Waning immunity against respiratory syncytial virus during the COVID-19 pandemic

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Abstract

Health jurisdictions have seen a near-disappearance of Respiratory Syncytial Virus (RSV) during the first year of the COVID-19 pandemic. Over a corresponding period, we report a reduction in RSV antibody levels and neutralization in women and infants one year into the COVID-19 pandemic (February – June 2021) compared to earlier in the pandemic (May – June 2020), in British Columbia (BC), Canada. This supports that humoral immunity against RSV is relatively short-lived and its establishment in infants requires repeated viral exposure. Waned immunity in young children may explain the inter-seasonal resurgence of RSV cases in BC as seen also in other countries.

Introduction

Countries have seen a near disappearance of respiratory illnesses due to Respiratory Syncytial Virus (RSV) during the winter of 2020-2021 associated with mitigation measures to control Coronavirus Disease 2019 (COVID-19) pandemic [1, 2]. Before the pandemic, an average of 1,450 RSV cases were reported in British Columbia (BC), Canada during three preceding RSV seasons (October to April of 2017 to 2020). In contrast, only five cases were reported in 2020-2021 [3]. As mitigation measures were relaxed, RSV cases resurged during the summer of 2021 around the world, including BC [4]. The reasons for this atypical resurgence of RSV cases are unclear, and the pandemic offers an opportunity to study RSV immunity after a prolonged (nearly one-year) lack of viral exposure.

Infants are immunologically naïve and dependent on maternal antibodies to avoid severe RSV infections at birth. IgG antibodies against the prefusion RSV F protein are responsible for the majority of neutralizing antibodies against RSV [5]. Here, we report prefusion RSV F IgG and neutralization titers in women of childbearing age and infants before and one year into the COVID-19 pandemic.

Methods

Study cohort: Paired serum samples were prospectively collected from healthy women of childbearing age (18 to 51 years old) in 2020 (February to May) and 2021 (May to June), at the BC Children's & Women's Health Centre and its affiliated Research Institute, and retrospectively obtained from age-matched healthy women who underwent prenatal screening at the BC Centre for Disease Control Public Health Laboratory in 2018 (April to May) and 2019 (April to June). Sera were also collected between July and August from infants born after March 31, 2019 (for 2020 samples) and between April and June from infants born after March 31, 2020 (for 2021 samples). The blood samples from the women and infants were collected after typical peak RSV seasons each year, except for samples collected in 2021 (**Supplemental Fig. S1**).

For the paired samples from women of childbearing age in 2020 and 2021, an email was sent to the clinical departments of the BC Children's Hospital and affiliated research institute, inviting adults

(>18 years of age) for a seroprevalence study of common respiratory virus exposures (including COVID-19) during the pandemic. More than >350 individuals replied, signed written consent and provided a blood sample. Of those, 18 paired samples from healthy women meeting age criteria were randomly selected to be included in this report (>70% were healthcare workers). The 2018 and 2019 samples in women of childbearing age were selected from a bank of thousands of residual prenatal sera based on matching for age, in steps (±1 year, ±2 year, ±3 year), to a maximum of ±3 years. Infants were enrolled from: i) infants followed in the BC RSV Immunoprophylaxis Program (https://www.childhealthbc.ca) (**Supplemental Table 1**) and ii) by posting advertisement in pediatric clinics within Vancouver, and Surrey Memorial and Royal Columbia Hospitals. Infants who had previously received immunoglobulins or palivizumab within the last 3 months were excluded.

Blood processing: Prospective blood samples for antibody measures were collected in gold-top serum separator tubes with polymer gel (BD Biosciences) in adults, and in red-top tubes without polymer gel (BD Biosciences) in infants. Blood samples for measurement of RSV T cell responses (in adults only) were collected in EDTA vacutainers (BD Biosciences). After blood collection, sera were left for 30 minutes at room temperature for clotting, before centrifugation at 1400 x g for 10 minutes, followed by aliquoting and freezing of sera at -80°C within 4 hours of collection.

RSV-specific antibody outcomes: Prefusion RSV F protein IgG levels (reported as Arbitrary Units [AU] per mL) were quantified using the VPLEX Respiratory Panel 1 IgG Kit (Meso Scale Diagnostics, K15365U) at dilutions of 1:5,000 to 1:10,000 for women's samples and 1:1,000 for infants' samples. RSV antibody neutralization was assayed using a live virus plaque assay using a green-fluorescent protein-expressing recombinant RSV A strain, in batches (**Supplemental Fig. S2**), as we described previously [6]. Results were expressed as serum titers to prevent 95% viral syncytial formation compared to virus-free sera (NT95) and were externally validated (**Supplement**). Palivizumab in serial dilutions (starting at 25 µg/mL) was used as a positive control (**Supplement**).

RSV-specific T cell outcomes: RSV T cell responses were measured on peripheral blood mononuclear cells (PBMCs) by flow cytometry (**Supplemental Fig. S3**). A single lot of a pool of 15-mer peptide with 11 amino acids overlap covering the sequence of the Nucleoprotein (protein N) of the RSV B1 (UniProt ID: O42053) was used for stimulation (RSV Peptivator, Miltenyi Biotech, Bergisch Gladbach, Germany) to stimulate RSV-specific T cells in batch experiments. After 48h stimulation, cells were stained using CD19-PE (clone: HIB19), CD14-PE (clone: M5E2), CD4-PE-Cy7 (clone: L200), CD69 BV786 (clone: FN50) from BD and CD3 FITC (clone: OKT3), CD8α PerCPCy5.5 (clone: RPA-T8), CD137 APC (clone: 4B4-1), OX40 BV421 (clone: Ber-ACT35; all from BioLegend). Data were acquired on a BD LSR Fortessa™ X-20 Cell Analyzer equipped with a UV laser, gating on singlet live cells, and CD137/OX40-positive CD4/CD3-expressing cells and excluding CD14-PE/CD19-PE-labeled cells. Compensations were set during the analysis, using signals obtained from single fluorescent antibody-conjugated CompBeads (BioLegends).

Statistics: A convenience sample size was used, expecting to detect greater than 4-fold differences in RSV neutralization between groups. Antibody outcomes are expressed as geometric means (GM) ± geometric standard deviation factor (GSD), with neutralization expressed as the reciprocal of the titer. Unpaired 2-sided student t tests were used for statistical comparisons, except for the paired women's samples where a paired 2-sided student t test was used. Welch's correction was applied for differences in variance between infant samples, for RSV neutralization. Spearman correlation was used for correlations. Prefusion RSV F protein-specific IgG levels and RSV neutralization were adjusted for postnatal age using a linear model with cohort (2020 *vs*. 2021) used as a co-variate.

Ethics: The study was approved by the University of British Columbia Children's and Women's, and the Fraser Health Research Ethics Boards (certificates number: H20-01205, H18-01724 and H18-01724). Written informed consent was obtained from all participants.

Results

Prefusion RSV F IgG levels were significantly reduced (GM ± GSD: 148,858 ± 2.4 vs. 197,806 ± 2.2 AU/mL; p = 0.0232) in women of childbearing age in the spring of 2021 (n=18 women, median

age 37, IQR 28 – 41 years), compared to the same individuals in 2020, but no statistical difference was observed when compared to age-matched women in 2018 (n = 14, median age 34, IQR 28 – 42 years; 141,563 ± 2.0; p=0.8620 comparing 2021 vs. 2018) or age-matched women in 2019 (n = 14, median age 37, IQR 28-45 years; 164,375 ± 1.8; p = 0.7236 comparing 2021 vs. 2019) (**Fig. 1a**). Strikingly, prefusion RSV F IgG levels were ~15-fold lower (4,258 ± 8.8 vs. 63,530 ± 4.4 AU/mL; p < 0.0001) in infants sampled in 2021 (n = 65, median age 6.7 months, IQR 4 – 11 months; median gestation: 39, IQR 33 – 40 weeks) compared to infants sampled in 2020 (n = 20, median age 7.5 months, IQR 7 – 12 months; median gestation: 32, IQR 27 – 34 weeks) (**Fig. 1a**). Prefusion RSV F IgG levels were comparable between term (4061 ± 6.3; n = 44) and preterm infants in 2021 (4704 ± 16; n = 21; **Fig. 1b**), and inversely correlated with post-natal age (Spearman R = -0.4360; p = 0.0003). Prefusion RSV F IgG antibody levels did not correlate with gestational age in infants in 2020 (Spearman r = -0.05475; p=0.8238) and in infants in 2021 (Spearman r = -0.1406; p=0.2641). RSV F IgG did not differ between infants born before vs. after 27 weeks gestation (**Supplemental Fig. S4**).

RSV neutralizing titers in women in 2021 were 12-fold lower compared to women in 2020 (10.3 ± 2.0 vs 120.9 ± 2.9; p < 0.0001), and were also lower compared to women in 2019 (28.7 ± 2.8; p = 0.0026) and 2018 (78.4 ± 2.9; p < 0.0001). The decrease in RSV neutralization from 2020 to 2021 was independently confirmed by a different laboratory, reporting results as serum titers to prevent 50% plaques compared to serum-free virus (PRNT50) (**Supplemental Fig. S5**). Prefusion RSV F IgG levels, combining all women and infant sera, strongly correlated with neutralizing titers (Spearman R = 0.6709, p < 0.0001) (**Supplemental Fig. S6**). In contrast, RSV-specific CD4 T cell responses in women were comparable between women in 2020 and 2021 (p = 0.4770) (**Fig. 1d**).

RSV neutralizing titers in infants in 2021 were 3.4-fold lower compared to infants in 2020 (6.7 ± 1.8 vs 22.8 ± 2.0; p < 0.0001) (**Fig. 1c**). Both prefusion RSV F IgG and neutralizing titers remained significantly lower in infants in 2021, compared to 2020, after adjusting for post-natal age (**Table 1**).

Discussion

This study showed profoundly reduced RSV antibody levels and function in women of childbearing age and infants, after a year of the COVID-19 pandemic, in absence of viral exposure. The data were independently validated externally by two laboratories. The reduction in prefusion RSV F protein IgG and neutralization titers in infants was likely due to a combination of waning maternal antibodies with increased post-natal age and a lack of RSV exposure. The lack of correlation between RSV antibodies and gestational age is expected as the bulk of maternal antibodies have waned in infants after 6 months age who formed the majority of our cohort.

Surveillance studies report infrequent infections in adults, although this is based on clinical detection of cases that come to medical attention [7]. These observations support the paradigm that RSV antibody immunity is stable in adults. However, other data support that the majority of RSV infections are not clinically detected [8]. Whereas data obtained prior to the COVID-19 pandemic showed that most children have been infected with RSV by 2 years of age [9], the current study supports that ongoing viral exposure is necessary to maintain high RSV antibody immunity in both adults and infants, and may also be necessary for optimal maternal transfer of RSV antibodies to infants. Overall, these findings suggest that RSV antibody levels wane rapidly in absence of viral exposure. This is supported by other studies showing a reduction in antibody neutralization to baseline within 5 months after intranasal RSV challenge in healthy adult volunteers [10]. A previous study examined the waning of antibody outcomes after documented RSV infection [11]. However, these data were more likely to be confounded by undetected RSV infections. Our study shed important light on the stability of RSV antibody outcomes in the context of little expected viral exposure for at least a year in BC.

These data have clinical implications. Seasonal RSV epidemics in temperate climates follow a seasonal biennial pattern linked to changes in population immunity, and supporting a half-life for optimal RSV protection between 6 and 12 months [12]. In adults, reduced antibody protection may have only moderate clinical relevance due to long-lived T cell immunological memory brought on

by life-long exposure to the virus. However, infants who do not have B or T cell memory may be more dependent on maternally-derived antibodies for protection against RSV in infancy. Data herein suggest that a population-level deficit in RSV immune protection may worsen subsequent seasonal RSV epidemics. This may also explain the inter-seasonal resurgence and increased median age for infants hospitalized for RSV in Australia as social distancing measures were relaxed [13]. Indeed, infants born during the pandemic may have remained susceptible at an older age as they were unable to acquire memory T and B cell immunity in absence of viral exposure. Children born during the pandemic, under two years of age, could be particularly vulnerable after a prolonged viral absence, so increased vigilance is warranted until RSV immunity levels are restored within populations.

It is important to acknowledge two main limitations of this study, which are i) that the observations presented herein were made in cohorts from a single regional health authority, and using a relatively limited population size and ii) that we don't know definitively to what extent passive maternal antibodies are required for protection against severe RSV infections in infants.

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Figure 1: Prefusion RSV F protein IgG and live virus neutralization in women and infants, and RSV-specific T cell responses in women from 2018 to 2021. (**A**) RSV IgG levels [AU/ml] in women of childbearing age (black dots; n = 64) and infants (open circles; n= 85) sampled post-winter season; (**B**) RSV antibody levels in 2021, subdivided between term (n = 44, gestational age range: 36-42 weeks) and preterm infants (n = 21; gestational age range: 24 to 34 weeks); (**C**) Live RSV antibody neutralization in women (black dots, n = 64) and infants (open circles, n = 52), as determined by the reciprocal of the lowest serum dilution to inhibit 95% viral cell syncytia formation *in vitro* (NT95) with a lower limit of detection of 8, in duplicate measures with data below lower limit of detection set to a 1:4 dilution (dotted line shows the limit of detection of the assay); (**D**) CD4 T cell activation in response to RSV nucleocapsid peptides in women of childbearing age 2021 and 2020 (n = 12, each year) (control = saline). Data are presented as boxes (25-75 percentiles) and whiskers, showing only relevant p values. Unpaired 2-sided t tests were used for comparison with infant samples in 2020 and 2021, and adult samples in 2018 and 2019, whereas paired 2-sided t tests were used when comparing between women samples in 2020 and 2021. Log-transformed data were used for all comparisons.

Table 1: Post-natal age-adjusted prefusion RSV F protein IgG and neutralization in infants collected in 2021.

	Unadjusted		Adjusted for post-natal age	
Outcome	Mean difference (95% CI)	p-value	Mean difference (95% CI)	p-value
prefusion RSV F IgG levels (AU/mL)	-74647 (-97285, -52009)	< 0.001	-73883 (-97179, -50586)	< 0.001
RSV neutralization (NT95)	-19.7 (-25.5, -14.0)	< 0.001	-19.6 (-25.5, 13.7)	< 0.001

AU/mL: Arbitrary Units per mL serum; NT95: Serum titers needed to prevent 95% viral syncytial formation compared to virus-free sera; For adjustment, a linear model was used, including cohort (infants 2020 *vs* .2021) and post-natal age as co-variates.

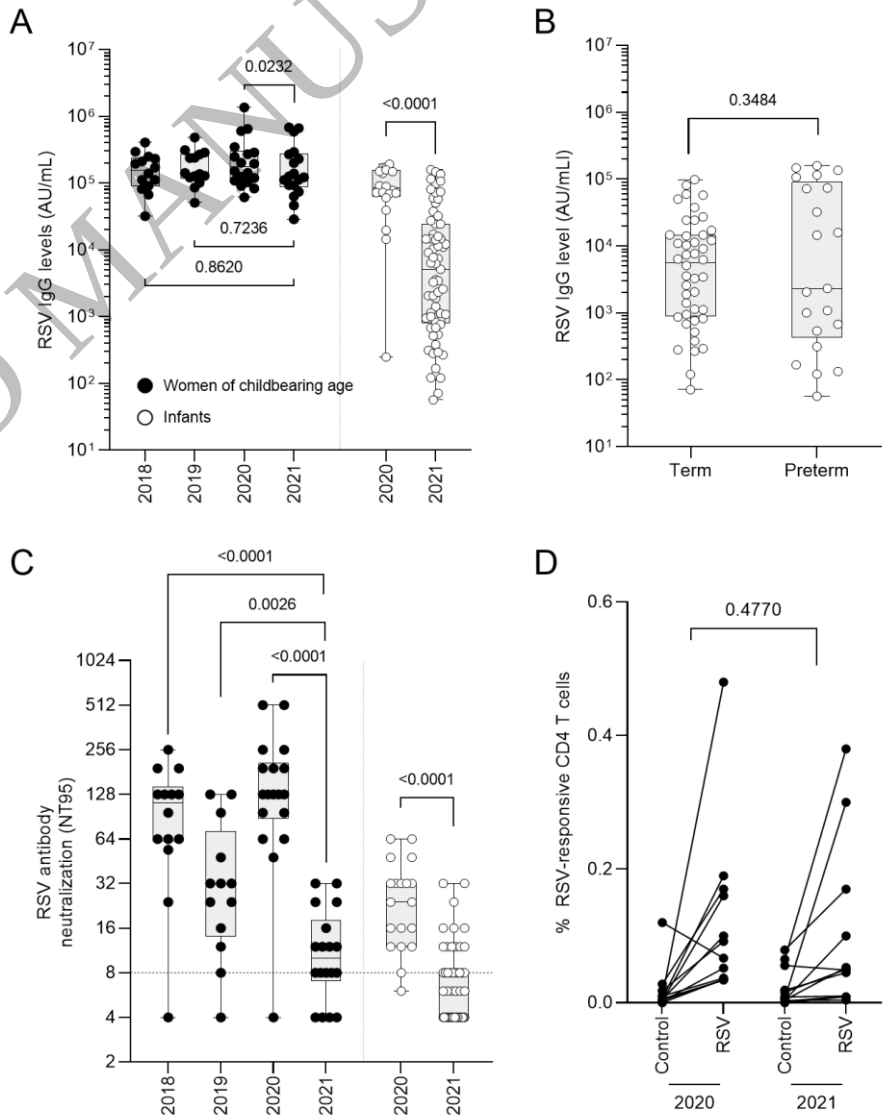

Figure 1
132x170 mm (.29 x DPI)