Supporting Information

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SI Text

Methods

Histamine Challenge. For bronchodilator reversibility, sputum induction and histamine challenge subjects used a Vitalograph Dry Wedge Bellows Spirometer. Histamine challenge (maximum concentration 32 mg/ml) was performed according to guidelines by using the 2-min tidal breathing method (1). The asthmatic group was required to have a PC_{20} <8 mg/ml, and the normal group had >8 mg/ml. The provocative concentration of histamine causing a 20% reduction in FEV_1 (PC_{20}) was calculated (1), and as eight normal subjects did not achieve a 20% reduction, a PC_{10} was also calculated for both groups.

Skin Prick Testing and Serum IgE. Atopy was determined by skin prick testing to common aeroallergens: six grass pollen mix; house dust mite; cat; dog; *Aspergillus fumigatus*; *Cladosporium herbarum*; *Alternaria alternata*; and birch, three tree and nettle pollen (ALK Abello). One wheal 3 mm greater than negative control or total IgE >110 units/ml was considered diagnostic of atopy.

Diary Cards for Symptom Scores and Spirometry Recording. Symptom assessment was by daily diary cards, for 2 weeks before, during, and 6 weeks after infection. The daily cold score was summated from individual scores (sneezing, headache, malaise, chilliness, nasal discharge, nasal obstruction, sore throat, cough, fever) graded 0 (absent) to 3 (severe). A clinical cold was defined as reported (2). The daily chest score was calculated from symptom scores (cough on waking; wheeze on waking; daytime cough; daytime wheeze; daytime shortness of breath; nocturnal cough, wheeze or shortness of breath), also graded 0–3. The same diary cards recorded medication usage and home spirometry (microDL; MicroMedical) recording the best of three recordings of PEF and FEV_1.

Nasal Lavage. A total of 2.5 ml of sterile normal saline was instilled into each nostril and fluid was collected into a sterile Petri dish, then aliquoted for storage at −80°C.

Peripheral Blood Analyses. Blood was collected at baseline, day 0, day 3, day 4, day 7, and 6 weeks. Serum was prepared and aliquoted for storage at −80°C. Blood taken at baseline, day 4, day 7, and 6 weeks was analyzed by flow cytometry; 50 ml in heparinized tubes was diluted 1:1 with PBS then layered over lymphoprep. After centrifugation mononuclear cells were washed, counted and assessed for viability (0.1% trypan blue) by hemocytometer.

Bronchoscopy. Bronchoscopies were performed (3) in the endoscopy unit at St Mary's Hospital by using a Keymed P100 bronchoscope. BAL was performed by instillation of sterile normal saline in 8 × 30-ml aliquots with a 10-s dwell time. At baseline and 6 weeks BAL was obtained from the medial segment and at day 4 from the lateral segment of the right middle lobe. The BAL fluid was centrifuged and the BAL cell pellet was used for cytospin preparations for differential cell counting. BAL cells were processed and analyzed by flow cytometry as for PBMCs.

Sputum Induction. Sputum was induced by 4 × 5-min inhalations of 4.5% saline from a DeVilbiss UltraNeb99 (4). Sputum plugs were selected, weighed, and homogenized by using 0.1% DTT (5) filtered and centrifuged. The cell pellet was washed, total cell count was determined, and cytospins were prepared for differential counts (% nonsquamous epithelial cells). Four subjects (two asthmatic, two normal) were excluded from analysis because of squamous epithelial cell counts >20% of total cells.

Virologic Confirmation of RV16 Infection. RV16 infection was confirmed by at least one of the following: positive nasal lavage standard or qPCR for RV, positive culture of RV16, or seroconversion defined as a titer of serum neutralizing antibodies to RV16 of at least 1:4 at 6 weeks.

Serology was performed at screening, day 0, and 6 weeks postinfection by microneutralization test for neutralizing antibody to RV16 (6). Virus was cultured at 37°C (and if negative repeated at 33°C) by adding 250 μl of nasal lavage (from the day of peak virus load by qPCR) to semiconfluent HeLa cells that were cultured for up to five passages. Cultured virus was confirmed as RV16 by microneutralization assay with RV16-specific antisera (ATCC; titer 1:600) (6).

RNA was extracted from samples (QIAamp viral RNA mini kit; Qiagen) and reverse-transcribed (omniscript RT kit, Qiagen) with random hexamers. Standard RV PCR (PerkinElmer 9600 GeneAmp) was performed from 2 μl of cDNA (7, 8).

qPCR was performed on 2 μl of cDNA to detect picornavirus in nasal lavage, an unprocessed plug of induced sputum, and unprocessed BAL, using AmplitaqGold DNA polymerase (PE Biosystems ABI Prism 7700) (9). A standard curve was produced by using serially diluted cloned product and results expressed as copies/ml. The sensitivity of this assay was 10^4 copies/ml.

PCR for Additional Respiratory Viruses. Infection with viruses other than RV was excluded by testing nasal lavage by PCR on random hexamer primed cDNA for *Mycoplasma* and *Chlamydia pneumoniae*, adenoviruses, respiratory syncytial virus, influenza AH1/AH3/B, parainfluenza 1–3, human metapnuemoviruses (HMPV), and coronaviruses 229E and OC43 as described (10), except HMPV, which was adapted from (11) and had a sensitivity of 5 $TCID_{50}$.

Analysis of Clinical Symptom Scores and Lung Function. Symptoms and lung function were analyzed in 2-week blocks as the baseline and acute infection stages both contained bronchoscopy on the fourth day of each 2-week block (Fig. S3*b*). Daily symptom scores during infection, corrected for baseline symptoms and the effects of bronchoscopy, were calculated by subtracting scores obtained during the baseline 2-week block from the corresponding days of the acute infection 2-week block. Total cold and total chest scores were calculated by summing the corrected daily scores for the 2-week infection period. Peak symptom scores were the maximum corrected daily scores in the 2-week block after infection. The % change in morning FEV_1 and PEF during infection was calculated for each subject as the % fall from the mean obtained during a separate 2-week screening period before baseline sampling. These were also corrected for the effects of bronchoscopy by subtracting the % changes from the same screening mean observed on the corresponding days in the 2-week baseline block. Maximal percent falls were the maximum corrected % fall in the 2-week block after infection.

Differential Cell Counting. Induced sputum and BAL cells were resuspended (2 × 10^5 cells/ml) in PBS and cytospin slides were

prepared (Shandon cytospin; 400 rpm, 5 min), stained (Shandon Diffquick), and coded, and 500 cells were counted and expressed as % nonsquamous epithelial cells. Absolute counts were generated from the differential and total counts.

Flow Cytometry for Lymphocyte Surface Marker Expression and Intracellular Cytokine Staining. Fresh PBMCs obtained by centrifugation on Lymphoprep and BAL cells were stained with conjugated antibodies (BD PharMingen) and analyzed for lymphocyte surface marker expression by three- and four-color flow cytometry (BD LSR flow cytometer) as described (12) to assess T cells ($CD3^+$), CD4 T cells ($CD3^+CD4^+CD8^-$), CD8 T cells ($CD3^+CD8^+CD4^-$), NK cells (CD3-CD16/56^+), B cells ($CD3^-CD19^+$). Intracellular type 1 (IFN-γ) and type 2 (IL-4, IL-5, IL-13) cytokines and IL-10 were determined in $CD4^+$ T cells as described (12). Analysis was performed on at least 10,000 lymphocyte events by using Cellquest and Winlist software.

BAL Cell Cultures. Cells from the BAL obtained at baseline bronchoscopy were washed and resuspended in RPMI medium 1640 with Glutamax (Life Technologies) containing 10% FCS and antibiotics (penicillin, streptomycin), at a final concentration of 2×10^6/ml. Cells were incubated with medium alone, 5 MOI rhinovirus-16, 0.1 μg/ml of *E. coli* 026:B6 LPS (Sigma), or 1 μg/ml PHA (Sigma). After 48 h supernatants were harvested and stored at -80°C for analysis for IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-17 and IFN-γ levels by using Luminex xMap assays (Antibody Bead Kit; BioSource International).

Statistical Analysis. Symptom scores and lung function were corrected for differences at baseline and effects of bronchoscopy (*SI Text*). Except where otherwise stated, data are presented as median and interquartile range. Differences during infection from baseline and convalescence were analyzed by using Friedman's test and, if significant, Wilcoxon tests. Differences between normal and asthmatic groups were analyzed by using Mann—Whitney tests and correlations by using Spearman's rank correlation. Results of stimulation of BAL cells were analyzed within groups by paired and between groups by unpaired parametric or nonparametric tests as determined by data distribution.

Results

Confirmation of RV16 Infection. All subjects were seronegative (neutralizing antibody titer <1:2) for RV16 at screening and on repeat serology performed on day 0 before inoculation and all subjects were negative to the PCR panel for common respiratory viruses in nasal lavage at baseline. For subjects 8 and 33, nasal lavage qPCR was positive for picornavirus but virus was not cultured and confirmed as RV16, and serologic responses increased from absent to 1:2 at 6 weeks. For subjects 16, 31, and 32 virus could not be cultured but these subjects all had positive serologic responses (Table 2).

RV was detected at 6 weeks in two subjects: subject 33 had clinical cold symptoms at 4 weeks and RV was also detected in nasal lavage taken at this time; subject 49 was asymptomatic. These two subjects were excluded from convalescent analyses. No additional respiratory viruses were detected by PCR panel in nasal lavage in any subject during the cold or at convalescence.

The relationship between peak nasal lavage and the titer of serum-neutralizing antibody to RV16 at 6 weeks postinoculation was examined. There was a highly significant positive correlation ($r = 0.628$, $P = 0.004$) implying that more severe infection with a higher virus load is associated with a stronger serum-neutralizing antibody response.

BAL Lymphocyte Subtypes During Infection in Asthmatic and Normal Subjects. At day 4 all lymphocyte subpopulations were more numerous in the asthmatic group for all subtypes; however, these increases were not statistically significant: CD4 T cells [9.45 (5.94,19.2) for asthmatic vs. 6.62 (3.90,13.1) for normal, $P = 0.179$], CD8 T cells [11.1 (5.33,14.8) vs. 4.02 (1.99,7.76) $P = 0.062$], B cells [0.282 (0.162, 0.503) vs 0.193 (0.098, 0.470) $P = 0.19$], and NK cells [1.59 (0.687,2.92) vs. 0.941 (0.392,1.64) $P = 0.126$].

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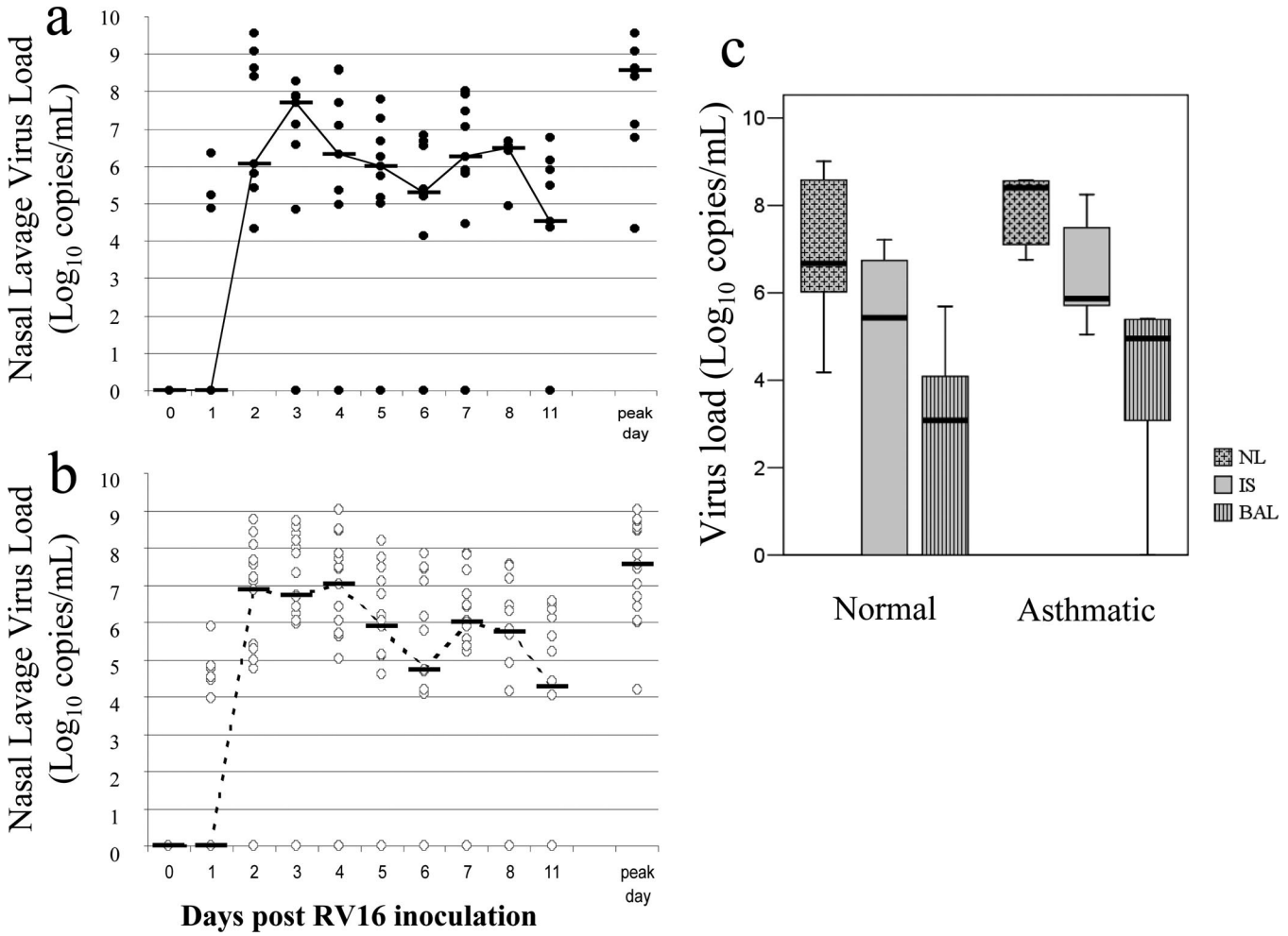

Fig. S1. Rhinovirus virus loads in the upper and lower respiratory tract after RV16 inoculation. (*a* and *b*) Rhinovirus virus load (Log_{10} copies/ml) was determined by qPCR during experimental RV16 infection of asthmatic ([circf[) and normal (○) subjects in upper [nasal lavage (NL)] and lower [induced sputum (IS) and BAL] respiratory tract samples. NL was obtained on days 1–8 and 11 after inoculation with RV16 on day 0. BAL was obtained on day 4; IS was obtained on days 3 and 7. The time course of virus loads in NL were similar in the asthmatic (*a*) and normal (*b*) groups. Individual data points are shown, the solid bar representing the median virus load on each day. Virus load was undetectable in most subjects on day 1 but increased rapidly from day 2 to peak around day 2–4, consistent with viral replication in the respiratory tract. (*c*) Median virus load was between ≈0.5–2 Logs greater in the asthmatic group in all three airway samples, but these differences were not statistically significant: NL, median 8.56 (IQR 6.93, 8.84) Logs/ml vs. 7.43 (6.43, 8.58) $P = 0.30$; IS, 5.79 (4.03, 7.61) vs. 5.14 (0, 6.74) $P = 0.33$; BAL, 4.96 (1.54, 5.40) vs. 3.08 (0, 4.26) $P = 0.29$.

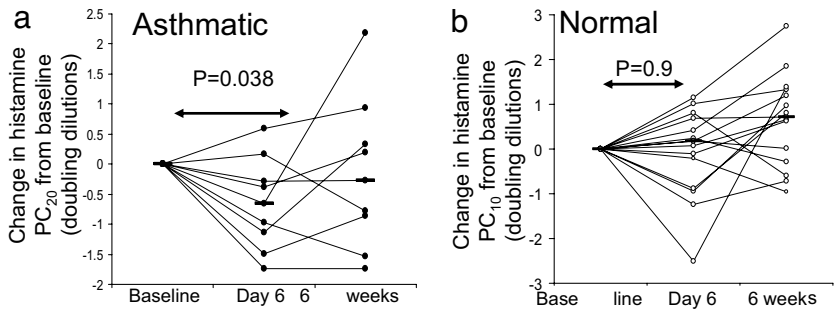

Fig. S2. Changes in bronchial reactivity during RV infection. Histamine PC_{10} and PC_{20} (in asthmatics only) were measured at baseline, day 6, and convalescence. (*a*) In the asthmatic group (filled circles), there were significant falls in both PC_{20} and PC_{10} (data not shown). (*b*) In constrast, in the normal group (open circles), there was no change in histamine PC_{10} after inoculation.

a

Day	-14	0	1	2	3	4	5	6	7	8	11	42
Nasal lavage	X	X	X	X	X	X	X	X	X	X	X	X
Serology	X	X										X
PBMCs	X				X	X			X			X
BAL	X					X						X
Induced sputum	X				X				X			X
Rhinovirus inoculation		X										
Histamine challenge	X							X				X
Symptom diaries	→	→	→	→	→	→	→	→	→	→	→	→
Home Spirometry	→	→	→	→	→	→	→	→	→	→	→	→

b

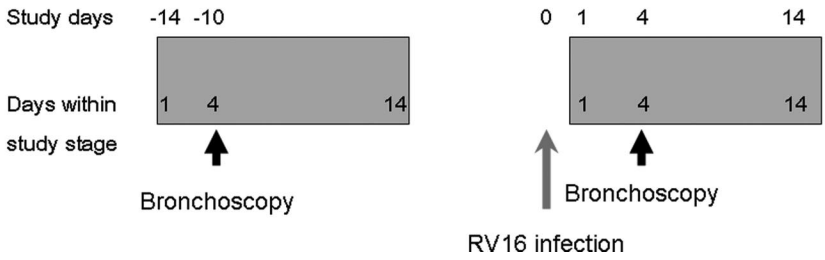

Fig. S3. Experimental infection. (*a*) Experimental RV16 infection: study design, clinical data, and sample collection. After baseline sampling, asthmatic and normal human volunteers underwent experimental inoculation with RV16 on day 0. Clinical sampling was performed after inoculation. Nasal lavage was collected on day 0 before inoculation then on days 1–8 and 11. Blood was taken for virus serology on day 0 and for full blood count and PBMC studies on days 0, 3, 4, and 7. Bronchoscopy was carried out on day 4 to obtain BAL. Induced sputum was performed on days 3 and 7. All clinical samples were also collected at baseline 2 weeks before and at convalescence 6 weeks after RV16 inoculation. Clinical data (symptom scores and home portable spirometry) was collected daily throughout the study. Bronchial reactivity was measured by histamine challenge on day 6 after RV16 infection and at baseline and convalescence. (*b*) Experimental rhinovirus infection: symptom and lung function analysis in 2-week blocks. Symptom scores and lung function were recorded by the subjects daily throughout the course of the study. Because baseline symptoms may be different between groups and because bronchoscopy is itself a cause of respiratory symptoms and falls in lung function, the symptom scores and lung function measurements recorded during the acute infection phase were corrected for baseline scores and the effects of bronchoscopy by subtracting the symptom scores recorded on the corresponding days during the baseline 2-week block. The resultant symptom scores and lung function changes therefore represent changes specifically caused by the rhinovirus infection.

Table S1. Baseline characteristics of recruited asthmatic and normal subjects

Subject no.	Age	Sex	IgE units/ml	Skin prick tests positive	Baseline FEV_1 % predicted	Baseline histamine PC_{20}, mg/ml*	Baseline histamine PC_{10}, mg/ml	Allergy syndromes	Completed study stages
				Atopic asthmatics					
1	19	F	50	g(5)h(3)b(6)3t(4)	104	3.5	2.71	A, R	Completed
4	28	M	553	g(2)h(6)alt(2)3t(2)c(3)d(4)	98	0.2	0.161	A	Completed
8	24	F	180	h(3)c(5)d(3)	114	1.1	0.543	A	Completed
17	19	F	271	g(6)h(7)	100	2.99	1.21	A	Completed
26	24	F	229	d(grade 3^C)latex(6)	83	0.228	0.139	A, E	Completed
39	19	M	162	Nil	105	2.83	0.794	A	Completed
53	22	F	212	h(5)	118	7.4	4.37	A	Completed
59	21	F	374	g(4)h(4)a(3)b(3)3t(3)c(6)d(4)	104	1.37	0.886	A, R	Completed
60	22	F	400	h(5)g(2)b(3)3t(2)d(4)	101	6.92	2.64	A, R	Baseline
62	22	F	355	h(5)c(2)	136	3.93	2.16	A	Completed
67	31	F	25	g(3)cl(3)alt(3)asp(3)3t(3)	97	3.66	1.04	A, R	Completed
Median	22	2 M/ 9F	229		104	2.89	1.04		
				Normal controls (nonatopic nonasthmatics)					
12	28	M	24	Nil	101	>32 (14.9)	9.77	Nil	Completed
16	29	M	115	Nil	90	26	4.46	Nil	Completed
18	21	F	22	Nil	103	>32 (17.4)	6.29	Nil	Completed
21	35	M	2	Nil	99	8.6	5.23	Nil	Completed
19	31	F	7	Nil	122	8.3	5.46	Nil	Completed
28	53	M	76	Nil	110	>32 (17.1)	11.72	Nil	Completed
20	47	F	8	Nil	126			Nil	Baseline
25	24	M	5	Nil	99	16.7	3.6	Nil	Completed
32	24	F	22	Nil	99	>32 (17.1)	5.78	Nil	Completed
33	28	F	14	Nil	136	>16 (7.8)	20	Nil	Completed
31	19	M	19	Nil	99	10.2	3.11	Nil	Completed
38	18	M	6	Nil	109	12.4	10.9	Nil	Completed
42	23	F	11	Nil	85	>16 (10.3)	15.26	Nil	Completed
49	18	F	16	Nil	86	>16 (12.9)	11.3	Nil	Completed
45	31	F	6	Nil	116	>32 (14.3)	7.58	Nil	Completed
57	21	F	6	Nil	105	14.4	5.73	Nil	Baseline
63	22	M	54	Nil	95	10.4	3.08	Nil	Completed
Median	24	8 M/9 F	14		101		6.035		

g, grass pollen; h, house mite; cl, cladosporium; a, aspergillus; b, birch; 3t, 3 trees; n, nettle; c, cat; d, dog; alt, alternaria; A, asthma; R, rhinitis; E, eczema.

*Five normal subjects had baseline histamine PC_{20} >32 mg/ml (the maximum concentration used in this study) and three were unable to tolerate a dose of histamine greater than 16 mg/ml. Where this was the case the percentage fall in FEV_1 is shown in parentheses alongside the maximum histamine concentration used.

Table S2. Virologic confirmation of RV16 infection

Subject no.	RV16 serology		Taqman RV RT-PCR, Log_{10} copies/ml, nasal lavage	Standard RV RT-PCR	Restriction enzyme analysis	Viral culture and RV16 neutralization	RV infection confirmed
	Day 0	6 weeks					
			Atopic asthmatics				
1	0	32	9.56	+	+	Cultured/neutralized	Yes
4	0	128	9.06	+	+	Cultured/neutralized	Yes
8	0	2	4.32	−	ND	Not cultured	Yes
17	0	8	8.61	+	+	Cultured/neutralized	Yes
26	0	8	7.1	+	+	Cultured/neutralized	Yes
39	0	<2	6.76	+	+	Cultured/neutralized	Yes
53	0	128	8.58	+	+	Cultured/neutralized	Yes
59	0	64	8.56	+	+	Cultured/neutralized	Yes
62	0	16	8.4	+	+	Cultured/neutralized	Yes
Median	0	24	8.56				
			Normal controls (nonatopic nonasthmatics)				
12	0	4	8.47	+	+	Cultured/neutralized	Yes
16	0	32	7.02	+	+	Not cultured	Yes
18	0	4	7.43	+	+	Cultured/neutralized	Yes
21	0	32	7.56	+	+	Cultured/neutralized	Yes
19	0	16	8.49	+	+	Cultured/neutralized	Yes
28	0	16	8.58	+	+	Cultured/neutralized	Yes
25	0	8	7.83	+	+	Cultured/neutralized	Yes
32	0	32	6	+	+	Not cultured	Yes
33	0	2	4.18	−	ND	Not cultured	Yes
31	0	8	6.02	+	+	Not cultured	Yes
38	0	64	9.01	+	+	Cultured/neutralized	Yes
42	0	4	6.43	+	+	Cultured/neutralized	Yes
49	0	0	8.72	+	+	Cultured/neutralized	Yes
45	0	2	5.67	+	+	Cultured/neutralized	Yes
63	0	64	6.67	+	+	Cultured/neutralized	Yes
Median	0	8	7.43				

Titre is serum dilution at which rhinoviral CPE is neutralized in a standardized assay for neutralizing serum antibody (see *Methods* for details). ND, assay not performed.