Supplementary Information

Supplementary Methods

Sequence processing and taxonomic assignment

After filtering the sequences in MOTHUR, reads with ambiguous base calls, average quality scores below 25 and homopolymers longer than 10 were eliminated. Bacterial V1-V2 regions of the 16S rRNA gene were verified and extracted using V-XTRACTOR 2.0 [1]. High quality sequences were queried against GREENGENES [2] using a naïve Bayesian classifier implemented in MOTHUR and a minimum bootstrap support of 60%. Similar sequences were clustered into operational taxonomic units (OTUs) using an unsupervised Bayesian clustering approach implemented in CROP [3] and the default threshold for species level. The consensus taxonomy of each OTU was determined using MOTHUR, as the taxonomic path represented by at least 51% of the sequences within the OTU. Abundance of OTUs at specific taxonomic ranks, species, genus, family, order, class, and phylum was determined and used to generate rank-specific matrices permitting downstream analyses of indicators at each taxonomic rank.

Multivariate analysis of community structures and diversity

Data were standardized by dividing the number of reads in each taxonomic unit by the total number of reads in each sample. A resemblance matrix was calculated using Bray-Curtis similarities [4] based on the standardized data. Principal Coordinate Analysis (PCO, [5]) implemented in the PRIMER6+ was used to display similarities in microbial community structures among all fecal and ileal samples. Tests of the multivariate null hypotheses of no differences among *a priori* defined groups were examined using

permutational multivariate analysis of variance (PERMANOVA, [6]). PERMANOVA was performed with 9,999 permutations and run in the PRIMER6+ software package. Residuals of raw data were permuted under a reduced model and partitioning was performed using Type III sums of squares. Pseudo-F values were reported to indicate the effects strength (F>1 indicates an effect) and P(Perm) values were reported to indicate the permutation-based p-value, which is the accurate value based on the 9,999 permutations run as described above. Bray Curtis similarity indices were reported to describe the average pairwise distance between the treatment groups analyzed. PCO ordination was plotted with STATISTICA 8.0 (StatSoft, Tulsa, OK). Simpson's diversity index [7] was used to calculate community diversity between treatment groups.

Identification of bacterial indicators

Taxa-treatment association analysis was based on the point biserial correlation coefficient [8] and used to determine the association strength (R) of each taxonomic unit with *a priori* groups. The analysis was based on fecal data obtained for each group (control, vancomycin and streptomycin-treated). This analysis was performed at each taxonomic level, from phylum to individual OTU. All possible combinations of *a priori* groups were analyzed to account for different niche breadths of the taxonomic units [9]. Correlation analysis was performed on each taxonomic unit using the diagnostic species analysis routine implemented in GINKGO [10] with 999,999 permutations. False discovery rate (FDR) correction was used to re-calculate these probabilities and draw experiment-wide conclusions [11]. Based on the P value distribution, Q values were determined using the

software QVALITY [12] and associations were considered significant with an FDR of 5% ($q < 0.05$).

Supplementary references

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Supplementary Figure Legends

Fig S1. Adult antibiotic treatment has no effect on allergic asthma. (**A**) Eosinophil numbers in the bronchoalveolar lavage (BAL) fluid of control or antibiotic-treated mice challenged with ovalbumin (OVA) or PBS. (**B**) Serum OVA-specific IgE responses measured by ELISA. (**C**) Total pathological scores and representative H&E stained lung sections. Scale bar, 300μm. All assessments were made on day 26. The data are shown as means of 5-7 mice per group±SEM and represent two independent experiments. Statistics shown are based on comparisons to OVA-challenged controls. The antibiotic-treated animals were treated with antibiotics at 7 weeks of age. Vanco, vancomycin; Strep, streptomycin; n.s.= not significant, n.d.= none detected.

Fig S2. Antibiotic treatment alone has no effect on lung histopathology. Total pathological scores and representative H&E stained lung sections. Scale bar, 300μm. All assessments were made on day 26. The data are shown as means of 4 mice per group±SEM and represent two independent experiments. Statistics shown are based on comparisons to PBS-treated controls. The antibiotic-treated animals were treated using the neonatal antibiotic regime. Vanco, vancomycin; Strep, streptomycin.

Fig S3. Intestinal communities shift differently after neonatal and adult antibiotic treatments. Bacterial communities from feces of OVA-challenged mice treated with antibiotics neonatally or only as adult were compared using principal coordinate analysis (PCO). Corresponding OVA-challenged controls for each experiment are also shown. Vanco, vancomycin; Strep, streptomycin.

Supplementary Data Sets

Data Set S1. Representative sequences from OTUs that were classified within a Greengenes OTU identified in Table 1. Sequences that could not be matched to a Greengenes OTU (gg_OTU) and thus remain "unclassified" in Table 1 have been listed under the headings corresponding to the closest-related taxon identified.

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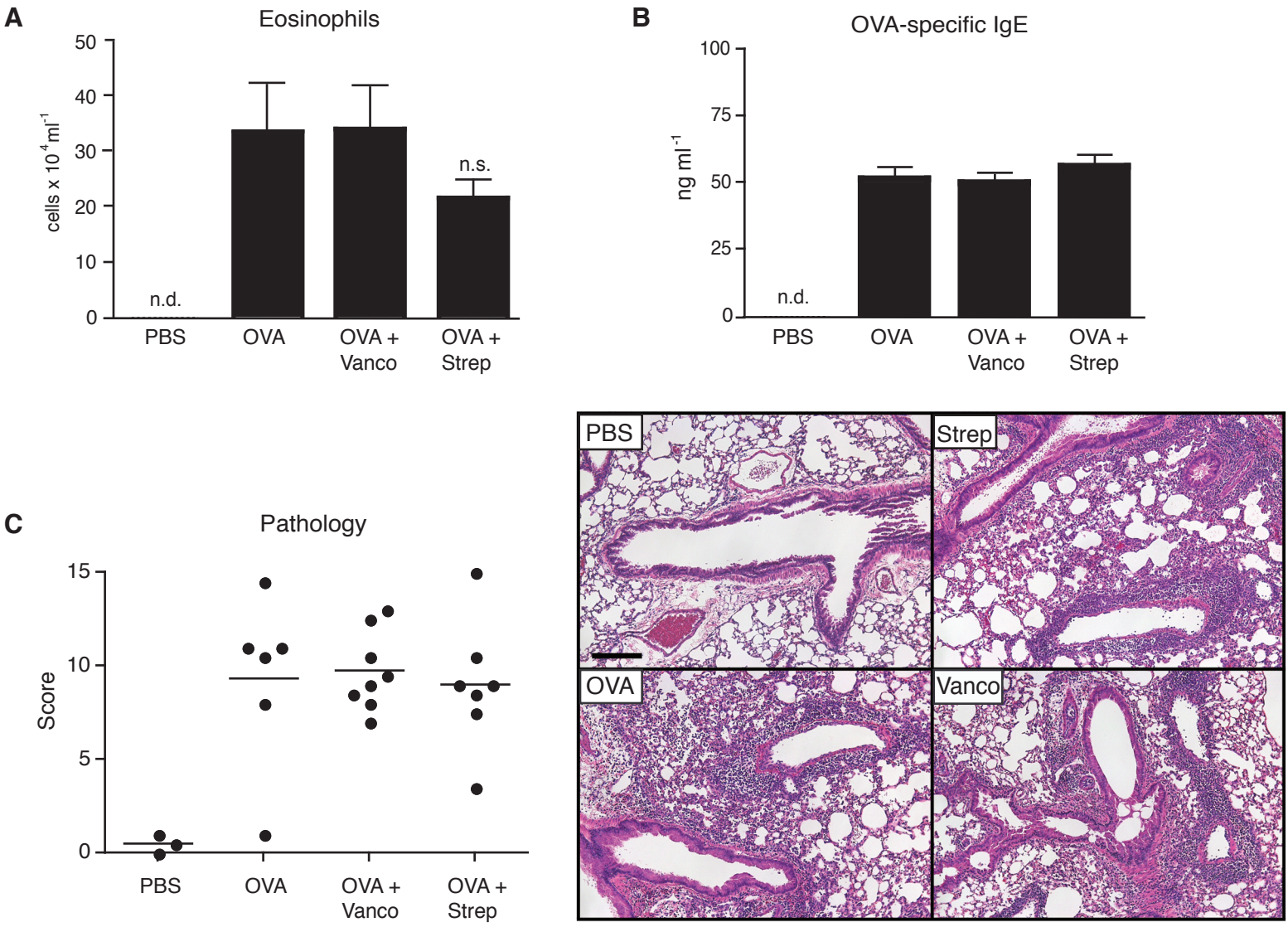
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Eosinophils
cells x 10^4 ml^{-1}
50
40
30
20
10
0
n.d.
n.s.
PBS
OVA
OVA + Vanco
OVA + Strep
B
OVA-specific IgE
ng ml^{-1}
100
75
50
25
0
n.d.
PBS
OVA
OVA + Vanco
OVA + Strep
C
Pathology
Score
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10
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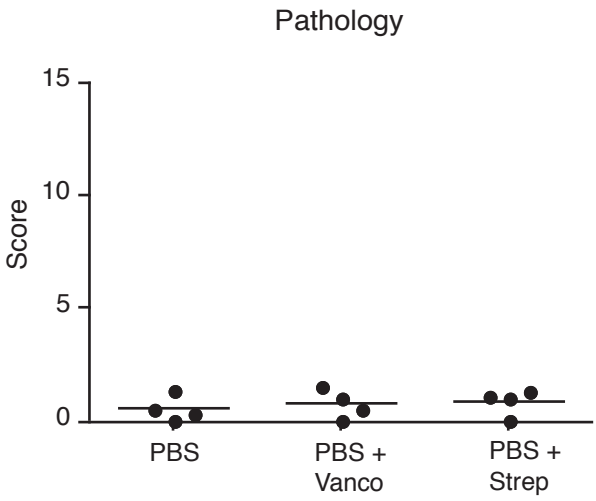
Pathology
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PBS +
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PBS +
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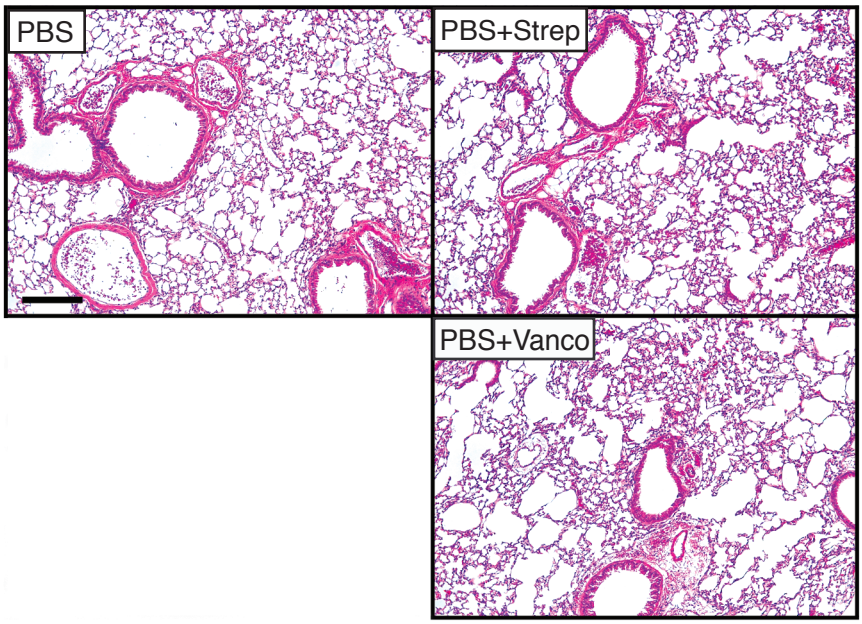
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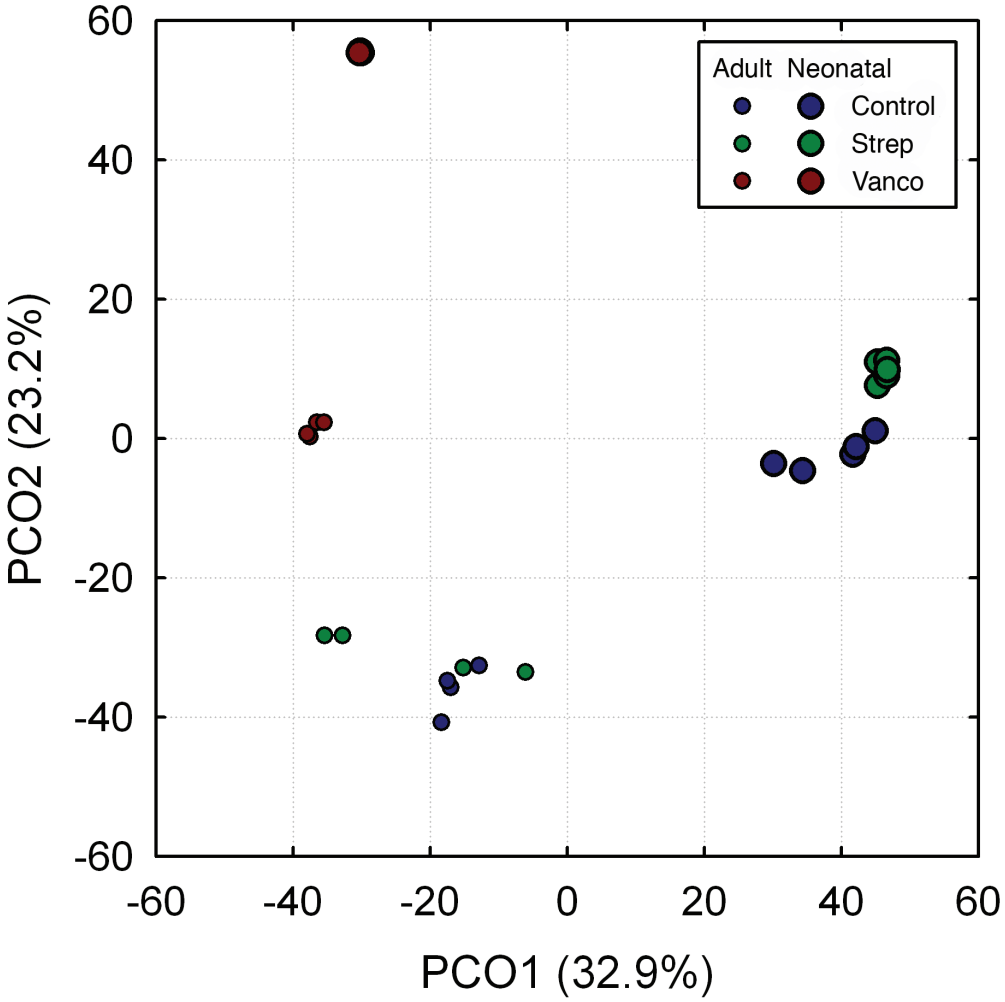
Adult Neonatal
Control
Strep
Vanco
PCO2 (23.2%)
PCO1 (32.9%)
60
40
20
0
-20
-40
-60
-60
-40
-20
0
20
40
60