*Main questions: What genes regulate eosinophil recruitment to the airway following an allergen challenge? What is the source of the recruitment: BAL, BE, or both?*

Table 1. Patient demographics

|  |  |  |  |
| --- | --- | --- | --- |
|  | Pre SBP-Ag | Post SBP-Ag | P-value\* |
| N | 26 | 26 |  |
| Age (years) | 26 ± 6.2 |  |  |
| Sex (% female) | 55 |  |  |
| Race (%)  White  Black  Mixed | 81.8  9.1  9.1 |  |  |
| FEV1 screening WLAC | 2.5 ± 0.46 |  |  |
| FEV1 PP (%) | 91 ± 11 | 91 ± 9.8 | 0.90 |
| FeNO | 46 ± 29 | 66 ± 41 | 4.5E-5 |
| BAL cell % \*\*  Eosinophil  Monocyte  Neutrophil  Lymphocyte  Epithelial | 0.68 ± 0.69  83 ± 9.2  2.1 ± 2.4  12 ± 6.9  2.5 ± 2.9 | 33 ± 29  46 ± 28  8.3 ± 12  13 ± 5.2  0.14 ± 0.35 | 9.1E-5  5.9E-6  2.0E-2  0.39  1.3E-3 |

Mean ± standard deviation \*Paired t-test \*\*BH corrected P-value across 5 cell types

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Description automatically generated with medium confidence

Figure X. Eosinophils and neutrophils associate with allergen challenge and lung function. (A) Eosinophil and neutrophil **percentages** increase with allergen challenge. (B) Eosinophils are positively correlated with FeNO both pre and post SBP-Ag while (C) neutrophils do not correlate with FeNO.

*Note: Significance holds for lgo2(FeNO). Data not shown here.*

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Description automatically generated with medium confidence

Figure X. Eosinophils and neutrophils associated with allergen challenge and lung function. (A) Eosinophil and neutrophil **cells per mL** increase with allergen challenge. (B) Eosinophils are positively correlated with FeNO both pre and post SBP-Ag while (C) neutrophils do not correlate with FeNO.

*Note: Trends hold for log2(FeNO) but pre SBP-Ag eosinophils are no longer significant (P = 0.054 vs 0.029)*

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Supplemental (?) Figure X. Pearson correlation of BAL cell percentages pre and post SBP-Ag. Eosinophils and neutrophils are not significantly correlated. Monocytes are negatively correlated with all other cell types pre allergen challenge and their inverse association with eosinophils strengthens post allergen challenge. \* P < 0.1 \*\*P < 0.01

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Supplemental (?) Figure X. (A) Eosinophils and (B) neutrophils do not correlate with FEV1.

A diagram of different types of data

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Supplemental Figure X. Global changes in gene expression in BAL and BE in response to SBP-Ag challenge.

Table X. Differentially expressed genes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample |  | Variable | FDR < | DEGs |
| BAL |  | SBP-Ag | 0.3 | 6863 |
| SBP-Ag and | Eosinophils | 0.1 | 2257 |
| Neutrophils | 0.1 | 8 |
| FeNO | 0.1 | 113 |
| BE |  | SBP-Ag | 0.3 | 2076 |
| SBP-Ag and | Eosinophils | 0.1 | 45 |
| Neutrophils | 0.1 | 0 |
| FeNO | 0.1 | 20 |

*Note: log2(FeNO) did not improve model fit. Thus, we should keep the original model results.*

*Note: No SBP-Ag significant genes were also significant for FEV1 (numeric or low/high at 87% cutoff)*

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Eosinophil** and FeNO association | DEGs FDR < 0.1 | DEGs FDR < 0.3 |
| BAL | Increase | 72 | ~~840~~ |
| BE | Increase | 5 | 58 |
| BAL | Decrease | 37 | ~~732~~ |
| BE | Decrease | 9 | 85 |

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Figure X. Post SBP-Ag gene expression associated with eosinophils and FeNO. BAL and BE genes significantly differentially expressed for SBP-Ag (FDR < 0.3) as well as eosinophil percentages and FeNO (**FDR < 0.1**). (A) Pearson’s correlation of genes associated with increased eosinophil recruitment and increased FeNO. (B) Pearson’s correlation of genes associated with decreased eosinophil recruitment and decreased FeNO.

A diagram of a string diagram

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Figure X. STRING network of eosinophil and FeNO associated genes (FDR < 0.1)

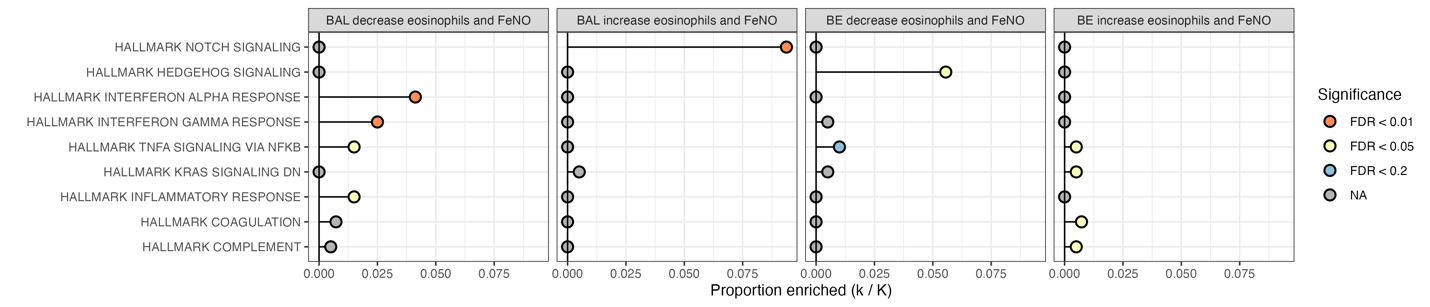


Figure X. Enrichment of eosinophil and FeNO associated genes. FDR < 0.1 and overlap > 1 in at least one group

GO FDR < 0.2, overlap>1

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BE increase eosinophils and FeNO

Ras protein signal transduction

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|  |  |  |  |
| --- | --- | --- | --- |
|  | **Neutrophil** and FeNO association | DEGs FDR < 0.1 | DEGs FDR < 0.3 |
| BAL | Increase |  |  |
| BE | Increase |  |  |
| BAL | Decrease |  |  |
| BE | Decrease |  |  |

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~~Supplemental (?) Figure X. Post SBP-Ag gene expression associated with neutrophils and/or FeNO. BAL and BE genes significantly differentially expressed for SBP-Ag (FDR < 0.3) as well as neutrophil percentages and/or FeNO (FDR < 0.1). (A) Pearson’s correlation of genes associated with increased neutrophil recruitment and/or increased FeNO. (B) Pearson’s correlation of genes associated with decreased neutrophil recruitment and/or decreased FeNO.~~

*Note that unlike the eos heatmaps, the neutrophil heatmaps show genes significant for neutrophils only, FeNO only, or both. Only BE genes negatively associated with both neutrophils and FeNO (columns in blue heatmap)*

**Higher BE FDR cutoff DEGs**

Table X. Differentially expressed genes (**higher FDR**)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample |  | Variable | FDR < | DEGs |
| BAL |  | SBP-Ag | 0.3 | 6863 |
| SBP-Ag and | Eosinophils | 0.3 | 3546 |
| Neutrophils | 0.3 | 23 |
| FeNO | 0.3 | 1758 |
| BE |  | SBP-Ag | 0.3 | 2076 |
| SBP-Ag and | Eosinophils | 0.3 | 229 |
| Neutrophils | 0.3 | 0 |
| FeNO | 0.3 | 193 |

A screen shot of a chart

Description automatically generated

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Description automatically generated with medium confidence

Figure X. Post SBP-Ag gene expression associated with eosinophils and FeNO. BAL and BE genes significantly differentially expressed for SBP-Ag (FDR < 0.3) as well as eosinophil percentages and FeNO (**FDR < 0.1 in BAL, FDR < 0.3 in BE**). (A) Pearson’s correlation of genes associated with increased eosinophil recruitment and increased FeNO. (B) Pearson’s correlation of genes associated with decreased eosinophil recruitment and decreased FeNO.

A diagram of a network

Description automatically generated with medium confidence

Figure X. STRING network of eosinophil and FeNO associated genes (**FDR < 0.1 for BAL, FDR < 0.3 for BE**)