weight loss and inflammatory cell infiltration observed in diseased animals.

Control of chemically induced inflammation

Experimental colitis and human IBD result from an initial inflammatory response that—lacking repression—advances in an uncontrolled fashion and ultimately leads to intestinal pathology and disease. To elucidate how PSA affects these primary inflammatory responses, we used an animal model of chemically induced colonic inflammation. Rectal administration of trinitrobenzene sulphonic acid (TNBS) to wild-type mice mimics the initiation of colitis by

eliciting inflammatory T-cell responses. Disease was induced by the administration of TNBS (vehicle was used as a negative control), and oral treatment of PSA was evaluated. TNBS-treated animals exhibited the greatest amount of weight loss, and were unable to recover as rapidly in comparison to either vehicle-treated or PSA-treated animals. (Fig. 3a). Histological analysis confirmed PSA protection of colonic tissues against the massive epithelial hyperplasia and loss of colonocyte organization seen after TNBS treatment (Fig. 3b). Studies have shown that pathogenic T-helper (T_H)17 cells, which produce IL-17, mediate the induction of TNBS experimental colitis³⁰. We found that *Il17* expression was increased among purified $CD4^+$ T cells from mesenteric lymph nodes (MLNs; Fig. 3c) of diseased animals but not from those receiving PSA treatment. The increased expression of *Tnfa* among $CD4^+$ T cells from MLNs of TNBS-treated animals was also reduced in PSA-treated groups (Fig. 3d). Transcriptional analysis of TNBS-treated colons demonstrated that the expression of both *Il17* and *Tnfa* was highly elevated in diseased but not in PSA-protected animals (Fig. 3e, f). Therefore, PSA inhibits intestinal pathology and inflammation in a chemically induced model of experimental colitis.

PSA induces production of IL-10

Protection from experimental colitis is engendered through anti-inflammatory processes that prevent undesirable reactions against the intestinal microbiota²³. Interleukin-10-deficient (*Il10*^{-/-}) animals develop colitis³¹. IL-10 is one of the most potent anti-inflammatory cytokines and is required for protection in many animal models of inflammation^{21,27,32}. As assayed by quantitative real-time polymerase chain reaction (qPCR), transcriptional expression of *Il10* within colons of PSA-treated mice was significantly higher than in control and TNBS-treated mice (Fig. 4a). IL-10 is produced by many cell types; however, $CD4^+$ T cells that express IL-10 have immunosuppressive activities that inhibit inflammation during experimental colitis³³. We purified fresh $CD4^+$ T cells from MLNs of PSA-treated mice in which inflammation was reduced, and we found greatly elevated expression of the *Il10* transcript (Fig. 4b). We assessed whether PSA was sufficient to induce IL-10 *in vitro*; a specific increase in IL-10 production occurred when bone-marrow-derived dendritic cells (BMDCs) and naive $CD4^+$ T cells were treated with purified PSA (Fig. 4c). When BMDCs and naive $CD4^+$ T cells were infected with *H. hepaticus* co-cultured with *B. fragilis*, we found specific expression of IL-10 from culture supernatants, whereas co-culture with *B. fragilis* Δ PSA induced significantly lower expression of IL-10 (Supplementary Fig. 5). As PSA induces expression of IL-10 *in vitro*, we speculated whether this molecule is required for inhibition of inflammatory responses to *H. hepaticus*. We infected BMDC-T-cell co-cultures with live *H. hepaticus* and measured production of

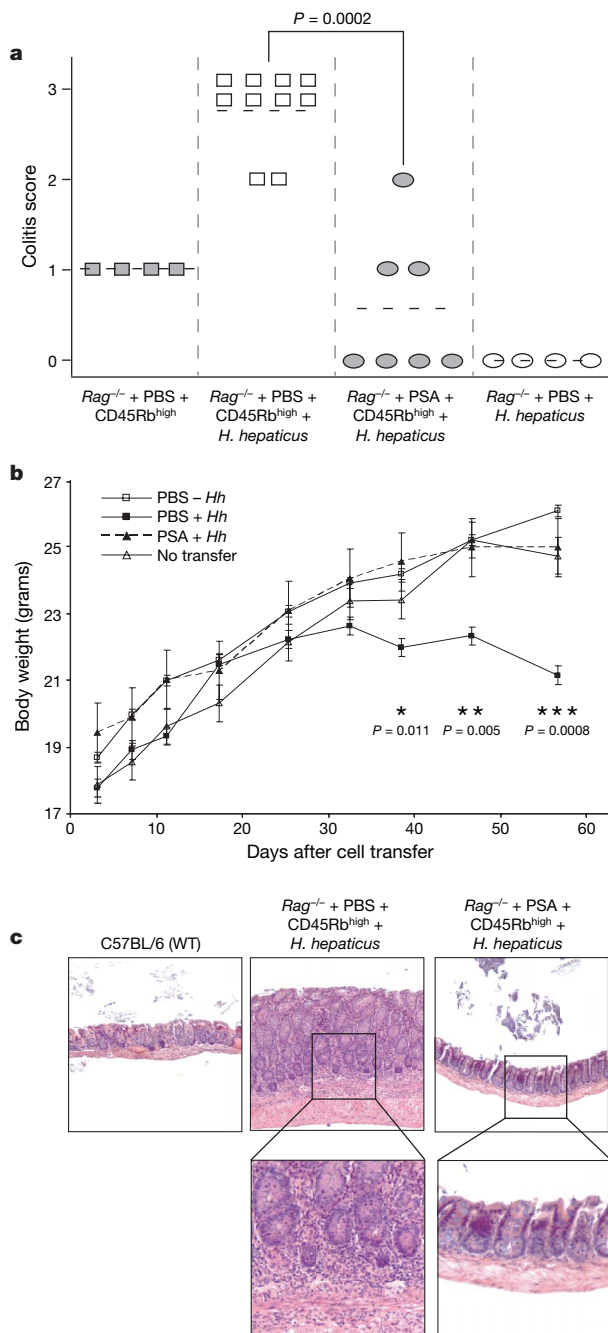


Figure 2 | Purified PSA protects against experimental colitis.

a, Colonization with *H. hepaticus* drives the onset of severe colitis (second column). PSA-treated animals either do not develop colitis or develop only mild disease (third column) ($P > 0.05$ for dissimilar results, $P < 0.01$ for similar results, Kruskal–Wallis comparison of all groups; $P = 0.0002$, two-tailed Mann–Whitney *U*-test). The data shown are the pooled results from duplicate experiments. **b**, Wasting disease in *Rag2*^{-/-} animals results from transfer of $CD4^+$ $CD45Rb^{\text{high}}$ T cells and colonization with *H. hepaticus* (PBS + *Hh*). PSA treatment by gavage protects animals against wasting (PSA + *Hh*). Two-factor analysis of variance (ANOVA) indicates that comparisons between all indicated groups (asterisks) are statistically significant. Error bars represent s.d. between four animals per group; experiments were performed in duplicate. **c**, Architecture of colonic sections from wild-type animals (first panel). $CD4^+$ $CD45Rb^{\text{high}}$ T-cell transfer into *H. hepaticus*-colonized *Rag2*^{-/-} mice resulted in severe colitis, as evidenced by massive epithelial hyperplasia and pronounced inflammation (second panel); the higher magnification below shows inflammatory cell infiltration into colonic tissues. Oral PSA treatment protects *H. hepaticus*-colonized animals (third panel) from colitis. Images in each row are the same magnification; original magnification $\times 10$ for top, $\times 40$ for bottom row. WT, wild type.

the pro-inflammatory cytokine TNF- α . Addition of increasing amounts of the pathogenic commensal bacterium resulted in a dose-dependent increase in TNF- α concentration, as determined by enzyme-linked immunosorbent assay (ELISA) of culture supernatants (Fig. 4d; left three bars). Treatment of cells with purified PSA decreased TNF- α production in response to *H. hepaticus* (Fig. 4d; middle three bars). Most notably, co-incubation of cell cultures with *H. hepaticus* and PSA in the presence of a neutralizing IL-10 receptor antibody completely reversed this phenotypic effect and increased expression of TNF- α (Fig. 4d; right three bars). The results are similar for the related pro-inflammatory cytokine IL-1 β (Supplementary Fig. 6). Thus, IL-10 produced in response to PSA is required for inhibition of inflammatory reactions in cell cultures.

IL-10-producing T cells suppress colitis

We investigated the requirement for IL-10 in suppression of intestinal inflammation. Initially, *Il10*^{-/-} animals were colonized with *H. hepaticus* alone or in combination with *B. fragilis* (either wild type or Δ PSA). We subsequently collected MLNs and re-stimulated cells in culture with soluble *H. hepaticus* antigens using an assay previously developed to measure antigen-specific responses to this pathogen²⁷. *H. hepaticus*-colonization resulted in increased production of TNF- α

and IL-17 by MLN cells; however, in the absence of IL-10 production in colonized animals, *B. fragilis* co-colonization did not reduce the concentration of these pro-inflammatory molecules (Fig. 5a and b, respectively). As expected, the absence of PSA had no effect. Again using the cell transfer model of colitis, we transferred CD4⁺CD45Rb^{high} T cells to *H. hepaticus*-colonized *Rag*^{-/-} animals. Administration of an anti-IL-10 receptor antibody to mice (to block IL-10 signalling) during oral treatment with PSA abrogated protection from colitis (Fig. 5c). When *Il10*^{-/-} animals were treated with TNBS in the presence or absence of PSA, weight and histology data (Supplementary Figs 7 and 8) indicate that IL-10 production is required for PSA-elicited reduction of intestinal immune responses.

Our data suggest that PSA-mediated protection entails the generation and/or expansion of IL-10-producing CD4⁺ T cells. To determine whether IL-10 production by CD4⁺ T cells is required for protection, we transferred CD4⁺CD45Rb^{high} T cells from *Il10*^{-/-} donor mice into *Rag*^{-/-} recipients and then colonized the recipients with *H. hepaticus*. As expected, groups of mice receiving *Il10*^{-/-} T cells along with *H. hepaticus* developed severe colitis (Fig. 5d; first column) and were not protected by PSA (Fig. 5d; second column). This result, supported by histological findings in colons, suggests that PSA induces protection from 'previously pathogenic' CD4⁺CD45Rb^{high} T cells in an IL-10-dependent manner (Fig. 5e). Weight analysis at death shows that colitic PBS- and PSA-treated animals receiving *Il10*^{-/-} CD4⁺CD45Rb^{high} T cells (unlike control animals receiving no transferred cells) developed wasting disease (Fig. 5f). Thus, IL-10 production by CD4⁺ T cells is required for PSA-mediated protection from experimental colitis. These results constitute the first reported evidence of a symbiotic bacterial molecule that networks with the immune system to coordinate anti-inflammatory responses required for mammalian health.

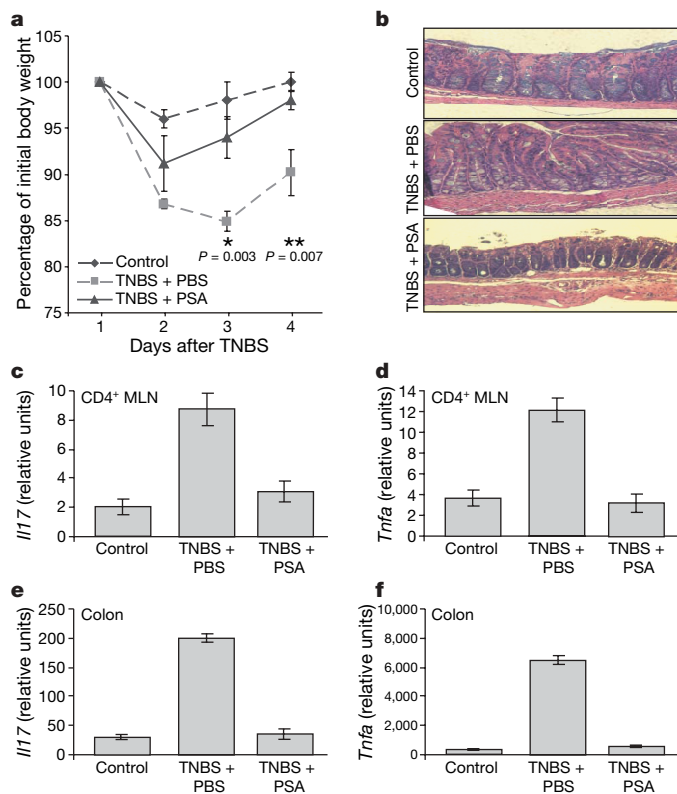


Figure 3 | Intestinal immune responses are modulated by a beneficial bacterial molecule. **a**, Oral PSA administration causes a statistically significant increase in body weight related to TNBS-treated PBS controls. Two-factor ANOVA values for all indicated groups (asterisks) are statistically significant. Error bars represent s.d. between four animals per group. **b**, Colons from TNBS plus PBS-treated groups show severe pathology, whereas those from TNBS and PSA-treated animals have histological architecture similar to that seen in untreated controls. The images shown are representative sections from animals in two independent experiments. Original magnification $\times 20$. **c**, **d**, qPCR of purified CD4⁺ T cells from MLNs with *Il17a*- and *Tnfa*-specific primers demonstrates that oral PSA administration reduces *Il17a* (**c**) and *Tnfa* (**d**) expression during disease. **e**, **f**, Transcriptional expression of *Il17a* (**e**) and *Tnfa* (**f**) from homogenized colons. Error bars are from duplicate runs of three independent experiments (**c–f**).

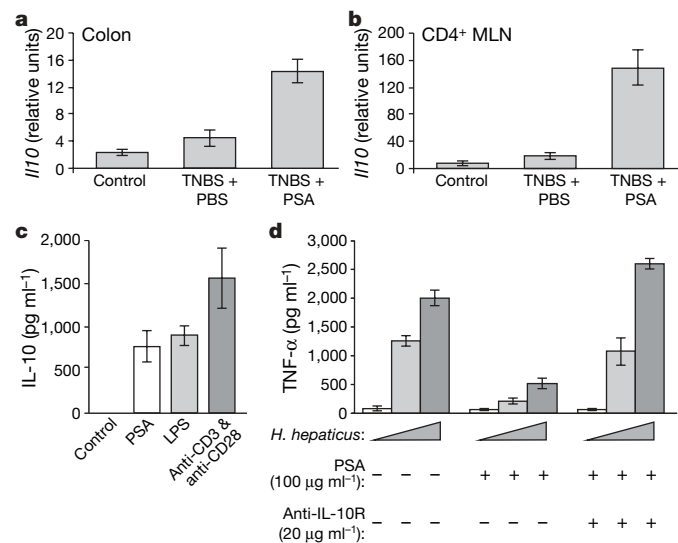


Figure 4 | PSA induces *Il10* expression in TNBS-treated animals and inhibits TNF- α production in primary cultured cells through IL-10 production. **a**, **b**, Wild-type mice were treated with ethanol (control), TNBS, or TNBS and PSA. qPCR assay of colons (**a**) and CD4⁺ T cells purified from MLNs (**b**) show elevated *Il10* expression in response to PSA. **c**, Incubation of BMDC-T cell co-cultures with purified PSA specifically induces IL-10 production at concentrations comparable to those induced by LPS or by anti-CD3 and anti-CD28 antibodies. **d**, Infection of BMDC-T cell co-cultures with increasing concentrations of *H. hepaticus* (multiplicity of infection: 0.1, 1.0 and 10, as depicted by triangles) results in increased TNF- α release. Treatment of infected cells with PSA reduces amount of TNF- α (middle three bars). Addition of an anti-IL-10 receptor antibody (anti-IL-10R) alleviates suppression of inflammatory responses, resulting in increased TNF- α production (right three bars). Error bars show s.d. for triplicate samples in all cases.

Summary and implications

According to the 'hygiene hypothesis' put forth nearly two decades ago, reduced exposure to infections in early childhood—owing to diminishing family size and improvements in living standards and personal hygiene, for example—may increase the risk of allergic and autoimmune disease³⁴. This concept is supported by epidemiological and clinical reports documenting increased incidences of IBD, colon cancer, asthma, type 1 diabetes and multiple sclerosis over the past 50 years in societies with improved medical care and hygiene (for example, Europe, the United States and Japan) but not in undeveloped countries³⁵. However, the application of major interventions, including vaccination, sanitation, and antibacterial and antiviral therapies, often does not permit discrimination between

infectious and non-infectious microorganisms and has undoubtedly led to changes in human association with the microbial world as a whole. The hygiene hypothesis does not address humanity's primary relationship with bacteria: the harbouring of multitudes of microbial species during commensalism. Our studies show that symbiotic bacteria residing in the mammalian gastrointestinal tract produce molecules that mediate healthy immune responses and protect the host from inflammatory disease. We propose that the mammalian genome does not encode for all functions required for immunological development but rather that mammals depend on critical interactions with their microbiome (the collective genomes of the microbiota) for health.

As mammals have harboured their commensal partners for millennia, adaptive co-evolution has formed an inextricable bond between microbe and host³⁶. Imbalances in the microbiota may contribute to some human diseases, and altered composition of the gut bacteria has been implicated in obesity³⁷. We show that *B. fragilis* protects its host from inflammatory disease caused by *H. hepaticus* in an animal model of experimental colitis. The implication that intestinal bacteria actively network with the host's immune system highlights the importance of the composition of the microbiota for overall health. If specific classes of bacteria have indeed evolved to promote the host's health, then disease may well result from the absence of these organisms and their beneficial molecules (for example, as a result of improved hygiene). Inflammation resulting from dysbiosis between symbionts and pathobionts may lay the molecular foundations for many intestinal—and perhaps non-intestinal—diseases. The exploration of probiotics (bacteria such as lactobacilli and bifidobacteria that promote health) has thus far failed to identify specific bacterial molecules or host mechanisms required for protection³⁸. Here we present evidence that a single bacterial molecule can ameliorate inflammatory disease in animals. Our observations suggest that many other symbiosis factors—bacterial molecules that have evolved to promote human health—remain to be discovered. The finding that PSA from *B. fragilis* is a natural anti-inflammatory molecule that actively promotes mammalian health may provide a platform for the development of therapies based on the fundamental relationship between humans and their beneficial microbial partners.

METHODS SUMMARY

Three models of intestinal inflammation were used: (1) CD4⁺CD45Rb^{high} T cells were purified from the spleens of wild-type or *Il10*^{-/-} donor mice by flow cytometry and transferred into *Rag*^{-/-} (C57BL/6) recipients as described; (2) TNBS colitis was induced by pre-sensitization of wild-type (C57BL/6) mice on the skin with a TNBS and acetone mix. Seven days after sensitization, 2.5% TNBS in ethanol was administered rectally; mice were killed 3–6 days later; and (3) *Il10*^{-/-} mice were colonized (by oral gavage) with *H. hepaticus* alone or in combination with wild-type *B. fragilis* or *B. fragilis* ΔPSA. *B. fragilis* NCTC 9343 and *H. hepaticus* ATCC 51149 were obtained from the American Type Culture Collection. Cytokines from the spleens, colons, or MLNs were assayed by ELISA, qPCR, or flow cytometry. Colitis was assessed with tissue sections (fixed, paraffin embedded, sectioned onto a slide and stained with haematoxylin and eosin) and was scored by a pathologist in a blinded experimental set-up (R. T. Bronson) according to a standard scoring system: 0, no thickening of colonic tissues and no inflammation (infiltration of lymphocytes); 1, mild thickening of tissues but no inflammation; 2, mild thickening of tissues and mild inflammation; 3, severe thickening and severe inflammation. BMDCs were purified from femurs of mice after extraction and washing in PBS. Cells were cultured for 8 days in C-RPMI-10 in the presence of GM-CSF (20 ng ml⁻¹; Biosource). CD4⁺ T cells were purified by negative selection over a magnetic column (Miltenyi or R&D Systems).

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- Poxton, I. R., Brown, R., Sawyerr, A. & Ferguson, A. Mucosa-associated bacterial flora of the human colon. *J. Med. Microbiol.* 46, 85–91 (1997).
- Sellon, R. K. et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect. Immun.* 66, 5224–5231 (1998).

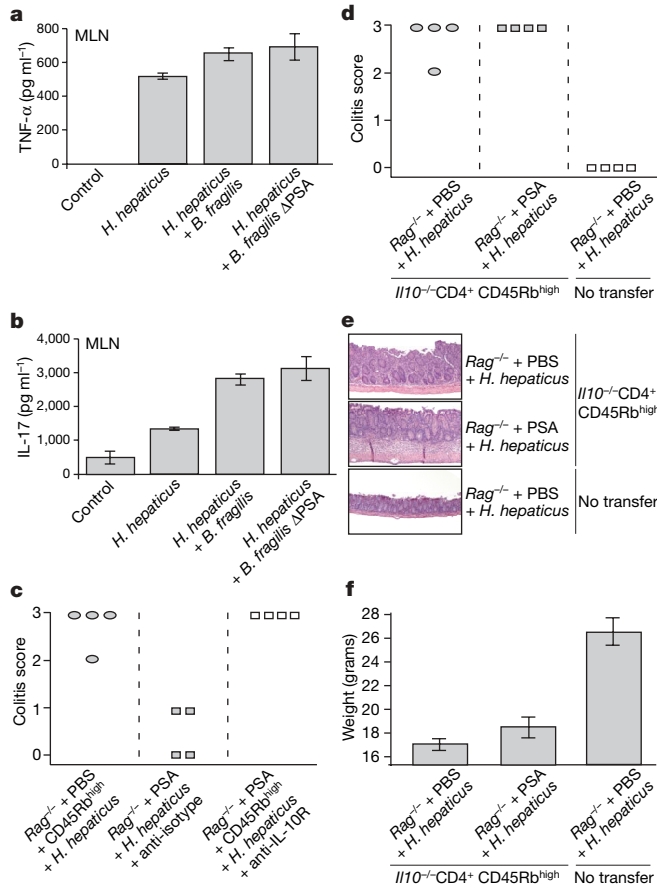


Figure 5 | IL-10 is required for PSA-mediated protection from intestinal inflammation and experimental colitis. **a, b**, *Il10*^{-/-} mice were left uncolonized (control) or were colonized with *H. hepaticus* (to induce inflammation) either alone or in combination with *B. fragilis* (wild-type or ΔPSA). MLNs from experimental groups were pooled and re-stimulated with soluble *H. hepaticus* antigen (5 μg ml⁻¹) for 48 h. Secretion of the pro-inflammatory cytokines TNF-α (**a**) and IL-17A (**b**) was analysed by ELISA. Error bars show s.d. for triplicate samples. **c**, Colitis scores show that PSA protection requires IL-10 signalling, as treatment with anti-IL-10 receptor antibody (anti-IL-10R) abrogates the PSA-mediated protection. Data represent two independent experiments. **d**, PSA-mediated protection from disease requires IL-10-producing CD4⁺ T cells. Treatment with PSA does not reduce colitis when CD4⁺CD45Rb^{high} T cells are transferred from *Il10*^{-/-} mice. Control animals without cell transfer do not develop colitis. Results are shown for one representative trial of two independent experiments. **e**, Histological colon sections show that PSA does not protect animals from experimental colitis when CD4⁺CD45Rb^{high} T cells cannot produce IL-10. All images are from the same magnification. Original magnification ×10. **f**, Mean body weights for groups of animals (*n* = 4) when culled demonstrate that IL-10 is required for PSA-mediated protection from wasting. Error bars show s.d. between 4 animals per group from one representative trial of two independent experiments.

3. Elson, C. O. Commensal bacteria as targets in Crohn's disease. *Gastroenterology* **119**, 254–257 (2000).
4. Sartor, R. B. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nature Clin. Pract. Gastroenterol. Hepatol.* **3**, 390–407 (2006).
5. Videla, S. *et al.* Role of intestinal microflora in chronic inflammation and ulceration of the rat colon. *Gut* **35**, 1090–1097 (1994).
6. Taurog, J. D. *et al.* The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *J. Exp. Med.* **180**, 2359–2364 (1994).
7. Kullberg, M. C. *et al.* Induction of colitis by a CD4⁺ T cell clone specific for a bacterial epitope. *Proc. Natl Acad. Sci. USA* **100**, 15830–15835 (2003).
8. O'Hara, A. M. & Shanahan, F. The gut flora as a forgotten organ. *EMBO Rep.* **7**, 688–693 (2006).
9. Frank, D. N. *et al.* Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl Acad. Sci. USA* **104**, 13780–13785 (2007).
10. Gill, S. R. *et al.* Metagenomic analysis of the human distal gut microbiome. *Science* **312**, 1355–1359 (2006).
11. Ley, R. E., Peterson, D. A. & Gordon, J. I. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* **124**, 837–848 (2006).
12. Smith, K., McCoy, K. D. & Macpherson, A. J. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin. Immunol.* **19**, 59–69 (2006).
13. Mazmanian, S. K., Liu, C. H., Tzianabos, A. O. & Kasper, D. L. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* **122**, 107–118 (2005).
14. Pamer, E. G. Immune responses to commensal and environmental microbes. *Nature Immunol.* **8**, 1173–1178 (2007).
15. Dethlefsen, L., McFall-Ngai, M. & Relman, D. A. An ecological and evolutionary perspective on human–microbe mutualism and disease. *Nature* **449**, 811–818 (2007).
16. Bell, E. B. Function of CD4 T cell subsets *in vivo*: expression of CD45R isoforms. *Semin. Immunol.* **4**, 43–50 (1992).
17. Izcue, A., Coombes, J. L. & Powrie, F. Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. *Immunol. Rev.* **212**, 256–271 (2006).
18. Maloy, K. J. *et al.* CD4⁺CD25⁺ T_R cells suppress innate immune pathology through cytokine-dependent mechanisms. *J. Exp. Med.* **197**, 111–119 (2003).
19. Cahill, R. J. *et al.* Inflammatory bowel disease: an immunity-mediated condition triggered by bacterial infection with *Helicobacter hepaticus*. *Infect. Immun.* **65**, 3126–3131 (1997).
20. Scheinin, T., Butler, D. M., Salway, F., Scallan, B. & Feldmann, M. Validation of the interleukin-10 knockout mouse model of colitis: antitumour necrosis factor-antibodies suppress the progression of colitis. *Clin. Exp. Immunol.* **133**, 38–43 (2003).
21. Kullberg, M. C. *et al.* Bacteria-triggered CD4⁺ T regulatory cells suppress *Helicobacter hepaticus*-induced colitis. *J. Exp. Med.* **196**, 505–515 (2002).
22. Bregenholt, S. Cells and cytokines in the pathogenesis of inflammatory bowel disease: new insights from mouse T cell transfer models. *Exp. Clin. Immunogenet.* **17**, 115–129 (2000).
23. Powrie, F. & Maloy, K. J. Immunology. Regulating the regulators. *Science* **299**, 1030–1031 (2003).
24. Xavier, R. & Podolsky, D. K. Commensal flora: wolf in sheep's clothing. *Gastroenterology* **128**, 1122–1126 (2005).
25. Rutgeerts, P. *et al.* Infliximab for induction and maintenance therapy for ulcerative colitis. *N. Engl. J. Med.* **353**, 2462–2476 (2005).
26. Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. & Medzhitov, R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* **118**, 229–241 (2004).
27. Kullberg, M. C. *et al.* IL-23 plays a key role in *Helicobacter hepaticus*-induced T cell-dependent colitis. *J. Exp. Med.* **203**, 2485–2494 (2006).
28. Hue, S. *et al.* Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J. Exp. Med.* **203**, 2473–2483 (2006).
29. Tzianabos, A. O. *et al.* The capsular polysaccharide of *Bacteroides fragilis* comprises two ionically linked polysaccharides. *J. Biol. Chem.* **267**, 18230–18235 (1992).
30. Elson, C. O. *et al.* Monoclonal anti-interleukin 23 reverses active colitis in a T cell-mediated model in mice. *Gastroenterology* **132**, 2359–2370 (2007).
31. Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K. & Muller, W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* **75**, 263–274 (1993).
32. Asseman, C., Mauze, S., Leach, M. W., Coffman, R. L. & Powrie, F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J. Exp. Med.* **190**, 995–1004 (1999).
33. Groux, H. *et al.* A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **389**, 737–742 (1997).
34. Strachan, D. P. Hay fever, hygiene, and household size. *Br. Med. J.* **299**, 1259–1260 (1989).
35. Bach, J. F. The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med.* **347**, 911–920 (2002).
36. Liu, C. H., Lee, S. M., Vanlare, J. M., Kasper, D. L. & Mazmanian, S. K. Regulation of surface architecture by symbiotic bacteria mediates host colonization. *Proc. Natl Acad. Sci. USA* **105**, 3951–3956 (2008).
37. Turnbaugh, P. J. *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031 (2006).
38. Mazmanian, S. K. & Kasper, D. L. The love-hate relationship between bacterial polysaccharides and the host immune system. *Nature Rev. Immunol.* **6**, 849–858 (2006).

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