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(54) **IMMUNOMODULATING COMPOUNDS AND RELATED COMPOSITIONS AND METHODS**

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(57) **ABSTRACT**

Provided herein are compounds, compositions and methods for balancing a T-helper cell profile and in particular Th1, Th2, Th17 and Treg cell profiles, and related methods and compositions for treating or preventing an inflammatory condition associated with an imbalance of a T-helper cell profile.

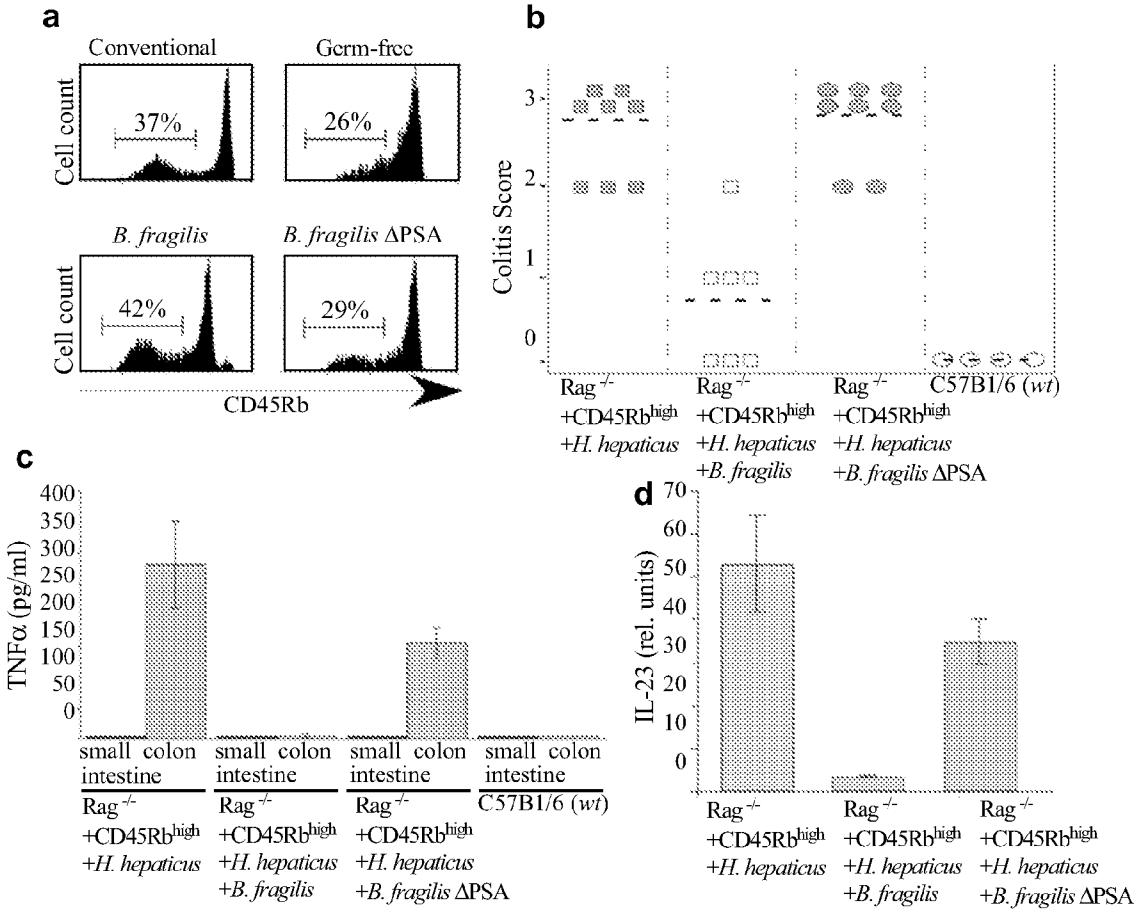
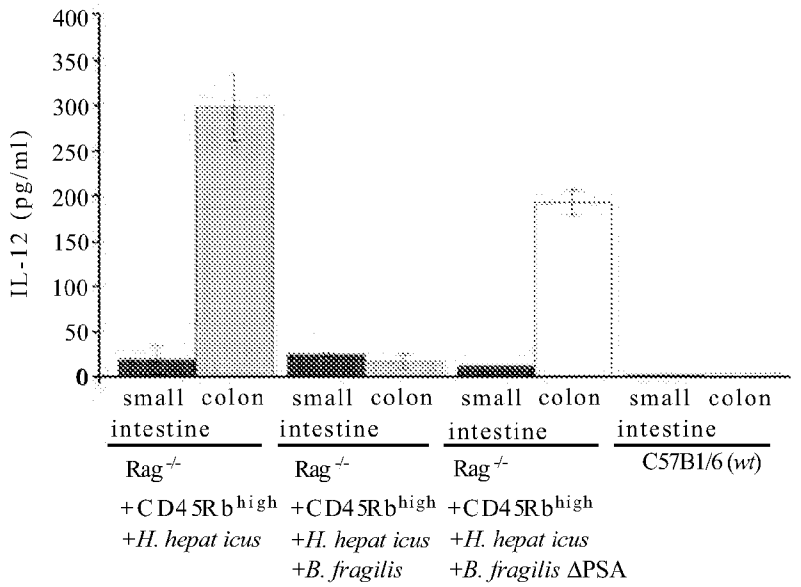

a
Conventional
Germ-free
Cell count
37%
26%
B. fragilis
B. fragilis ΔPSA
Cell count
42%
29%
CD45Rb
b
Colitis Score
0
1
2
3
Rag -/- +CD45Rb high +H. hepaticus
Rag -/- +CD45Rb high +H. hepaticus +B. fragilis
Rag -/- +CD45Rb high +H. hepaticus +B. fragilis ΔPSA
C57Bl/6 (wt)
c
TNFα (pg/ml)
0
50
100
150
200
250
300
350
400
small intestine
colon
Rag -/- +CD45Rb high +H. hepaticus
Rag -/- +CD45Rb high +H. hepaticus +B. fragilis
Rag -/- +CD45Rb high +H. hepaticus +B. fragilis ΔPSA
C57Bl/6 (wt)
d
IL-23 (rel. units)
0
10
20
30
40
50
60
70
Rag -/- +CD45Rb high +H. hepaticus
Rag -/- +CD45Rb high +H. hepaticus +B. fragilis
Rag -/- +CD45Rb high +H. hepaticus +B. fragilis ΔPSA

FIG. 1


IL-12 (pg/ml)
400
350
300
250
200
150
100
50
0
small colon intestine
small colon intestine
small colon intestine
small colon intestine
Rag -/- +CD45RbHigh +H. hepat icus
Rag -/- +CD45RbHigh +H. hepat icus +B. fragilis
Rag -/- +CD45RbHigh +H. hepat icus +B. fragilis ΔPSA
C57B1/6 (wt)

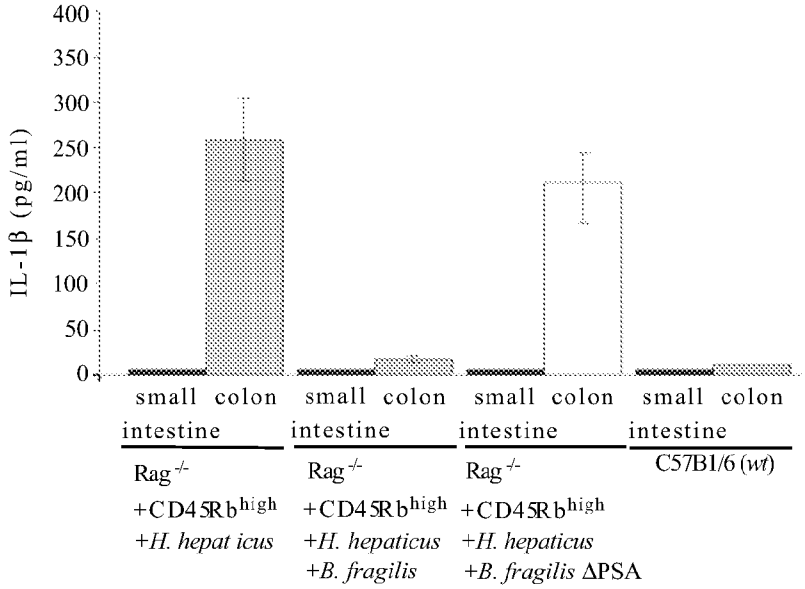

IL-1β (pg/ml)
400
350
300
250
200
150
100
50
0
small colon intestine
small colon intestine
small colon intestine
small colon intestine
Rag -/- +CD45RbHigh +H. hepat icus
Rag -/- +CD45RbHigh +H. hepaticus +B. fragilis
Rag -/- +CD45RbHigh +H. hepaticus +B. fragilis ΔPSA
C57B1/6 (wt)

FIG. 2

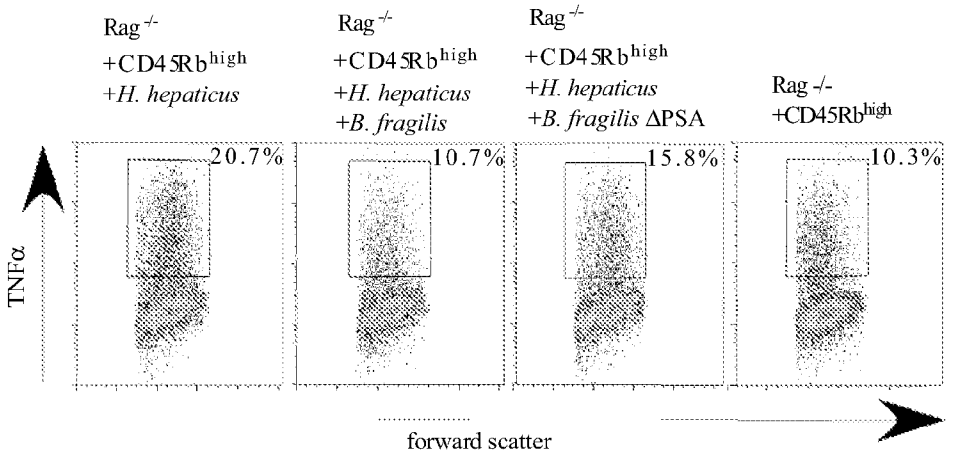

Rag -/- +CD45RbHigh +H. hepaticus
Rag -/- +CD45RbHigh +H. hepaticus +B. fragilis
Rag -/- +CD45RbHigh +H. hepaticus +B. fragilis ΔPSA
Rag -/- +CD45RbHigh
20.7%
10.7%
15.8%
10.3%
TNFα
forward scatter

FIG. 3

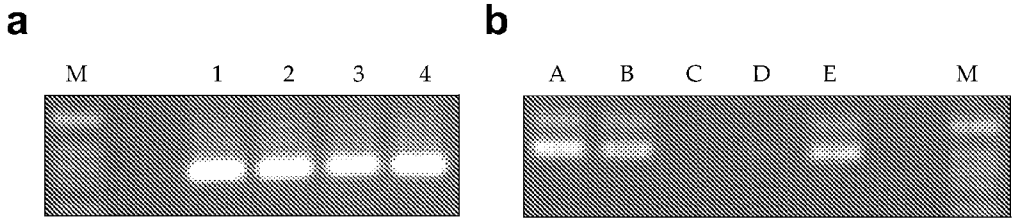

a
b
M
1
2
3
4
A
B
C
D
E
M

FIG. 4

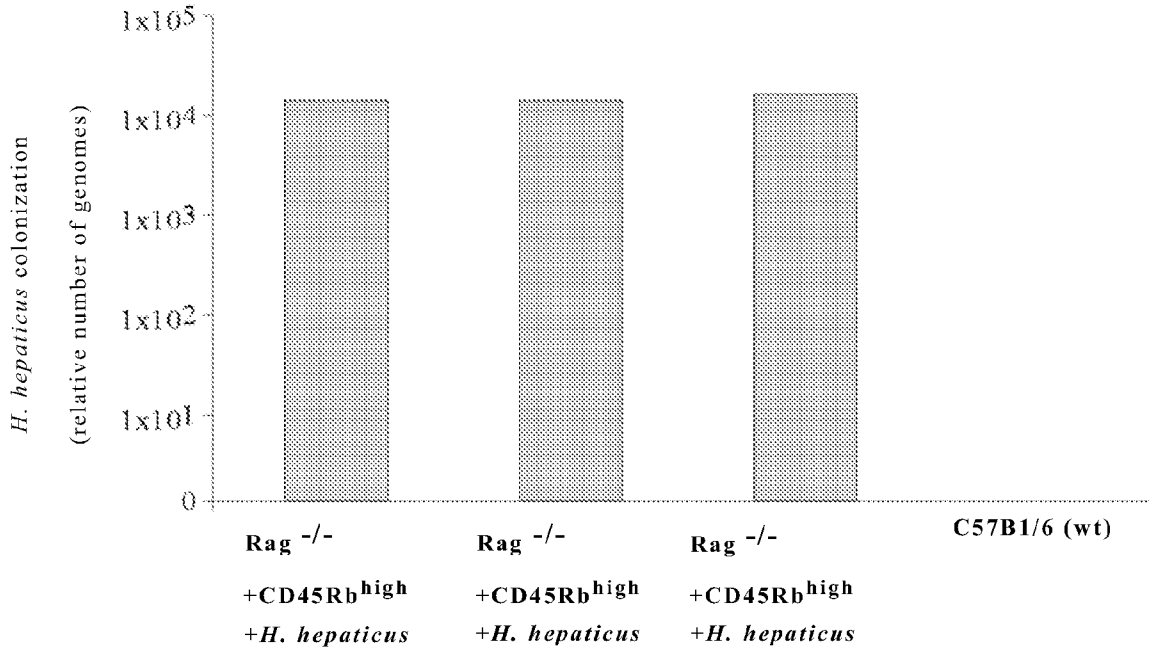

H. hepaticus colonization
(relative number of genomes)
1x10^5
1x10^4
1x10^3
1x10^2
1x10^1
0
Rag -/-
+CD45Rb^high
+H. hepaticus
Rag -/-
+CD45Rb^high
+H. hepaticus
Rag -/-
+CD45Rb^high
+H. hepaticus
C57B1/6 (wt)

FIG. 5

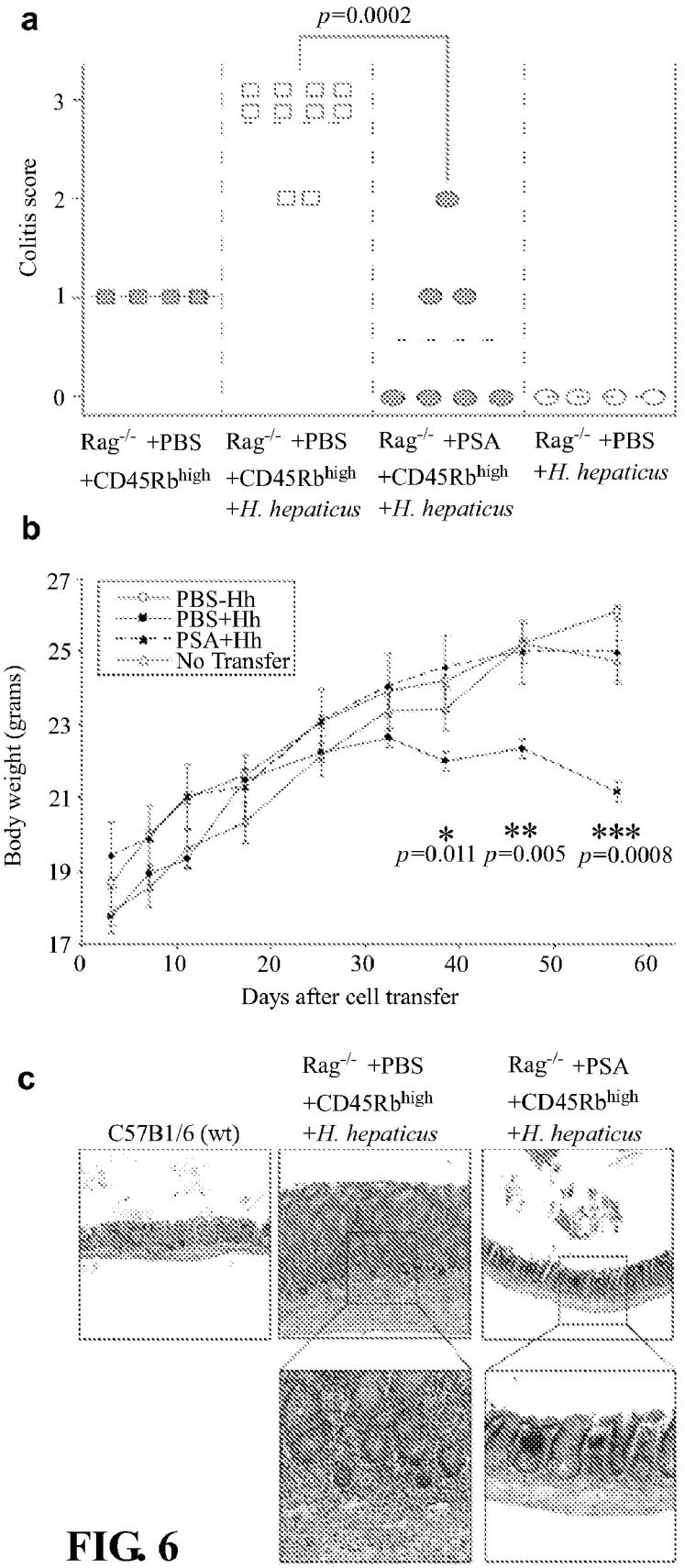

a
p=0.0002
Colitis score
0
1
2
3
Rag-/- +PBS +CD45RBhigh
Rag-/- +PBS +CD45RBhigh +H. hepaticus
Rag-/- +PSA +CD45RBhigh +H. hepaticus
Rag-/- +PBS +H. hepaticus
b
PBS-Hh
PBS+Hh
PSA+Hh
No Transfer
Body weight (grams)
17
19
21
23
25
27
0
10
20
30
40
50
60
Days after cell transfer
* p=0.011
** p=0.005
*** p=0.0008
c
C57B1/6 (wt)
Rag-/- +PBS +CD45RBhigh +H. hepaticus
Rag-/- +PSA +CD45RBhigh +H. hepaticus

FIG. 6

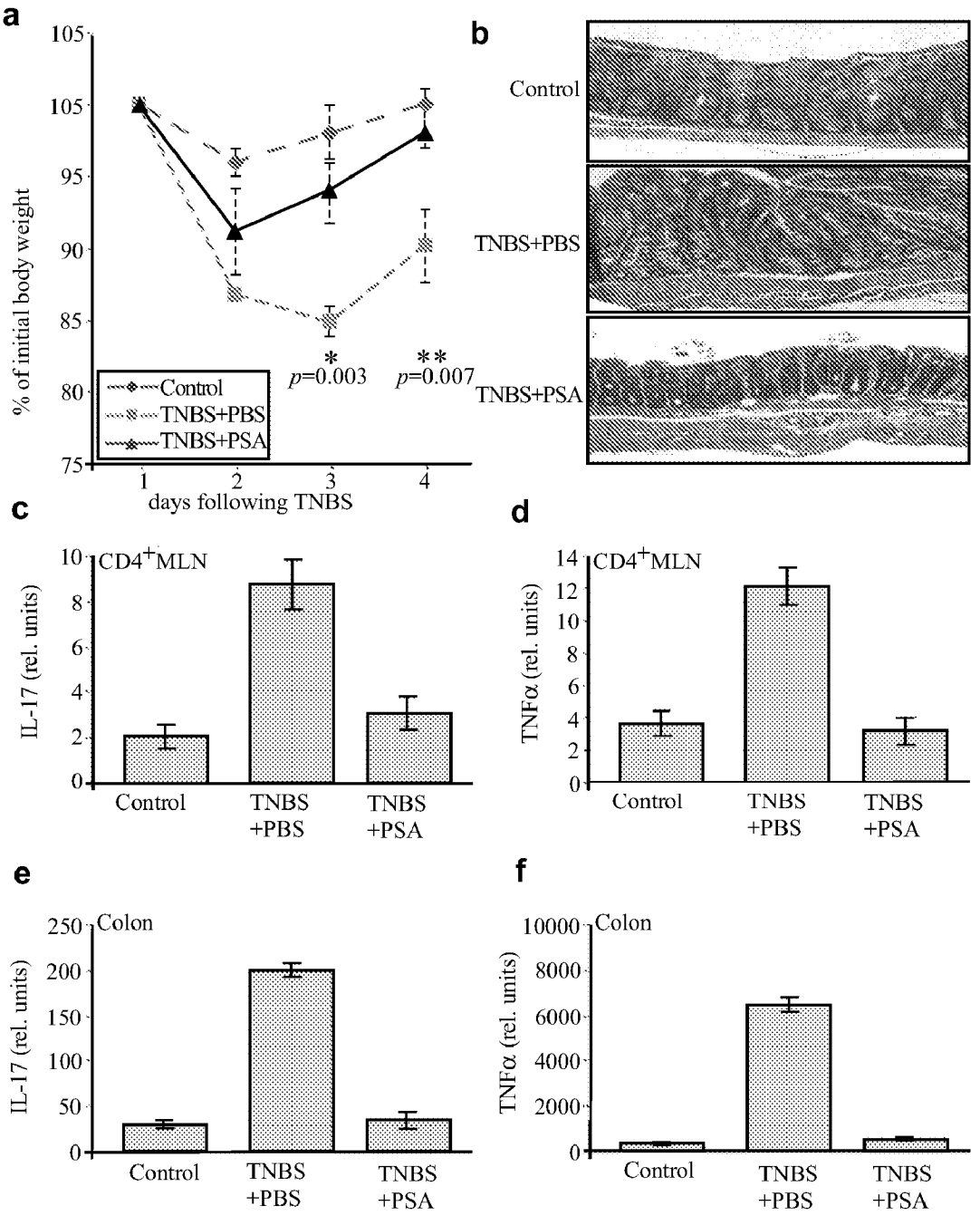

a
105
105
95
90
85
80
75
% of initial body weight
1
2
3
4
days following TNBS
*
p=0.003
**
p=0.007
Control
TNBS+PBS
TNBS+PSA
b
Control
TNBS+PBS
TNBS+PSA
c
CD4+MLN
IL-17 (rel. units)
10
8
6
4
2
0
Control
TNBS +PBS
TNBS +PSA
d
CD4+MLN
TNFα (rel. units)
14
12
10
8
6
4
2
0
Control
TNBS +PBS
TNBS +PSA
e
Colon
IL-17 (rel. units)
250
200
150
100
50
0
Control
TNBS +PBS
TNBS +PSA
f
Colon
TNFα (rel. units)
10000
8000
6000
4000
2000
0
Control
TNBS +PBS
TNBS +PSA

FIG. 7

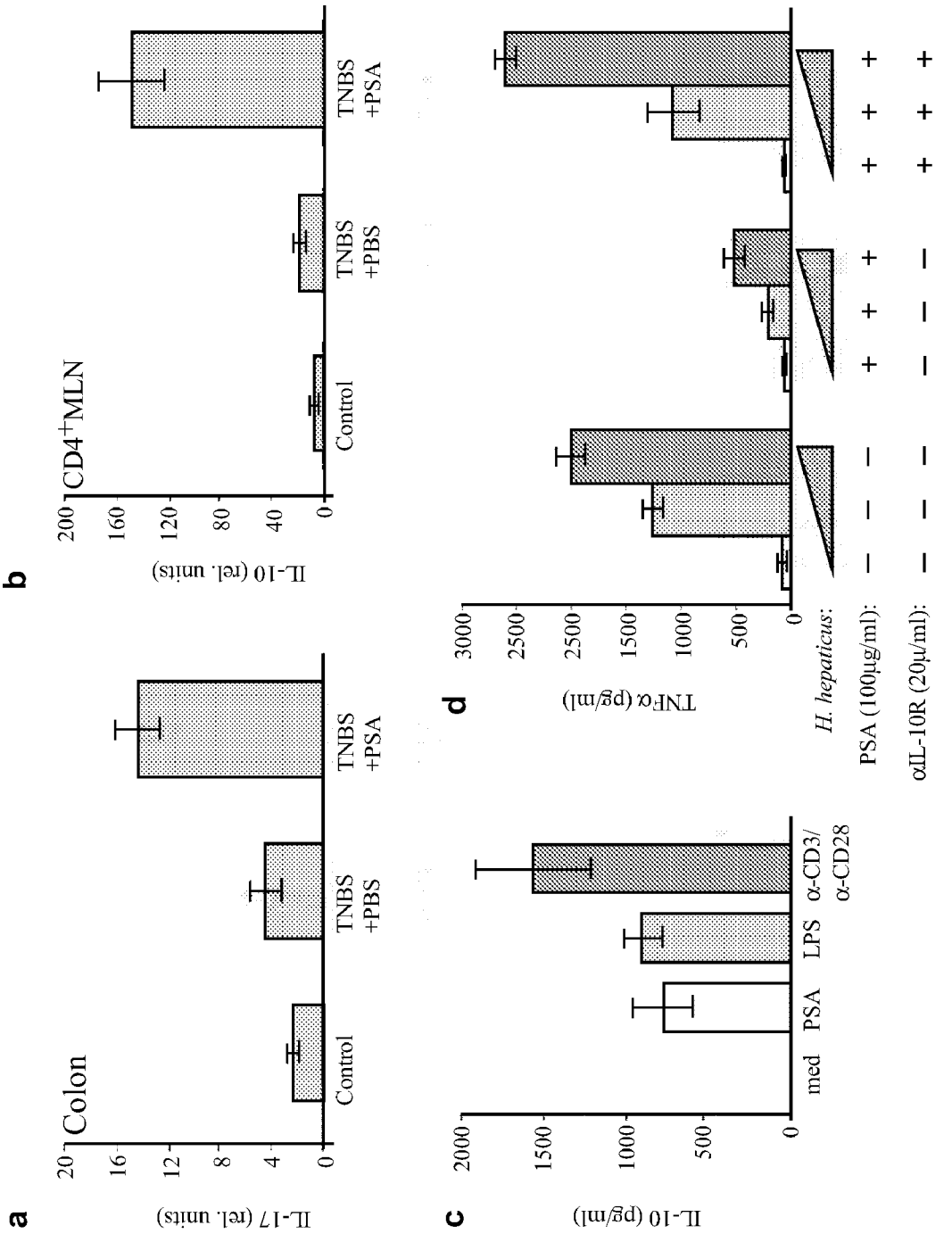

a
Colon
IL-17 (rel. units)
Control
TNBS +PBS
TNBS +PSA
b
CD4+MLN
IL-10 (rel. units)
c
IL-10 (pg/ml)
med
PSA
LPS
α-CD3/ α-CD28
d
TNFα (pg/ml)
H. hepaticus:
PSA (100μg/ml):
αIL-10R (20μ/ml):

FIG 8

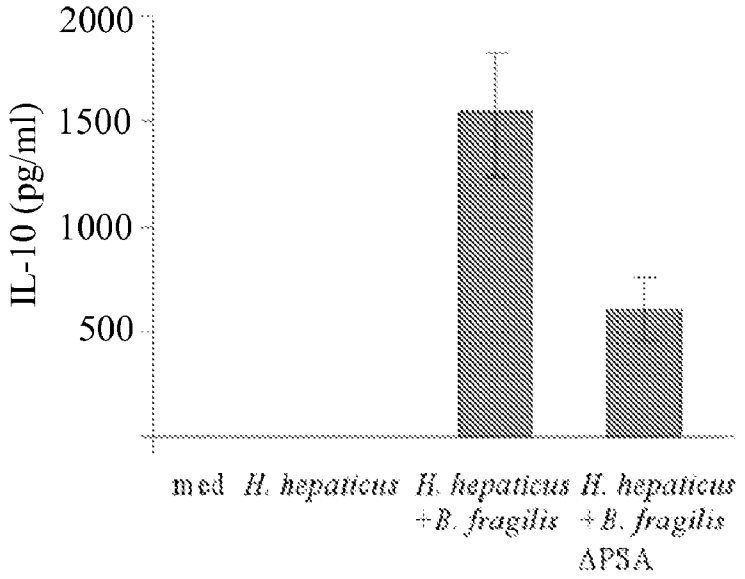

2000
1500
1000
500
IL-10 (pg/ml)
med
H. hepaticus
H. hepaticus +B. fragilis
H. hepaticus +B. fragilis ΔPSA

FIG. 9

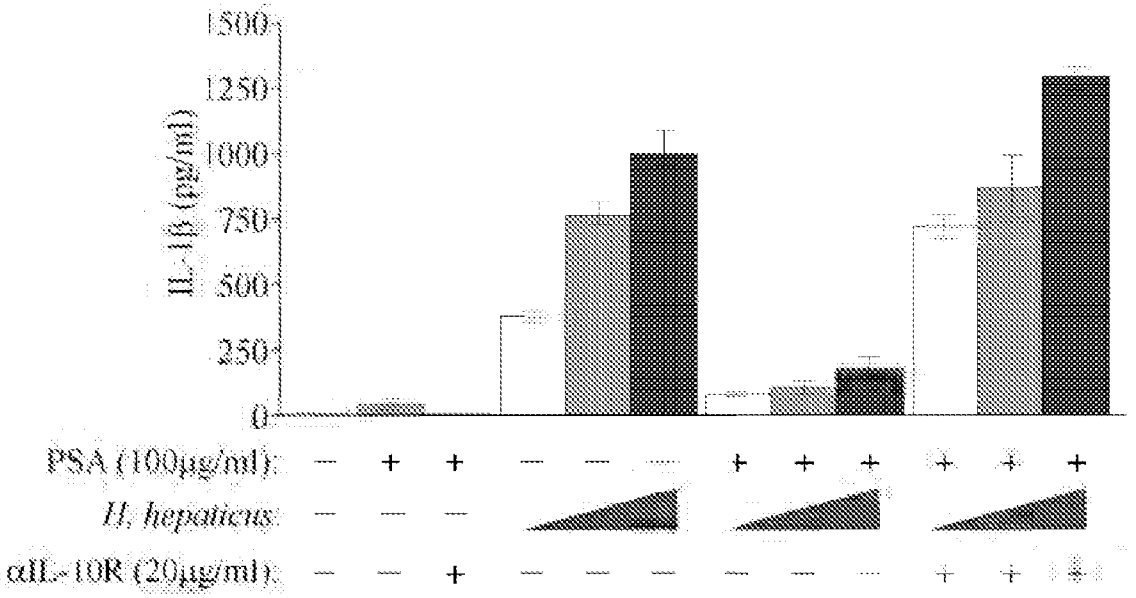

1500
1250
1000
750
500
250
0
IL-1β (pg/ml)
PSA (100μg/ml):
H. hepaticus:
αIL-10R (20μg/ml):

FIG. 10

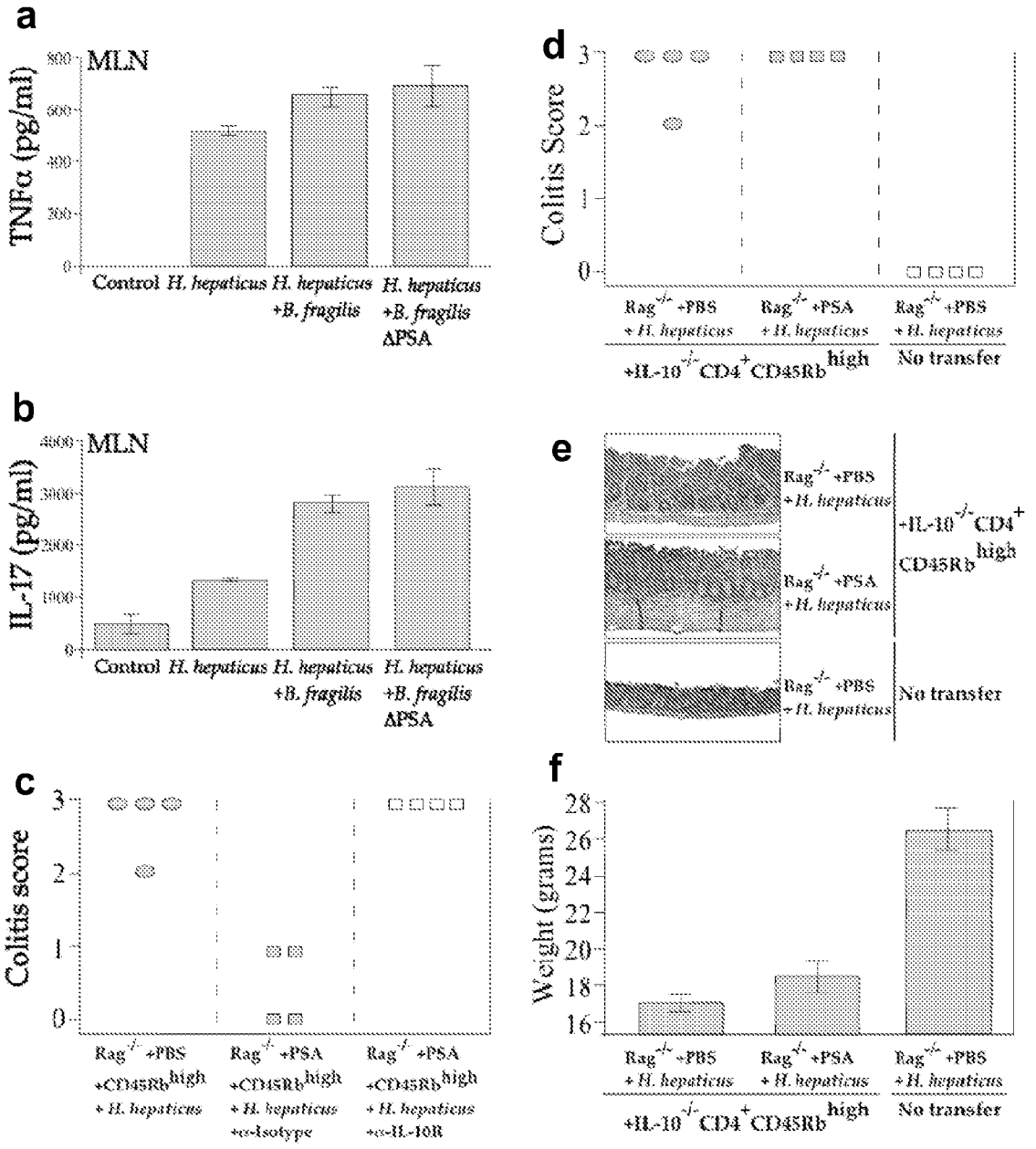

a
MLN
TNFα (pg/ml)
800
600
400
200
0
Control
H. hepaticus
H. hepaticus +B. fragilis
H. hepaticus +B. fragilis ΔPSA
b
MLN
IL-17 (pg/ml)
4000
3000
2000
1000
0
Control
H. hepaticus
H. hepaticus +B. fragilis
H. hepaticus +B. fragilis ΔPSA
c
Colitis score
3
2
1
0
Rag-/- +PBS +CD45Rbhigh + H. hepaticus
Rag-/- +PSA +CD45Rbhigh + H. hepaticus +α-Isotype
Rag-/- +PSA +CD45Rbhigh + H. hepaticus +α-IL-10R
d
Colitis Score
3
2
1
0
Rag-/- +PBS + H. hepaticus
Rag-/- +PSA + H. hepaticus
Rag-/- +PBS + H. hepaticus
+IL-10-/- CD4+ CD45Rbhigh
No transfer
e
Rag-/- +PBS + H. hepaticus
Rag-/- +PSA + H. hepaticus
Rag-/- +PBS + H. hepaticus
+IL-10-/- CD4+ CD45Rbhigh
No transfer
f
Weight (grams)
28
26
24
22
20
18
16
Rag-/- +PBS + H. hepaticus
Rag-/- +PSA + H. hepaticus
Rag-/- +PBS + H. hepaticus
+IL-10-/- CD4+ CD45Rbhigh
No transfer

FIG. 11

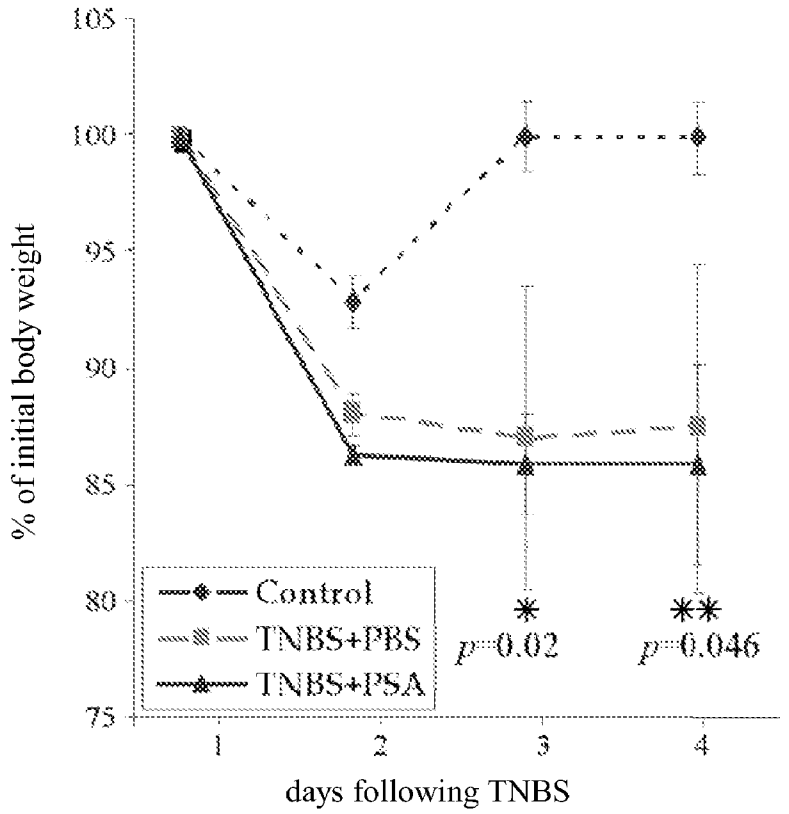

105
100
95
90
85
80
75
% of initial body weight
Control
TNBS+PBS
TNBS+PSA
p=0.02
p=0.046
1
2
3
4
days following TNBS

FIG. 12

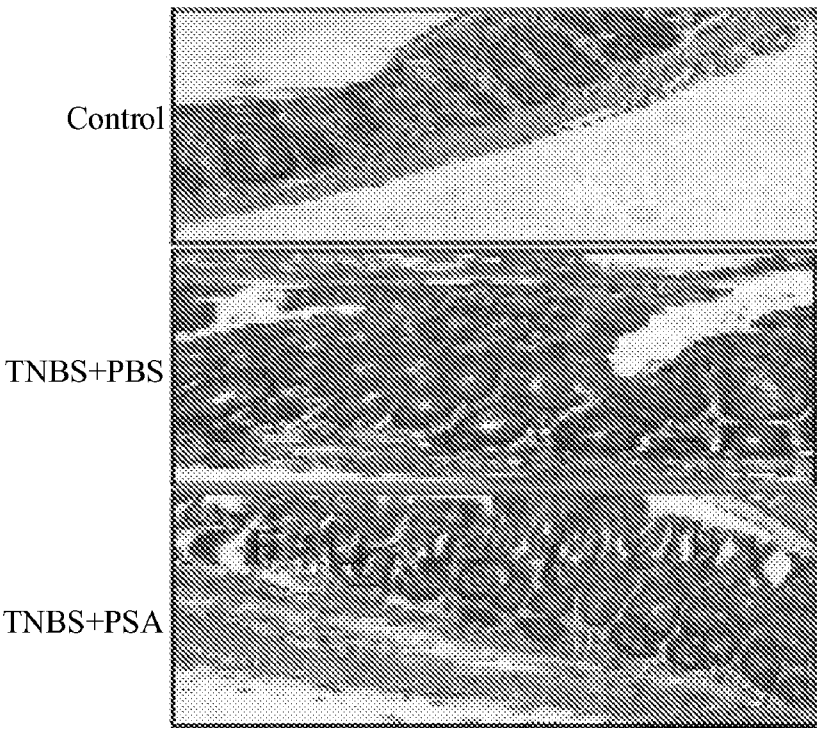

Control
TNBS+PBS
TNBS+PSA

FIG. 13

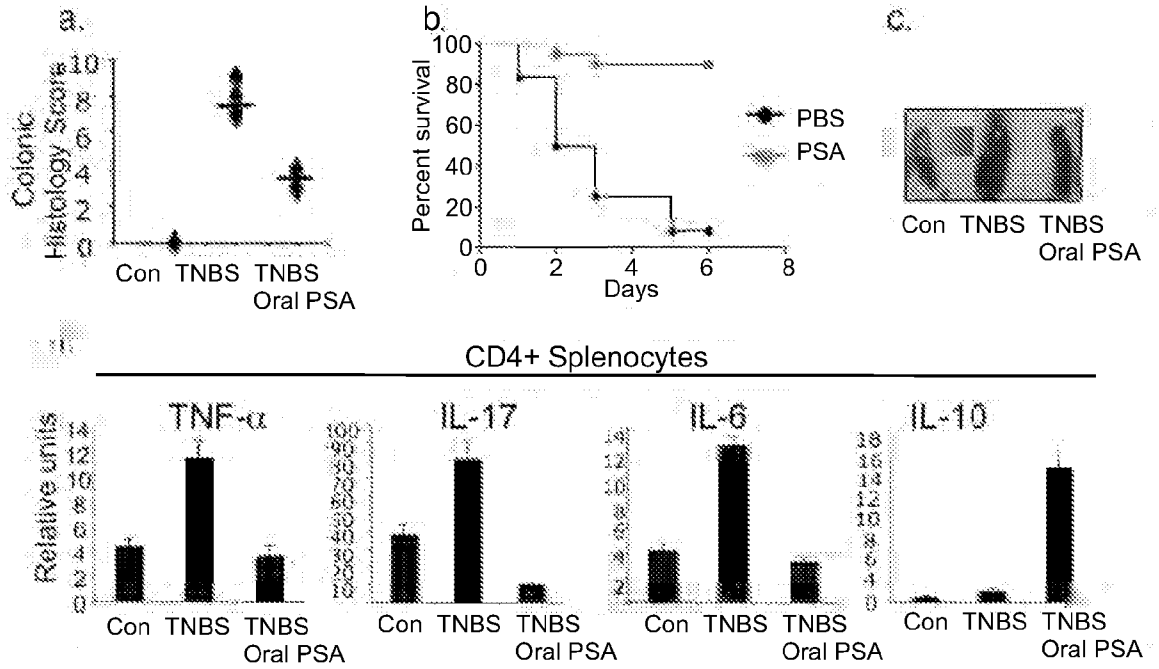

a.
Colonic Histology Score
Con TNBS TNBS Oral PSA
b.
Percent survival
Days
PBS
PSA
c.
Con TNBS TNBS Oral PSA
CD4+ Splenocytes
TNF-α
IL-17
IL-6
IL-10
Relative units
Con TNBS TNBS Oral PSA

FIG. 14

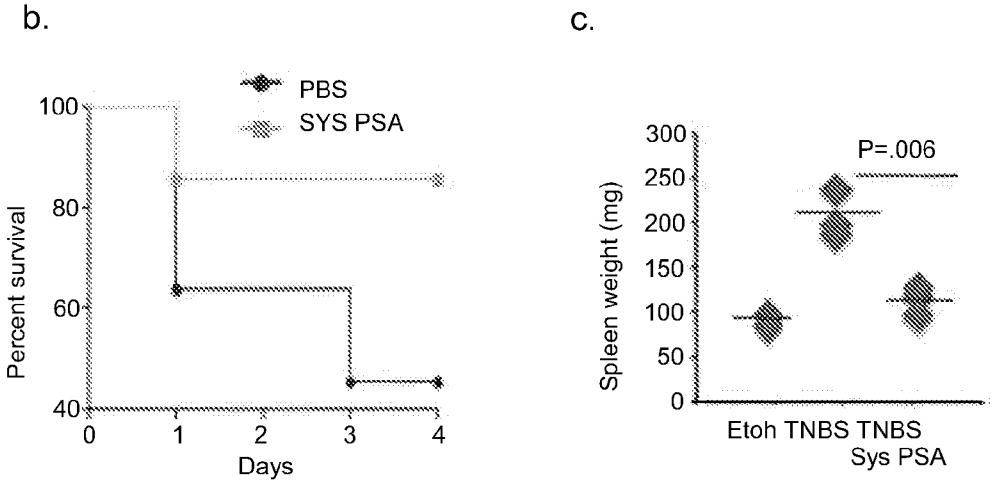

b.
PBS
SYS PSA
Percent survival
Days
c.
P=.006
Spleen weight (mg)
Etoh TNBS TNBS Sys PSA

FIG. 15

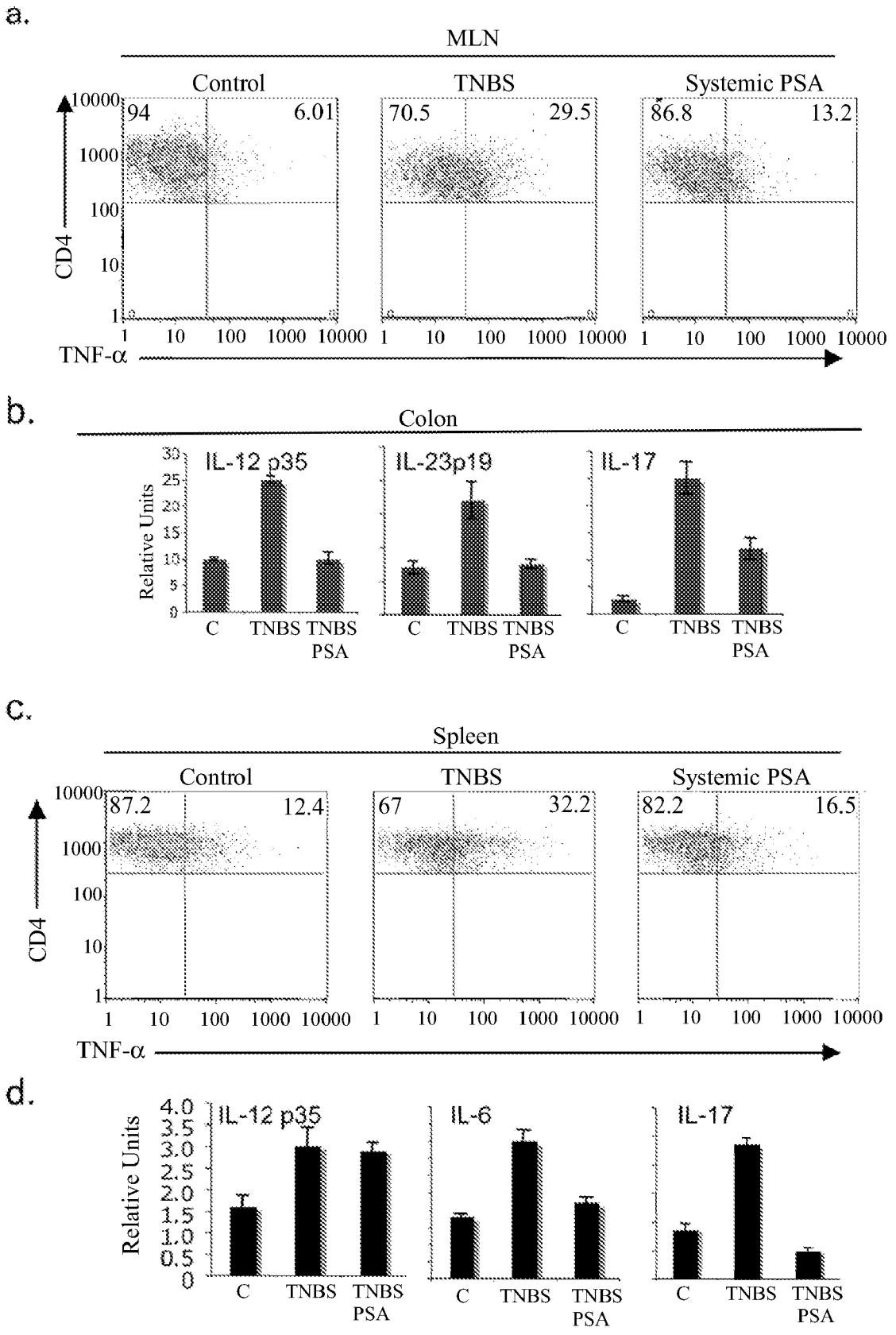

a.
MLN
Control
TNBS
Systemic PSA
94
6.01
70.5
29.5
86.8
13.2
CD4
TNF-α
b.
Colon
IL-12 p35
IL-23p19
IL-17
Relative Units
C
TNBS
TNBS PSA
c.
Spleen
Control
TNBS
Systemic PSA
87.2
12.4
67
32.2
82.2
16.5
CD4
TNF-α
d.
IL-12 p35
IL-6
IL-17
Relative Units
C
TNBS
TNBS PSA

FIG. 16

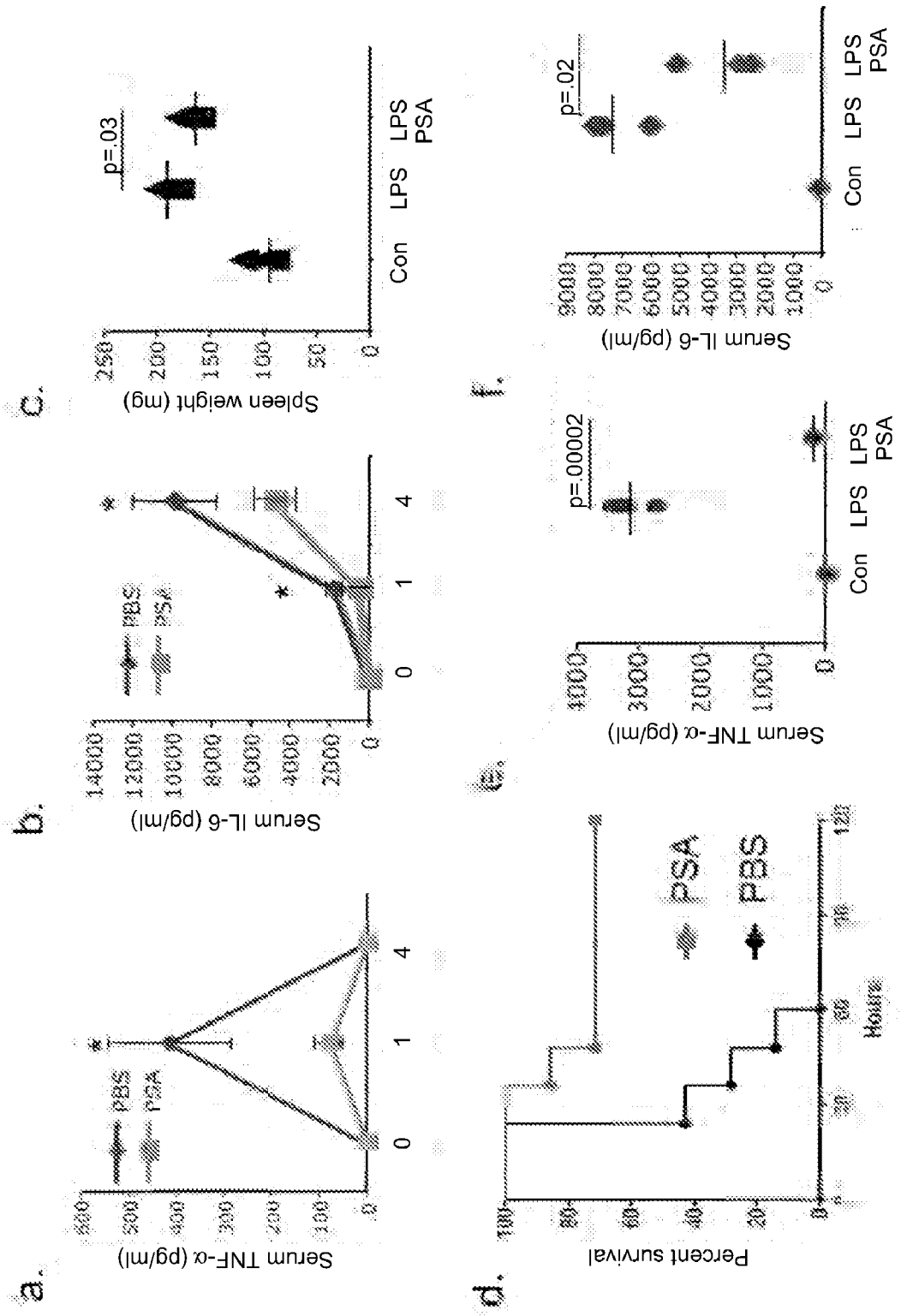

a.
Serum TNF-α (pg/ml)
PBS
PSA
b.
Serum IL-6 (pg/ml)
PBS
PSA
c.
Spleen weight (mg)
p=.03
Con
LPS
LPS PSA
d.
Percent survival
PSA
PBS
Hours
e.
Serum TNF-α (pg/ml)
p=.00002
Con
LPS
LPS PSA
f.
Serum IL-6 (pg/ml)
p=.02
Con
LPS
LPS PSA

FIG 17

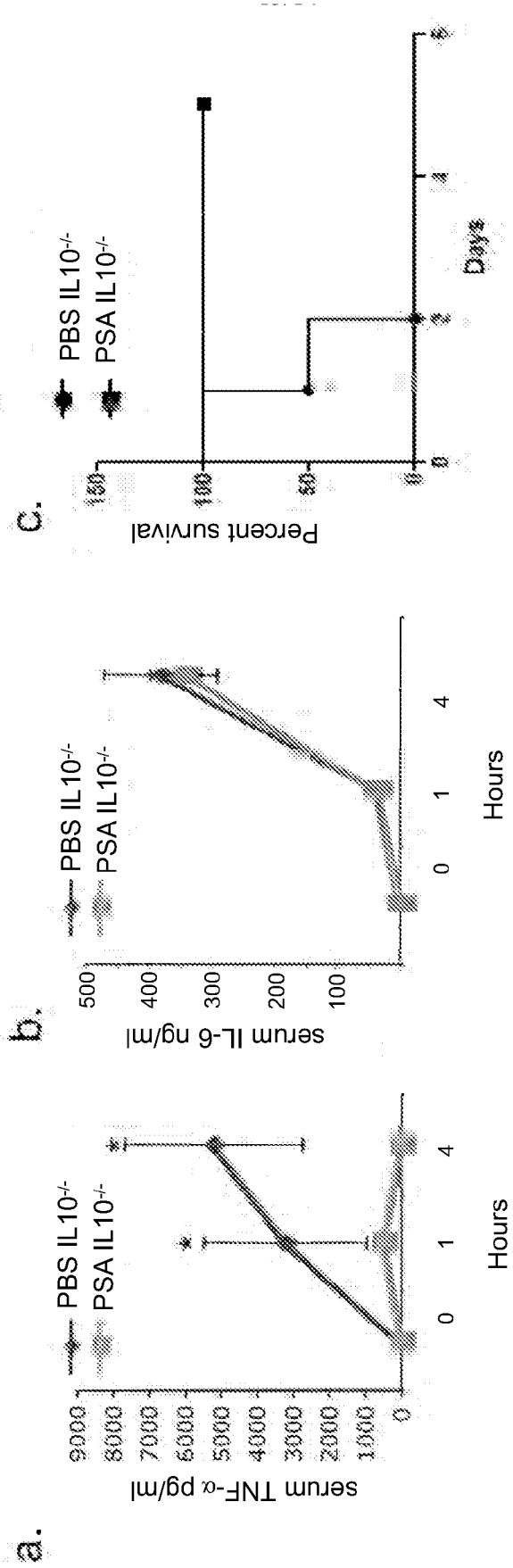
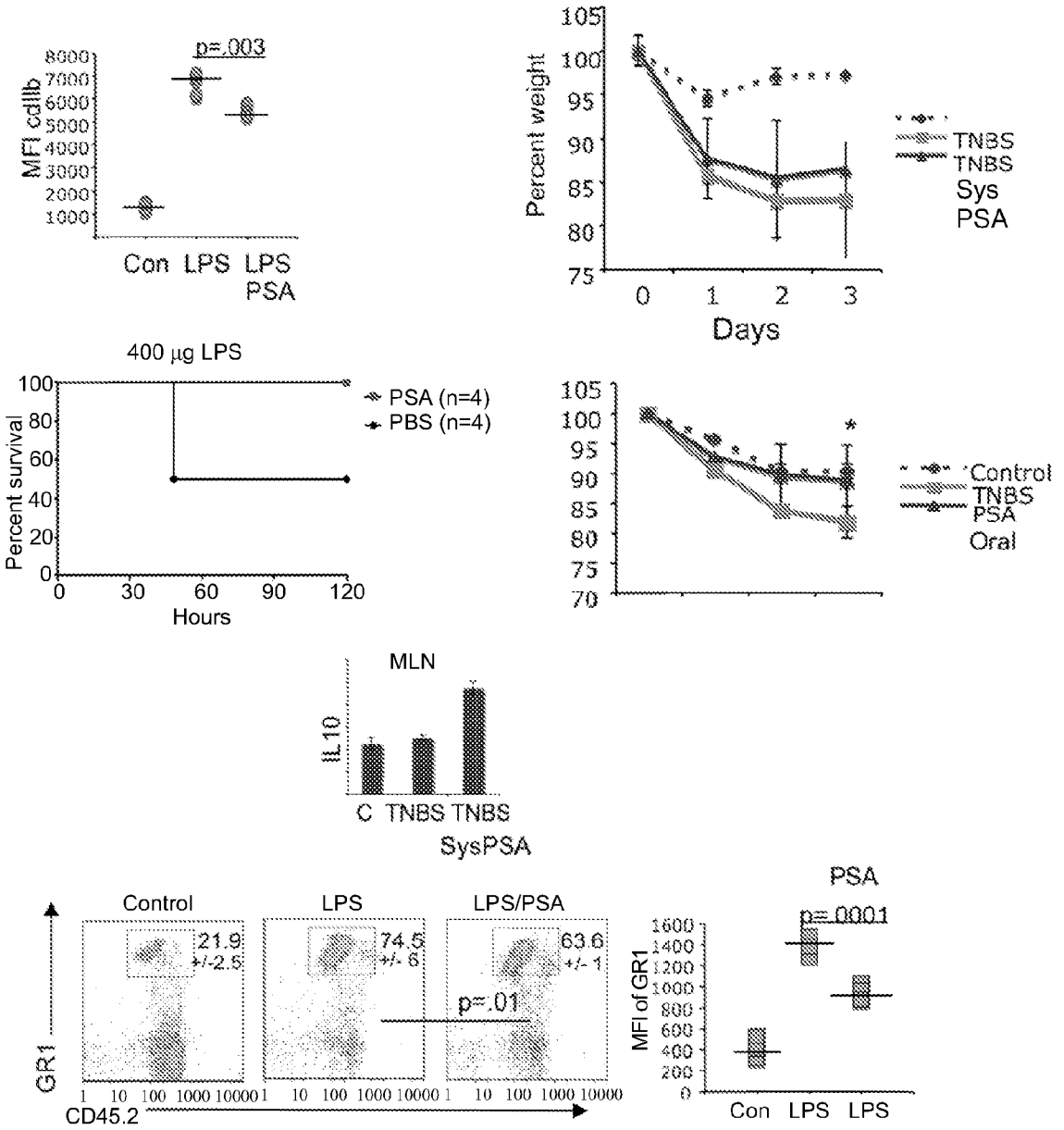

a.
serum TNF-α pg/ml
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
PBS IL10-/-
PSA IL10-/-
0
1
4
Hours
b.
serum IL-6 ng/ml
500
400
300
200
100
PBS IL10-/-
PSA IL10-/-
0
1
4
Hours
c.
Percent survival
150
100
50
0
PBS IL10-/-
PSA IL10-/-
0
2
4
Days

FIG. 18



Io Dose LPS-neutrophil recruitment in serum

FIG. 19

IMMUNOMODULATING COMPOUNDS AND RELATED COMPOSITIONS AND METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application entitled "Host-Bacterial Mutualism by a Microbial Symbiosis Factor Prevents Inflammatory Disease" Ser. No. 61/002,705, filed on Nov. 9, 2007 Docket No. CIT5031-P, U.S. Provisional Application entitled "Host-Bacterial Mutualism by a Microbial Symbiosis Factor Prevents Inflammatory Disease" Ser. No. 61/008,407, filed on Dec. 20, 2007 Docket No. CIT5031-P2, and to U.S. Provisional Application entitled "A Molecule from a Symbiotic Gut Bacteria Controls Systemic Inflammation", Ser. No. 61/196,046, filed on Oct. 13, 2008 Docket No. CIT5250-P, the disclosure of each of which is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT GRANT

[0002] The U.S. Government has certain rights in this invention pursuant to Grant No. AI039576 awarded by the National Institutes of Health.

FIELD

[0003] The present disclosure relates to the immune system, and, in particular, to an immunomodulating compound able to control T cell differentiation and/or cytokines production associated with an immunitary response in an individual.

BACKGROUND

[0004] T cells belong to a group of white blood cells known as lymphocytes, and play a central role in cell-mediated immunity. In particular, T helper cells (also known as effector T cells or Th cells) are a sub-group of lymphocytes (a type of white blood cell or leukocyte) that plays an important role in establishing and maximizing the capabilities of the immune system and in particular in activating and directing other immune cells. More particularly, Th cells are essential in determining B cell antibody class switching, in the activation and growth of cytotoxic T cells, and in maximizing bactericidal activity of phagocytes such as macrophages.

[0005] Different types of Th cells have been identified that originate in outcome of a differentiation process and are associated with a specific phenotype. Following T cell development, matured, naïve (meaning they have never been exposed to the antigen to which they can respond) T cells leave the thymus and begin to spread throughout the body. Once the naïve T cells encounter antigens throughout the body, they can differentiate into a T-helper 1 (Th1), T-helper 2 (Th2), T-helper 17 (Th17) or regulatory T cell (Treg) phenotype.

[0006] Each of these Th cell types secretes cytokines, proteins or peptides that stimulate or interact with other leukocytes, including T_h cells. However, each cell type has a peculiar phenotype and activity that interferes and often conflict with the other.

[0007] Th1, Th2, and Th17 (inflammatory T-helper or inflammatory Th), promote inflammation responses trough secretion of pro-inflammatory cytokines, such as IL-1, IL-6, TNF-a, IL-17, IL21, IL23, and/or through activation and/or inhibition of other T cell including other Th cells (for example Th1 cell suppresses Th2 and Th17, Th2 suppresses Th1 and Th17). Tregs instead, are a component of the immune system that suppresses biological activities of other cells associated to an immune response. In particular, Tregs can secrete immunosuppressive cytokines TGF-beta and Interleukin 10, and are known to be able to limit or suppress inflammation.

[0008] An imbalance in the profile of any of the inflammatory T-helper cells is usually associated with a condition in an individual. For example, an increase profile for Th1 or Th17 leads to autoimmunity, whereas an increased Th2 cell profile leads to allergies and asthma. In particular, imbalance of Th17 cell profile has been associated with several autoimmunitary conditions. Treg cells suppress inflammation induced by all 3 other T cell lineages, and thus are crucial for preventing uncontrolled inflammation, which leads to disease. Therefore, a balanced T-helper profile is critical for health in individuals.

SUMMARY

[0009] Provided herein, are immunomodulating compounds and related methods and compositions that are suitable to balance a T-helper cell profile, and in particular to balance the cell profile of at least one of Th1, Th2, Th17 and Treg cells in an individual. More particularly, provided herein are methods and compositions based on the surprising immunomodulating properties of PSA polysaccharide A (PSA) and other zwitterionic polysaccharides (ZPs) that make those polysaccharides suitable for treatment, prevention and control of inflammations and inflammatory conditions in an individual.

[0010] According to a first aspect, a method to balance a T-helper cell profile in an individual is disclosed. The method comprises administering to the individual an effective amount of a zwitterionic polysaccharide.

[0011] According to a second aspect, a method to balance a cell profile of at least one Th cell selected from the group consisting of Th1, Th2, Th17 and Treg, in an individual is disclosed. The method comprises administering to the individual an effective amount of a zwitterionic polysaccharide.

[0012] According to a third aspect, a method to control cytokine production in an individual, is disclosed, the cytokine being at least one of IL-1, IL-6, TNF-a, IL-17, IL21, IL23. The method comprises administering to the individual an effective amount of a zwitterionic polysaccharide.

[0013] According to a fourth aspect, a method to control inflammation associated with a Th-cell profile imbalance in an individual is disclosed. The method comprises administering to the individual an effective amount of a zwitterionic polysaccharide.

[0014] According to a fifth aspect, a method to treat or prevent conditions associated with an imbalanced cell profile of at least one Th cell selected from the group consisting of Th1, Th2, Th17 and Treg in an individual is disclosed. The method comprises administering to the individual an effective amount of a zwitterionic polysaccharide.

[0015] According to a sixth aspect, a method to treat or prevent conditions associated with production of at least one of IL-1, IL-6, TNF-a, IL-17, IL21, IL23 cytokines in an individual, is disclosed. The method comprises administering to the individual an effective amount of a zwitterionic polysaccharide.

[0016] According to a seventh aspect, an anti-inflammatory composition is disclosed. The anti-inflammatory composition comprises a zwitterionic polysaccharide and a suitable vehicle, wherein the zwitterionic polysaccharide is comprised in an amount of from about 1 to about 100 μg.

[0017] The compositions and methods herein disclosed can be used in several embodiments to simultaneously control and balance the profile of Th1, Th2, Th17 and Treg cells in an individual, thus preventing or treating conditions associated with an imbalanced profile for those cytokines in the individual.

[0018] The compositions and methods herein described can be used in connection with medical, pharmaceutical, veterinary applications as well as fundamental biological studies and various applications, identifiable by a skilled person upon reading of the present disclosure, wherein investigating the possible role of a zwitterionic polysaccharide is desirable

[0019] The details of one or more embodiments of the disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The accompanying drawings, which are incorporated into and constitute a part of this specification, illustrate one or more embodiments of the present disclosure and, together with the detailed description, serve to explain the principles and implementations of the disclosure.

[0021] FIG. **1** shows an exemplary ZP mediated protection from experimental colitis in individuals according to some embodiments herein disclosed. Panel (a) shows diagrams summarizing the results of mono-association of germ-free mice with wild-type *B. fragilis* and *B. fragilis* DPSA (mean percentages±Standard Deviation (SD) for 3 experiments: conventional, 38.4%±2.2; germ-free, 26.7%±1.3; *B. fragilis,* 40.8%±3.1; *B. fragilis* DPSA, 28.8%±2.6). All cells gated on $CD4^+$ splenocytes. Panel (b) shows a diagram illustrating the results of co-colonization experiments of *H. hepaticus* with *B. fragilis* and *B. fragilis* DPSA (two-tailed p value, 0.004; Mann-Whitney U test). Combined data from 2 independent experiments are shown. Error bars show SD for triplicate samples. Panel (c) shows a diagram illustrating the results of ELISA test of colon organ cultures to detect TNFa levels in animals co-colonized with *H. hepaticus* and wild-type *B. fragilis* or *B. fragilis* DPSA. Panel (d) shows a diagram illustrating the results of a Q-PCR for IL-23p19 performed on splenocytes, normalized to L32 expression. Error bars show SD for triplicate samples.

[0022] FIG. **2** shows an exemplary ZP mediated cytokine control according to some embodiments herein disclosed. In particular, FIG. **2** shows diagrams illustrating the results of ELISA tests for the detection of the pro-inflammatory cytokines IL-12p40 (left) and IL-1b (right) in animals co-colonized with *H. hepaticus* and wild-type *B. fragilis* or *B. fragilis* DPSA over those in control animals (C57BL/6). Results are from one trial of 2 independent experiments. Error bars indicate SD values from studies of colons recovered from 4 animals per group.

[0023] FIG. **3** shows an example of ZP mediated control of TNFa expression by $CD4^+$ T cells according to some embodiments herein disclosed. In particular FIG. **3** $CD4^+$ cells were purified from pooled splenocytes from each group (4 mice per group) and restimulated in vitro with PMA and ionomycin in the presence of brefeldin A for 4 hours. Cells were stained for intracellular TNFα. Cells within the lymphocyte gate were included in the analysis, and numbers indicate the percentage of cells producing TNFα. Purified cells were >90% $CD4^+$. Animals colonized with PSA-producing *B. fragilis* during protection displayed lower TNFa levels than diseased animals.

[0024] FIG. **4** shows control experiments supporting various embodiments herein described. Panel (a) shows an ethidium bromide-stained gel electrophoresis of *H. hepaticus*-specific Q-PCR performed following co-colonization with wild-type and various mutants of *B. fragilis* after 8 weeks. M: Marker. 1: $Rag2^{-/-}$ animals with $CD4^+$ $CD45Rb^{high}$ T cell transfer colonized with *H. hepaticus* alone. 2: $Rag2^{-/-}$ animals with $CD4^+$ $CD45Rb^{high}$ T cell transfer colonized with *H. hepaticus* and *B. fragilis* 9343 (wt). 3: $Rag2^{-/-}$ animals with $CD4^+$ $CD45Rb^{high}$ T cell transfer colonized with *H. hepaticus* and *B. fragilis* DPSA. 4: C57BL/6 mice colonized with *H. hepaticus* alone. Note: *H. hepaticus* readily colonized animals but did not induce disease (FIG. **1**). Primers for *H. hepaticus* 16S rDNA: (HB-15) 5'-GAAACTGTTACTCTG-3' (SEQ ID NO: 1) and (HB-17) 5'-TCAAGCTCCCCGAAGGG-3' (SEQ ID NO: 2). Panel (b) shows ethidium bromide-stained gel electrophoresis of *B. fragilis*-specific Q-PCR performed following co-colonization with wild-type and various mutants of *B. fragilis* after 8 weeks. A: $Rag2^{-/-}$ animals with $CD4^+$ $CD45Rb^{high}$ T cell transfer colonized with *H. hepaticus* and *B. fragilis* 9343 (wt). B: $Rag2^{-/-}$ animals with $CD4^+$ $CD45Rb^{high}$ T cell transfer colonized with *H. hepaticus* and *B. fragilis* DPSA. C: $Rag2^{-/-}$ animals with $CD4^+$ $CD45Rb^{high}$ T cell transfer colonized with *H. hepaticus* alone. D: C57BL/6 mice colonized with *H. hepaticus* alone. E: *B. fragilis* genomic DNA (positive control). M: Marker. Primers for *B. fragilis* ssr3 (finB) gene: (ssr3-F) 5'-TATTTGCGAGAAGGTGAT-3' (SEQ ID NO: 3) and (ssr3-r) 5'-TAAACGCTTTGCTGCTAT-3' (SEQ ID NO: 4).

[0025] FIG. **5** effects associated to a ZP-mediated protection according to some embodiments herein disclosed. In particular, FIG. **5** shows a diagram illustrating the results of Q-PCR experiments directed to quantitate *H. hepaticus* in animals co-colonized with *H. hepaticus* and wild-type *B. fragilis* or *B. fragilis* DPSA. The results was assessed according to Young et al., 2004[1] as $\log^{10}$ number of copies of a known gene (cytolethal distending toxin). Animals contained equivalent levels of *H. hepaticus* at the end of the experiment.

[0026] FIG. **6** shows a ZPs mediated protection according to some embodiments herein disclosed. Panel (a) shows a diagram illustrating the results of a colonization with *H. hepaticus* in absence (second column) or in presence of purified PSA (third column) (Kruskal-Wallis comparisons of all groups: $p>0.05$ for dissimilar results, $p<0.01$ for similar results; Mann-Whitney U test: two-tailed p value, 0.0002). Panel (b) shows a diagram illustrating results of experiments directed to detect wasting disease in $Rag2^{-/-}$ animals following transfer of $CD4^+$ $CD45Rb^{high}$ T cells and colonization with *H. hepaticus* (PBS+Hh) in presence or absence of PSA as indicated. ANOVA indicates that comparisons between all indicated groups (asterisks) are statistically significant. Panel (c) shows the architecture of colonic sections from wild-type animals (left panel); following transfer of $CD4^+$ $CD45Rb^{high}$ T cell into *Helicobacter*-colonized $Rag2^{-/-}$ mice (middle panel); oral PSA treatment of *Helicobacter*-colonized animals (right panel). Images in each row are the same magnification.

[0027] FIG. **7** shows a ZP modulated immune response according to some embodiments herein disclosed. Panel (a) shows a diagram illustrating the correlation between oral PSA

administration and body weight related to TNBS-treated PBS controls. ANOVA values for all indicated groups (asterisks) are statistically significant. Error bars show SD between 4 animals per group. Panel (b) shows colon sections from TNBS+PBS-treated groups, from TNBS+PSA-treated animals and from a control (representative sections from animals in 2 independent experiments). Panels (c, d) show diagram illustrating the results of Q-PCR of purified $CD4^+$ T cells from MLNs with IL-17A (Panel c) and TNFa (Panel d) in presence or absence of PSA during disease. Error bars are from duplicate runs of 3 independent experiments. Panels (e, f) show diagrams illustrating transcriptional expression of IL17A (Panel e) and TNFα (Panel f) from homogenized colons of TNBS+PBS-treated groups, from TNBS+PSA-treated animals and from a control. Error bars are from duplicate runs of 3 independent experiments.

[0028] FIG. **8** shows a ZP mediated control of cytokine expression according to some embodiments herein disclosed. Panel (a) shows a diagram illustrating the results of Q-PCR assay of colons for IL-10 in wild type mice treated with ethanol (control), TNBS, or TNBS and PSA. Error bars show SD for triplicate samples. Panel (b) shows a diagram illustrating Q-PCR results for IL-10 expression in $CD4^+$ T cells purified from MLNs of TNBS-treated groups. Error bars show SD for triplicate samples. Panel (c) shows a diagram illustrating the effects of incubation of BMDC/T cell co-cultures with purified PSA LPS and a-CD3/a-CD28 on IL-10 production. Error bars show SD for triplicate samples. Panel (d) shows a diagram illustrating the results of an 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) on TNFa release in presence (middle three bars) or absence (left three bars) of PSA and following the addition of aIL-10R right three bars. Error bars show SD values of experiments run in triplicate.

[0029] FIG. **9** shows a ZP mediated control of cytokine expression according to some embodiments herein disclosed. In particular, FIG. **9** shows a diagram illustrating the results for an IL-10 ELISA of supernatants of primary BMDC-T cell co-cultures incubated for 48 hours with *H. hepaticus* alone or with *H. hepaticus* and *B. fragilis* (wild-type or ΔPSA) at a multiplicity of infection of 5. Error bars show SD values for samples run in duplicate and represent 3 independent experiments.

[0030] FIG. **10** shows a ZP mediated control of cytokine expression according to some embodiments herein disclosed. In particular, FIG. **10** shows a diagram illustrating the results of an infection of BMDC-T cell co-cultures with increasing concentrations of live *H. hepaticus* (multiplicity of infection: 0.1, 1.0, and 10, as depicted by triangles) on release of the cytokine IL-1b in presence (middle three bars) or absence (left three bars) of PSA and following the addition of aIL-10R right three bars. Error bars show SD values for experiments run in triplicate.

[0031] FIG. **11** shows a ZP mediated protection from inflammation according to some embodiments herein disclosed. Panels (a, b) show diagrams illustrating results of ELISA detection for pro-inflammatory cytokines TNFα (Panel a) and IL-17A (Panel b) in $IL\text{-}10^{-/-}$ mice left uncolonized (control) or colonized with *H. hepaticus* (to induce inflammation) either alone or in combination with *B. fragilis* (wild-type or ΔPSA). Error bars show SD for triplicate samples. Panel (c) shows a diagram illustrating the colitis scores in $Rag^{-/-}$ animals with $CD4^+$ $CD45Rb^{high}$ T cell transfer colonized with *H. hepaticus* with or without PSA and in presence of neutralizing antibodies to IL-10 block (α-IL10R). Data represent 2 independent experiments. Panel (d) shows a diagram illustrating colitis scores in $Rag^{-/-}$ animals with $CD4^+$ $CD45Rb^{high}$ T cell transferred from $IL\text{-}10^{-/-}$ mice colonized with *H. hepaticus* with PSA or PBS. Results are shown for 1 representative trial of 2 independent experiments. Panel (e) shows histologic colon sections $Rag^{-/-}$ animals with $CD4^+$ $CD45Rb^{high}$ T cell transferred from $IL\text{-}10^{-/-}$ mice colonized with *H. hepaticus* with PSA or PBS. All images are the same magnification. Panel (f) shows a diagram illustrating the mean body weights for groups of $Rag^{-/-}$ animals (n=4) with $CD4^+$ $CD45Rb^{high}$ T cell transferred from $IL\text{-}10^{-/-}$ mice colonized with *H. hepaticus* with PSA or PBS.

[0032] FIG. **12** shows effect of a ZP administration supporting embodiments herein disclosed. In particular, FIG. **12** shows a diagram illustrating the variation on body weight in groups of 4 C57BL/6 mice treated with PSA (or PBS) and then subjected to rectal administration of TNBS or vehicle (control). Mean body weights (shown as percentages of initial weight) are shown for each group; SD values indicate that, in the absence of IL-10, PSA cannot restore TNBS-induced weight loss. ANOVA demonstrates that weight loss in both TNBS-treated groups is statistically different from that in control animals.

[0033] FIG. **13** shows effects of a ZP administration supporting some embodiments herein disclosed. In particular, FIG. **13** shows results of histologic analysis of H&E-stained sections from a representative animal of groups of 4 C57BL/6 mice treated with PSA (or PBS) and then subjected to rectal administration of TNBS or vehicle (control). Results represent 2 independent experiments.

[0034] FIG. **14** shows inhibition of inflammation within extra-intestinal immune compartments following oral administration of ZPS according to some embodiments herein disclosed. In particular, Panel (a) shows a diagram illustrating the colonic histological score detected in untreated mice (control) and in mice treated with TNBS or TNBS/PSA. Each dot represents an individual mouse and the line indicates the average score of the group. Panel (b) shows a diagram illustrating the percent of survival in time of Balb/c mice undergoing TNBS induced colitis. n=16 mice in each group. Panel (c) shows an image of the spleen of untreated mice (control) and mice treated with TNBS or TNBS/PSA Panel (d) shows a diagram illustrating the relative units of TNF-α, IL-6, IL-17A and IL-10 within $CD4^+$ splenocytes in untreated mice (control) and in mice treated with TNBS or TNBS/PSA. These data are representative of three independent experiments.

[0035] FIG. **15** shows protection from TNBS induced intestinal colitis following administration of ZPS to extra-intestinal sites according to some embodiments herein disclosed. In particular, Panel (a) shows a diagram illustrating the percent survival of mice undergoing TNBS induced colitis. n=10 mice in each group. Panel (b) shows a diagram illustrating variation of the spleen weight in untreated mice (Etoh) and in mice treated with TNBS or TNBS/PSA systemically administered. The weight of the spleen was used as an indicator of size. Each diamond represents the weight of the spleen from an individual animal. The bar indicates the average weight of the group. P values were determined by students T test.

[0036] FIG. **16** shows inhibition of inflammatory cytokines at both intestinal and systemic immune compartments following systemic administration of ZPS during TNBS induced

colitis according to some embodiments herein disclosed. Panel (a) shows a diagram illustrating TNF-α production in $CD4^+$ T lymphocytes residing within the mesenteric lymph nodes (MLN) splenocytes in untreated mice (control) and in mice treated with TNBS or TNBS/PSA. Cells were collected from the MLN and stained with antibodies recognizing CD4 or TNF-a. Numbers within quadrants represent the percentage of cells. Panel (b) shows a diagram illustrating analysis of the expression of IL-12, IL-23, and IL-17 in colon of untreated mice (control) and mice treated with TNBS or TNBS/PSA. Panel (c) show a diagram illustrating TNF-α production in $CD4^+$ T lymphocytes residing within the spleen of untreated mice (control) and of mice treated with TNBS or TNBS/PSA. Numbers within quadrants represent the percentage of cells. Panel (d) a diagram illustrating analysis of the expression of IL-12, IL-6, and IL-17 in spleen of untreated mice (control) and mice treated with TNBS or TNBS/PSA.

[0037] FIG. **17** shows inhibition of inflammation and death associated with systemic septic shock following administration of ZPS according to some embodiments herein disclosed. Panel (a): shows a diagram illustrating TNF-a serum levels in mice 1 and 4 hours post-administration of 100 μg of LPS alone. Mice were either pre-treated with PBS or 50 ug of PSA three times every other day before LPS administration. * indicates statistical significance as determined by a students t test. SD was determined from the serum of individual mice. These data are representative of three independent experiments. Panel (b): shows a diagram illustrating IL-6 serum levels in mice 1 and 4 hours post-administration of 100 μg of LPS. Pre-treatment as in panel a * indicates statistical significance as determined by a students t test. SD was determined from the serum of individual mice. These data are representative of three independent experiments. Panel (c): shows a diagram illustrating variation of the spleen weight in untreated mice (con) and in mice administered LPS within the intraperitoneal cavity (LPS) and pre-treated with PBS or PSA as in panel a. Each dot represents the weight of the spleen from an individual mouse. P values were determined by a students T test. Panel (d): shows a diagram illustrating the survival rate of animals undergoing septic shock induced by high dose (500 μg) administration of LPS and pre-treated with PSA or PBS. N=12 mice in each group. Panel (e): shows a diagram illustrating the serum concentrations of TNF-a in mice post-administration of 500 μg of LPS alone or pre-treated with PSA or PBS. p values were determined by students T test. Each dot represents an individual mouse. Panel (f) shows a diagram illustrating the serum concentrations of IL-6 in mice post-administration of 500 g of LPS alone and pre-treated with PSA or PBS. p values were determined by students T test. Each dot represents an individual mouse.

[0038] FIG. **18** shows inhibition of inflammation and death associated with systemic septic shock following administration of ZPS according to some embodiments herein disclosed. Panel (a): shows a diagram illustrating TNF-a serum levels in mice pre-treated with PBS or PSA and administered LPS. Serum was collected 1 and 4 hours post-administration of LPS in IL-$10^{-/-}$ mice. * indicates statistical significance as determined by a students t test. SD was determined from the serum of individual mice. Panel (b): shows a diagram illustrating IL-6 serum level in mice pre-treated with PSA or PBS. Serum was collected 1 and 4 hours post-administration of LPS in IL-$10^{-/-}$ mice. * indicates statistical significance as determined by a students t test. SD was determined from the serum of individual mice Panel (c): shows a diagram illustrating percent survival in mice post-administration of LPS alone or together with PSA in IL-$10^{31/-}$ mice. N=8 mice in each group.

[0039] FIG. **19** shows a diagram illustrating additional effects of ZPS administration on inflamed tissues according to some embodiments herein disclosed.

DETAILED DESCRIPTION

[0040] Methods and compositions are herein disclosed that allow balancing a T-helper cell profile in an individual, based on the use of PSA or another zwitterionic polysaccharide.

[0041] The term "T-helper" as used herein with reference to cells indicates a sub-group of lymphocytes (a type of white blood cell or leukocyte) including different cell types identifiable by a skilled person. In particular, T-helper cell according to the present disclosure include effector T_h cells (such as Th1, Th2 and Th17)-i.e. Th cells that secrete cytokines, proteins or peptides that stimulate or interact with other leukocytes, including T_h cells—and suppressor Th cells (such as Treg) i.e. Th cells that suppress activation of the immune system and thereby maintain immune system homeostasis and tolerance to self-antigens. Mature T_h cells are believed to always express the surface protein CD4. T cells expressing CD4 are also known as $CD4^+$ T cells. $CD4^+$ T cells are generally treated as having a pre-defined role as helper T cells within the immune system, although there are known rare exceptions. For example, there are sub-groups of suppressor T cells, natural killer T cells, and cytotoxic T cells that are known to express CD4 (although cytotoxic examples have been observed in extremely low numbers in specific disease states, they are usually considered non-existent).

[0042] The term "cell profile as used herein indicates a detectable set of data portraying the characterizing features of a cell that distinguish the characterized cell from another. In particular, when referred to a T helper cell, the wording "cell profile" indicates a detectable set of data related to a marker cytokine that is produced by the Th cell and characterizes the Th cell with respect to another. For example, marker cytokine for Th1 cell is Interferon-g; marker cytokine for Th2 is IL-4, marker cytokine for Th7 is 11-17 and marker cytokine for Treg is IL-10. Accordingly, the wording "Th17 cell profile" as used herein indicates the detectable set of data, such as presence and amount, related to production of IL-17 in a certain organ or tissue of the individual wherein the presence and/or activity of Th1 cell is investigated. Similar definitions apply to the other Th cell types. On the other hand, when the wording "cell profile" is referred to a subset of Th cell including more then one Th cell type, the wording "T-helper cell profile" indicates a detectable set of data related to each marker cytokine that is produced by and characterizes each, of the T-helper cells of the subset.

[0043] The term "balance" as used herein with reference to a "Th cell profile" as used herein indicates the activity of bringing the cell profile to a status associated with absence of an inflammatory response. Similarly the term "balanced Th profile" indicates the Th cell profile status associated with absence of an inflammatory response and in particular to the detectable set of data related to a marker cytokine that is produced by the T helper cell and characterizes the T helper cell with respect to another in absence of an inflammatory response. When the term "T-helper cell" profile refers to a subset of Th cell including more then one Th cell type, the term "balanced Th profile" refers instead to the relative ratio between the detectable set of data related to each marker

cytokine that is produced by and characterizes each, of the T-helper cells. For example, a "balanced Th cell profile" referred to a Th cells subset comprising Th1, Th2 and Th17 indicates the relative ratio of data related to Interferon-gamma, IL-4 and IL17 associated with absence of an inflammatory response.

[0044] The term "zwitterionic polysaccharide" as used herein indicates synthetic or natural polymers comprising one or more monosaccharides joined together by glicosidic bonds, and including at least one positively charged moiety and at least one negatively charged moiety. Zwitterionic polysaccharides include but are not limited to polymers of any length, from a mono- or di-saccharide polymer to polymers including hundreds or thousands of monosaccharides. In some embodiments, a zwitterionic polysaccharide can include repeating units wherein each repeating unit includes from two to ten monosaccharides, a positively charged moiety (e.g. an free positively charged amino moiety) and a negatively charged moiety (such as sulfonate, sulfate, phosphate and phosphonate). In some embodiment ZPs can have a molecular weight comprised between 500 Da and 2,000,000 Da. In some embodiments, the ZPs can have a molecular weight comprised between 200 and 2500. Exemplary ZPS include but are not limited to PSA and PSB from *Bacteroides Fragilis*, CP5/CD8 from *Staphylococcus aureus*, and Sp1/CP1 from *Streptococcus pneumonia*. Zwitterionic polysaccharides can be isolated from natural sources, and in particular from bacterial sources, e.g. by purification. Zwitterionic polysaccharides can also be produced by chemical or biochemical methods, as well as by recombinant microorganism technologies all identifiable by a skilled person. Thus, those methods and technologies will not be further described herein in detail.

[0045] The wording "polysaccharide A" as used herein indicates a molecule produced by the PSA locus of *Bacteroides Fragilis* and derivatives thereof which include but are not limited to polymers of the repeating unit {→3)α-d-AATGalp (1→4)[β-d-Galf(1→3)]-d-GalpNAc(1 3)β-d-Galp(1→}, where AATGal is acetamido-amino-2,4,6-trideoxygalactose, and the galactopyranosyl residue is modified by a pyruvate substituent spanning O-4 and O-6. The term "derivative" as used herein with reference to a first polysaccharide (e.g., PSA), indicates a second polysaccharide that is structurally related to the first polysaccharide and is derivable from the first polysaccharide by a modification that introduces a feature that is not present in the first polysaccharide while retaining functional properties of the first polysaccharide. Accordingly, a derivative polysaccharide of PSA, usually differs from the original polysaccharide by modification of the repeating units or of the saccharidic component of one or more of the repeating units that might or might not be associated with an additional function not present in the original polysaccharide. A derivative polysaccharide of PSA retains however one or more functional activities that are herein described in connection with PSA in association with the anti-inflammatory activity of PSA.

[0046] In some embodiments, the zwitterionic polysaccharide can be PSA and/or PSB. In some embodiments, the effective amount of ZP and in particular PSA and/or PSB is from about 1-100 micrograms to about 25 grams of body weight and the T-helper cell profile is balanced by balancing at least one of Th1, Th2, Th17 and Treg, in particular at least one of Th1, Th 2 and Treg and Th17. More particularly, in some embodiments, balance Th cell profile can be performed by balancing the Th17 cell profile

[0047] In some embodiments, a ZP can be used to control cytokine production associated with inflammation in an individual. In particular, in some embodiments, ZPs can be administered to inhibit production of pro-inflammatory cytokine molecules such as TNF-a, IL1 or IL-6, IL21, IL23 and IL17.

[0048] The term "control" as used herein indicates the activity of affecting and in particular inhibiting a biological reaction or process, which include but are not limited to biological and in particular biochemical events occurring in a biological system, such as an organism (e.g. animal, plant, fungus, or micro-organism) or a portion thereof (e.g. a cell, a tissue, an organ, an apparatus).

[0049] The terms "inhibiting" and "inhibit", as used herein indicate the activity of decreasing the biological reaction or process. Accordingly, a substance "inhibits" a certain biological reaction or process if it is capable of decreasing that biological reaction or process by interfering with said reaction or process. For example, a substance can inhibit a certain biological reaction or process by reducing or suppressing the activity of another substance (e.g. an enzyme) associated to the biological reaction or process, e.g. by binding, (in some cases specifically), said other substance. Inhibition of the biological reaction or process can be detected by detection of an analyte associated with the biological reaction or process. The term "detect" or "detection" as used herein indicates the determination of the existence, presence or fact of an analyte or related signal in a limited portion of space, including but not limited to a sample, a reaction mixture, a molecular complex and a substrate. A detection is "quantitative" when it refers, relates to, or involves the measurement of quantity or amount of the analyte or related signal (also referred as quantitation), which includes but is not limited to any analysis designed to determine the amounts or proportions of the analyte or related signal. A detection is "qualitative" when it refers, relates to, or involves identification of a quality or kind of the analyte or related signal in terms of relative abundance to another analyte or related signal, which is not quantified.

[0050] The term "cytokine" as used herein indicates a category of signaling proteins and glycoproteins extensively used in cellular communication that are produced by a wide variety of hematopoietic and non-hematopoietic cell types and can have autocrine, paracrine and endocrine effects, sometimes strongly dependent on the presence of other chemicals. The cytokine family consists mainly of smaller, water-soluble proteins and glycoproteins with a mass between 8 and 30 kDa. Cytokines are critical to the development and functioning of both the innate and adaptive immune response. They are often secreted by immune cells that have encountered a pathogen, thereby activating and recruiting further immune cells to increase the system's response to the pathogen.

[0051] Detection of inhibition of cytokine production can be performed by methods known to a skilled person including but not limited to ELISA, Q-PCR and intracellular cytokine staining detected by FACs and any other methods identifiable by a skilled person upon reading of the present disclosure.

[0052] In some embodiments, a ZP can be administered to inhibit production of at least one of TNF-a, IL-6, IL-17, IL-21 and IL-23. In particular, in some of those embodiments ZP can be administered systemically and in particular, orally, sub cutaneously, intra peritoneally, and intravenously. In some

embodiments ZP can be administered in an amount between about 1 and about 100 micrograms/25 grams of body weight.

[0053] Methods and compositions are herein disclosed that allow control of an inflammation associated with an imbalanced Th cell profile and or to production of at least one of the pro-inflammatory cytokines IL-1, IL-6, TNF-a, IL-17, IL21, IL23, and TGF-β in an individual.

[0054] The term "inflammation" and "inflammatory response as used herein indicate the complex biological response of vascular tissues of an individual to harmful stimuli, such as pathogens, damaged cells, or irritants, and includes secretion of cytokines and more particularly of pro-inflammatory cytokine, i.e. cytokines which are produced predominantly by activated immune cells such as microglia and are involved in the amplification of inflammatory reactions. Exemplary pro-inflammatory cytokines include but are not limited to IL-1, IL-6, TNF-a, IL-17, IL21, IL23, and TGF-β. Exemplary inflammations include acute inflammation and chronic inflammation. The wording "acute inflammation" as used herein indicates a short-term process characterized by the classic signs of inflammation (swelling, redness, pain, heat, and loss of function) due to the infiltration of the tissues by plasma and leukocytes. An acute inflammation typically occurs as long as the injurious stimulus is present and ceases once the stimulus has been removed, broken down, or walled off by scarring (fibrosis). The wording "chronic inflammation" as used herein indicates a condition characterized by concurrent active inflammation, tissue destruction, and attempts at repair. Chronic inflammation is not characterized by the classic signs of acute inflammation listed above. Instead, chronically inflamed tissue is characterized by the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes, and plasma cells), tissue destruction, and attempts at healing, which include angiogenesis and fibrosis. An inflammation can be controlled in the sense of the present disclosure by affecting and in particular inhibiting anyone of the events that form the complex biological response associated with an inflammation in an individual. In particular, in some embodiments, an inflammation can be controlled by affecting and in particular inhibiting cytokine production, and more particularly production of pro-inflammatory cytokines, following administration of a zwitterionic polysaccharide.

[0055] More particularly, in some embodiments, a ZP can be used to control an inflammation associated with IL-1, IL-6, TNF-a, IL-17, IL21, IL23, and/or TGF-β mediated inflammation in an individual. The wording "cytokine mediated inflammation" as used herein indicates an inflammation wherein the complex biological response to a harmful stimulus is controlled by cytokine molecules, such as pro-inflammatory cytokine molecules (e.g. TNF-a, IL1 and/or IL-6) and anti-inflammatory cytokine molecules (e.g. IL-10). Exemplary cytokine mediated inflammation include but are not limited to conditions mediated by IL-1, IL-6, TNF-α, IL-12p35, IL-17A, IL-21, IL-22, IFN-γ and/or IL-23p19.

[0056] In some embodiments, the cytokine is at least one of TNF-a, IL-17, IL-21, and IL-23 and the cytokine mediated inflammation is a IBD, asthma, type I diabetes, multiple sclerosis, obesity, type 2 diabetes, hay fever, food allergies, skin allergies, or rheumatoid arthritis. Reference is also made to Mazmanian et al 2008[43], in particular the figures and related portion of the paper herein incorporated by reference in its entirety.

[0057] In some embodiments, the inflammation is a systemic inflammation. Systemic inflammations include but are not limited to an inflammatory response in the circulatory system, an inflammatory response which is not confined in a specific organ, and an inflammatory response that extends to a plurality (up to all) tissues and organs in an individual.

[0058] In some embodiments, a ZP can be used to control an inflammation associated with an imbalance of T-helper cell profile and in particular to a Th17 cell profile, including but not limited to rheumatoid arthritis, respiratory diseases, allograft rejection, systemic lupus erythematosis, tumorgenesis, multiple sclerosis, systemic sclerosis and chronic inflammatory bowel disease.

[0059] In some embodiments, PSA can be administered systemically to the individual. The wording "systemic administration" as used herein indicates a route of administration by which PSA is brought in contact with the body of the individual, so that the desired effect is systemic (i.e. non limited to the specific tissue where the inflammation occurs). Systemic administration includes enteral and parenteral administration. Enteral administration is a systemic route of administration where the substance is given via the digestive tract, and includes but is not limited to oral administration, administration by gastric feeding tube, administration by duodenal feeding tube, gastrostomy, enteral nutrition, and rectal administration. Parenteral administration is a systemic route of administration where the substance is given by route other than the digestive tract and includes but is not limited to intravenous administration, intra-arterial administration, intramuscular administration, subcutaneous administration, intradermal, administration, intraperitoneal administration, and intravesical infusion.

[0060] In some embodiments, administration is performed intravenously by introducing a liquid formulation including a ZP in a vein of an individual using intravenous access methods identifiable by a skilled person, including access through the skin into a peripheral vein. In some embodiments, administration of a ZP is performed intraperitoneally, by injecting a ZP in the peritoneum of an individual, and in particular of animals or humans. Intraperitoneal administration is generally preferred when large amounts of blood replacement fluids are needed, or when low blood pressure or other problems prevent the use of a suitable blood vessel for intravenous injection. In some embodiments administration is performed intragastrically, including administration through a feeding tube. In some embodiments, administration of a ZP is performed intracranially. In some embodiments a ZP can be administered topically by applying the ZP usually included in an appropriate formulation directly where its action is desired. Topical administration include but is not limited to epicutaneous administration, inhalational administration (e.g. in asthma medications), enema, eye drops (E.G. onto the conjunctiva), ear drops, intranasal route (e.g. decongestant nasal sprays), and vaginal administration.

[0061] In some embodiments, the inflammation is an inflammation of in a tissue and in particular in pancreas, lungs, joints, skin, brains and central nervous system, and eyes. In some embodiments, PSA is used in a method of treating or preventing a condition associated with inflammation in an individual. The method comprises administering to the individual a therapeutically effective amount of the PSA. The term "individual" as used herein includes a single biological organism wherein inflammation can occur including

but not limited to animals and in particular higher animals and in particular vertebrates such as mammals and in particular human beings.

[0062] The term "condition" as used herein indicates a usually the physical status of the body of an individual, as a whole or of one or more of its parts, that does not conform to a physical status of the individual, as a whole or of one or more of its parts, that is associated with a state of complete physical, mental and possibly social well-being. Conditions herein described include but are not limited disorders and diseases wherein the term "disorder" indicates a condition of the living individual that is associated to a functional abnormality of the body or of any of its parts, and the term "disease" indicates a condition of the living individual that impairs normal functioning of the body or of any of its parts and is typically manifested by distinguishing signs and symptoms. Exemplary conditions include but are not limited to injuries, disabilities, disorders (including mental and physical disorders), syndromes, infections, deviant behaviors of the individual and atypical variations of structure and functions of the body of an individual or parts thereof.

[0063] The wording "associated to" as used herein with reference to two items indicates a relation between the two items such that the occurrence of a first item is accompanied by the occurrence of the second item, which includes but is not limited to a cause-effect relation and sign/symptoms-disease relation.

[0064] Conditions associated with an inflammation include but are not limited to inflammatory bowel disease, including but not limited to Chron's disease and ulcerative colitis, asthma, dermatitis, arthritis, myasthenia gravis, Grave's disease, sclerosis, psoriasis.

[0065] The term "treatment" as used herein indicates any activity that is part of a medical care for or deals with a condition medically or surgically.

[0066] The term "prevention" as used herein indicates any activity, which reduces the burden of mortality or morbidity from a condition in an individual. This takes place at primary, secondary and tertiary prevention levels, wherein: a) primary prevention avoids the development of a disease; b) secondary prevention activities are aimed at early disease treatment, thereby increasing opportunities for interventions to prevent progression of the disease and emergence of symptoms; and c) tertiary prevention reduces the negative impact of an already established disease by restoring function and reducing disease-related complications.

[0067] An effective amount and in particular a therapeutically effective amount of PSA is for example in the range of between about 1 μg to about 100 μg of PSA per 0.025 kilograms of body weight. In some embodiments, the effective amount is in a range from about 001 to about 1,000 μg per 25 grams of body weight.

[0068] In some embodiments, PSA is comprised in a composition together with a suitable vehicle. The term "vehicle" as used herein indicates any of various media acting usually as solvents, carriers, binders or diluents for PSA comprised in the composition as an active ingredient.

[0069] In some embodiments, where the composition is to be administered to an individual the composition can be a pharmaceutical anti-inflammatory composition, and comprises PSA and a pharmaceutically acceptable vehicle.

[0070] In some embodiments, PSA can be included in pharmaceutical compositions together with an excipient or diluent. In particular, in some embodiments, pharmaceutical compositions are disclosed which contain PSA, in combination with one or more compatible and pharmaceutically acceptable vehicle, and in particular with pharmaceutically acceptable diluents or excipients.

[0071] The term "excipient" as used herein indicates an inactive substance used as a carrier for the active ingredients of a medication. Suitable excipients for the pharmaceutical compositions herein disclosed include any substance that enhances the ability of the body of an individual to absorb PSA. Suitable excipients also include any substance that can be used to bulk up formulations with PSA to allow for convenient and accurate dosage. In addition to their use in the single-dosage quantity, excipients can be used in the manufacturing process to aid in the handling of PSA. Depending on the route of administration, and form of medication, different excipients may be used. Exemplary excipients include but are not limited to antiadherents, binders, coatings disintegrants, fillers, flavors (such as sweeteners) and colors, glidants, lubricants, preservatives, sorbents.

[0072] The term "diluent" as used herein indicates a diluting agent which is issued to dilute or carry an active ingredient of a composition. Suitable diluent include any substance that can decrease the viscosity of a medicinal preparation.

[0073] In certain embodiments, compositions and, in particular, pharmaceutical compositions can be formulated for systemic administration, which includes enteral and parenteral administration.

[0074] Exemplary compositions for parenteral administration include but are not limited to sterile aqueous solutions, injectable solutions or suspensions including PSA. In some embodiments, a composition for parenteral administration can be prepared at the time of use by dissolving a powdered composition, previously prepared in lyophilized form, in a biologically compatible aqueous liquid (distilled water, physiological solution or other aqueous solution).

[0075] Exemplary compositions for enteral administration include but are not limited to a tablet, a capsule, drops, and suppositories.

[0076] The Examples section of the present disclosure illustrates examples of the compositions and methods herein described as well as the studies carried out by applicants in order to investigate the functional and physical interactions of PSA

[0077] Further advantages and characteristics of the present disclosure will become more apparent hereinafter from the following detailed disclosure in the Examples given by way or illustration only with reference to an experimental section.

EXAMPLES

[0078] The methods and system herein disclosed are further illustrated in the following examples, which are provided by way of illustration and are not intended to be limiting.

[0079] In particular, in the following examples, the following materials and methods were used.

[0080] Bacterial strains and animals. *B. fragilis* NCTC9343 and *H. hepaticus* ATCC51149 were obtained from the American Type Culture Collection. Conventionally reared SPF mice of strains C57BL/6NTac, C57BL/6NTac IL-$10^{-/-}$, and B6.129S6-$Rag2^{tm/Fwa}$ N12 ($Rag2^{-/-}$) were purchased from Taconic Farms (Germantown, N.Y.) and screened negative for *B. fragilis* and *H. hepaticus*. Swiss-Webster germ-free (SWGF) mice were purchased from Taconic Farms. Upon delivery in sterile shipping containers, the mice were trans-

ferred to sterile isolators (Class Biologically Clean, Madison, Wis.) in our animal facility. Animals were screened weekly for bacterial, viral, and fungal contamination as previously described[40]. All animals were cared for under established protocols and the IACUC guidelines of Harvard Medical School and the California Institute of Technology.

[0081] Model of inflammation: Three models of intestinal inflammation were used: 1) $CD4^+$ $CD45Rb^{high}$ T cells were purified from the spleens of wild-type or IL-$10^{-/-}$ donor mice by flow cytometry and transferred into $Rag^{-/-}$ (C57B1/6) recipients as described. 2) TNBS colitis was induced by pre-sensitization of wild-type (C57B1/6) mice on the skin with a TNBS/acetone mix. Seven days after sensitization, 2.5% TNBS in ethanol was administered rectally; mice were sacrificed 3-6 days later. 3) $IL10^{-/-}$ mice were colonized (by oral gavage) with *H. hepaticus* alone or in combination with wild-type *B. fragilis* or *B. fragilis* ΔPSA.

[0082] Assays and scoring systems: Cytokines from the spleen, colons, or MLNs were assayed by ELISA, Q-PCR, or flow cytometry. Colitis was assessed with tissue sections (fixed, paraffin embedded, sectioned onto a slide, and stained with hematoxylin and eosin) and was scored by a blinded pathologist (Dr. R. T. Bronson, Harvard Medical School) 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; Biosource, Camarillo, Calif.). $CD4^+$ T cells were purified by negative selection over a magnetic column (Miltenyi or R& D Systems).

[0083] Flow cytometry, fluorescence-activated cell sorting (FACS), and staining. Lymphocytes were isolated from mouse spleens that were mechanically disrupted into single-cell preparations. Red blood cells were lysed, and splenocytes (1×10^6) were incubated with various combinations of antibodies (BD Pharmingen, San Diego, Calif.) at 2 mg/mL for 30 min at 4° C. Cells were then washed and either fixed or used directly. For intracellular cytokine flow cytometry, samples were analyzed on a model FC500 cytometer (Beckman Coulter, Fullerton, Calif.) or a FacsCalibur (Becton Dickson), and data were analyzed with RXP Analysis Software (Beckman Coulter) or FlowJO. FACS was performed on a BD FACSAria, and cell purity was always >99%.

[0084] In vitro cytokine assays. For colon organ cultures, procedures were followed as previously reported[41]. For co-culture, $CD4^+$ T cells were purified from splenic lymphocytes (prepared as described above) with a $CD4^+$ T Cell Subset Kit (R&D Systems, Minneapolis, Minn.) used as instructed by the manufacturer. Cell purity was always >95%. 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; Biosource, Camarillo, Calif.). Medium was replaced after 4 days, and adherent cells were cultured for an additional 4 days, at which point nonadherent cells were recovered, washed, and used directly. Cells were >95% $CD11c^+$ at the time of use. Purified $CD4^+$ T cells (1×10^6) were mixed with purified $CD11c^+$ BMDCs (1×10^6) in a 48-well plate and were incubated at 37° C. in an atmosphere containing 5% CO_2. Various stimuli were used, as described in Results. ELISA was performed with pre-coated plate kits (BD Pharmingen) according to the manufacturer's guidelines. In some assays, *H. hepaticus*, with or without wild-type *B. fragilis* or *B. fragilis* ΔPSA, was added at various concentrations.

[0085] Induction of experimental colitis. As assessed by PCR, $Rag2^{-/-}$ and control C57Bl/6 mice were negative for *H. hepaticus* colonization at the time of delivery. Splenic lymphocytes were harvested from wild-type donor mice, and $CD4^+$ $CD45Rb^{high}$ cells were purified from lymphocyte populations by FACS as described above. Cells were washed with PBS, and 3×10^5 cells were injected intraperitoneally in a volume of 0.2 mL into recipient *H. hepaticus*-colonized $Rag2^{-/-}$ animals. For colonization experiments, both *H. hepaticus* (1×10^8 organisms) and *B. fragilis* (1×10^8 organisms) were introduced at the time of cell transfer. Throughout PSA treatment studies, animals received 50 μg of PSA by gavage 3 times per week. Animals were weighed throughout the experiment until sacrifice at 8 weeks.

[0086] Induction of intestinal inflammation-TNBS colitis. The backs of wild-type (C57BL/6) male mice were shaved, and pre-sensitization solution (150 μL; acetone with olive oil in a 4:1 ratio mixed with 5% TNBS in a 4:1 ratio) was slowly applied. Seven days after sensitization, mice were anesthetized with isofluorene and TNBS solution (100 μL; 1:1 5% TNBS with absolute ethanol) administered rectally through a 3.5 F catheter (Instech Solomon; SIL-C35). Mice were analyzed 4-6 days after TNBS administration.

[0087] Histologic tissue analysis. Mouse tissues in Bouin's fixative (VWR, West Chester, Pa.) were embedded in paraffin, sectioned (6-μm slices), mounted onto slides, and stained with hematoxylin and eosin. Sections were evaluated in blinded fashion by a single pathologist (Dr. R. T. Bronson, Harvard Medical School).

[0088] Quantitative real-time PCR. RNA was extracted with Trizol per the manufacturer's instructions (Invitrogen). RNA (1 μg) was reverse transcribed into cDNA with an iScript cDNA synthesis kit (Bio-Rad). cDNA was diluted by addition of 60 μL of water, and a 2-μL volume of this solution was used for Q-PCR. Q-PCR was performed using IQ SYBR Green supermix (Bio-Rad) and primers were used at 0.2 μm. Q-PCR was performed on a Bio-Rad iCycler IQ5. Sequences of Q-PCR primers were as follows 5'-3': IL-23 (p19) F: AGC TAT GAA TCT ACT AAG AGA GGG ACA (SEQ ID NO: 5) R: GTC CTA GTA GGG AGG TGT GAA GTT G (SEQ ID NO: 6). IL-17A F: TTA AGG TTC TCT CCT CTG AA (SEQ ID NO: 7) R: TAG GGA GCT AAA TTA TCC AA. (SEQ ID NO: 8) TNFα F: ACG GCA TGG ATC TCA AAG AC (SEQ ID NO: 9) R: GTG GGT GAG GAG CAC GTA GT (SEQ ID NO: 10). IL-10 F: CTG GAC AAC ATA CTG CTA ACC G (SEQ ID NO: 11) R: GGG CAT CAC TTC TAC CAG GTA A (SEQ ID NO: 12) RORγT F: CCG CTG AGA GGG CTT CAC (SEQ ID NO: 13) R: TGC AGG AGT AGG CCA CAT TAC A (SEQ ID NO: 14) IL-21 F: ATC CTG AAC TTC TAT CAG CTC CAC (SEQ ID NO: 15) R: GCA TTT AGC TAT GTG CTT CTG TTT C (SEQ ID NO: 16) IL-27 F: CTG TTG CTG CTA CCC TTG CTT (SEQ ID NO: 17) R: CAC TCC TGG CAA TCG AGA TTC (SEQ ID NO: 18).

Example 1

PSA Balances the Th1/Th2 Profile of the Mammalian Immune System

[0089] The two subtypes of effector $CD4^+$ T cells, T_H1 and T_H2, are defined by expression of the cytokines interferon g (IFNg) and interleukin 4 (IL-4), respectively (Janeway et al.,

2001). As shown above, PSA induces CD4+ T cell expansion in *B. fragilis*-colonized mice and in vitro. To further characterize the effects of PSA-mediated T cell activation, we assessed cytokine profiles using purified cellular components. Co-culture of DCs and CD4+ T cells in the presence of PSA yields dose-dependent up-expression of the T_H1 cytokine IFNg. The level of IFNg production associated with PSA is comparable to that associated with several known potent IFNg inducers (a-CD3, LPS, and staphylococcal enterotoxin A [SEA]) and requires both DCs and T cells. Specificity is evidenced by the lack of T_H1 cytokine production after NAc-PSA treatment.

[0090] T_H1 cytokine production suppresses T_H2 responses; conversely, T_H2 cytokine expression inhibits T_H1 responses. Normal immune responses require a controlled balance of these opposing signals. Examination of IL-4 expression in response to PSA treatment reveals no cytokine production by purified CD4+ T cells. a-CD3 and the superantigen SEA are potent stimulators of both classes of cytokine. As T_H2 cytokine production is a "default pathway" in many systems (Kidd, 2003; Amsen et al. 2004)) and T_H1 cytokine production is antagonistic to T_H2 expression, the specific stimulation of IFNg by PSA in vitro may provide a mechanism for establishing commensal-mediated homeostasis of the host immune system by balancing T_H1/T_H2 responses.

Example 2

PSA is Required for Appropriate CD4+ T-Helper Cytokine Production During Colonization

[0091] A proper T_H1/T_H2 balance is critical for human and animal health; over- or underproduction of either response is associated with immunologic disorders. We investigated the effects of PSA on T_H1/T_H2 cytokine responses in colonized animals, again using germ-free mice. CD4+ T cells from mouse spleens were purified and tested by ELISA for cytokine production. Overproduction of the T_H2 cytokine IL-4 in spleens of germ-free mice compared with levels in conventional mice. This result is consistent with previous reports of the appreciably T_H2-skewed profile of mice devoid of bacterial contamination and reflects the human neonatal (precolonization) cytokine profile (Kirjavainen and Gibson, 1999; Prescott et al., 1998; Adkins, 2000; Kidd, 2003). This "default" T_H2-bias in the absence of bacterial colonization again highlights the profound contributions of the microflora to immune development and provides a model to test the effects of symbiotic bacteria on the establishment of appropriate host cytokine production.

[0092] Mice colonized with wild-type *B. fragilis* alone display a level of IL-4 production similar to that in conventional mice with a complex microflora; this similarity shows the organism's sufficiency to correct systemic immune defects. Moreover, mice colonized with *B. fragilis* DPSA produce T_H2 cytokines at elevated levels similar to those in germ-free mice. Thus the expression of a single bacterial antigen allows *B. fragilis* to correct the IL-4 cytokine imbalance found in uncolonized animals.

[0093] Examination of IFNg production by purified splenic CD4+ T cells reveals that germ-free mice, which are T_H2-skewed, are deficient in production of this prototypical T_H1 marker when compared to conventional mice. Colonization with wild-type *B. fragilis* alone is sufficient to correct the defect in IFNg expression in germ-free mice, with levels nearly as high as those in conventional mice. Lack of PSA production by the *B. fragilis* mutant during colonization of germ-free mice results in low-level production of T_H1 cytokines. These results were corroborated by intracellular cytokine staining of splenic lymphocytes from each group, which confirms that IFNg production is attributable to CD4+ T cells. The production of IL-2, another T_H1 cytokine, by CD4+ T cells in gnotobiotic mice also requires PSA production data not shown) Together, these results demonstrate that intestinal colonization of germ-free mice by *B. fragilis* alone is sufficient to establish a proper systemic T_H1/T_H2 balance within the host a fundamental aspect of the mammalian immune response required for health.

Example 3

PSA Suppresses Th-17 Induced Inflammation

[0094] 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, Applicants employed 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 administration of TNBS (or vehicle, as a negative control), and oral treatment of PSA was evaluated.

[0095] The results illustrated in FIG. **7** show that the intestinal immune response are beneficially modulated by PSA. In particular, the results illustrated in FIG. **7***a* show that TNBS-treated animals display weight loss that is statistically significant relative to figures for vehicle-treated and PSA-treated animals, although partial weight loss is observed in the PSA group (FIG. **7***a*). Histological analysis confirmed PSA protection of colonic tissues against the massive epithelial hyperplasia and loss of colonocyte organization seen after TNBS treatment (FIG. **7***b*). Studies have shown that pathogenic T_H17 cells, which produce IL-17, mediate the induction of experimental colitis[30]. Indeed, IL-17 levels are increased among purified CD4+ T cells from mesenteric lymph nodes (MLNs; FIG. **7***c*) of diseased animals but not from those of PSA-treated animals. The increased level of TNFa among CD4+ T cells from MLNs of TNBS-treated animals is also reduced in PSA-treated groups (FIG. **7***d*). Transcriptional analysis of TNBS-treated colons revealed that expression of both IL-17 and TNFa is highly elevated in diseased but not in PSA-protected animals (FIGS. **7***e* and **7***f*).

[0096] Therefore, the above results show that PSA inhibits intestinal pathology and inflammation in a chemically induced model of experimental colitis.

Example 4

PSA Induces the Differentiation of IL-10 Producing Treg to Suppress Inflammation

[0097] Protection from experimental colitis is engendered through anti-inflammatory processes that prevent undesirable reactions against the intestinal microbiota[23]. Interleukin-10-deficient (IL-10−/−) animals develop colitis[31]. IL-10, one of the most potent anti-inflammatory cytokines, is required for protection in many animal models of inflammation[21,27,32].

[0098] The results of a series of experiments directed to test the effect of PSA on IL-10 production are illustrated in FIG. **8**, and show that PSA induces IL-10 expression in TNBS-

treated animals and inhibits pro-inflammatory cytokine production in primary cultured cells through IL-10 production. In particular, as assayed by real-time PCR, transcriptional levels of IL-10 within colons of PSA-treated mice are significantly higher than those in control and TNBS-treated mice (FIG. **8***a*). IL-10 is produced by many cell types. However, since $CD4^+$ T cells that express IL-10 display immunosuppressive activities that inhibit inflammation during experimental colitis[33], Applicants tested the IL-10 production in $CD4^+$ T. When fresh $CD4^+$ T cells were purified from MLNs of PSA-treated mice (in which inflammation is reduced), highly elevated levels of the IL-10 transcript were observed (FIG. **8***b*). Applicants then assessed whether PSA is sufficient to induce IL-10 in vitro; when bone marrow-derived dendritic cells (BMDCs) and naïve $CD4^+$ T cells were treated with purified PSA, a specific increase in IL-10 production was observed (FIG. **8***c*).

[0099] A further series of experiments illustrated in FIG. **9**, shows that PSA from *B. fragilis* induces expression of IL-10 in vitro. In particular, BMDCs and naïve $CD4^+$ T cells were infected with *H. hepaticus* co-cultured with *B. fragilis*, and a specific expression of IL-10 from culture supernatants was observed; co-culture with *B. fragilis* DPSA induces significantly lower levels of IL-10 (FIG. **9**). Since PSA induces expression of IL-10 in vitro, to test whether this molecule is required for inhibition of inflammatory responses to *H. hepaticus*, BMDC-T cell co-cultures were infected with live *H. hepaticus* and measured expression of the critical pro-inflammatory cytokine TNFa. Addition of increasing concentrations of the pathogenic commensal causes a dose-dependent increase in TNFa production, as measured by ELISA of culture supernatants (FIG. **8***d*; left three bars). Treatment of cells with purified PSA markedly decreases TNFa production in response to *H. hepaticus* (FIG. **8***d*; middle three bars). Most importantly, co-incubation of cell cultures with *H. hepaticus* and PSA in the presence of a neutralizing IL-10 receptor antibody (aIL-10R) completely reverses this phenotypic effect and increases expression of TNFa (FIG. **8***d*; right three bars).

[0100] The results are similar for the related pro-inflammatory cytokine IL-1b, as shown by the results of experiments illustrated in FIG. **10**. In particular, infection of BMDC-T cell co-cultures with increasing concentrations of live *H. hepaticus* (see FIG. **10** multiplicity of infection: 0.1, 1.0, and 10, as depicted by triangles) results in release of the cytokine IL-1b Treatment of infected cells with PSA reduces IL-1b levels, as shown in the middle three bars. Neutralization of IL-10 signaling by addition of an IL-10 receptor antibody (aIL-10R) alleviates suppression of in vitro inflammatory responses, resulting in increased levels of IL-1b FIG. **10** left three bars.

[0101] Thus, the results illustrated in the present example support the conclusion that IL-10 produced in response to PSA is required for inhibition of inflammatory reactions in cell cultures.

Example 5

PSA Administration Results in Differentiation of Treg, Inhibition of TNF-a and IL-17 Cytokine Production and in Colitis Suppression

[0102] Applicants investigated the requirement for IL-10 in suppression of intestinal inflammation. Initially, $IL\text{-}10^{-/-}$ animals were colonized with *H. hepaticus* alone or in combination with *B. fragilis* (wild-type or DPSA). Applicants subsequently harvested MLNs and re-stimulated cells in culture with soluble *Helicobacter* antigens in an assay previously developed to measure antigen-specific responses to *H. hepaticus*[27]. In particular, $IL\text{-}10^{-/-}$ 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 ←PSA). MLNs from experimental groups were pooled and re-stimulated with soluble *Helicobacter* antigen (5 μg/ml) for 48 hours. Secretion of pro-inflammatory cytokines TNFα (a) and IL-17A (b) was analyzed by ELISA

[0103] The results of these experiments, illustrated in FIG. **11***a*-**11***c*, show that *Helicobacter*-colonized animals display increased production of TNFa and IL-17; however, in the absence of IL-10 production in colonized animals, *B. fragilis* co-colonization dos not reduce levels of these pro-inflammatory molecules (FIGS. **11***a* and *b*, respectively). As expected, the absence of PSA has no effect. Using the cell transfer model of colitis (see Examples 6 to 8 below, Applicants transferred $CD4^+$ $CD45Rb^{high}$ T cells to *Helicobacter*-colonized $Rag^{-/-}$ animals. Administration of aIL-10R to mice (to block IL-10 signaling) during oral treatment with PSA abrogates protection from colitis (FIG. **11***c*). In particular, colitis scores show that PSA protection requires aIL-10 signaling, as neutralizing antibodies to IL-10 block PSA's suppressive activity. Treatment with IL-10 OR abrogates PSA-mediated protection. (FIG. **11***c*).

[0104] Additionally, when $IL\text{-}10^{-/-}$ animals were treated with TNBS in the presence or absence of PSA, weight and histology data illustrated in FIGS. **12** and **13**, indicated that IL-10 production is required for PSA-elicited reduction of intestinal immune responses. In particular, in a first series of experiments, groups of 4 C57BL/6 mice were treated with PSA (or PBS) and then subjected to rectal administration of TNBS or vehicle (control). SD values illustrated in FIG. **12**, indicate that, in the absence of IL-10, PSA cannot restore TNBS-induced weight loss. ANOVA demonstrates that weight loss in both TNBS-treated groups is statistically different from that in control animals and that PSA does not prevent weight loss in TNBS-treated $IL\text{-}10^{-/-}$ animals (FIG. **12**).

[0105] In a second series of experiments, groups of 4 C57BL/6 mice were treated with PSA (or PBS) and then subjected to rectal administration of TNBS or vehicle (control). Histologic analysis of H&E-stained sections from a representative animal from each group is shown in FIG. **13**. Thickening of the colon and epithelial hyperplasia are noted in both TNBS-treated groups of $IL\text{-}10^{-/-}$ animals, regardless of PSA treatment. Thus, the results illustrated in FIG. **13** show that in the absence of IL-10, PSA does not reduce intestinal injury in TNBS-treated $IL\text{-}10^{-/-}$ mice.

[0106] The above 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, Applicants transferred $CD4^+$ $CD45Rb^{high}$ T cells from $IL\text{-}10^{-/-}$ donor mice into $Rag^{-/-}$ recipients and then colonized the recipients with *H. hepaticus.*

[0107] The results illustrated in FIGS. **11***d*-**11***f* show that, as expected, groups of mice receiving $IL\text{-}10^{-/-}$ T cells along with *H. hepaticus* develop severe colitis (FIG. **11***d*; left bar) and are not protected by PSA (FIG. **11***d*; middle bar). This result, supported by histological findings in colons, indicates that PSA induces protection from "previously pathogenic"

$CD4^+$ $CD45Rb^{high}$ T cells in an IL-10-dependent manner (FIG. **11***e*). Weight analysis at sacrifice shows that colitic PBS- and PSA-treated animals receiving $IL\text{-}10^{-/-}$ $CD4^+$ $CD45Rb^{high}$ T cells (unlike control animals receiving no transferred cells) develop wasting disease (FIG. **11***f*). 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.

Example 6

PSA Balances the $CD4^+$ $CD45Rb^{high}$/$CD4^+$ $CD45Rb^{low}$ T Cells Ratio

[0108] $CD4^+$ T cells of the mammalian immune system can be generally divided into a naïve ('uneducated') $CD4^+$ $CD45Rb^{high}$ population and an antigen-experienced ('educated') CD4 $CD45Rb^{low}$ population[16].

[0109] In a first series of experiments, mono-association of germ-free mice with wild-type *B. fragilis* was performed to analyze the effect on the $CD4^+$ $CD45Rb^{low}$ T cells v. $CD4^+$ $CD45Rb^{high}$ proportion. In particular, the ability of *B. fragilis* to correct deficiencies in the $CD4^+$ $CD45Rb^{low}$ T cell population in spleen.

[0110] The results illustrated in FIG. **1***a* show that association of *B. fragilis* expands the proportions of $CD4^+$ $CD45Rb^{low}$ T cells in a PSA-dependent manner Remarkably, Applicants found that splenic cells from germ-free animals include a smaller proportion of $CD4^+$ $CD45Rb^{low}$ T cells than do those from age-matched conventional mice with a complete bacterial microbiota (FIG. **1***a*). Additionally, it appears that mono-colonization of germ-free mice with wild-type *B. fragilis* alone restores the $CD4^+$ CD45Rb profile in animals with a complete bacterial microbiota (FIG. **1***a*; left panels). Most notably, colonization with a mutant strain defective in the ability to produce PSA (*B. fragilis* DPSA) does not generate an expansion of the $CD4^+$ $CD45Rb^{low}$ T cell population (FIG. **1***a*; lower right). It is well established that the latter population possesses potent anti-inflammatory properties and confers protection in animal models of inflammation[17]. These results suggested that PSA mediate protection from inflammation.

Example 7

PSA Controls IL23, IL1b and TNF-a Production in Inflamed Tissues, Thus Controlling Th17 and Th1-Mediated Cytokine Production

[0111] The well-established $CD4^+$CD45Rb transfer model of experimental colitis[18] was employed 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 (SPF) $Rag^{-/-}$ mice. Upon cell transfer, mice are colonized with *Helicobacter* hepaticus[8,19], a pathobiont that is a benign commensal in wild-type animals but an opportunistic pathogen causing colitis in immuncompromised mice. After 8 weeks, animals are sacrificed and colitis is assessed with a standard scoring system[20].

[0112] The pathology scores illustrated in FIG. **1***b*, show that *H. hepaticus* colonization and $CD4^+$ $CD45Rb^{high}$ T cell transfer are sufficient to induce severe colitis in $Rag^{-/-}$ mice (FIG. **1***b*; first column), as previously reported[19,21]. Co-colonization with wild-type *B. fragilis* results in significant protection from disease (FIG. **1***b*; second column), whereas co-colonization with *B. fragilis* DPSA does not (FIG. **1***b*; third column).

[0113] Tissue damage in colitis is widely believed to result from production of inflammatory cytokines in response to commensal bacteria[22]. The pro-inflammatory cytokines tumor necrosis factor a (TNFa, interleukin-1b (IL-1b and IL-23 are central to disease initiation and progression in this experimental model of colitis[23]. Furthermore, levels of these cytokines are elevated in patients with IBD[24], and therapies neutralizing TNFa have yielded promising results in clinical trials in patients with Crohn's disease[25]. Accordingly, Applicants decided to test the inflammatory cytokine levels during disease by directly culturing intestinal tissues of T cell recipient colonized animals[26]. The results illustrated in FIGS. **1***c*, **1***d*, **2** and **3** show that PSA alters cytokine levels in affected tissue.

[0114] In particular, the results of ELISA experiments of colon organ cultures illustrated in FIG. **1***c* show an increased expression of pro-inflammatory cytokine TNFa in diseased colons, with significant reductions in animals co-colonized with wild-type *B. fragilis* but not with *B. fragilis* DPSA.

[0115] The results of Q-PCR for IL-23p19 performed on splenocytes, normalized to L32 expression illustrated in FIG. **1***d* show that increases in IL-23 production by splenocytes following disease induction are completely suppressed by intestinal colonization with PSA-producing *B. fragilis*.

[0116] ELISA results for the pro-inflammatory cytokines IL-12p40 and IL-1b in colon and small intestines shown in FIG. **2** show a specific increase in pro-inflammatory cytokines in diseased colons but not in small intestines. This increase is significantly reduced in animals co-colonized with PSA-producing *B. fragilis*. Conversely, animals colonized with *B. fragilis* DPSA express greatly increased pro-inflammatory cytokine levels over those in control animals (C57BL/6) (FIG. **2**).

[0117] The results of experiments illustrated in FIG. **3** show that the expression of the TNFa by $CD4^+$ T cells is reduced by wild-type *B. fragilis* colonization during experimental colitis. $CD4^+$ cells were purified from pooled splenocytes from each group (4 mice per group) and restimulated in vitro with PMA and ionomycin in the presence of brefeldin A for 4 hours. Cells were stained for intracellular TNFα. Cells within the lymphocyte gate were included in the analysis, and numbers indicate the percentage of cells producing TNFα. Purified cells were >90% $CD4^+$. Animals colonized with PSA-producing *B. fragilis* during protection displayed lower TNFa levels than diseased animals.

[0118] Overall these above results show that PSA performs its effect by altering cytokine levels in affected tissues. In particular, levels of the pro-inflammatory cytokines TNFa (FIG. **1***c*), IL-12p40, and IL-1b (FIG. **2**) are elevated in the colons of $Rag^{-/-}$ recipient mice colonized with *H. hepaticus* but not in sections of small intestine (a site not affected in this model). Consistent with the protection observed by pathophysiologic analysis of experimental colitis, TNFa levels are not elevated when these animals are co-colonized with wild-type *B. fragilis*. T cell transfer plus co-colonization with *H. hepaticus* and *B. fragilis* DPSA 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, display increased TNFa production; this condition is corrected by colonization with wild-type *B. fragilis* but not with the PSA deletion strain (FIG. **3**). Expression of IL-23 is critical in the cascade of events leading to experimental colitis[27,28]. Applicants found that increases in IL-23 production by splenocytes following disease induction are completely suppressed by intestinal colonization with PSA-producing *B. fragilis* (FIG. **1***d*).

[0119] Experiments directed to rule out bacterial clearance were performed to show whether, over the course of the experiments, levels of *H. hepaticus* and *B. fragilis* colonization did differ between groups. The results illustrated in FIGS. **4** and **5** show that protection is not the result of bacterial clearance.

[0120] In particular, the results shown in FIG. **4** show that experimental animals remain colonized with *H. hepaticus* and *B. fragilis* throughout the course of disease. More particularly, the ethidium bromide-stained gel electrophoresis of *H. hepaticus*-specific Q-PCR of FIG. **4***a* shows that co-colonization with *B. fragilis* does not induce clearance of bacteria after 8 weeks. The primers used for *H. hepaticus* 16S rDNA were: (HB-15) 5'-GAAACTGTTACTCTG-3' (SEQ ID NO: 1) and (HB-17) 5'-TCAAGCTCCCCGAAGGG-3' (SEQ ID NO: 2). Ethidium bromide-stained gel electrophoresis of *B. fragilis*-specific Q-PCR of FIG. **4***b* show stable bacterial colonization after 8 weeks; the primers used for *B. fragilis* ssr3 (finB) gene were: (ssr3-F) 5'-TATTTGCGAGAAGGTGAT-3' (SEQ ID NO: 3) and (ssr3-r) 5'-TAAACGCTTTGCTGCTAT-3' (SEQ ID NO: 4).

[0121] In an additional series of experiments, quantitation of *H. hepaticus* was performed to verify whether PSA administration affected the presence of the organism. The results of quantitation of *H. hepaticus* colonization experiments of FIG. **5** demonstrate that the organism is present in equal numbers regardless of PSA-mediated protection. In particular, in the experiments of FIG. **5** fecal samples were collected from each experimental group, and total DNA was extracted (Qiagen DNAeasy tissue kit). Equal amounts of DNA (50 ng) were used in Q-PCR (Bio-rad) with *H. hepaticus*-specific primers. Q-PCR for *H. hepaticus* colonization was assessed according to Young et al., 2004[1] as $\log^{10}$ number of copies of a known gene (cytolethal distending toxin). Animals contained equivalent levels of *H. hepaticus* at the end of the experiment.

[0122] The results illustrated in this example support the conclusion that PSA is a specific immunomodulatory molecule that orchestrates beneficial immune responses to prevent *B. Fragilis* host from developing experimental colitis.

Example 8

PSA Suppresses Inflammation Associated with $CD4^+$ $CD45Rb^{high}$ T Cells

[0123] To determine whether PSA is sufficient for protection in the absence of the intact *B. fragilis* organism, Applicants purified PSA to homogeneity[29] and administered it by gavage (orally) to $Rag^{-/-}$ mice. Disease progression was then measured by various pathologic and histologic criteria.

[0124] The results of related experiments illustrated in FIG. **6**, show that purified PSA orally administered protects against experimental colitis.

[0125] In particular, in a first series of experiments illustrated in FIG. **6***a*, colitis scores after $CD4^+$ $CD45Rb^{high}$ T cell transfer in the absence of *H. hepaticus* colonization indicated the development of very mild colitis due to inflammation elicited by the animals' SPF microbiota (FIG. **6***a*; first column). However, *Helicobacter*-colonized $Rag^{-/-}$ animals that receive $CD4^+$ $CD45Rb^{high}$ T cell transfers develop severe colitis (FIG. **6***a*; second column). Oral PSA administration almost completely protects animals against *H. hepaticus*-induced colitis (FIG. **6***a*; third column), reducing disease to levels of control animals without T cell transfer, that known not to develop colitis (FIG. **6***a*; fourth column).

[0126] A second set of experiments was then performed to test the inability to gain weight, a hallmark of colitis in this experimental setting[4]. In particular, transfer of $CD4^+$ $CD45Rb^{high}$ T cells and colonization with *H. hepaticus* (PBS+Hh) in $Rag2^{-/-}$ animals was performed and the animals were subsequently tested for wasting disease. The results illustrated in FIG. **6***b* show that wasting disease in $Rag^{-/-}$ animals follows transfer of $CD4^+$ $CD45Rb^{high}$ cells and colonization with *H. hepaticus* (FIG. **6***b*; PBS+Hh). These animals also develop intestinal pathology and express pro-inflammatory cytokines (as described above). Oral administration of PSA from the outset completely protects animals against *H. hepaticus*-mediated wasting disease (PSA+Hh). *H. hepaticus* provides the necessary antigens for inflammation induction; no pathology is observed in uncolonized animals (PBS−Hh) or in animals without cell transfer. Therefore these experiments show that oral administration of PSA protects animals against wasting (PSA+Hh).

[0127] In a further set of experiments, histologic sections of colons of wild-type animals and animals subjected to transfer of $CD4^+$ $CD45Rb^{high}$ T cells and colonization with *H. hepaticus* (PBS+Hh) were examined to verify the presence of inflammation resulting in experimental colitis. The results illustrated in FIG. **6***c* show that transfer of $CD4^+$ $CD45Rb^{high}$ T cells into *Helicobacter*-colonized $Rag^{-/-}$ mice results in onset of severe colitis, as evidenced by massive epithelial cell hyperplasia and gross thickening of the gut wall (FIG. **6***c*; second panel). Furthermore, consistent with previous studies, the combination of $CD4^+$ $CD45Rb^{high}$ T cell transfer plus *H. hepaticus* colonization results in infiltration of affected tissues by leukocytes—a hallmark of inflammation and disease (FIG. **6***c* second panel, bottom)[19,21]. Additionally, oral administration of PSA to *H. hepaticus*-colonized cell transfer recipients confers complete protection against experimentally induced colonic hyperplasia (FIG. **6***c*; third panel); furthermore, PSA-treated animals display no leukocyte infiltration in colonic tissues (FIG. **6***c* third panel, bottom)—a result indicating protection against inflammation.

[0128] Taken together, these results indicate that oral administration of PSA prevents colitis and protects mice against the associated weight loss and inflammatory cell infiltration observed in diseased animals.

Example 9

PSA is Effective in Systemic Immune Compartments Suppressing Cytokine Production by Th1 and Th17 Cells

[0129] In further series of experiments, mice were treated with TNBS or TNB/PSA, orally administered to the mice. The relevant colonic sections were subsequently analyzed by a blinded pathologist who provided a histological score. The results illustrated in FIG. **14***a* provide further evidence that oral PSA administration reduces colitis.

[0130] While oral treatment with purified PSA protects from experimental colitis (FIG. **14***a*), colonization by a *B. fragilis* mutant that does not make PSA (*B. fragilis* ΔPSA) is unable to protect. During the course of the experiments exemplified in Examples 1 to 8, Applicants noted strong effects of PSA in systemic immune compartments. To further understand these systemic responses Applicants utilized the TNBS induced model in the susceptible Balb/c mouse strain. As this model allows for disease induction in an immune-competent animal, it permits analysis of all immune cells involved in both disease induction and protection.

[0131] Balb/c mice were orally administered purified PSA before induction of colitis. Indeed, oral treatment of PSA protected from weight loss associated with experimental colitis and inflammation within the intestine (not shown).

[0132] Additionally, pre-treatment of Balb/c mice undergoing TNBS induced colitis, with PSA dramatically increases the survival of animals with disease from 40% to 90%, (see FIG. **14***b*), further attesting to the powerful anti-inflammatory effects of PSA. Since splenomegaly is commonly seen in this model of IBD and demonstrates the systemic nature of this disease, the Applicants analyzed the spleen of mice treated with TNBS, and TNBS/PSA. The results illustrated in FIG. **14***c* show that oral administration of ZPS is protects from the splenomegaly. Furthermore, analysis of cytokine expression showed that animals undergoing TNBS induced colitis have severe splenomegaly with increases in the expression of inflammatory cytokines from CD4+ T lymphocytes residing within the spleen, as illustrated in FIG. **14***d*. Orally administered PSA significantly reduces splenomegaly and the expression of TNF-α, IL-17 and IL-21 in CD4+ T lymphocytes from the spleen during mucosal disease (FIG. **14***d*). The experiments outlined in Example 5 demonstrate that PSA is able to protect from colitis through induction of IL-10 from CD4+ T cells residing within the intestinal compartments. Consistent with previous data, Applicants find that IL-10 levels are elevated within the CD4+ T lymphocytes in spleen (FIG. **14***d*). Taken together, these data suggest that PSA residing within the intestine is capable of effecting systemic immunity. In particular these results show that oral administration of ZPS can not only protect from intestinal disease but also suppresses inflammation within extra-intestinal immune compartments, such as the spleen.

Example 10

Parenteral Administration of PSA Protects from Inflammation and Controls TNF-a, IL-17 and IL23 Production in Intestine and Spleen

[0133] Distinct subsets of cells reside within the intestinal compartment, including CD8αα T cells, mucosal γδ T cells and CD103+ dendritic cells. Recent studies have demonstrated that these various cell types have distinct functions from their systemic immune counterparts. To determine whether PSA acts specifically within the intestine, purified PSA was administered intravenously and mucosal inflammation was induced. In a first series of experiments illustrated in FIG. **15**, PSA was administered before inflammation was induced. In a second series of experiments, illustrated in FIG. **16**, PSA was administered after inflammation was induced.

[0134] The results illustrated in FIG. **15**, show that delivery of ZPS to extra-intestinal sites is able to protect from induced intestinal colitis. In particular, systemic administration of PSA enhances the survival of diseased animals and protects from splenomegaly (60% survival vs. 90%) (FIGS. **15***a* and **15***b*). Additionally, it is expected that colons of animals that treated with PSA systemically, have significantly less hyperplasia and inflammatory infiltrate.

[0135] The results illustrated in FIG. **16** show that while disease is exacerbated by the increased production of inflammatory cytokines at the site of induction, systemic administration of PSA during TNBS induced colitis suppresses inflammatory cytokines at both intestinal and systemic immune compartments. In particular, TNF-α from Mesenteric Lymph Nodes (MLN) CD4+ T cells is increased in expression during TNBS induced colitis, but is reduced by PSA systemically administered (FIG. **16***a*). Additionally, inflammatory cytokines IL12p35 IL-23p19 and IL-17 are elevated in the colons of diseased mice, as shown by analysis of transcripts from RNA extracted from colons of mice undergoing TNBS induced colitis, but are reduced by administration of PSA (FIG. **16***b*). Also in spleen, systemic administration of ZPS reduces the production of TNF-α from CD4+ T lymphocytes within the spleen as shown by the results illustrated in FIG. **16***c*. Furthermore, systemic administration of ZPS reduces expression of the transcripts IL-17, and IL-6 within the spleen as shown by the results illustrated in FIG. **16***d*.

[0136] Additional experiments also demonstrated that while PSA decreases expression of inflammatory cytokines, intravenous treatment with PSA leads to an elevation in the production of IL-10 within the intestine (supplementary data). These data indicate that systemically administered PSA is capable of extending to mucosal sites and protecting from inflammatory bowel disease.

[0137] The data illustrated in this example also show that systemic administration of PSA during TNBS induced colitis suppresses inflammatory cytokines at both intestinal and systemic immune compartments.

Example 11

Parenteral Administration of PSA Modulates Cytokine Expression and Protects from Systemic Inflammation Caused by Th1 and Th17 Cells

[0138] Endotoxic shock occurs during severe gram-negative bacterial infections and is characterized by hypotension, multi-organ failure and potentially death. This syndrome results from the production of multiple inflammatory cytokines, including TNF-a and IL-6, in response to the lipopolysaccharides (LPS) found in the cell wall of gram negative bacteria. IL-10 has been demonstrated to be a central regulator of the inflammatory response to LPS, indeed a single dose of IL-10 prevents death in murine models of endotoxic shock[42]. The dramatic effects of PSA within the systemic immune compartments lead us to investigate whether PSA could ameliorate systemic inflammation.

[0139] To determine whether PSA was capable of suppressing inflammation associated with endotoxic shock Applicants injected Balb/c mice with a low dose (100) ug) of LPS and monitored serum levels of the cytokines TNF-α and IL-6. In particular, serum was collected from mice 1 and 4 hours post-administration of 100 μg or 500 μg of LPS and TNF-α and IL-6 protein levels in the serum were determined by ELISA.

[0140] The results illustrated in FIG. **17***a* to *c*, show that untreated mice had undetectable levels of serum TNF-α and IL-6 at both time points collected. In particular, consistent

with previous studies, in absence of PSA administration, LPS treated mice experienced an over 300 fold increase in serum TNF-α levels that peaked at one hour post injection and decreased to basal levels by four hours post injection (FIG. **17***a*). In absence of PSA treatment, also IL-6 levels in the serum of LPS injected mice was detectable as early as 1 hour and continues to increase in expression by 4 hours, (FIG. **17***b*). Remarkably, mice that had been pre-treated with PSA had a significant reduction in serum levels of TNF-α and IL-6 at both time points (FIGS. **17***a* and **17***b*), indicating that PSA is able to prevent the early induction of inflammatory cytokines in response to LPS.). Additionally, in absence of PSA treatment, splenomegaly occurs within three days of LPS injection and results from the recruitment of inflammatory cell types. Animals pre-treated with PSA, have smaller spleens and express lower levels of inflammatory cytokines at this site (data not shown and FIG. **17***c*).

[0141] This data demonstrates that PSA is capable of suppressing systemic inflammatory responses induced by a low dose administration of LPS.

Example 12

Parenteral Administration of PSA Results in TNF-a Modulation and Treatment Systemic Inflammation

[0142] Death occurring during endotoxic shock is a result of the elevated levels of inflammatory cytokines that occur within hours of the response to LPS. Indeed, blockage of the inflammatory mediator TNF-α completely rescues animals from LPS induced mortality. That PSA had such a dramatic effect on the levels of the cytokines expressed during low dose administration of LPS, suggested that PSA might prevent death associated with endotoxic shock. Applicants therefore administered high dose levels of LPS (500 that cause death with 24-96 hours and accessed both cytokine levels within the serum and monitored survival.

[0143] The results illustrated in FIGS. **17***d* and **17***e*, show that while animals that were administered PBS all die within 60 hours of administration of LPS, those animals that received PSA treatment have a significantly increased survival rate (FIG. **17***d*). Remarkably, while PBS animals have an over 3000 fold induction of TNF-α when administered LPS, those mice receiving PSA have very little TNF-a induction (FIG. **17***e*). These data demonstrate that PSA is able to suppress the systemic inflammatory response that ensues in response to LPS and is able to protect from septic shock.

[0144] As shown in the exemplary experiments of Example 7 PSA mediated protection from IBD is reliant on IL-10 production from a $CD4^+$ T lymphocyte. To determine whether IL-10 is required for protection from LPS induced death Applicants pretreated IL10 deficient animals with PBS or purified PSA and administer levels of LPS that would result in septic shock. The cytokine level and percentage survival were monitored.

[0145] The results illustrated in FIG. **18**, show that consistent with previous data IL10-deficient animals were more sensitive to lower doses of LPS and TNF alpha levels continue increase (FIG. **18***a*). Interestingly, PSA treated animals have a drastic decrease in the levels of serum TNF-a in response to LPS that drops to negligible levels by 4 hours post LPS administration (FIG. **18***a*), indicating that decreased TNF-a levels by PSA is not dependent on the ability of PSA to induce IL-10. Strikingly, decreased IL-6 production by PSA is IL-10 dependent as levels are similar to PBS treated animals, indicating multiple mechanisms are employed by PSA to alleviate endotoxic shock (FIG. **18***b*). Finally, IL10 deficient mice receiving PSA are completely protected from LPS induced death (FIG. **18***c*).

[0146] Additional experiments were performed to detect additional effects of PSA administration in connection with low dose LPS administration in mice. The results illustrated in FIG. **19** show that other effects of ZPS administration during low dose LPS administration include a reduction in CD11b and GR1 expression on the surface of neutrophils as well as reduced neutrophil recruitment in the blood.

[0147] Taken together, the data of this example and of example indicate that PSA is capable of blocking extra-intestinal disease and is expected to be a novel therapeutic agent to reduce systemic inflammation.

[0148] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the embodiments of the compounds compositions and methods of the disclosure, and are not intended to limit the scope of what the inventors regard as their disclosure. Modifications of the above-described modes for carrying out the disclosure that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the disclosure pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

[0149] The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background, Summary, Detailed Description, and Examples is hereby incorporated herein by reference.

[0150] Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties.

[0151] It is to be understood that the disclosures are not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. The term "plurality" includes two or more referents unless the content clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0152] Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the specific examples of appropriate materials and methods are described herein.

[0153] A number of embodiments of the disclosure have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the present disclosure. Accordingly, other embodiments are within the scope of the following claims.

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What is claimed is:

1. A method to balance a T-helper cell profile in an individual, the method comprising administering to the individual an effective amount of a zwitterionic polysaccharide.

2. The method of claim **1**, wherein the T-helper cell is a subset of T-helper cells, the subset consisting of at least one of Th1, Th2, Th17, and Treg.

3. The method of claim **1**, wherein the T-helper cell is a subset of T-helper cells, the subset consisting of Th17 and at least one of Th1, Th2, and Treg.

4. The method of claim **1**, wherein the T-helper cell is Th17.

5. The method of claim **1**, wherein the zwitterionic polysaccharide is a naturally occurring bacterial capsular polysaccharide.

6. The method of claim **1**, wherein the zwitterionic polysaccharide is a *B fragilis* capsular polysaccharide A (PSA) or polysaccharide B (PSB).

7. The method of claim **1**, wherein the effective amount is in a range from about 1 μg to about 100 μg of zwitterionic polysaccharide per 0.025 kilograms of body weight.

8. The method of claim **1**, wherein the effective amount is in a range from about 0.001 μg to about 1,000 μg per 0.25 kilograms of body weight.

9. A method to control an inflammation associated with an imbalance of a T-helper cell profile in an individual, the method comprising administering to the individual an effective amount of a zwitterionic polysaccharide.

10. The method of claim **9**, wherein the T-helper cell is Th17.

11. The method of claim **9**, wherein the zwitterionic polysaccharide is a *B fragilis* capsular polysaccharide A (PSA) or polysaccharide B (PSB).

12. The method of claim **9**, wherein the method is a therapeutic method for treating the cytokine mediated inflammation and the effective amount of PSA is a therapeutically effective amount of a zwitterionic polysaccharide.

13. A method to control cytokine production in an individual, the cytokine being at least one of IL-1, IL-6, TNF-a, IL-17, IL21, IL23, the method comprising administering to the individual an effective amount of a zwitterionic polysaccharide.

14. The method of claim **13**, wherein the cytokine is Il-17.

15. The method of claim **13**, wherein the zwitterionic polysaccharide is a *B fragilis* capsular polysaccharide A (PSA).

16. A method to control an inflammation associated with production of at least one of IL-1, IL-6, TNF-a, IL-17, IL21, IL23 in an individual, the method comprising administering to the individual an effective amount of a zwitterionic polysaccharide.

17. The method of claim **16**, wherein the method is a therapeutic method for treating the inflammation and the effective amount of zwitterionic polysaccharide is a therapeutically effective amount of zwitterionic polysaccharide.

18. An anti-inflammatory composition comprising a zwitterionic polysaccharide and a suitable vehicle, wherein the zwitterionic polysaccharide is comprised in an amount of from about 1 μg to about 100 μg.

19. The anti-inflammatory composition of claim **18**, wherein the zwitterionic polysaccharide is comprised in an amount of from about 0.01 μg to about 1,000 μg.

20. The anti-inflammatory composition of claim **18**, wherein the composition is a pharmaceutical composition and wherein the suitable vehicle is a pharmaceutically acceptable vehicle.

21. The anti-inflammatory composition of claim **18**, wherein the zwitterionic polysaccharide is a *B fragilis* capsular polysaccharide A (PSA) or polysaccharide B (PSB).

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