Adults 65 Years Old and Older Have Reduced Numbers of Functional Memory T Cells to Respiratory Syncytial Virus Fusion Protein

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Respiratory syncytial virus (RSV) infects elderly (≥65 years) adults, causing medically attended illness and hospitalizations. While RSV neutralizing antibody levels correlate inversely with RSV-associated hospitalization in the elderly, the role of RSV-specific T cells in preventing disease in the elderly remains unclear. We examined RSV-specific humoral, mucosal, and cellular immune profiles in healthy elderly (65 to 85 years) and young (20 to 30 years) adults. RSV neutralization antibody titers in the elderly (10.5 ± 2.2 log₂) and young (10.5 ± 2.1 log₂) were similar. In contrast, levels of RSV F protein-specific gamma interferon (IFN-γ)-producing T cells were lower in elderly (180 ± 80 spot-forming cells [SFC]/10⁶ peripheral blood mononuclear cells [PBMC]) than in young adults (1,250 ± 420 SFC/10⁶ PBMC). Higher levels of interleukin-13 (IL-13; 3,000 ± 1,000 pg/ml) in cultured PBMC supernatants and lower frequency of RSV F-specific CD107a⁺ CD8⁺ T cells (3.0% ± 1.6% versus 5.0% ± 1.6%) were measured in PBMC from elderly than young adults. These results suggest that deficient RSV F-specific T cell responses contribute to susceptibility to severe RSV disease in elderly adults.

MATERIALS AND METHODS

Study cohort. Thirty young adults who were 20 to 30 years old (median age, 26 years) and 30 elderly individuals who were 65 to 85 years old (median age, 74 years) were enrolled. All subjects were healthy and free of respiratory illness and had no hospitalization episodes for a 2-month period prior to sample collection by SeraCare Life Sciences, Inc. (Milford, MA), and Bioreclamation (Hicksville, NY). Informed consents given by all subjects were approved by Bioreclamation’s Independent Institutional Review Board. Since the amount of available peripheral blood mononuclear cells (PBMC) was insufficient to perform every assay on every donor sample, we used the indicated number of donor samples in each assay to enable reasonable comparisons between the age cohorts. The subjects’ demographic characteristics and the number and type of samples assessed in each immunological assay are shown in Table 2.

Specimen collection and processing. All specimens (whole blood, plasma, and nasal washes) were collected between the months of May and July and transported at ambient temperature to the processing site within 2 h of sample draw. PBMC were isolated from fresh whole blood using serum-free medium conditions, and frozen PBMC were placed at −80°C overnight before being transferred to liquid nitrogen for storage. Samples were shipped overnight on dry ice to MedImmune within 2 weeks of freezing and were thawed for enzyme-linked immunosorbent spot (ELISPOT) assay evaluation within 3 days of arrival. Plasma and nasal wash samples were aliquoted into 1-ml cryovials (Thermo Scientific, Rochester, NY) and frozen at −80°C at the processing site within 2 h of collection. Samples were shipped overnight on dry ice to MedImmune within 2 weeks of freezing and were thawed for evaluation within 1 week of arrival.

RSV antigens. The soluble fusion F protein of RSV A2 was expressed in Chinese hamster ovary (CHO) cells and immunoaffinity purified with
TABLE 1 RSV epidemiology in U.S. elderly (≥65 years)

| Parameter                        | Incidence rate (%) | Total no. of cases
|----------------------------------|--------------------|-------------------|
| RSV illnesses/year               | ~30/1,000 (~3)     | ~1,200,000        |
| RSV medically attended illnesses/year | ~10/1,000 (~1)   | ~420,000          |
| RSV-associated hospitalizations/year | ~1.5/1,000 (~0.15) | ~45,000–50,000   |
| RSV-associated deaths/year       | ~0.25/1,000 (~0.025) | ~10,000          |

*Totals cases based on a U.S. population of ~40,000,000 elderly, i.e., ≥65 years of age.*

an anti-RSV F monoclonal antibody (palivizumab; MedImmune, Gaithersburg, MD) to >95% purity. The sequence of the soluble RSV F protein lacking the transmembrane and cytoplasmic tail has been previously described (19). Wild-type (wt) RSV A2 and recombinant green fluorescent protein (GFP)-tagged RSV A2 were produced in Vero cells, RSV-specific CD4+ and CD8+ T cell immunodominant peptides described elsewhere (20–23) were custom designed and ordered at >90% purity from Proimmune (Oxford, United Kingdom). Although the major histocompatibility complex (MHC) restriction of some of the peptides in the pools was unknown, we utilized the information available in the published reports (20–23) to optimize our RSV-specific CD4+ and CD8+ T cell peptide pools for use in ELISPOT and cytokine multiplex assays.

**RSV microneutralization assay.** Donor plasma samples were heat inactivated at 56°C and serially diluted 2-fold in growth medium. Equal volumes of the diluted plasma were mixed with GFP-tagged RSV A2 virus at a concentration of 500 PFU/well. Samples were preincubated at 33°C for 1 h to facilitate antibody neutralization of the GFP-tagged RSV A2 virus. Samples were used to infect confluent monolayers of Vero cells (ATCC, Manassas, VA), and the cell plates were incubated at 33°C for 22 h. Fluorescent viral foci were enumerated using an IsoCyte Reader (Blue-shift Biotechnologies, Sunnyvale, CA), and a 50% reduction in fluorescent viral foci was calculated using a 4-parameter curve-fit algorithm. Data were expressed as log10 neutralization titers.

**RSV F-specific IgG by ELISA.** RSV F-specific IgG antibody titers in plasma were measured by enzyme-linked immunosorbent assay (ELISA). The plasma samples were serially diluted 2-fold and incubated on RSV F-coated plates for 1 h at 37°C. Horseradish peroxidase (HRP)-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch, West Grove, PA) was diluted 1:2,000, and incubated with the samples for 1 h at 37°C. The plates were washed, and goat anti-human IgG secondary antibody (KPL, Gaithersburg, MD) to detect antigen-specific antibody-secreting cells (ASC) or total IgG/IgA-producing ASC, respectively. Spots were enumerated using an ImmunoSpot analyzer (Cellular Technologies Ltd., Cleveland, OH).

**RSV-F-specific IFN-γ ELISPOT assay.** Human gamma interferon (IFN-γ) ELISPOT kits containing anti-IFN-γ precoated plates and anti-IFN-γ detection antibodies were purchased from Mabtech, Inc. (Mariemont, OH), and the assay was carried out per the manufacturer’s instructions. PBMC were incubated for 24 h at 37°C on the ELISPOT plates together with wt RSV A2 virus (1 PFU/10 cells) or 5 μg/ml of RSV F or 2 μg/ml of RSV F-specific CD4+ /CD8+ T cell peptide pools (ProImmune, Oxford, United Kingdom). The peptide pools were custom designed as described in the review by Olson and Varga to represent 12 CD8+ T cell epitopes and 21 CD4+ T cell epitopes of RSV F, respectively (21). Cells incubated with CTL-test medium (Cellular Technology Ltd., Cleveland, OH) or phorbol 12-myristate 13-acetate (PMA) and ionomycin (Sigma, St. Louis, MO) served as the negative and positive controls, respectively.

Data were expressed as spot-forming cells (SFC)/10^6 PBMC after background subtraction of medium wells to indicate a positive response. Valid samples were defined as those with viabilities of ≥70%, medium background of ≤50 SFC/10^6 PBMC, and PMA/ionomycin response of ≥500 SFC/10^6 PBMC.

**Th1/Th2 cytokine profiling by Luminex.** PBMC were incubated with 5 μg/ml of RSV F for 72 h. Medium alone or PMA with ionomycin stimulation served as the negative and positive control for each sample, respectively. Human cytokine multiplex kits were custom designed to include IFN-γ, interleukin-2 (IL-2), IL-4, IL-5, IL-10, IL-12 (p70), IL-13, IL-17, IP-10, and tumor necrosis factor alpha (TNF-α; Millipore, Billerica, MA). The assay was performed per the manufacturer’s instructions. The culture supernatants were analyzed on a Bio-Rad BioPlex 2200 (Bio-Rad, Hercules, CA), and cytokine concentrations are expressed in pg/ml.

**CD107a expression on CD8+ T cells.** Donor samples that were tested in the IFN-γ ELISPOT assay were also stained for CD107a expression following RSV F-specific stimulation. Cells were incubated with 5 μg/ml of RSV F or 2 μg/ml of RSV F-specific CD8+ T cell peptide pool for 8 h. Phycocerythrin (PE)-labeled CD107a or isotype control (BD Biosciences, San Jose, CA) antibody was added to the wells. Four hours later, 0.5 μg/ml of the Golgi plug, brefeldin A (BD Biosciences), Millipore, Billerica, MA), was added for the last 8 h of incubation. Medium alone or PMA with ionomycin-treated cells served as the negative and positive controls, respectively. Cells were surface stained with an antibody cocktail (BD Biosciences) to label CD3, CD8, CD19, and CD56 for 30 min. Cells were fixed, permeabilized, and incubated with PE-labeled CD107a or isotype control antibody for 30 min. All antibody incubations were carried out at 4°C. Samples were analyzed on a FACs Canto (BD Biosciences), and data are expressed as percent CD107a+ CD8+ T cells, fold change relative to the medium, and mean fluorescence intensity (MFI).

**Statistical analysis.** The Pearson’s correlation coefficient was used to measure the strength of the correlation between the different serological and cellular markers evaluated in the study cohorts. Statistical significance of the difference in the means between the young and elderly IFN-γ SFC/10^6 PBMC was determined using analysis of variance (ANOVA) and the two-sample t test.

**RESULTS**

Young and elderly adults have comparable titers of neutralizing antibodies to RSV. To determine whether the higher incidence of severe RSV disease in the elderly was related to the neutralizing antibody titers against RSV, we first examined RSV neutralizing antibody levels in plasma from 30 healthy young versus 30 elderly adults. Neutralizing antibody titers to RSV were determined using a microneutralization assay to a GFP-RSV A2 virus. The distribution range of the GFP-RSV A2 virus-specific neutralizing antibody titers across donors in the two cohorts demonstrated that the majority of the donors had RSV neutralizing antibody titers between 10 and 10 log2, with the elderly cohort showing slightly higher...
numbers of donors with titers between 12 and 15 log₂ (Fig. 1A). An average neutralizing antibody titer of 10.5 ± 2.2 log₂ was observed in both cohorts.

Since neutralizing antibody titers can include antibodies to both RSV F and G proteins, we next examined whether there were measurable differences in the RSV F-specific IgG titers between the young and elderly donors. The young and elderly donors also had equivalent titers of RSV F-specific plasma IgG antibodies (Fig. 1B), and there was a strong correlation (r² = 0.855) between RSV A2 neutralizing antibody and RSV F-specific IgG titers (Fig. 1C). Presence of RSV F-specific nasal IgA antibodies has been associated with protection from severe RSV disease in the elderly; therefore, we compared RSV F-specific IgA titers in the nasal washes of the young and elderly donors (Fig. 1D). We also measured the total IgA and protein concentration levels (data not shown) in the nasal washes and did not find significant differences between the young and elderly samples. Importantly, the RSV F-specific IgA titers were equivalent between the young and elderly donors (Fig. 1D), suggesting that other deficits in the immune system account for susceptibility to RSV disease in the elderly.

**RSV F-specific memory B cell frequencies are comparable in the young and elderly.** We next explored the RSV F-specific memory B cell frequency in PBMC to determine if there were quantitative differences between young and elderly donors. Young adults showed an RSV F-specific IgG ASC frequency ranging from 0.05 to 0.5% of the total IgG ASC, whereas elderly adults showed an RSV F-specific IgG ASC frequency ranging from 0 to 0.68% of the total IgG ASC frequency (Fig. 2). However, an overall trend of reduced RSV F-specific IgG ASC frequency between the two cohorts was calculated by the unpaired t test was not statistically significant (P = 0.2413). However, an overall trend of reduced RSV F-specific IgG ASC frequency in the elderly was noted, except for two donors that possessed relatively high F-specific memory IgG ASC frequency (Fig. 2). Taken together, these data indicated that the elderly were not deficient in neutralizing antibody titers or RSV F-specific memory B cell responses.

**Elderly adults have lower levels of RSV F-specific IFN-γ-producing cells than young adults.** We decided to evaluate whether RSV F-specific memory T cell responses would be significantly different between the young and elderly. Findings on the frequency of RSV F-specific T cell immune responses in PBMC from

### Table 2 Demographic characteristics of the study cohort and assays performed

| Demographic or assay parameter | Demographic or assay result by age | P value |
|-------------------------------|-----------------------------------|---------|
|                               | 20–30 yr old (young) | 65–85 yr old (elderly) | |
| **Demographics**              |                              |         |
| Age (yr; median ± SD)         | 26 ± 3                       | 74 ± 6             | <0.0001 |
| Age (yr; range)               | 20–30                         | 65–85          |
| Gender (no. [%])              |                               |         |
| Male                          | 14 (46.7)                     | 12 (40)            | 0.6021 |
| Female                        | 16 (53.3)                     | 18 (60)            | 0.6020 |
| Race (no. [%])                |                               |         |
| Caucasian                     | 8 (26.7)                      | 7 (23.3)            | 0.3903 |
| Hispanic                      | 11 (36.7)                     | 7 (23.3)            | 0.4196 |
| African-American              | 11 (36.7)                     | 16 (53.3)           | 0.2776 |
| **Assay parameters [mean ± SD (no. of samples/assay)]** |         |
| Sample                        |                              |         |
| Plasma                        | RSV neutralization (log₂ titer) | 10.5 ± 2.2 (30)   | 10.5 ± 2.1 (30) | 0.7758 |
|                               | RSV F IgG ELISA (×10⁴ titer)   | 0.90 ± 0.31 (20)  | 0.93 ± 0.36 (20) | 0.8821 |
| Nasal wash                    | RSV F IgA ELISA (RLU)          | 1.4 × 10⁴ ± 1.2 × 10⁴ (10) | 1.0 × 10⁴ ± 1.1 × 10⁴ (20) | 0.7694 |
| PBMC                          | Memory B cell ELISPOT (% F-specific ASC/total IgG ASC) | 0.22 ± 0.25 (10) | 0.18 ± 0.5 (20) | 0.2413 |
|                               | Memory T cell ELISPOT (SFC/10⁶ PBMC) | 360 ± 130 (12) | 93 ± 41 (20) | 0.0262 |
|                               | RSV A2 virus                  | 1,250 ± 420 (20) | 180 ± 80 (20) | <0.0001 |
|                               | RSV F protein                 | 450 ± 180 (12) | 100 ± 60 (12) | <0.0001 |
|                               | CD4⁺ T cell peptides          | 400 ± 210 (12) | 90 ± 55 (12) | <0.0001 |
|                               | CD8⁺ T cell peptides          |                   |               |         |
| CD107a flow cytometry (% CD107a⁺ CD8⁺ T cells) | RSV F protein | 5.0 ± 1.6 (14) | 3.0 ± 1.7 (14) | 0.0173 |
|                               | CD4⁺ T cell peptides          | 4.7 ± 1.9 (14) | 2.3 ± 1.8 (14) | 0.0045 |
|                               | Th1-Th2 cytokines (pg/ml)     | 7,500 ± 3,100 (10) | 3,000 ± 800 (10) | 0.0004 |
|                               | RSV F IFN-γ                   | 475 ± 450 (10) | 5,000 ± 4,000 (10) | 0.0017 |
|                               | RSV F IL-13                   | 1,000 ± 560 (10) | 3,000 ± 1,000 (10) | 0.0033 |
the young and elderly donors are summarized in Table 2. In contrast to findings for plasma neutralizing antibody titers and memory B cell responses, PBMC from elderly donors had reduced RSV-specific IFN-γ responses to whole virus (Fig. 3A), F antigen (Fig. 3B), and F-specific immunodominant CD4+ and CD8+ T cell peptide pools (Fig. 3C and D) compared to PBMC from young donors. RSV A2 virus-specific IFN-γ-producing memory T cell frequency/10^6 PBMC was 360 ± 130 SFC in the young and 93 ± 41 SFC in the elderly (P = 0.0262) (Fig. 3A). Total RSV F-specific IFN-γ-producing cell frequency in the young cohort was 1,250 ± 420 SFC, while that observed in the elderly was only 180 ± 80 SFC (P < 0.0001) (Fig. 3B). Further examination of the RSV F-specific memory T cell responses revealed that the IFN-γ-producing CD4+ and CD8+ T cell frequency in the young was 450 ± 180 SFC, while that seen in the elderly was 100 ± 60 SFC (P < 0.0001) (Fig. 3C). The CD8+ T cell frequency in the young was 400 ± 210 SFC and 90 ± 55 SFC in the elderly (P < 0.0001) (Fig. 3D). Finally, there existed a strong correlation (r^2 = 0.887) between the RSV F-specific IFN-γ-producing CD4+ and CD8+ T cell numbers in both of these cohorts (Fig. 3E). Thus, the observed deficit in the numbers of functional memory T cells in the elderly existed in both the CD4+ and CD8+ T cell compartments. Taken together, our results suggest that the reduced numbers of RSV F-specific IFN-γ-producing T cells contribute to the lack of complete immunity against RSV, predisposing elderly adults to disease.

Elderly humans have significantly lower numbers of degranulating RSV F-specific CD107a+ CD8+ T cells. The comparative cytolytic potential of the RSV F-specific CD8+ T cells in PBMC from the two cohorts was evaluated by quantifying the number of CD107a-expressing degranulating CD8+ T cells following an in vitro stimulation of PBMC with RSV F or a peptide pool containing RSV F-specific immunodominant CD8+ T cell peptides. The CD107a or LAMP1 (for lysosomal associated membrane protein-1) protein is intracellularly expressed in activated cytotoxic T cells (25). The gating strategy utilized in the study and acquisition plots for medium, PMA/ionomycin, RSV F protein, or CD8+ T cell peptide pool stimulation are illustrated for representative young (24 years) and elderly (76 years) donor PBMC.

FIG 1 Equivalent RSV-specific antibody levels in plasma of young and elderly donors. (A) RSV-specific antibody titers in plasma from young (n = 30) and elderly (n = 30) donors were compared by microneutralization of GFP-RSV A2 virus. (B) F-specific IgG ELISA. (C) Correlation analysis between neutralizing antibody titers and F-specific IgG titers. (D) RSV F-specific and total IgA titers in nasal washes of young (n = 10) and elderly (n = 20) adults.

FIG 2 RSV F-specific memory B cells in young and elderly donors. RSV F-specific memory B cells were measured in young (n = 10) and elderly (n = 20) donor PBMC. PBMC cultures were stimulated ex vivo with a mixture of pokeweed mitogen, S. aureus protein A, and CpG DNA. The expanded RSV F-specific antibody-secreting cells (ASC) were measured in a memory B cell ELISPOT assay. Data are expressed as percent RSV F-specific IgG ASC/total IgG ASC.
Different degrees of background CD107a expression were observed in all samples (Fig. 4B). However, upon incubation with RSV F protein or CD8^{x/H11001} T cell peptide pool, this number increased from a baseline of 1.5 to 5% of the parent CD8^{x/H11001} T cell population in the young cohort compared to an increase from 2.0 to 3.0% of the parent CD8^{x/H11001} T cell population following antigen incubation in PBMC from the elderly (P < 0.005) (Fig. 4B). The fold change relative to medium in the percentage of CD107a^{x/H11001}CD8^{x/H11001} T cells following RSV F or CD8^{x/H11001} peptide pool stimulation was calculated and showed an increase of 2- to 22-fold in young and 0- to 2.5-fold in elderly donor PBMC, respectively (P < 0.005) (Fig. 4C). The mean fluorescence intensity (MFI) of the RSV F-specific CD107a^{x/H11001}CD8^{x/H11001} T cells was equivalent in the elderly and young donor PBMC samples (data not shown), suggesting that the difference between the young and elderly was the frequency of CD8^{x/H11001} T cells expressing CD107a rather than the number of CD107a molecules expressed on each RSV F-specific CD8^{x/H11001} T cell. The lower numbers of RSV F-specific CD107a^{x/H11001}CD8^{x/H11001} T cells in the elderly suggests a potential mechanism for the higher susceptibility to RSV disease in this population.

Elderly donor PBMC secrete relatively high basal levels of IL-13 ex vivo. The functional activity of the RSV F-specific T cells was examined ex vivo by measuring cytokine production in PBMC in response to stimulation with RSV F antigen or a peptide pool containing RSV F-specific immunodominant CD4^{x/H11001} T cell peptides. The cytokines that were undetectable in the cell supernatants of PBMC cultures from either cohort included IL-2, IL-4, IL-5, IL-17, IL-12(p70), and TNF-α. In contrast, cytokines that were present in measurable quantities in the cell supernatants included IFN-γ, IP-10, IL-10, and IL-13. Basal levels of the Th1 cytokine, IFN-γ, were secreted at low levels in both cohorts. However, upon RSV F stimulation, considerably higher levels of IFN-γ (7,500 ± 3,100 pg/ml) were produced by PBMC from young compared to elderly donors (3,000 ± 1,000 pg/ml) (P < 0.0004) (Fig. 5A). This finding was consistent with the results obtained in the IFN-γ T cell ELISPOT assay, where significantly higher numbers of RSV F-specific IFN-γ-producing cells were observed in young donors compared to elderly donors (Fig. 4A).

FIG 3 Elderly have significantly lower numbers of RSV F-specific IFN-γ-producing cells. PBMC from young and elderly donors were stimulated ex vivo using (A) wt RSV A2 at 1 PFU/10 cells (n = 12 young and n = 20 elderly), (B) 5 μg/ml of RSV F protein (n = 20 young and n = 20 elderly), (C) 2 μg/ml of RSV F-specific CD4^{x/H11001} T cell peptide pools (n = 12 young and n = 12 elderly), or (D) 2 μg/ml of RSV F-specific CD8^{x/H11001} T cell peptide pools (n = 12 young and n = 12 elderly). The IFN-γ secreted by T cells was measured by ELISPOT assay, and data are expressed as spot-forming cells (SFC)/10^6 PBMC. (E) Correlation between RSV F-specific CD4^{x/H11001} and CD8^{x/H11001} T cell frequencies.
of IFN-γ-producing T cells were observed in PBMC from the young cohort (Fig. 3). Interestingly, PBMC from elderly donors secreted relatively high basal levels of the Th2 cytokine, IL-13 (2,500 ± 2,000 pg/ml), compared to the levels produced by PBMC from young donors (800 ± 600 pg/ml) (Fig. 5B). Stimulation of elderly or young PBMC with RSV F slightly increased IL-13 secretion; however, the increase above basal levels was not statistically significant in either cohort (Fig. 5B). The inability of elderly PBMC to secrete high levels of IFN-γ upon RSV F stimulation seemed to reflect the inherently weak Th1 antigenic responses observed in aged individuals. Moreover, the high basal levels of IL-13 are consistent with a Th2 polarization of the CD4+ T cell compartment in this population. Both of these factors may contribute to the increased susceptibility to RSV disease observed in the elderly.

**DISCUSSION**

In this study, we explored immunological factors that could contribute to the higher susceptibility of elderly adults to severe disease by measuring the RSV F-specific humoral and cellular im-

![Image](https://example.com/image.png)
mune responses in healthy elderly and young adults. Compared to the overall U.S. population (26), the African-American and Hispanic numbers in both our study cohorts appeared relatively over-represented, while the Caucasian numbers appeared relatively under-represented (Table 2). However, analysis of the RSV F-specific responses obtained in the different assays by race indicated that the results were not influenced by racial or ethnic origin of the donors (data not shown).

RSV infection in adults elicits a robust serum antibody response (27, 28). The neutralizing antibody titers to GFP-tagged RSV A2 measured in the elderly donors in our study were consistent with previous findings (27–30). RSV F- and G-specific IgA antibodies measured in nasal washes of older adults ≥65 years of age have been shown to correlate with protection from RSV disease (31). We expect a successful RSV vaccine candidate that prevents disease in elderly adults to elicit robust and durable humoral, mucosal, and T cell-mediated immunity (31). It has been shown that RSV-specific IgE antibodies can be detected in the nasopharyngeal secretions of patients with acute RSV bronchiolitis, and interestingly, the levels correlated inversely with the number of circulating RSV-specific CD8+ T cells (32). Evidence indicates that RSV-specific IgE-mediated mast cell degranulation and histamine release in the airway epithelium, along with the induction of Th2-type cytokines in the respiratory tract, potentiates the clinical symptoms of severe RSV disease (33). Our data showed no measurable RSV-specific serologic, mucosal, or memory B cell deficits in elderly adults (Table 2), implying that these immune responses are insufficient to fully protect against RSV disease in this population.

We hypothesized that T cell responses to RSV proteins play an important role in clearing RSV infections, and at least one report has suggested that the persistence of RSV-specific memory CD8+ T cells following primary infection is important in preventing severe RSV disease (16). Moreover, RSV-specific CD8+ T cells have been detected in the peripheral blood of previously infected adults in whom these responses have been associated with decreased clinical symptoms (34, 35). The contribution of the different antigen-specific T cell subsets to recovery from RSV infection has been extensively studied in mouse models, and long-lived RSV-specific cytotoxic T cell function has been demonstrated to be critical for the accelerated clearance of virus from the lungs of infected animals (36–40). Recently, it was demonstrated that RSV-specific CD8+ T cells induced upon vaccination protected mice against RSV infection and pathogenesis, and the authors reported that waning protection correlated with reduced CD8+ T cell cytokine expression (41).

To determine if impaired RSV-specific T cell numbers and/or function could account for the susceptibility to severe RSV illness in the elderly, we compared the RSV F-specific CD4+ and CD8+ T cells in PBMC from elderly and young adults. We utilized previously published RSV F T cell epitope mapping results (20–23) to custom synthesize T cell peptide pools consisting of 12mers or 9mers to evaluate putative CD4+ or CD8+ T cell responses, respectively, realizing that the lack of a response in our ELISPOT assay may also be attributed to HLA mismatch between the stimulating peptide and the donor PBMC. Notably, the young-adult PBMC produced significantly higher IFN-γ responses to the same peptide pools than elderly PBMC. Furthermore, elderly PBMC appeared to have comparable numbers of total CD3+ and CD8+ T cells and CD19+ B cells compared to young-adult PBMC based on our flow cytometry studies (data not shown). Future studies will focus on delineating the CD4+ versus CD8+ T cell responses in the ELISPOT assay by performing T cell subset depletions of whole PBMC or using flow cytometry to identify relevant cytokine-producing T cell populations.

Cell surface expression of CD107 has been correlated with the secretion of cytolytic molecules, such as perforin and granzyme B, following cytotoxic T lymphocyte (CTL) activation (42). In vivo, CD107a and CD107b are expressed on the surface of CD8+ T cells following activation with the cognate antigen leading to IFN-γ production and cytolytic activity directed against the target cells (25). Our studies suggest that since the amount of CD107a expressed on CD8+ T cells of elderly PBMC is relatively similar to that seen in young adults, the elderly may benefit from a vaccine that can boost the numbers of RSV F-specific degranulating cytotoxic T cells.

Aging individuals experience deficits in both innate and adaptive immune responses to pathogens, reflecting changes at the molecular, cellular, and tissue levels that accumulate over time (21, 43, 44). As a result, immune responses to vaccination in the

FIG 5 Elderly PBMC secrete relatively low levels of IFN-γ in conjunction with secreting high basal levels of IL-13 \textit{ex vivo}. PBMC cultures (n = 10 young and n = 10 elderly) were incubated in medium alone or with RSV F protein, and the resulting cytokines secreted into the supernatant were measured using a multiplex Th1-Th2 cytokine bead array panel (Luminex). (A) IFN-γ. (B) IL-13.
elderly are diminished compared to those in young adults, posing serious challenges to the development of effective vaccination strategies.

Taken together, results from the studies presented here demonstrate that elderly individuals possess relatively high titers of RSV neutralizing antibodies that are comparable to those seen in young adults. However, the elderly are deficient in RSV F-specific CD4+ Th1 and/or CD8+ memory T cell responses that may contribute to their higher susceptibility to RSV disease. A longitudinal evaluation in elderly adults involving systematic correlation of RSV F-specific T cell responses with the clinical outcomes of RSV disease should provide significant insight into the observations made in the current study. Further work will be required to elucidate the precise mechanisms governing the distinct RSV F-specific memory T cell responses observed in elderly versus young individuals.

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