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RESEARCH ARTICLE

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# Rhinoviruses A and C elicit long-lasting antibody responses with limited cross-neutralization

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## Abstract

Rhinoviruses (RVs) can cause severe wheezing illnesses in young children and patients with asthma. Vaccine development has been hampered by the multitude of RV types with little information about cross-neutralization. We previously showed that neutralizing antibody (nAb) responses to RV-C are detected twofold to threefold more often than those to RV-A throughout childhood. Based on those findings, we hypothesized that RV-C infections are more likely to induce either cross-neutralizing or longer-lasting antibody responses compared with RV-A infections. We pooled RV diagnostic data from multiple studies of children with respiratory illnesses and compared the expected versus observed frequencies of sequential infections with RV-A or RV-C types using log-linear regression models. We tested longitudinally collected plasma samples from children to compare the duration of RV-A versus RV-C nAb responses. Our models identified limited reciprocal cross-neutralizing relationships for RV-A (A12–A75, A12–A78, A20–A78, and A75–A78) and only one for RV-C (C2–C40). Serologic analysis using reference mouse sera and banked human plasma

For affiliations refer to page 8.

**Abbreviations:** COAST, Childhood Origins of ASThma birth cohort; nAb, neutralizing antibody; RV, rhinovirus; URI, upper respiratory illness; VP, viral protein.

samples confirmed that C40 infections induced nAb responses with modest heterotypic activity against RV-C2. Mixed-effects regression modeling of longitudinal human plasma samples collected from ages 2 to 18 years demonstrated that RV-A and RV-C illnesses induced nAb responses of similar duration. These results indicate that both RV-A and RV-C nAb responses have only modest cross-reactivity that is limited to genetically similar types. Contrary to our initial hypothesis, RV-C species may include even fewer cross-neutralizing types than RV-A, whereas the duration of nAb responses during childhood is similar between the two species. The modest heterotypic responses suggest that RV vaccines must have a broad representation of prevalent types.

**KEYWORDS**
cross-neutralization, duration, neutralizing antibodies, rhinovirus, vaccine

## 1 | INTRODUCTION

Rhinovirus (RV) is the most common virus detected in acute respiratory illnesses. All three RV species (RV-A, RV-B, and RV-C) can cause upper respiratory illnesses (URIs); however, RV-A and RV-C are more likely to cause wheezing illnesses in preschool-aged children and in children and adults with asthma.[1–3] While no options are currently available to treat or prevent RV infection, new developments suggest that a polyvalent RV vaccine could be feasible.[4] Young children, especially those with a polymorphism (rs6967330) in the RV-C receptor gene *CDHR3*, represent a high-risk group that could benefit from a vaccine.[5]

After natural infection, neutralizing antibody (nAb) responses to RV-A and RV-B develop in the serum and mucosal secretions of most infected persons and can persist in serum for at least 1 year.[6–8] High-serum RV nAb titers protect against RV infection.[6,9,10] A total of 100 serotypes of RV-A and RV-B were identified by 1987.[11] More recently, the numbering system has been extended to 112 types.[12] Neutralization tests with RV reference rabbit and guinea pig sera showed limited cross-neutralization between serotypes of these two species in vitro. Of 90 tested RV serotypes, 50 were classified into 16 antigenic groups.[13] However, it is unknown whether these heterotypic nAb responses enable cross-protection against RV infections in vivo.

Discovered in 2006, the RV-C species is classified into 57 types based on the sequence identity thresholds in the VP1 capsid gene,[14] but there is limited information about their serological relationships. Total immunoglobulin G responses to synthetic RV-C peptides were measured in several prior studies,[15,16] and assays for detection of nAbs to RV-C have recently been developed.[17,18] We reported that nAbs to RV-C in children develop earlier and are two to three times more prevalent than nAbs to RV-A throughout childhood.[17] These findings suggest that RV-C infections can induce cross-neutralizing antibodies or antibody responses of longer duration than those elicited by RV-A. Identifying cross-neutralization patterns could inform the development of a polyvalent RV-C vaccine.

Our previous study pooled diagnostic virology data from over 10 000 RV-positive clinical samples obtained from children in the United States, Finland, and Australia.[17] Many children in this pooled cohort contracted a series of illnesses caused by various RV-A and RV-C types. The main goal of this study was to analyze the chronological sequences of RV illnesses to infer potential patterns of cross-neutralization and to test whether RV-C infections are more likely to induce cross-neutralizing antibody responses compared with RV-A infections. We reasoned that if a specific order of infections with two RV types occurred less often than expected, this could indicate that the first virus induced a cross-neutralizing antibody response that reduced the risk of infection and illness with the second virus. To experimentally verify these findings, we immunized mice with specific RV-C types and then used those reference sera and banked human plasma specimens to test for cross-neutralizing antibody responses. In addition, we tested plasma specimens obtained from the same birth cohort participants multiple times throughout childhood to determine nAb persistence following natural RV-A and RV-C illnesses.

## 2 | MATERIALS AND METHODS

### 2.1 | Statistical analysis of sequential patterns of RV infections

Viral diagnostic data[19,20] were pooled from 14 studies of respiratory health in children[17] to estimate the relative frequency of illnesses caused by specific RV types. Study designs included prospective birth or infant cohorts and asthma treatment studies that involved a broad range of ages and demographics. Data from 11 studies included serial sampling of nasal secretions for RV diagnostics across more than one illness, and the results from these studies were included in the main analysis (Table S1). In addition, several cohorts collected specimens when children were well, but only the illness specimens were considered in the main analysis of sequential illnesses. Samples were collected over a 21-year time span. We limited this analysis to specific RV-A or RV-C types with at least 40 infections across all

studies, reasoning that less common viruses would not be informative in this analysis. In addition, since RV antibody responses likely fade with time after infection, we considered sequences in which two or more RV illnesses occurred within 5 years of each other. Separate analyses were conducted for RV-A and RV-C illnesses because we did not expect to find inter-species cross-reactivity. The expected number of sequential illnesses was calculated using log linear Poisson regression models of daily incident RV illness with each RV-A or RV-C type as a function of age and study cohort alone; daily expected counts for each subsequent RV-A or RV-C type were then obtained by summing overall individuals and all days at risk for each initial RV-A or RV-C type. The incidence of infections as a function of previous infections with other RV-A or RV-C types was modeled using a LASSO (least absolute shrinkage and selection operator) penalized log linear Poisson regression model after adjustment for age and study cohort.[21] The LASSO is a penalized regression algorithm that simultaneously performs variable selection and regularization; the regression parameters for previous infections with other RV-A or RV-C types were penalized, while the regression parameters for age and study cohort were not penalized. The LASSO penalty parameter was chosen based on the Akaike information criterion.[22] Analyses were performed using the glmnet package[23] in R Statistical Software (v4.1.2; R Core Team 2021).

### 2.2 | RV-C and mouse reference serum production

RV-C2[24] and RV-C40 were isolated from the Childhood Origins of Asthma (COAST) study nasal lavage specimens identified by RV molecular typing assay,[20] and the RV-C47 genomic copy was cloned in a plasmid vector pMJ3[25] using the published sequence (GenBank accession number MF806525) from three overlapping synthetic cDNA gBlocks (Integrated DNA Technologies). RV-C2 and RV-C47 were produced from infectious cDNA clones in human embryonic lung fibroblasts (WisL cells) by reverse genetics as described,[24,26] whereas RV-C40 was isolated directly from a nasal secretion specimen by infection of HeLa-E8 cells transduced to express CDHR3 [27] and propagated by infection. Viruses were purified and concentrated by ultracentrifugation (100 000 × *g*, 4 h, 10°C) and resuspended in phosphate-buffered saline.[24,26,28] Mouse reference serum samples to RV-C2, C40 and C47 types were obtained after intramuscular immunization of BALB/cJ mice ($n = 30$ per RV type) twice with purified, formalin-inactivated viral preparations using alum adjuvant as previously described.[4]

### 2.3 | Virus neutralization assay

An RT-qPCR-based RV neutralization assay in HeLa-E8 cells was performed as previously described[17] and included an RV-C2 variant with HeLa-E8 adaptive mutation in nonstructural 3A gene.[29] Since the assay requires optimal viral replication, we adapted RV-C40 and RV-C47 clinical isolates to efficient replication in HeLa-E8 cells by serial passaging as described.[29] Plasma samples from participants with documented infection with RV-C2 and RV-C40 in the COAST study were selected to experimentally test for the presence of cross-neutralizing antibodies.[3,17]

## 3 | RESULTS

### 3.1 | Sequential patterns of RV illnesses

We pooled RV diagnostic information (species and type) from 11 studies and 3199 children (Table S1). These data were used to calculate the incidence of infections with specific RV types ("initial detection") as a function of previous infections with other RV types of the same species ("follow-up"). A total of 2187 RV-A illnesses and 2138 RV-C illnesses were considered (Table 1). Overall, 27 unique RV-A types and 34 unique RV-C types were included in the final dataset (Table S2).

We identified negative relationships (LASSO), indicating that fewer sequential infections were observed than expected (potential cross-neutralization) for both RV-A ($n = 30$; Figure 1A) and RV-C ($n = 16$; Figure 2A). Several one-way and five reciprocal negative relationships were observed involving four RV-A types (A12–A75, A12–A78, A20–A78, and A75–A78) and two RV-C types (C2–C40) (Table S3). One (A12–A78) of the four predicted RV-A reciprocal relationships had been observed experimentally,[13] whereas the RV-C types had previously not been tested for cross-neutralization. The results of the group-wise comparison of expected versus observed infection sequences suggest that RV-C viruses are less likely to be cross-neutralizing compared with RV-A viruses ($p = 0.0002$, chi-squared test).

RV-A infections induce nAb responses that inhibit homotypic reinfections.[7–9] Our dataset included a small number of sequential infections with the same RV types (Table S4). We therefore calculated the rates of their expected versus observed homotypic sequences to validate our model. Most RV types (except for A54) had an observed frequency of re-infections with the same virus lower than the expected frequency ($p < 0.0001$, sign test) (Table S5). These results demonstrate that our model can detect antibody-mediated protection against infection with the same virus (Figures 1B and 2B).

### 3.2 | Capsid protein sequence analysis of RV types

When the cross-relationships among 90 RV types were reported in 1982 by analyzing rabbit reference antisera, their genome sequences were not available.[13] To determine whether cross-neutralization is more likely to occur in RV with high capsid protein sequence similarity, we analyzed the RV-A and RV-B types tested in that study. The phylogenetic analysis of the VP1 protein, the most variable among the four RV capsid proteins, demonstrated that antigenically related types are phylogenetically related (Figure 3). Similar results were obtained by analyzing the other three capsid proteins (data not shown). VP1 pairwise distances (p-distances) between 17 pairs of cross-neutralizing RV-A types ($n = 28$) were significantly lower

**TABLE 1** Comparing the frequency of expected versus observed sequential infections for RV-A and RV-C species.

| Interactions | RV-A | RV-C |
|---|---|---|
| Nonsignificant | 659 (93.9%) | 1097 (97.8%) |
| Significant one-way (−) | 26 (3.7%) | 15 (1.3%) |
| Significant one-way (+) | 9 (1.3%) | 8 (0.7%) |
| Reciprocal (−) | 8 (1.1%) | 2 (0.2%) |
| Total sequential infections | 702 | 1122 |

Abbreviations: RV-A, rhinovirus A; RV-C, rhinovirus C.

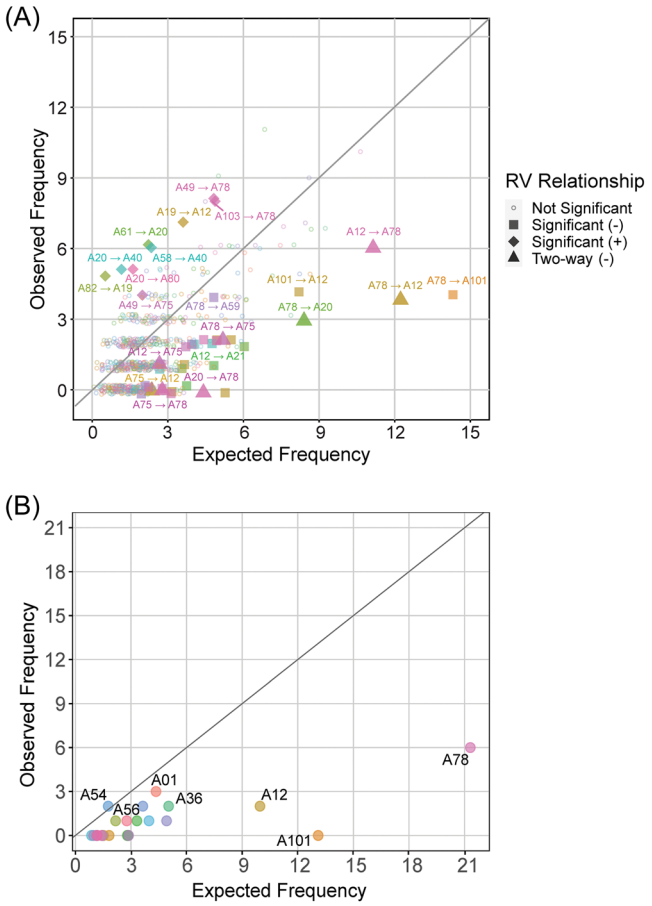


**FIGURE 1** Analysis of sequential RV-A illnesses by linear regression modeling. (A) The expected frequency of heterotypic sequential infections is on the *x*-axis while the observed frequency is on the *y*-axis. Statistically significant coefficients in those models are shown by different point shapes. Significant negative (−) coefficients, shown by squares (one-way) or triangles (reciprocal) indicate that we observed less sequential infections than expected and potentially cross-neutralization between paired RV types, whereas significant positive (+) coefficients, shown by diamonds, may indicate a decreased likelihood of finding an antigenic relationship. Color represents the follow-up RV type in illness sequences. (B) The expected and observed frequencies of re-infections with the same virus type were plotted as in Panel A to validate the statistical model. RV, rhinovirus.

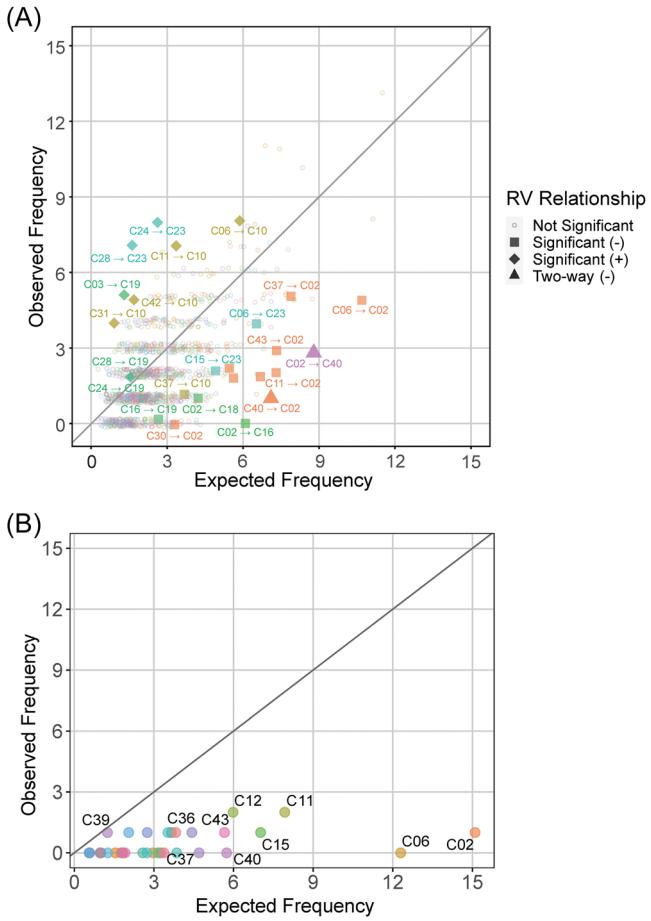


**FIGURE 2** Analysis of sequential RV-C illnesses by linear regression modeling. (A) The expected frequency of heterotypic sequential infections is on the *x*-axis while the observed frequency is on the *y*-axis. Statistically significant coefficients in those models are shown by different point shapes. Significant negative (−) coefficients, shown by squares (one-way) or triangles (reciprocal), indicate that we observed less sequential infections than expected and potentially cross-neutralization between paired RV types, whereas significant positive (+) coefficients, shown by diamonds, may indicate a decreased likelihood of finding an antigenic relationship. Color represents the follow-up RV type in illness sequences. (B) The expected and observed frequencies of re-infections with the same virus type were plotted as in Panel A to validate the statistical model. RV, rhinovirus.

($p < 0.001$) than those between the remaining 40 RV-A types without evidence of cross-neutralization (Figure 4).

We compared 30 pairs of RV-A types and 16 pairs of RV-C types that were identified as potentially cross-neutralizing by statistical modeling (Table S3). Viruses with one-way reactivity had VP1 p-distances similar to those without (Figure S1). However, p-distances between two pairs (A12–A78 and A20–A78) of reciprocally related RV-A (0.22 and 0.28, respectively) and one pair (C2–C40) of RV-C (0.11) types were lower than the overall mean within-species VP1 p-distances (0.29 and 0.34, respectively). In contrast to those viruses, A75 type, involved in reciprocal

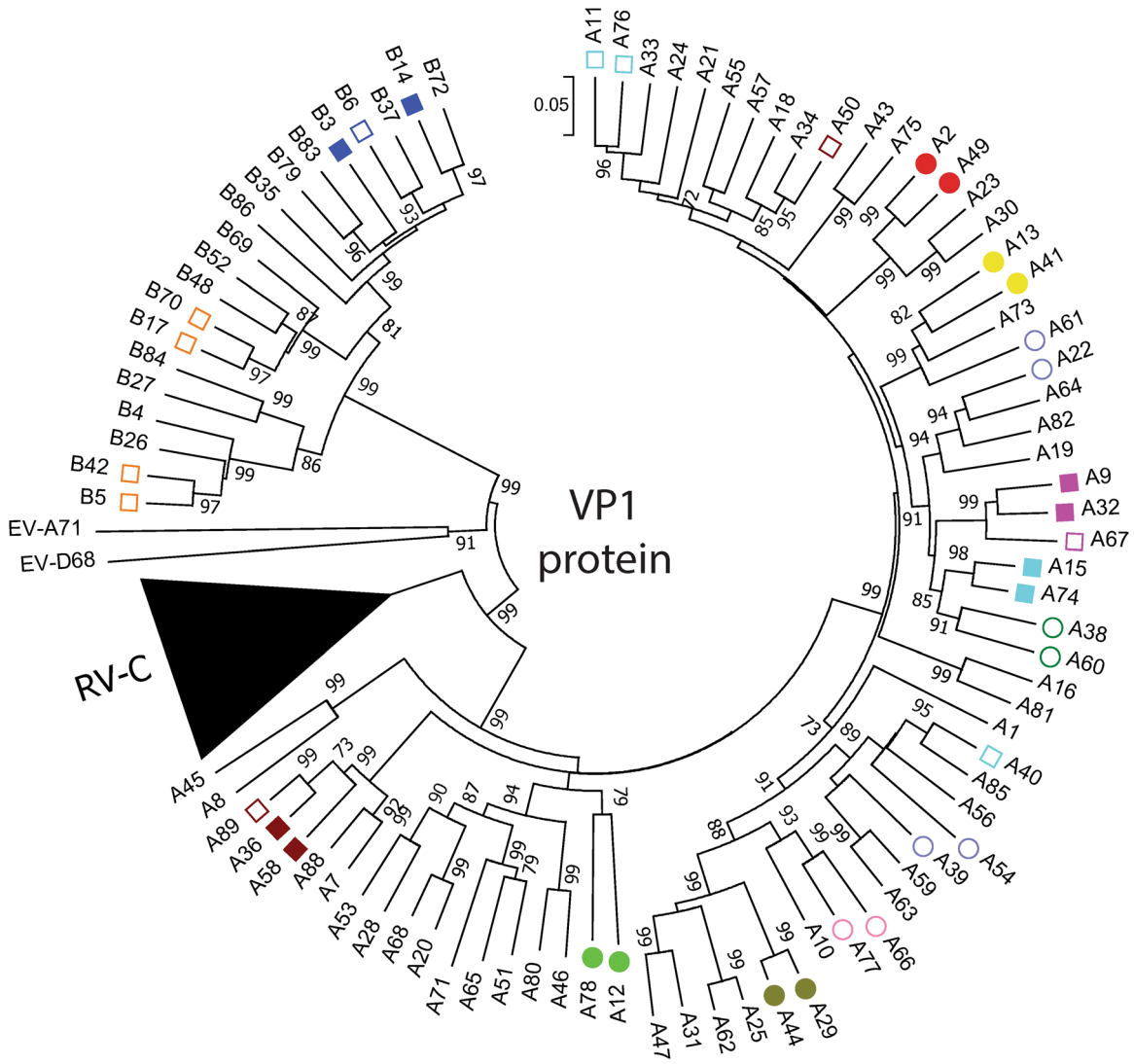


**FIGURE 3** VP1 phylogenetic tree showing relationships between antigenically cross-reactive RV-A and RV-B serotypes. A neighbor-joining phylogenetic tree was constructed using MEGA version 7.[30] The evolutionary distances were computed using the p-distance method. The distances are presented in the units of the number of amino acid differences per site. All major nodes are labeled with bootstrap values (500 replicates, with its value more than 75%). Branch lengths are proportional to amino acid similarity (p-distance). The analysis included 88 out of 90 RV-A and RV-B types previously tested for cross-reactivity using rabbit reference sera[13] because RV-A1a and A1b were reassigned as a single type RV-A1,[14] and RV-87 was reassigned as enterovirus D68.[31] The tree branch representing RV-C types was condensed. Enteroviruses (EV-A71 and EV-D68) were included as an outgroup. Filled and empty shapes of the same color show reciprocal and one-way antigenic cross-reactivity, respectively. Circles and squares indicate cross-reactivity with one and ≥two heterologous RV type(s), respectively. EV, enterovirus; RV, rhinovirus.

relationships with A12 and A78, was more distant from them (0.35–0.36) in VP1 (Figure 5).

### 3.3 | Serologic validation of predicted RV-C cross-reactivity

We used monospecific reference sera from mice and banked human plasma samples to test the cross-reactivity of antibodies specific for the C2 and C40 types, which were identified as potentially cross-neutralizing in our analysis of sequential infections. These RV-C types had a high VP1 protein sequence identity (87%) and clustered together with C47 by phylogenetic analysis (Figure 5). We immunized mice with C2, C40, and C47 whole-virus antigens to obtain reference sera with high-level homotypic antibody titers (1:1553, 1:473, and 1:5724, respectively; Table 2). Virus cross-neutralization experiments with mouse reference sera confirmed a one-way antigenic relationship between C40 and C2 (1:17) and identified reciprocal relationships between C2 and C47 with modest heterotypic nAb titers (1:12 and 1:16, respectively). In addition, specific mouse antisera to six viruses (C6, C11, C15, C25, C41, and C49) that had less phylogenetic similarity (Figure 5) showed no neutralizing activity against C2 (data not shown).

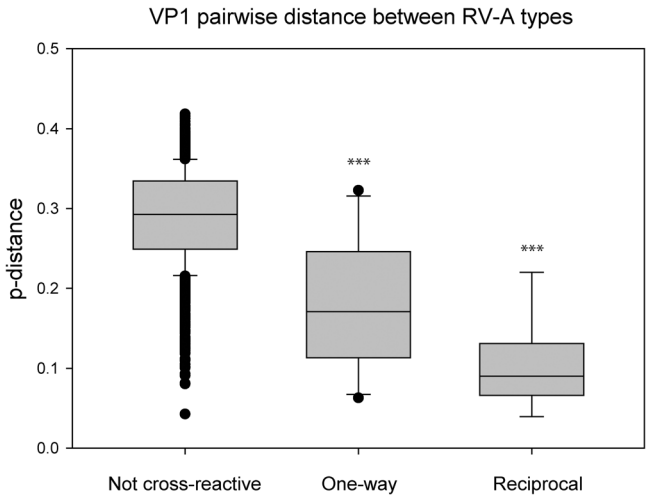


**FIGURE 4** VP1 pairwise distances (p-distances) between antigenically cross-reactive RV-A types. The p-distances between VP1 amino acid sequences of 68 RV-A types previously tested for cross-reactivity using rabbit reference sera[13] were computed using MEGA version 7.[30] RV-A types with cross-reactivity (one-way or reciprocal) were compared with the remaining types (not cross-reactive) using one-way ANOVA on ranks and multiple comparisons were done using Dunn's method (SigmaPlot 13, Systat Software). ***$p < 0.001$ versus not cross-reactive types. RV, rhinovirus; VP1, viral protein 1.

To test patterns of cross-neutralization in human sera, we identified three banked plasma samples from children participating in the COAST birth cohort study who had documented C2 or C40 illnesses (Table 2) preceding blood collection by at least 2 months. No participants had confirmed C47 illness with available blood specimens in the COAST study. We tested these samples for neutralizing activity against C2, C40, and C47 viruses. In these specimens, the C40 convalescent plasma sample (#255) neutralized the C2 virus (1:301) in addition to the C40 virus (1:2936), whereas two C2 plasma samples (#76 and #93) neutralized only the homologous C2 virus (1:34 and 1:50).

### 3.4 | Analysis of the duration of nAb responses

We previously measured responses at 2, 10, and 16 years of age.[17] For this study, we analyzed nAb responses in additional plasma specimens collected from the same children at 12, 14, and 18 years of age to provide more precise estimates of the antibody response duration (mixed-effects regression modeling). Detecting nAb responses to a particular virus type at one visit was highly predictive of a continued response to that same virus type at the next visit (odds ratio 30.7, 95% confidence interval 14.3–65.8); however, this effect did not differ between the tested RV-A and RV-C types (Figure 6).

## 4 | DISCUSSION

Children at increased risk for RV-C wheezing illnesses include preschoolers, children with a polymorphism (rs6967330) in the RV-C receptor gene *CDHR3*, and children with asthma.[5,32–35] Preventive treatments, such as vaccines, are needed but remain challenging due to the many RV-C types and the knowledge gaps related to this viral species. For example, while relationships between genotype and serotype are well-established for RV-A and RV-B, no such data are available for RV-C. Our analysis of the expected versus observed frequency of RV-A and RV-C illnesses within a 5-year time frame in a large, pooled dataset suggested that RV infections largely induce type-specific responses and that cross-neutralizing antibody responses are uncommon for both RV-A and RV-C. Thus, each RV genotype likely corresponds to a serotype with only limited cross-neutralization. For RV-A and RV-B types, the limited potential to induce cross-neutralizing responses was confirmed experimentally decades ago.[11,13,36] Our study provides strong evidence that antigenic relationships among the RV-C types are similar to those of other RVs and that RV-C types defined by genetic criteria[14] correspond to serotypes. In fact, this pooled data analysis indicated that RV-C types are even less likely to induce cross-neutralizing responses than RV-A.

Our study demonstrates that cross-neutralization is more likely to occur among types with high sequence homology. For example, previous studies that analyzed monospecific rabbit sera to 68 out of 80 currently assigned RV-A types revealed 10 one-way and 8 reciprocal cross-relationships involving 30 RV-A types.[13] We phylogenetically reanalyzed these data and found that, in general, cross-neutralization was more likely to occur between types with high homology in capsid sequences. The significant one-way relationships that we identified in the sequential infection analysis, which involved 26 pairs of RV-A types and 15 pairs of RV-C types, had low intra-species sequence homology. In contrast, the few reciprocal relationships that we identified involved two pairs of RV-A and one pair of RV-C types with relatively high sequence homology, although the numbers were too low for statistical analysis. These results suggest that predicted reciprocal relationships between phylogenetically similar types are more likely to indicate cross-protective nAb responses in vivo, whereas one-way relationships could be less informative. Interestingly, two reciprocal relationships (A12–A75 and A75–A78) were identified between phylogenetically distant virus types. Since neutralizing epitopes on the virus surface are usually formed by only a few amino acid residues in VP1, VP2, and VP3,[37–40] the cross-neutralizing relationships could be due to structural and sequence similarities in key neutralizing epitopes.

Cross-neutralizing antibody responses to RV-C types have not been previously studied due to the lack of plaque or $TCID_{50}$ assays for this species. Using monospecific mouse sera, defined specimens of convalescent human sera, and a PCR-based neutralization assay,[17]

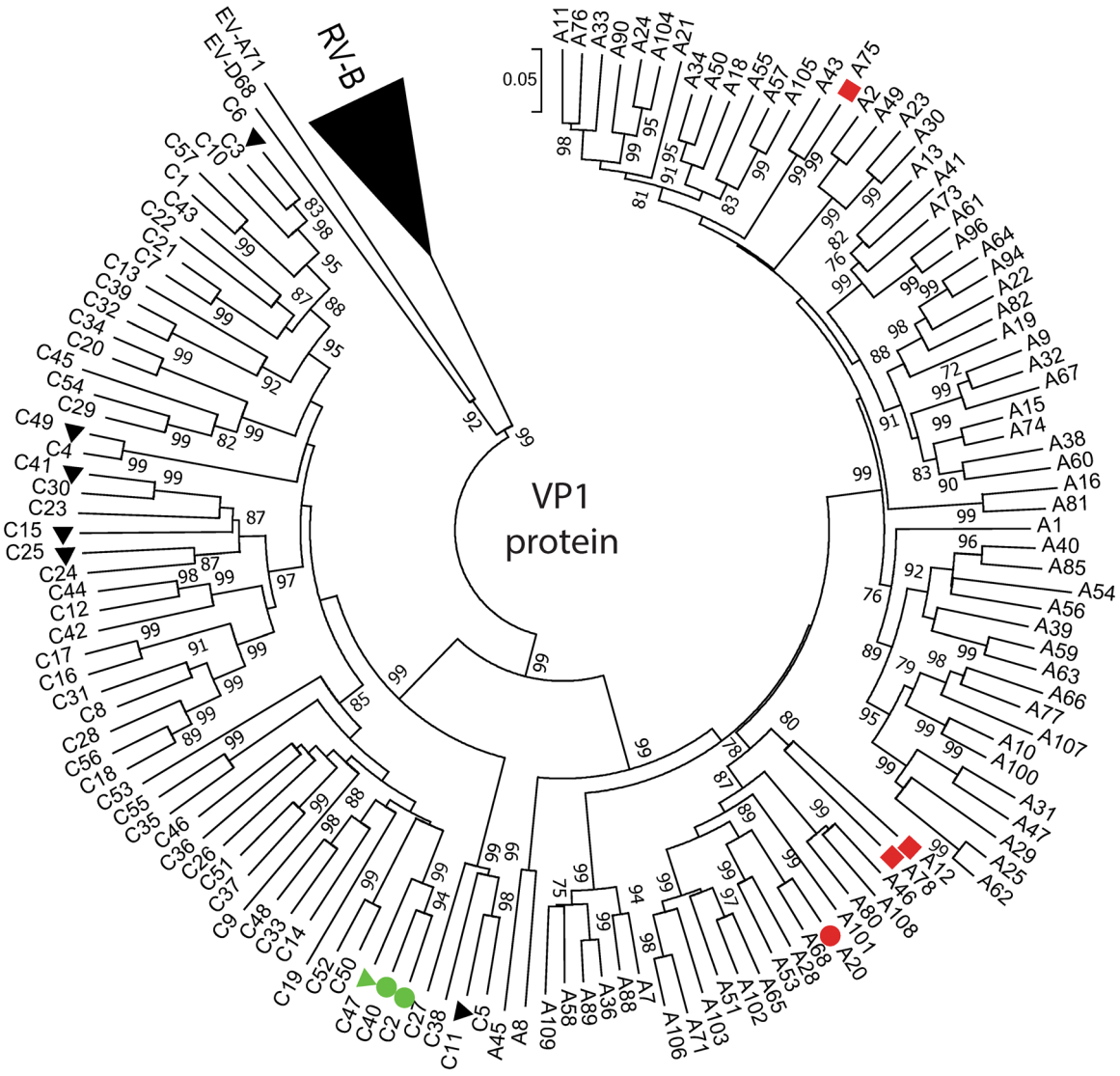


**FIGURE 5** VP1 phylogenetic tree showing relationships between RV-A and RV-C types identified to reciprocally cross-react by statistical modeling. A neighbor-joining phylogenetic tree was constructed in MEGA version 7[30] as in Figure 3 using reference sequences (Table S6) of all currently assigned RV types ($n = 169$). The tree branch representing RV-B types was condensed. Circles and squares of the same color indicate predicted cross-reactivity with one and ≥two heterologous RV type(s), respectively. Green and black triangles show the presence or absence of cross-reactivity with RV-C2 mouse serum, respectively. RV, rhinovirus; VP1, viral protein 1.

we verified the predicted relationships among a trio of RV-C types that were identified in the observational study and had high sequence homology. These serological tests demonstrated a one-way relationship between C40 and C2 and found a reciprocal relationship between C2 and C47. The titers for cross-neutralization were modest and much lower than those for homotypic neutralization. In contrast, antisera to other more phylogenetically distant RV-C types had no neutralizing activity against C2.

Neutralizing antigenic sites have not yet been identified for RV-C; however, at least one antigenic site was predicted in the VP1 external loop of the "finger" region that forms a major protrusion from the RV-C15 virus surface.[41] Dominant neutralizing antigenic sites in RV-C may differ from those in RV-A (and RV-B) based on major structural differences in RV-C capsids;

**TABLE 2** Cross-neutralizing antibody responses in human plasma and mouse serum samples.

| | Viruses | | |
|---|---|---|---|
| **Specificity of serum sample** | **C2** | **C40** | **C47** |
| Mouse sera | | | |
| C2 | 1/1553 | Negative | 1/12 |
| C40 | 1/17 | 1/473 | Negative |
| C47 | 1/16 | Negative | 1/5724 |
| Human plasma | | | |
| C2 (#76) | 1/34 | Negative | Negative |
| C2 (#93) | 1/50 | Negative | Negative |
| C40 (#255) | 1/301 | 1/2936 | Negative |

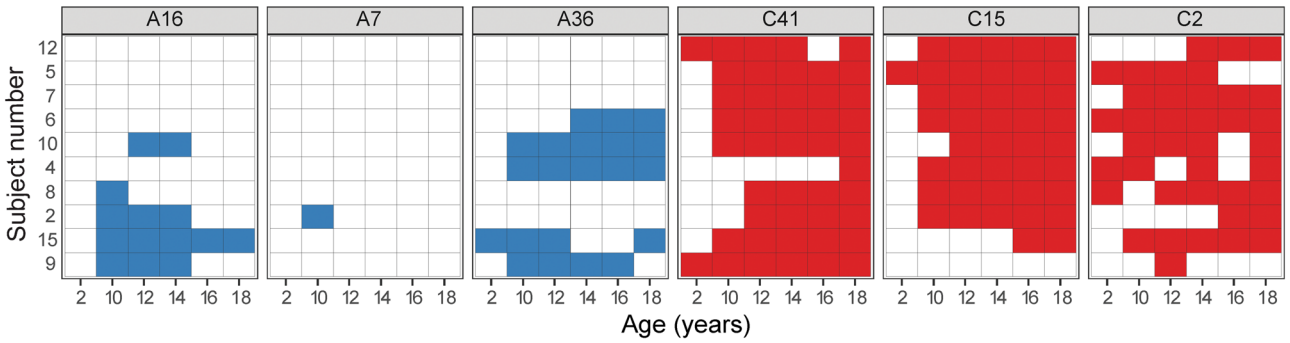


**FIGURE 6** Both RV-A and RV-C neutralizing antibody (nAb) responses are very likely to persist from one age to the next. Heatmaps show the presence of nAbs to indicated RV-A (blue) and RV-C (red) types in plasma (ages 2, 10, 12, 14, 16, and 18 years; each row represents serial sampling from the same subjects) from COAST study participants ($n = 10$). Odds ratio (OR) refers to the odds of finding a continued nAb response to RV type from one age to the next (OR 30.7, 95% confidence interval 14.3–65.8). COAST, Childhood Origins of ASThma; RV, rhinovirus; VP1, viral protein 1.

however, as with RV-A, high sequence homology in VP1 could still help identify antigenic relationships between RV-C types.

Our prior analysis of samples from 20 children participating in the COAST birth cohort study suggested that nAbs were likely to persist from one age to the next (2, 10, and 16 years).[17] While RV species was not significantly associated with the persistence of nAbs ($p = 0.58$), this analysis lacked precision because only three age groups were studied. We extended our analysis of antibody duration by analyzing samples from three additional age groups (ages 12, 14, and 18 years) for 10 of the previous 20 children in the original study. The results demonstrate a consistently higher prevalence of nAbs to RV-C types throughout childhood compared with RV-A types ($p = 0.0001$). Analysis of plasma samples from these six age groups (2, 10, 12, 14, 16, and 18 years) confirmed that RV-A and RV-C elicit similarly durable antibody responses. Therefore, the increased prevalence of RV-C nAbs throughout childhood may indicate that either RV-C infections are more likely to elicit systemic nAb responses or induce a higher titer of nAbs. We are now working to test these additional hypotheses.

Our study has several strengths and limitations. The strengths of this study include an extensive pooled dataset of RV illnesses and viral diagnostics from studies that were diverse in participant age, geographic location, study design, and season (Table S1). RV species and type were confirmed using similar molecular typing methods for all samples.[19,20] A limitation of this study is that our analysis only included RV types that were detected at least 40 times (27 of 80 RV-A types and 34 of 57 RV-C types); therefore, potentially cross-neutralizing types with low detection frequency could not be studied. The sequential analysis only considered viruses detected during illnesses. Asymptomatic infections can also induce antibody responses, but they are generally smaller in magnitude[42,43] and thus less likely to influence future infections. Finally, it is uncertain how many cross-related RV types identified by serological analysis in vitro would enable cross-protection against RV infection in vivo.

In conclusion, analysis of sequential RV infections in an extensive clinical dataset and nAb responses in immunized laboratory animals demonstrated that intra-species cross-neutralizing relationships are relatively infrequent and are limited to types with high capsid sequence similarity. The low heterotypic nAb titers suggest a limited capacity to protect against clinical infection. These results have implications for vaccine development. RV-C polyvalent vaccines may require multiple clinically important types representing all major phylogenetic clades of RV-C species to induce broad protection.

## AUTHOR CONTRIBUTIONS

Yury A. Bochkov drafted the manuscript. Yury A. Bochkov, Mark Devries, Sujin Lee, Ramyani De, Tressa Pappas, and Kristine Grindle performed laboratory analyses. Kaitlin Tetreault, Timothy Choi, and Ronald Gangnon performed the statistical analysis. James E. Gern and Yury A. Bochkov designed the study, analyzed data, and edited the manuscript. Other authors contributed samples and data and reviewed the manuscript. All authors have read and approved the final manuscript.

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## CONFLICT OF INTEREST STATEMENT

Yury A. Bochkov has patents on production methods of rhinoviruses. Leonard B. Bacharier reports consulting fees from GlaxoSmithKline, Genentech/Novartis, DBV Technologies, AstraZeneca, WebMD/Medscape, Sanofi/Regeneron, Vertex, OM Pharma, Recludix; and royalties from Elsevier. William W. Busse receives consulting fees/honoraria from Arrowhead, GlaxoSmithKline, Sanofi, Regeneron, and royalties from Elsevier. Tina Hartert reports consulting fees from Sanofi and personal fees for data and safety monitoring board (DSMB) membership from Pfizer. Daniel J. Jackson receives grants from GlaxoSmithKline and Regeneron, consulting fees from Avillion, AstraZeneca, Genentech, GlaxoSmithKline, Regeneron, Sanofi, and personal fees for DSMB from Pfizer. James E. Gern has served as a paid consultant for AstraZeneca, Meissa Vaccines Inc., and Via Nova Therapeutics Inc., has stock options in Meissa Vaccines Inc. and has patents on production methods of rhinoviruses. Other authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon request from the corresponding author.

## ETHICS STATEMENT

Each of the 14 human studies was approved by the local human research ethics committees, and participants provided written informed consent. All experiments involving mice were performed at Emory University in accordance with guidelines established by the Animal Welfare Act and the NIH Guide for the Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee (IACUC) of Emory University approved these studies.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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