Supplementary appendix

Clinical study design

28 atopic volunteers with mild-to-moderate asthma and 11 non-atopic healthy controls were recruited to a previously reported human rhinovirus infection study investigating the pathogenesis of rhinovirus-induced asthma exacerbations(1). The volunteers gave no recent history of a viral illness and had no serum-neutralizing antibodies to rhinovirus(RV)-16 at screening. Volunteers with asthma were excluded if they had severe asthma, a recent asthma exacerbation, or current symptoms of allergic rhinitis.

The clinical characteristics of the participants have been previously described but because bronchial epithelial cell samples for the present study were only available for a subset (25 out of 28 subjects with asthma; all 11 healthy controls), those are shown in Table S1.

Participants were inoculated with RV-16 at a dose of 100 $TCID_{50}$ (tissue culture infective dose 50%) diluted in 250µl of 0.9% saline, administered via an atomizer into both nostrils. All were successfully infected, as confirmed by positive virus copies in nasal lavage and/or seroconversion. Concomitant infection with another virus was excluded by the absence of symptoms, confirmed by the clinical team at enrolment when the baseline bronchoscopy samples were taken, and by PCR on bronchoalveolar lavage sampled at the same time as the bronchial epithelial cells. Nasal lavage was furthermore tested by PCR immediately prior to rhinovirus inoculation.

Symptom scores and home spirometry were recorded daily in a diary card, with assessments and sampling taking place in the clinic at the timepoints shown in

Figure S1. Bronchoscopies were performed using an Olympus PF260 bronchoscope. Subjects were administered 2.5mg nebulised salbutamol pre-procedure and sedated with intravenous midazolam. Intubation was via the mouth not the nose. Bronchial brushings were performed from the right middle and lower lobe sub-segmental bronchi to collect bronchial epithelial cells using disposable cytology brushes (Olympus, Japan). Brushes were immediately transferred to pre-warmed culture medium where they were mixed vigorously to dislodge the cells from the brush prior to pelleting the cells via centrifugation. Bronchoalveolar lavage (BAL) was performed by instillation of 150 mL sterile normal saline in five 30 mL aliquots with a 10 second dwell time into the right upper lobe bronchus; the returned lavage fluid was subsequently pelleted via centrifugation.

Table S1 Baseline characteristics of study volunteers with Bronchial Epithelial Cell (BEC) samples

Characteristics	Healthy (n=11)	Asthma (n=25)	*P*
Age, years[1]	31 ± 12	35 ± 11	NS
Sex Female, n (%) Male, n (%)	 4 (36%) 7 (74%)	 14 (56%) 11 (44%)	NS
Baseline FEV_1, % predicted	104 ± 8	86 ± 12	<0.0001
Baseline histamine PC_{20}, mg/ml	>16	1.11 ± 1.69	-
ICS use, n (%)	-	14 (56%)	-
IgE, IU/ml; median (IQR)	16 (14–19)	121 (68–623)	<0.0001

BAL fluid eosinophilia, %; median (IQR)	0 (0)	0.5 (0–1.7)	0.001

BAL = bronchoalveolar lavage; ICS = inhaled corticosteroid; IQR = interquartile range; NS = not significant.
Data shown are mean ± standard deviation unless otherwise stated. Parametric data compared by unpaired t test, non-parametric by Mann-Whitney.

Table S2 Results of gene set enrichment analysis

Gene set (Hallmark)	Number of genes	Enrichment score (ES)	Normalized ES	Nominal P value	False discovery rate Q value	Familywise-error rate P value	Rank at maximal ES	Leading edge
Allograft rejection	199	0.666	1.685	0.036	0.258	0.131	3252	tags=58%, list=14%, signal=67%
IL6 JAK STAT3 signaling	87	0.707	1.632	0.018	0.219	0.176	2078	tags=48%, list=9%, signal=53%
Interferon γ response	200	0.849	1.628	0.004	0.152	0.181	1954	tags=78%, list=9%, signal=84%
Inflammatory response	199	0.673	1.625	0.024	0.117	0.184	2384	tags=50%, list=10%, signal=55%
Interferon α response	97	0.906	1.580	0.012	0.145	0.240	1427	tags=89%, list=6%, signal=94%
Complement	200	0.577	1.575	0.035	0.125	0.242	4088	tags=49%, list=18%, signal=59%
Apoptosis	161	0.504	1.571	0.044	0.111	0.249	2402	tags=32%, list=10%, signal=35%
G2M checkpoint	198	0.465	1.569	0.051	0.100	0.252	3061	tags=33%, list=13%, signal=38%
IL2 STAT5 signaling	197	0.503	1.560	0.041	0.097	0.267	2885	tags=37%, list=13%, signal=42%
PI3K AKT MTOR signaling	104	0.451	1.540	0.087	0.103	0.290	5469	tags=44%, list=24%, signal=58%
TNF α signaling via NFkB	198	0.624	1.530	0.072	0.104	0.312	3294	tags=50%, list=14%, signal=58%
KRAS signaling up	197	0.467	1.448	0.073	0.184	0.470	3909	tags=39%, list=17%, signal=46%
Apical junction	198	0.357	1.420	0.070	0.204	0.527	3218	tags=27%, list=14%, signal=31%
Coagulation	138	0.379	1.418	0.064	0.191	0.530	4812	tags=36%, list=21%, signal=45%
Apical surface	44	0.385	1.361	0.077	0.246	0.626	4323	tags=32%, list=19%, signal=39%
Estrogen response late	200	0.379	1.351	0.091	0.246	0.645	3954	tags=31%, list=17%, signal=37%
UV response up	158	0.364	1.328	0.162	0.258	0.677	3336	tags=27%, list=15%, signal=32%
MTORC1 signaling	200	0.453	1.327	0.219	0.245	0.679	4351	tags=40%, list=19%, signal=49%
Reactive oxygen species pathway	49	0.434	1.291	0.185	0.277	0.729	3335	tags=39%, list=15%, signal=45%
Angiogenesis	36	0.410	1.278	0.140	0.281	0.746	3853	tags=39%, list=17%, signal=47%
E2F targets	200	0.370	1.224	0.281	0.335	0.816	3423	tags=29%, list=15%, signal=34%
P53 pathway	199	0.356	1.213	0.235	0.337	0.833	3086	tags=28%, list=13%, signal=32%
Hypoxia	200	0.330	1.199	0.212	0.341	0.851	3723	tags=29%, list=16%, signal=34%
TGF β signaling	54	0.360	1.197	0.263	0.330	0.853	5717	tags=37%, list=25%, signal=49%
Epithelial mesenchymal transition	200	0.303	1.124	0.278	0.411	0.910	2827	tags=24%, list=12%, signal=27%
Glycolysis	200	0.318	1.106	0.319	0.419	0.924	4421	tags=33%, list=19%, signal=41%
MYC targets V2	58	0.346	1.081	0.412	0.440	0.941	3318	tags=26%, list=14%, signal=30%
Notch signaling	32	0.351	1.064	0.398	0.448	0.949	1327	tags=22%, list=6%, signal=23%
Androgen response	100	0.357	1.049	0.400	0.454	0.953	2436	tags=26%, list=11%, signal=29%
Estrogen response early	200	0.287	1.037	0.413	0.457	0.965	3908	tags=29%, list=17%, signal=35%
Mitotic spindle	199	0.292	1.017	0.448	0.468	0.973	3742	tags=25%, list=16%, signal=29%
MYC targets V1	199	0.331	0.918	0.555	0.593	0.992	4434	tags=28%, list=19%, signal=35%
Unfolded protein response	112	0.307	0.897	0.549	0.604	0.994	2736	tags=21%, list=12%, signal=23%
Myogenesis	200	0.187	0.821	0.824	0.697	0.998	2391	tags=13%, list=10%, signal=14%
Cholesterol homeostasis	74	0.282	0.796	0.716	0.712	0.998	2164	tags=20%, list=9%, signal=22%
Heme metabolism	198	0.207	0.770	0.818	0.728	0.999	4347	tags=23%, list=19%, signal=28%
Oxidative phosphorylation	200	0.223	0.588	0.823	0.911	1.000	5599	tags=29%, list=24%, signal=39%

Highlighted rows indicate gene sets with a false discovery rate <25%, which is the estimated probability that a gene set (rather than a specific gene) with a given normalised enrichment score (ES) represents a false positive finding.

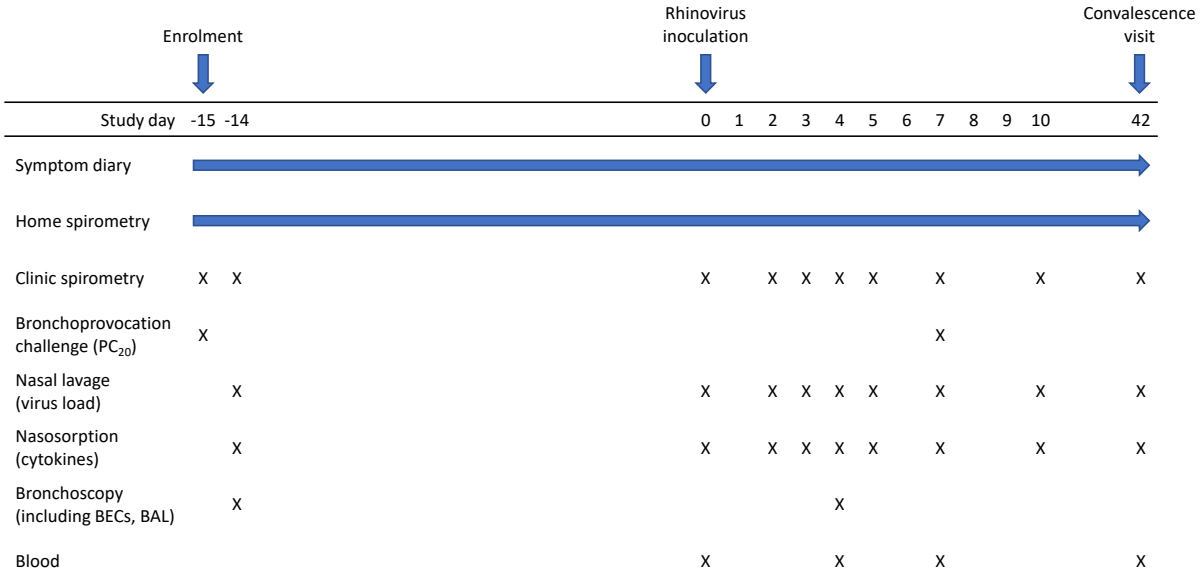


Figure S1: Study design and timing of assessments

REFERENCES

1. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J, et al. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. American journal of respiratory and critical care medicine. 2014;190(12):1373-82.