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Obesity and disease severity magnify disturbed microbiome-immune interactions in asthma patients

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In order to improve targeted therapeutic approaches for asthma patients, insights into the molecular mechanisms that differentially contribute to disease phenotypes, such as obese asthmatics or severe asthmatics, are required. Here we report immunological and microbiome alterations in obese asthmatics (n = 50, mean age = 45), non-obese asthmatics (n = 53, mean age = 40), obese non-asthmatics (n = 51, mean age = 44) and their healthy counterparts (n = 48, mean age = 39). Obesity is associated with elevated proinflammatory signatures, which are enhanced in the presence of asthma. Similarly, obesity or asthma induced changes in the composition of the microbiota, while an additive effect is observed in obese asthma patients. Asthma disease severity is negatively correlated with fecal Akkermansia muciniphila levels. Administration of A. muciniphila to murine models significantly reduces airway hyper-reactivity and airway inflammation. Changes in immunological processes and microbiota composition are accentuated in obese asthma patients due to the additive effects of both disease states, while A. muciniphila may play a non-redundant role in patients with a severe asthma phenotype.

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hanges in lifestyle, diet, weight, environment and micro-
biome have all been associated with an increased risk and
severity of chronic inflammatory disorders such as aller-
gies and asthma.12,13 However, the complex interactions between
these factors and host immunoregulatory processes are still
poorly understood at a mechanistic level in humans. In particular,
it is unclear if changes in microbiota composition contribute to
disease pathology or if these changes reflect altered immune
reactivity within host tissues.14 The microbiota contributes sig-
ificantly to host health via multiple mechanisms15. Both the
composition and metabolic activity of microbiota have profound
effects on proinflammatory activity and the induction of immune
effector functions or tolerance within mucosal tissues16–19. A
diverse microbiota contributes to a balanced homeostatic
immunological state, while microbial dysbiosis has been associ-
ated with many inflammatory diseases including asthma and
obesity20–22.

Asthma is characterized by recurrent and reversible airflow
obstruction with airway inflammation central to its pathogen-
esis.12 Several different molecular mechanisms can lead to similar
clinical outcomes, which has led to the concept of linking asthma
endotypes (an asthma subtype defined by a distinct pathophys-
iological mechanism) with asthma phenotypes13. However, to
fully understand the complex interactions between innate and
adaptive immune cells in different endotypes, additional factors
such as the microbiome, nutrition and host metabolic activity
need to be considered so that therapeutic options can be identi-
fied and dysregulated mechanisms appropriately targeted.

Since 1980, the number of obese individuals has doubled in
more than 70 countries.24 Correspondingly, the number of obese
patients with asthma has also risen dramatically.15 Obesity has
been found to be a distinguishing variable for clustering and
classifying asthma subtypes (e.g., enriched in women with adult
onset) and the obese asthmatic is more likely to become corti-
costeroid resistant, has a higher risk of being hospitalized and
more frequently presents with severe disease.16–21 Severe asthma
is defined as asthma that requires treatment with high-dose
inhaled corticosteroids combined with a second controller and/or
systemic corticosteroids to maintain control or, asthma that
remains uncontrolled despite this therapy.22 However, both obese
and non-obese asthma patients can present with severe disease
and it is currently unknown if similar or divergent factors, such as
the microbiome, might contribute to asthma severity independent
of obesity.23,24

In this study, our aim is to comprehensively characterize the
immunological and microbiota changes that occur in obese
asthma patients and to determine if these changes are related to
asthma, to obesity, or both. In addition, we determine if changes
in the microbiota associated with asthma severity are shared
between obese and non-obese asthma patients. We describe here
substantial additive effects of obesity and asthma on host
immunological responses and the microbiota. In addition, both
obese and non-obese asthma patients with severe disease have
reduced fecal levels of Akkermansia muciniphila, which may have
a causal relationship as suggested by murine models of acute and
chronic airway inflammation.

Results
Systemic inflammation is enhanced by obesity and asthma.
Patient demographic details and comorbidities are detailed in
Table 1 and Supplementary Table 1, respectively. Inflammatory
markers were measured in serum of obese asthmatics (n = 50),
non-obese asthmatics (n = 52), obese non-asthmatics (n = 50),
and their non-obese non-asthmatic healthy counterparts (n =
47). The liver acute phase proteins C-reactive protein (CRP),
serum amyloid A (SAA) and fibrinogen were significantly elev-
ated in the serum of obese non-asthma and obese asthma
patients (Fig. 1a and Supplementary Fig. 1). Similarly, proin-
flammatory cytokines and chemokines were significantly elev-
ated in the serum of both obese patient groups. Gene expression
analysis of whole blood identified differentially expressed genes
(DEGs) in obese asthmatics (n = 50), non-obese asthmatics (n =
53), obese non-asthmatics (n = 51) relative to healthy volunteers
(n = 48, Supplementary Fig. 2). Significant enrichments in on-
tologies related to inflammatory and innate immune responses
(Fig. 1b) were accentuated in obese asthmatics (n = 50), sugges-
ting an additive effect between obesity and asthma.

Airway inflammation is influenced by obesity and asthma. We
obtained bronchoalveolar lavage (BAL) fluid and bronchial
biopsies from obese asthmatics (n = 10), non-obese asthmatics
(n = 12), obese non-asthmatics (n = 11) and their non-obese
non-asthmatic healthy counterparts (n = 8). Acute phase pro-
teins, ICAM-1 and VCAM-1 levels were elevated in BALs from
obese patients (Fig. 2a and Supplementary Fig. 3). Non-obese
asthma patients (n = 12) had the highest levels of chemokines
within BAL. However, BAL IL-5 levels were significantly elev-
ated for both obese (n = 10) and non-obese asthma (n = 12) patients.
The total number of inflammatory cells (including eosinophils,
neutrophils and lymphocytes) in BAL cytopsins was significantly
different between the groups (p = 0.042, ANOVA). However,
while elevated eosinophils, neutrophils, and lymphocytes were
observed in specific obese non-asthma, non-obese asthma and
obese asthma patients, none of the inflammatory cell types alone
were statistically significantly different between the groups
(Fig. 2b). The presence of eosinophils and neutrophils was con-
firmed by H&E staining in available biopsies (Supplementary
Fig. 4).

Transcriptomic analysis of bronchial biopsies revealed a
number of genes and related pathways that were differentially
expressed in obese and asthmatic individuals. The top ten most
significant gene ontology pathway enrichments for each group are
illustrated in Supplementary Fig. 5a, while expanded heatmaps of
immunologically relevant DEGs are illustrated in Supplementary
Fig. 5b. Asthma-related gene ontology pathways were enriched
in both non-obese asthmatics (n = 12) and obese asthmatics
(n = 10), but not obese non-asthmatics (n = 11), compared to non-
obese non-asthmatic controls (n = 8, Fig. 2c). Both obese groups
displayed significant enrichments in pathways relating to airway
remodeling and inflammatory responses (Fig. 2c). In BALs, the
top ten most significant gene ontology pathway enrichments for
each group are illustrated in Supplementary Fig. 6a, while
expanded heatmaps of immunologically relevant DEGs are
illustrated in Supplementary Fig. 6b. Asthma-related gene
ontology pathway enrichments were evident in BALs from non-
obese asthmatics, while enrichments in gamma-secretase prote-
ytic targets, epithelial-to-mesenchymal transition and WNT
signaling were observed in both obese groups (Fig. 2d).

Obesity and asthma influence microbiome composition. To
investigate the relationship between obesity, asthma and the
microbiome, we performed 16S rRNA profiling of samples from
the lower gastrointestinal tract (gut, n = 202), upper gastro-
intestinal tract (oral, n = 41), lower respiratory tract (BAL, n =
41) and upper respiratory tract (nasal, n = 41). The composition
of the microbiota showed significant differences based on body
site (Fig. 3a). Specific differences at the genus taxonomic level
were observed between groups for the lower respiratory tract
(BALs, Fig. 3b), upper respiratory tract (nasal, Fig. 3c), upper
gastrointestinal tract (oral, Fig. 3d) and lower gastrointestinal


tract (fecal, Fig. 3e), after correcting for known covariates, age and gender (Supplementary Table 2). Interestingly, multiple differences were shared between the obese groups, or the asthma groups, especially within the BAL samples, suggesting that obesity and asthma have an additive effect on the microbiome alterations associated with asthma in obese individuals. Of note, the relative abundance of *Dehalobacterium* was increased for all asthma patients, independent of BMI. A random-forest classifier model of

### Table 1 Patient demographics.

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (48)</th>
<th>Non-obese asthma (53)</th>
<th>Obese non-asthma (51)</th>
<th>Obese asthma (50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (S.D.)</td>
<td>39.4 (11.9)</td>
<td>39.5 (11.3)</td>
<td>44.4 (13.5)</td>
<td>44.6 (11.6)</td>
<td>0.078</td>
</tr>
<tr>
<td>BMI (S.D.)</td>
<td>22.2 (1.4)</td>
<td>22.9 (1.5)</td>
<td>35.6 (4.6)</td>
<td>34.6 (5.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male/female</td>
<td>19/29</td>
<td>22/31</td>
<td>18/33</td>
<td>17/33</td>
<td>0.817</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>18 (38)</td>
<td>8 (15)</td>
<td>20 (40)</td>
<td>9 (18)</td>
<td>0.011</td>
</tr>
<tr>
<td>Alcohol U/week (S.D.)</td>
<td>2.3 (22)</td>
<td>2.5 (3.5)</td>
<td>1.8 (2.4)</td>
<td>1.4 (2.0)</td>
<td>0.141</td>
</tr>
<tr>
<td>FEV1% (S.D.)</td>
<td>–</td>
<td>81.7 (15.5)</td>
<td>–</td>
<td>79.8 (17.3)</td>
<td>0.619</td>
</tr>
<tr>
<td>Allergy (%)</td>
<td>–</td>
<td>40 (75)</td>
<td>–</td>
<td>36 (72)</td>
<td>0.823</td>
</tr>
<tr>
<td>Daily ICS (%)</td>
<td>–</td>
<td>38 (72)</td>
<td>–</td>
<td>40 (80)</td>
<td>0.365</td>
</tr>
<tr>
<td>Daily ICS dose (S.D.)*</td>
<td>–</td>
<td>678 (585)</td>
<td>–</td>
<td>946 (710)</td>
<td>0.054</td>
</tr>
<tr>
<td>Oral steroids (%)</td>
<td>2 (4)</td>
<td>8 (16)</td>
<td>–</td>
<td>8 (16)</td>
<td>0.048</td>
</tr>
<tr>
<td>SABA (%)</td>
<td>–</td>
<td>22 (42)</td>
<td>–</td>
<td>26 (52)</td>
<td>0.327</td>
</tr>
<tr>
<td>LABA (%)</td>
<td>–</td>
<td>33 (62)</td>
<td>–</td>
<td>31 (62)</td>
<td>1.000</td>
</tr>
<tr>
<td>Daily LABA dose (S.D.)*</td>
<td>–</td>
<td>62 (56)</td>
<td>–</td>
<td>86 (78)</td>
<td>0.098</td>
</tr>
<tr>
<td>Male/female</td>
<td>–</td>
<td>32 (60)</td>
<td>–</td>
<td>30 (60)</td>
<td>1.000</td>
</tr>
<tr>
<td>Leukotriene receptor antagonist (%)</td>
<td>–</td>
<td>27 (51)</td>
<td>–</td>
<td>27 (54)</td>
<td>0.844</td>
</tr>
<tr>
<td>Daytime asthma symptoms &gt;2 times (%)</td>
<td>–</td>
<td>10 (19)</td>
<td>–</td>
<td>18 (36)</td>
<td>0.076</td>
</tr>
<tr>
<td>Exercise/activity limited due to asthma (%)</td>
<td>–</td>
<td>18 (34)</td>
<td>–</td>
<td>25 (50)</td>
<td>0.113</td>
</tr>
<tr>
<td>Waking at night due to asthma symptoms (%)</td>
<td>–</td>
<td>12 (23)</td>
<td>–</td>
<td>21 (42)</td>
<td>0.056</td>
</tr>
<tr>
<td>Use of rescue medications &gt;2 times (%)</td>
<td>–</td>
<td>14 (26)</td>
<td>–</td>
<td>12 (24)</td>
<td>0.824</td>
</tr>
</tbody>
</table>

*Note: Dose of inhaled glucocorticosteroids calculated as a budesonide equivalent microgram/day

### Fig. 1 Blood inflammatory profiles. a Fold change of cytokine levels in serum for obese non-asthma (n = 50), obese asthma (n = 50) and non-obese asthma (n = 52) patients relative to the non-obese non-asthmatic group (n = 47) are illustrated. All comparisons with a P < 0.05 (ANOVA with post hoc Tukey-kramer test) are labeled with asterisks. b Gene ontology pathway enrichments in whole blood for obese non-asthma (n = 51), obese asthma (n = 50) and non-obese asthma (n = 53) patients relative to the non-obese non-asthmatic group (n = 48) are illustrated using both the proportion of the pathway or ontology gene set that overlapped with the differentially expressed genes in that comparison and the FDR-corrected p-values as a label next to the bar. Source data are provided as a Source Data file.
Fig. 2 Lung inflammatory profiles. **a** Fold change of cytokine levels in bronchoalveolar lavages (BALs) for obese non-asthma \((n = 11)\), obese asthma \((n = 10)\) and non-obese asthma \((n = 12)\) patients relative to the non-obese non-asthmatic group \((n = 7)\) are illustrated. All comparisons with a \(P < 0.05\) (ANOVA with post hoc Tukey–Kramer test) are labeled with asterisks.

**b** Total inflammatory cell numbers and differential cell counts in BAL cytospins are illustrated (mean \(+/-\) standard error). Gene ontology enrichments in asthma-related pathways or airway remodeling/inflammation-related pathways in **c** lung biopsy and **d** BALs for each group (Non-obese asthma \((n = 12)\); Obese non-asthma \((n = 11)\); Obese asthma \((n = 10)\) compared to non-obese and non-asthma volunteers \((n = 8)\), are illustrated using both the proportion of the pathway or ontology gene set that overlapped with the differentially expressed genes in that comparison and the FDR-corrected \(p\)-values as a label next to the bar. Source data are provided as a Source Data file.
Fig. 3 Microbiota changes associated with obesity and asthma. a Distinct body sites show significantly different microbiota compositions ($P < 0.001$, PERMANOVA, Pseudo F-statistic $= 41.42$), independent of obesity or asthma ($n = 41$, all groups combined). Fold change of genera relative abundances in bronchoalveolar lavages (b, $n = 41$), nasal swabs (c, $n = 41$) and oral washes (d, $n = 41$) for non-obese asthma ($n = 12$), obese non-asthma ($n = 11$), obese asthma ($n = 10$) relative to the non-obese non-asthmatic group ($n = 8$) are illustrated. e Fold change of genera relative abundances in fecal samples for non-obese asthma ($n = 53$), obese non-asthma ($n = 51$), obese asthma ($n = 50$) relative to the non-obese non-asthmatic group ($n = 48$) are illustrated. All comparisons with a $P < 0.05$ (ANOVA with post hoc Tukey-kramer test) are labeled with asterisks.
the fecal microbiota had a reasonable ability to distinguish obesity (AUC = 0.76), but little predictive value for asthma (AUC = 0.53). No significant differences were observed for alpha diversity measures between the groups, except for the obese asthmatics who had a significantly increased Shannon diversity within the nose (Supplementary Fig. 7).

**Microbiome correlates with markers of inflammation.** Within the gut microbiota, three distinct bacterial enterotypes were evident, as already described by others. A Bacteroides-dominated enterotype (E1, Bacteroides:Prevotella ratio > 2.0), a Prevotella-dominated enterotype (E2, Bacteroides: Prevotella ratio < 0.50) and a mixed enterotype (Emix) were observed (Fig. 4a). Patients with a *Prevotella*-rich gut enterotype (E2, *n* = 39) displayed increased serum levels of the chemokines CCL22, CCL13, CCL17, and CXCL10 compared to individuals with an E1 (*n* = 116) or Emix (*n* = 37) enterotype (Fig. 4b). In addition, several serum biomarkers were significantly correlated (*p* < 0.01, Pearson’s test) with the levels of specific gut microbes in both obese (Supplementary Fig. 8a) and asthma patients (Supplementary Fig. 8b).

Among BAL samples (*n* = 40), significant correlations were observed between inflammatory cell numbers and microbiome composition (Supplementary Table 3). Of note, increased eosinophils in asthma patients, regardless of their BMI, was associated with an increased relative abundance of the genera *Rothia*, *Dorea*, *Lautropia*, and *Haemophilus*. We explored two major variables, smoking status and inhaled corticosteroids, for their effect on oral, nasal, and BAL microbiome, and observed no statistically significant differences between these groups in the microbiome composition at each airway site (PERMANOVA *p* > 0.05 for each). In contrast, significant associations (*p* < 0.01, Pearson’s test) between BAL cytokine levels with individual microbial genera were observed in obese patients (Supplementary Fig. 9a) and asthma patients (Supplementary Fig. 9b). In addition, focusing only on the taxa that were shown to be significantly decreased within the asthma BALs (Fig. 3b), their combined relative abundance inversely correlated with BAL IL-5, IFN-γ, and IL-15 levels (*n* = 40, Fig. 4c).

**Akkermansia muciniphila decreases in severe asthma patients.** In addition to the effects of obesity on the gut microbiota of asthma patients, we evaluated if asthma severity could also influence the composition of the gut microbiota. In patients with severe asthma (*n* = 41), a significant reduction in the family *Verrucomicrobiaceae* was observed, compared to patients with mild/moderate asthma (*n* = 53, Fig. 5a). The most common species within this family is *Akkermansia muciniphila*, which showed a significant decrease (*p* = 0.0041) in its relative abundance in severe asthma patients (Supplementary Fig. 10). The reduced level of *A. muciniphila* was specifically associated with severe asthma as the relative abundance of *A. muciniphila* was not significantly different between the asthma or obese groups (non-obese non-asthmatic 0.046 +/- 0.082, non-obese asthmatics 0.035 +/- 0.069, obese non-asthmatics 0.026 +/- 0.028, obese asthmatics 0.046 +/- 0.076, mean plus/minus (+/-) standard deviation, *p* > 0.05 ANOVA). Both obese and non-obese asthmatics with severe disease (*n* = 22 and *n* = 19, respectively) showed a significant decrease in *A. muciniphila* compared to obese and non-obese patients with mild/moderate asthma (*n* = 23 and *n* = 30, respectively, Fig. 5b). Using qPCR primers specific to *A. muciniphila*, we observed a strong concordance with the relative abundance data (r2 = 0.762) and we confirmed the reduced levels in *Akkermansia* associated with severe disease (Fig. 5c). In asthma patients, we also observed a negative correlation between *A. muciniphila* and circulating CRP levels (Fig. 5d). These data suggest that lower levels of *A. muciniphila* are associated with a higher risk of severe asthma symptoms.

**A. muciniphila reduces airway inflammation in animal models.** In order to determine if any of the microbial changes described above might play a causal role in influencing respiratory inflammation, we selected *A. muciniphila* to test further in murine models of allergen-induced respiratory airway disease. Oral administration of *A. muciniphila* induced a marked reduction in BAL eosinophil numbers in female animals sensitized and challenged with ovalbumin (OVA), compared to animals that received OVA alone (*n* = 5 mice per group, Fig. 6c). In addition, IL-4 and IL-5 secretion from OVA-stimulated isolated lung cells was reduced in animals treated with *A. muciniphila* (Fig. 6c). Heat killed *A. muciniphila* or cell free supernatants from *A. muciniphila* cultures did not reduce OVA-induced eosinophils or cytokine levels (Fig. 6c). *A. muciniphila* administration, but not heat killed *A. muciniphila* or its supernatant, was associated with an altered lymphocyte profile within lung tissue as the percentage of IL-4 and IFN-γ positive CD4 T cells were reduced, while IL-10 + Foxp3 + double positive lymphocytes were increased (*n* = 5 mice per group, Fig. 6d and Supplementary Fig. 11). *A. muciniphila* was equally effective in reducing BAL inflammatory cell numbers in male mice (*n* = 6 per group, Fig. 6e). Airway hyper-reactivity in response to methacholine was significantly reduced in animals administered *A. muciniphila* (*n* = 8–9 mice per group, Fig. 6f). Oral administration of *A. muciniphila* significantly reduced the number of BAL inflammatory cells in the acute house dust mite (HDM) extract challenge model (*n* = 7–8 mice per group, Fig. 6g). *A. muciniphila* was equally effective in reducing BAL inflammatory cell numbers in MyD88−/− animals (*n* = 5 mice per group, Fig. 6h). *A. muciniphila* levels in fecal samples increased 1000–10,000 fold in exposed mice, while *A. muciniphila* was not detected in the BALs from exposed animals (*n* = 3 mice per group at each time point, Fig. 6i).

The influence of *A. muciniphila* on airway inflammation was also assessed in a chronic model of HDM exposure, in which persistent airway inflammation was initiated prior to administration of *A. muciniphila*. Administration of *A. muciniphila* reduced the number of all innate and adaptive cell types examined within the BAL, suggesting that *A. muciniphila* accelerated the resolution of airway inflammation following cessation of HDM exposure (*n* = 6 mice per group, Fig. 7b and Supplementary Fig. 12). In additional groups of animals, mice chronically exposed to HDM were allowed to recover in the absence of HDM and were then re-challenged with a single high dose of HDM extract. A significant influx of eosinophils was observed in the BAL within 24 h post re-challenge (*n* = 6–8 mice per group at each time point, Fig. 7d). However, re-challenged animals that received *A. muciniphila* during the resolution phase displayed a significantly reduced eosinophil response (Fig. 7d). Of note was the reduction in infiltration of a sub-set of eosinophils expressing high levels of Sialic acid-binding immunoglobulin-type lectin-F (Siglec-FHI) (*n* = 6 mice per group, Fig. 7e). Representative dot-plots illustrating the presence of Siglec-FHI eosinophils in the lung are illustrated in Fig. 7f.

**Discussion**

In this study we describe immunological and microbiome alterations that are associated with obesity and asthma. In addition, we clearly identify an obese asthma phenotype that shares immunological and microbiome features of both obesity and asthma. Furthermore, we discovered that asthma severity was associated with reduced levels of *A. muciniphila* in the gut, which may be clinically relevant as this bacterium was protective in...
Fig. 4 Microbial community composition correlates with cytokine levels. a A Bacteroides-dominated enterotype, a Prevotella-dominated enterotype and a mixed enterotype were observed in 60%, 21%, and 19%, respectively of volunteer fecal samples (n = 202). Average abundances of the other dominant bacterial families (not Bacteroides or Prevotella) did not differ significantly between enterotypes. b Serum CCL22, CCL13, CCL17, and CXCL10 are significantly higher (ANOVA and Tukey correction) for individuals with a Prevotella-dominated gut microbiota (E2, n = 39), compared to individuals with a Bacteroides-dominated gut microbiota (E1, n = 116) or a mixed microbiota (Emix, n = 37). Box plots show median and whiskers represent 10–90 percentiles. c The combined relative abundance for taxa reduced in the asthmatic lung (Butyricimonas, Moraxella, Propionibacterium, Pasteurellaceae, Campylobacter, Faecalibacterium, Bacteroides, Parabacteroides, Mitsuokella, Megaplasma, Ruminococcaceae, Paraprevotella, Phascolarctobacterium, Roseburia, Enterococcus, Bifidobacterium, Aeromonadaceae, and Coriobacteriaceae) negatively correlated with BAL IL-5, IFN-γ, and IL-15 levels (n = 40, all groups combined) using linear regression analysis. Source data are provided as a Source Data file.
multiple murine models of both acute and chronic respiratory airway disease.

The combination of obesity and asthma had significant effects on host inflammatory and transcriptomic responses. This was clearly observed in the peripheral blood transcriptome, where enrichments in ontologies related to inflammatory and innate immune responses were accentuated in obese asthmatics, suggesting an additive effect between obesity and asthma. Within BALs and lung biopsies, non-obese asthma patients showed enrichments for TH2 and asthma-related ontologies, while obese non-asthma patients showed enrichments for tissue-remodeling related and inflammation-related ontologies. Obese asthmatics displayed enrichments in tissue remodeling-related, inflammation-related and TH2-related ontologies. However, the quantitative additive effect observed for specific ontology enrichments in peripheral blood was not observed for BALs or lung biopsies, rather the obese asthmatic displayed qualitative additive effects in gene ontologies representing both asthma and obesity signatures within the lung. This difference between systemic versus pulmonary-specific responses may reflect the contribution of multiple organs (e.g., liver and adipose tissue) to systemic inflammatory gene responses.

Previous studies have demonstrated that obesity is associated with an altered gut microbiota. Our data extends these findings in showing that the composition of the microbiota changes in both the obese and asthma states relative to healthy individuals, not only in gut and lung, but also in the nose and mouth. This suggests that the microbiota can be altered at sites distant to the diseased organ, perhaps due to the influence of diet, inflammation or medications. Certain microbiota changes in the obese asthmatic were shared by obese individuals or by patients with non-obese asthma. Thus, the additive effect of having asthma and being obese exaggerates the microbial changes present in these patients. In obese asthmatics, atopic status is likely to also play a role in better phenotyping this patient group, particularly their response to treatments such as weight loss. However, the majority of obese asthmatics described in this cohort are atopic and therefore it was not possible to further separate microbiome-host effects associated with atopic status in obese asthmatics. One additional finding is the identification of

Fig. 5 A. muciniphila is negatively correlated with severe asthma. a Relative abundances at the Family taxonomic level within the fecal samples of asthma patients with mild/moderate (n = 53) or severe (n = 41) disease are illustrated. Asterisk (*) denotes a P < 0.05 ANOVA with post hoc Tukey-kramer test. b Relative abundance of the genus Akkermansia is significantly decreased in non-obese severe (n = 19) compared to non-obese mild/moderate (n = 30) disease and obese severe (n = 22) compared to obese mild/moderate (n = 23) disease (Mann-Whitney test). c Absolute qPCR quantification of Akkermansia muciniphila in severe (n = 28) versus mild/moderate (n = 38) disease (Mann-Whitney test). d Decreased levels of Akkermansia muciniphila is associated (ANOVA with post hoc Tukey-kramer test) with increased levels of C-reactive protein (CRP). Box plots show median and whiskers represent 10–90 percentiles. Source data are provided as a Source Data file.
Dehalobacterium in obese and non-obese asthma patients. Dehalobacterium is a strictly anaerobic dichloromethane-degrading bacterium. Dichloromethane present in the environment is the result of industrial emissions and increased levels of Dehalobacterium may therefore indirectly indicate greater exposure to solvents containing this compound. However, further work is required to determine if an increase in this organism has any influence on host immune responses. Significant associations were observed between the composition of the microbiota and host inflammatory responses, reinforcing the hypothesis that there is a close relationship between immune regulatory mechanisms and the microbes that reside within us. For example, the highest serum chemokine levels were observed in those with a Prevotella-dominated gut microbiota. This association was observed regardless of obesity or asthma status, suggesting a causal relationship. In addition,
the close relationship between BAL microbiota composition and GM-CSF levels in asthma patients is intriguing as GM-CSF has been shown in murine models to influence lung TH2-associated sensitization. 

*A. muciniphila* is a mucin-degrading organism and has been previously associated with protective effects in obesity models. However, we found that *A. muciniphila* was reduced in asthma patients with severe disease, regardless of their BMI. In addition, the experimental models were performed using lean mice, suggesting an influence on lung inflammatory responses independent of its effects on obesity-associated inflammation. While all inflammatory cells were reduced in the murine inflamed lungs, a surprising finding was the significant reduction in eosinophils expressing high levels of Siglec F. These eosinophils are IL-5-dependent and have been shown to facilitate skewing towards a TH2 response. Thus, the inhibitory effect of *A. muciniphila* on this eosinophil population may have clinical relevance given the established relationship between IL-5 levels, eosinophils and asthma exacerbations. The *A. muciniphila* mechanism of action is not MyD88-dependent, but may involve other pattern recognition receptors that are MyD88 independent or previously described mechanisms such as improvement of gut barrier integrity or release of metabolites such as nicotinamide that can have systemic effects. The increase in IL-10+Foxp3+ lymphocytes may also mediate anti-inflammatory effects within the lung and *A. muciniphila* administration has previously been shown to increase Tregs in other murine models. In addition, heat killed *A. muciniphila* was not effective suggesting that either heat sensitive factors or viable bacteria are required for this protective effect and any metabolites secreted in vitro were not sufficient to reduce airway inflammation, at least for the concentrations tested.

An interesting finding of our study is that obese non-asthmatic patients show inflammatory changes within the lung. In addition, it is not only the gut microbiota, but also the microbiota of the mouth, nose, and lung that are altered in obese non-asthmatics. Obese individuals have a higher risk of developing asthma compared to lean individuals and these immune and microbiome changes may play a part in the increased susceptibility of obese individuals in the development of asthma. Indeed, some of these changes may represent pre-asthma features. For example, elevated BAL ICAM-1 and VCAM-1 levels in obese individuals are suggestive of significant vascular injury within the lung, while the increased level of *Moraxella* may be clinically relevant and should be investigated further.

In conclusion, the microbiome and host immune responses are intimately connected at multiple body sites and can be influenced by obesity, asthma and asthma disease severity. The presence of two diseases, obesity and asthma, are additive and contribute to exaggerated inflammatory and microbiota changes, suggesting that there’s a need to address obesity during asthma management.

While the obese asthma patient obviously needs to be treated differently than a non-obese asthma patient, severe asthma may benefit from similar microbiota interventions (*i.e.*, *A. muciniphila*), regardless of BMI. However, many of the other changes in microbiome composition that we identified in this study may also be biologically relevant and will need to be investigated in future studies for their relative contributions to the stratification and selection of patients for specific therapies.

**Methods**

**Patient groups.** A total of 202 volunteers were recruited for this study—obese asthma (n = 50), obese non-asthma (n = 51), non-obese asthma (n = 53), and non-obese non-asthma healthy controls (n = 48). Asthma patients had a physician diagnosis of asthma. Severe asthma was defined according to the American Thoracic Society (ATS) guidelines. Obesity was defined as having a body mass index (BMI) greater than 30 kg/m². Non-obese individuals had a BMI of 20–25 kg/m². All relevant ethical regulations for human participants were complied with, and informed consent was obtained from all participants. The human biological samples were sourced ethically and their research use was in accordance with the terms of the informed consents. Patients were recruited under informed consent at two centers, ALL-MED Medical Research Institute, Wroclaw, Poland and the Pulmonary Division, University Hospital of Zurich, Switzerland. Ethical approval was granted at both sites from the local ethical committee for all study procedures.

**Measurement of inflammatory mediators and cells.** Serum was obtained by allowing blood collection tubes (with no anti-coagulant) to rest at room temperature for 1 h and then tubes were centrifuged at 800g for 10 min. Serum was removed, aliquoted in 500 μl quantities and stored at −80 °C for later analysis. BAL was obtained and filtered through a 70 μm filter into sterile tubes. Aliquots of 500 μl were stored at −80 °C for later analysis. BAL cells were centrifuged onto slides and following air drying were stained using the Diff-Quik stain or remained unstained. Fixed slides were stored at −20 °C and differential cell counts were performed by two independent histopathologists. All soluble mediators were measured using the mesoscale discovery platform (MDS) kits according to manufacturer’s instructions. Data were analyzed using one-way ANOVA (correcting for gender) and Tukey’s correction.

**Gene expression analysis.** Peripheral blood was collected in Paxgene tubes and immediately frozen at −80 °C until later analysis. Total mRNA was extracted from peripheral blood using Qiagen RNeasy kit (Qiagen, Valencia, CA) and quantified by NanoDrop (Thermo Fisher Scientific, Waltham, MA). RNA integrity was confirmed using the Agilent 2100 BioAnalyzer (Agilent, Palo Alto, CA). Samples were normalized to the lowest concentration sample, and cDNA was made using Superscript Vilo cDNA synthesis Master Mix (Invitrogen Life Technologies, Grand Island, NY). The cDNA samples were then labeled with biotin with the FL-Ovation cDNA Biotin Module V2 and hybridized to a Human Genome U133 Plus 2.0 Array using a Genechip Hybridization Kit. The microarray chips were washed and stained using a Genechip Hybridization Wash and Stain Kit and then scanned using a Genechip Scanner. All reagents and readers were used according to the manufacturer’s instructions. The microarray gene expression data were analyzed using ArrayStudio 7.0 (OmicSoft, Cary, NC). Data from CEL files from Human Genome U133 Plus 2.0
**Fig. 7** *A. muciniphila* protects against airway inflammation in a chronic HDM murine model. **a** The timings for allergen and bacterial administration in the chronic HDM model assessing effects on disease resolution are illustrated. **b** Administration of *A. muciniphila* (HDM + Akk) reduced the number of eosinophils (CD45+CD11c−CD11b+MCHIIloCD24+Siglec−F+), neutrophils (CD45+CD11c−CD11b+MHCII+CD24−Ly6-G+), monocytes (CD45+CD11c−CD11b+CD20−MHCII+CD64+Ly6-C+), CD8 T cells (CD45+TCRβ+CD8+), B cells (CD45+CD19+), NK cells (CD45+CD49b+), and CD4 T cell subsets (CD45+TCRβ+CD4+CD62L+−/−CD196−/−) following chronic exposure to HDM (n = 6 per group). **c** The timings for allergen and bacterial administration in the chronic HDM model assessing effects on high dose allergen challenge following disease resolution are illustrated. **d** HDM re-challenged animals that received *A. muciniphila* during the resolution phase displayed a reduced eosinophil response at 24 h (n = 5 mice HDM group, n = 8 mice HDM + Akk group) and 7 days (n = 6 mice HDM group, n = 6 mice HDM + Akk group) following HDM rechallenge, especially for the Sialic acid-binding immunoglobulin-type lectin-F high (Siglec-Fhi) eosinophils (n = 6 per group). **f** Representative dot-plots illustrating the presence of Siglec-Fhi eosinophils in the lung. Statistical significance for two group comparisons were estimated using Mann-Whitney tests and results are shown as mean +/−SE. Source data are provided as a Source Data file.
Affymetrix chips were normalized using robust multiarray averaging and scaled to a mean target intensity of 150. Affymetrix image files were analyzed using the Genome Workbench: hierarchical cluster analysis of microarrays (Ward’s method). Significance at a 0.01 level was determined using the Bonferroni correction.

Animal models. Both acute and chronic models of airway inflammation were performed. Female and male wild-type and Mydd8−/− BALB/c mice aged 6–8 weeks were obtained from Charles River (Sulzfeld, Germany) and housed at AO Research Institute Davos for the acute ovalbumin (OVA) and acute house dust mite (HDM) models. Mice were housed 4–6 animals per cage in individually ventilated and simultaneously cycling 12:12 h light/dark cycles under conditions of controlled temperature at 22 °C and relative humidity at 50%. Food and water were ad libitum. All experimental procedures were carried out in accordance with Swiss law and ethical approval was obtained from Amt für Lebensmittelsicherheit und Tiergesundheit, Chur. Female wild-type BALB/c mice for the chronic HDM model were bred and housed at GlaxoSmithKline (GSK), Stevenage. Animal studies conducted at GSK were carried out under the UK Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals. All studies were performed using littermates.

Either ovalbumin (OVA) or house dust mite (HDM) extract were used in acute models of airway inflammation. In the OVA model, mice were sensitized by intraperitoneal (i.p.) injection of 20 µg of OVA grade VI (Sigma-Aldrich, Buchs, Switzerland) emulsified in 500 µg Alum (Pierce, Rockford, IL, USA) in 200 µl sterile 0.9% isotonic Sodium chloride (NaCl) on days 0, 7, and 21, followed by 20 min i.n. OVA grade V (Sigma-Aldrich, Buchs, Switzerland) aerosol exposures on days 26, 27, and 28. Negative control animals received NaCl and alum injections and were exposed to the OVA aerosolization. Analysis of mice occurred 24 h after the last aerosol challenge. In the acute HDM model, HDM extract (Greer labs, USA) was administered intra-nasally (i.n.) on day 0 (1 µg), followed by higher dose i.n. administrations on days 7, 8, 9, 10, and 11 (10 µg each day). Negative control animals received saline i.n. on the same days as the respective treatment groups. Mice received HDM extracts i.n. Animals were euthanized on day 12 for analysis. In both acute models, A. muciniphila and media control was administered daily (1 × 106 cells per dose) by oral gavage, beginning at day 5 until the end of the study. A. muciniphila was pre-grown in fresh cultures every day in 10 ml aliquots of anaerobic Mucin v3 media (10% inoculation) at 37 °C, and 80 °C until analysis. Pre-procedure bronchoscope wash sample (while of low read count) aligned predominantly (84%) to the families lower biomass starting material, they were subjected to additional quality control for 1 min.

Microbiota analysis. Fecal samples were collected by volunteers at their home, immediately chilled using ice-packs and delivered to the lab within 4 h. Upon receipt in the lab, fecal samples were separated into 1 g aliquots and stored at −80 °C until analysis. Pre-procedure bronchoscope wash fluid (10 ml), BAL (10 ml), and nasal aspirates (5–12 ml) were centrifuged (22,500 g) at 4 °C for 10 min (Hermle Z 231 M microcentrifuge; Hermle Labortechnik GmbH, Wehingen, Germany) in dolphin-nosed eppendorf tubes and the pellets stored at −80 °C until the time of DNA extraction. Nasal swabs of the middle meatus were stored in 1 ml of DPBS before the time of DNA extraction. DNA was isolated from stool, BAL, and nasal aspirates using the Qiagen PowerFecal DNA Kit (Qiagen Sciences, Germantown, MD). DNA was quantitated using Quant-iT PicoGreen dsDNA reagent (Cat # Q3427; Invitrogen, Eugene, OR).

Both acute and chronic models of airway inflammation were performed. A. muciniphila was heat killed by boiling at 100 °C for 15 min. Cell free supernatants were generated by filtering the culture broth, following A. muciniphila culture at 37 °C, through a 0.22 µm syringe filter (Sarlstedt). Two hundred microliter of the filtered supernatants were orally gavaged daily to the mice. Fecal and BAL levels of A. muciniphila were determined using PCR. Identical primers (i.e., AM1 5′-GTGTCAGCAGCGCCGGGAGTAAA-3′ and 800′-5′-GGGCTACHTGGGTGTTCAAT-3′) containing the illumina sequencing adapters and a 12 bp error-correcting Golay barcode sequence48,49. Each 50 µl PCR was conducted with primers, 515f (5′-GTGTCAGCAGCGCCGGGAGTAAA-3′), 800′-5′-GGGCTACHTGGGTGTTCAAT-3′) according to the manufacturer

Flow cytometry. Cells present in the broncho-alveolar lavage fluid (BALF) and lung tissue were quantified using multi-color flow cytometry. Analysis of myeloid and lymphoid subsets was performed on a FACSCanto II machine (Becton Dickinson) running FACSDiva software version 8. Data analysis was performed using Flowjo (Flowjo). Red blood cells were removed using a 1/10 dilution in sterile distilled water of 10× Test solution (Beckman Coulter, UK) for 5 min at room temperature. The

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lysing solution was then washed twice by centrifugation at 370×g for 5 min at 4 °C. Supernatants were discarded and cell pellets were resuspended in 20 μl Fc—Block™ to block non-specific Fcy binding sites for 10 min at 4 °C. Without washing off Fc—Block™, cells were stained with 50 μl surface marker antibody cocktails for 10 min at 4 °C in the dark after which cells were washed twice to remove any unbound antibodies. Cell pellets were then resuspended in 200 μl of FACS buffer (PBS containing 1% Fetal Calf Serum). For intracellular cytokine staining, freshly deposited in the NCBI Sequence Read Archive under accession number PRJNA434133.

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Author contributions


Competing interests

D.M., D.M., S.U., S.Y.H., K.A.S., K.D.S., J.R.B., and E.M.H. are full-time employees of GSK and hold company stock. L.O’M. has consulted for Alimentary Health Ltd. and has received research funding from GSK. C.A.A. has received research support from Novartis and Stallergenes and consulted for Actelion, Aventis, and Allergopharma. M.J. is a consultant to Allergopharma, G.E.R, Anergis, CH, Biomay and received lecture fees from GSK, Allergopharma, Stallergens.

Additional information

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