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Serum C-Reactive Protein and Congestive Heart Failure as Significant Predictors of Herpes Zoster Vaccine Response in Elderly Nursing Home Residents

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Background. Elderly long-term care residents often exhibit a myriad of risk factors for immune dysfunction, including chronic inflammation and multiple comorbid conditions, which undoubtedly contribute to their enhanced susceptibility to infection. Hence, understanding the factors required for optimal vaccine responsiveness is critical.

Methods. We examined 187 elderly nursing home residents (aged 80–102 years) and 50 community-dwelling seniors (aged 60–75 years) immunized with the live-attenuated varicella-zoster virus (VZV) vaccine. Specifically, we examined whether vaccine responsiveness was associated with serum C-reactive protein (CRP), tumor necrosis factor, interleukin 1β, 6, and 10, leukocyte telomere length, chronic disease status, and frailty.

Results. Elderly participants had significantly higher levels of CRP, tumor necrosis factor, and interleukin 6 and shorter leukocyte telomere length. Vaccine responsiveness was inversely related to the CRP level in elderly participants, but not seniors, and those with congestive heart failure were less likely to achieve a 2-fold response (odds ratio, 0.08). The latter relationship is probably due to immunosenescence, because heart failure was associated with increased senescent $CD4^+$ T cells, and reduced naive and effector and central memory $CD8^+$ T cells.

Conclusions. In summary, these data improve our understanding of vaccine responsiveness for those in long-term care, suggesting that certain risk factors are associated with a greater likelihood of vaccine failure.

Keywords. Vaccination; Varicella-zoster; inflammation; heart failure; immunosenescence.

Primary infection with varicella-zoster virus (VZV) usually occurs in young children, resulting in chickenpox. After illness, VZV becomes latent in the dorsal root ganglion, a cluster of nerve cell bodies in a dorsal root of the spinal nerve, and remains dormant until reactivation [1]. Reactivation often leads to herpes zoster (HZ), also known as shingles, which is characterized by a painful skin rash lasting days to weeks [2, 3]. Pain persisting >90–120 days after HZ rash onset is known as postherpetic neuralgia [4] and can require analgesic and neuroactive drugs for pain management as well as hospitalization [3].

VZV-specific immunity wanes with age [5–7], which is probably the major contributing factor for the elevated incidence of HZ and postherpetic neuralgia in older adults [8]. Elderly residents of long-term care are of particular concern with regard to development HZ, owing not only to their advanced age but also to a high degree of immunosenescence, malnutrition, and existing chronic conditions [9]. Lelic et al [10] showed that elderly nursing home residents do indeed have reduced VZV-specific immunity before and after vaccination, even though their vaccine response did not differ from that in community-dwelling older adults when measured as a fold change relative to baseline. That said, variability in the magnitude of this response was significantly greater in the elderly, suggesting the presence of underlying factors that influence the vaccine response.

In the previous study, Lelic et al [10] also showed that the frequency of cytomegalovirus-specific $CD4^+$ T cells and regulatory T cells, 2 lymphocyte subsets that potently suppress viral immunity [11, 12], were inversely related to the vaccine response of elderly nursing home residents. Other studies in community-dwelling adults found that chronic conditions and comorbid conditions such as diabetes [13] and depression [14], and changes in serum cytokines [15], significantly influence VZV vaccine responsiveness. Other risk factors or mechanisms that influence the response to VZV vaccination in older adults, whether community dwelling or in long-term care, remain to be discovered.

In the current study, we sought to expand our understanding of the risk factors related to VZV vaccine responsiveness in elderly long-term care residents. Specifically, we investigated the levels of serum C-reactive protein (CRP), tumor necrosis factor (TNF), interleukin 6, 1β, and 10 (IL-6, IL-1β, and IL-10),

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leukocyte telomere length, disease status, and frailty, comparing those relationships with vaccine responsiveness to findings in community-dwelling seniors (60-75 years).

METHODS

VZV Vaccine Study: Participants and Design

This study is based on participants from a previously published trial comparing VZV vaccine immunogenicity in community-dwelling seniors and elderly nursing home residents ([10]; Clinical Trials registration: NCT01328548). For this study, nursing home residents (n = 187) from 18 facilities in Hamilton and Toronto (Ontario, Canada) were recruited between January 2012 and February 2014; they were between 80 and 102 years of age (median, 89 years), 81% female, and all had ≥1 comorbid condition. Community-dwelling seniors (n = 50) from the same geographic location were recruited between the months of February and May 2012, and were between 60 and 75 years of age (median age, 68 years), 64% female, and had no more than 1 comorbid condition.

Frailty, defined using a frailty index score [10, 16], and comorbidity status, defined using a major condition score [10], were included in analyses, as well as the following comorbid conditions: congestive heart failure (CHF; prevalence in elderly nursing home residents, 13%), chronic pulmonary disease (11%), dementia (67%), diabetes (with or without end-organ damage, 23%), hemiplegia (12%), and peripheral vascular disease (64%); comorbid conditions with a prevalence <10% in the nursing home residents were not included. Written informed consent was obtained from all participants or their legally appointed guardians. The study protocol and consent procedures were approved by the McMaster Research Ethics Board and the participants' nursing homes.

All participants were subcutaneously administered the live-attenuated Oka strain of VZV (≥19 400 plaque-forming units; Zostavax, Merck Group). Heparinized venous blood was obtained before vaccination (baseline) and 6 weeks later (follow-up) and processed within 8 hours of blood collection; serum was collected only at baseline and stored at −20°C. Peripheral blood mononuclear cells (PBMCs) were isolated using a validated protocol [17] and were cryopreserved in vapor-phase liquid nitrogen (160°C) in 10% dimethyl sulfoxide/human AB serum (Lonza) freezing medium.

Interferon γ Enzyme-Linked Immunospot Assay

Vaccine responses were determined by interferon (IFN) γ enzyme-linked immunospot assay, as described elsewhere [10]. Briefly, cryopreserved PBMCs were thawed at 37°C, washed and resuspended in complete medium. Cells (5×10^5) were added to Multiscreen-IP membrane plates (Millipore) coated with anti-human IFN-γ and stimulated with VZV antigen for 20 hours at 37°C; VZV antigen was prepared from lysed, UV light–inactivated VZV-infected MRC-5 cells. Plates were subsequently incubated with biotinylated anti-human IFN-γ, followed by streptavidin-alkaline phosphatase. Spots were enumerated by CTL ImmunoSpot Image Analyzer system and counting software (Cellular Technologies), and VZV-specific responses were reported as absolute VZV spot-forming cells per 10^6 PBMCs. Fold-change responses were calculated as the VZV-specific spot-forming cell count at follow-up relative to the baseline count.

Serum Cytokines, CRP, and Telomere Length Quantification

Serum cytokines TNF, interleukin (IL)-6, IL-1β and IL-10 were measured using the Milliplex MAP Human High Sensitivity T Cell kit (Millipore) according to manufacturer's recommendations. CRP was measured in serum (1/500 diluted) by sandwich enzyme-linked immunosorbent assay using the monoclonal capture and detection antibody clones C5 (ab8279; 1 μg/mL) and C6 (ab24462; 1 μg/mL) (Abcam), respectively, and using native, purified CRP as a standard (Aviva Systems Biology). Cytokines and CRP were natural log-transformed in regression analyses to minimize the effect of outliers.

Telomere length was measured in heparinized peripheral blood. Briefly, genomic DNA was extracted from whole blood using the DNeasy Blood Mini Kit (Qiagen). Telomere length was measured using a validated quantitative polymerase chain reaction assay [18] at the Genetic and Molecular Epidemiology Laboratory in Hamilton, Ontario, and reported as the telomere to single-copy gene (*T*/*S*) ratio.

Analysis of Immunosenescence in Elderly Participants With CHF

To examine the effects of CHF on immunosenescence, we analyzed a large cohort of nursing home residents in whom T-cell immunosenescence markers were previously quantified [19–21]. This cohort includes 1072 participants from 32 nursing homes in 4 Canadian cities (including Hamilton, Ontario) and is similar to the current cohort with regard to median age (86 years), sex ratio (72% female), and CHF prevalence (14%). Written informed consent was obtained from all participants or their legally appointed guardians, and the study protocol and consent procedures were approved by the McMaster Research Ethics Board and the participants' nursing homes. The following $CD4^+$ and $CD8^+$ T-cell subsets were enumerated in cryopreserved PBMCs by flow cytometry: naive, $CD45RA^+CCR7^+$; central memory, CD45RACCR7$^+$; effector memory, $CD45RA^-CCR7^-$; terminally differentiated, $CD45RA^+CCR7^-$; and senescent, $CD28^-CD57^+$. Each subset was reported as the percentage of $CD3^+$ cells and natural-log-transformed to minimize the effects of outliers. To allow log-transformation of those subsets whose frequency was zero, 0.1% was added to all subsets.

Statistics

All analyses were performed using R software, version 3.3.2 (R Foundation for Statistical Computing). Pairwise comparisons were performed by means of Wilcoxon rank sum test or

Spearman correlation test. Associations with disease status were determined by using multiple logistic regression, adjusting for age and sex. For associations with continuous $\log_2$ VZV fold-change responses or having a ≥2-fold VZV vaccine response, multiple linear regression and logistic regression were performed, respectively, adjusting for age, sex, and $\log_2$ baseline VZV response. These models were performed in the elderly and senior cohorts separately, and the resulting trends were compared afterward; adjustment for the baseline response was deemed necessary, because it is significantly associated with the overall fold-change vaccine response in both elderly persons ($\beta = -.48$; $P < .001$) and seniors ($\beta = -.47$; $P < .001$), and such adjustment improved the fitness of the above models, as determined by the Akaike information criterion (data not shown). For tests of immunosenescence markers, linear mixed models were used, fitting age, sex, and CHF as fixed effects and nursing home residence as a random effect. Type II *P* values were calculated using the Wald χ^2 test.

RESULTS

Elevated Serum Cytokine and CRP Levels in Elderly Nursing Home Residents

The levels of TNF, IL-1β, IL-6, IL-10, and CRP were measured in the serum of elderly nursing home residents (n = 187) and community-dwelling seniors (n = 50). Elderly participants were older (median age [range], 89 [80–102] vs 68 [60–75] years), were more frail (median frailty index, 0.31 vs 0.03; $P < .001$), and had a higher comorbidity score (2 vs 0; $P < .001$). As expected, the levels of serum cytokines and CRP were also higher, as follows: CRP (median [interquartile range], 21.2 μg/mL [6.7–57.2] vs 4.1 μg/mL [1.9–13.3]; $P < .001$), TNF (7.9 pg/mL [6.1–10.0] vs 4.6 pg/mL [3.7–5.9]; $P < .001$), IL-6 (3.1 pg/mL [1.7–5.3] vs 0.95 pg/mL [0.49–1.76]; $P < .001$) (Figure 1A). Leukocyte telomere length was also measured in a subset of participants (elderly, n = 35; seniors, n = 39), and although the median length was shorter in the elderly (*T*/*S* ratio, median [interquartile range], 0.57 [0.51–0.71] vs 0.66 [0.54–0.96]), this difference did not reach statistical significance ($P = .058$) (Figure 1B).

The levels of serum cytokines and CRP were also compared between elderly participants with or without the following diseases, using logistic regression: CHF (prevalence, 13%), peripheral vascular disease (64%), dementia (67%), chronic pulmonary disease (11%), diabetes (23%), and hemiplegia (12%). These diseases were selected because they exhibited a prevalence >10% in our nursing home elderly cohort; no disease had >2% prevalence in the senior cohort; hence, seniors were not included in the analysis. Elderly participants with diabetes had significantly lower levels of CRP (natural-log-transformed

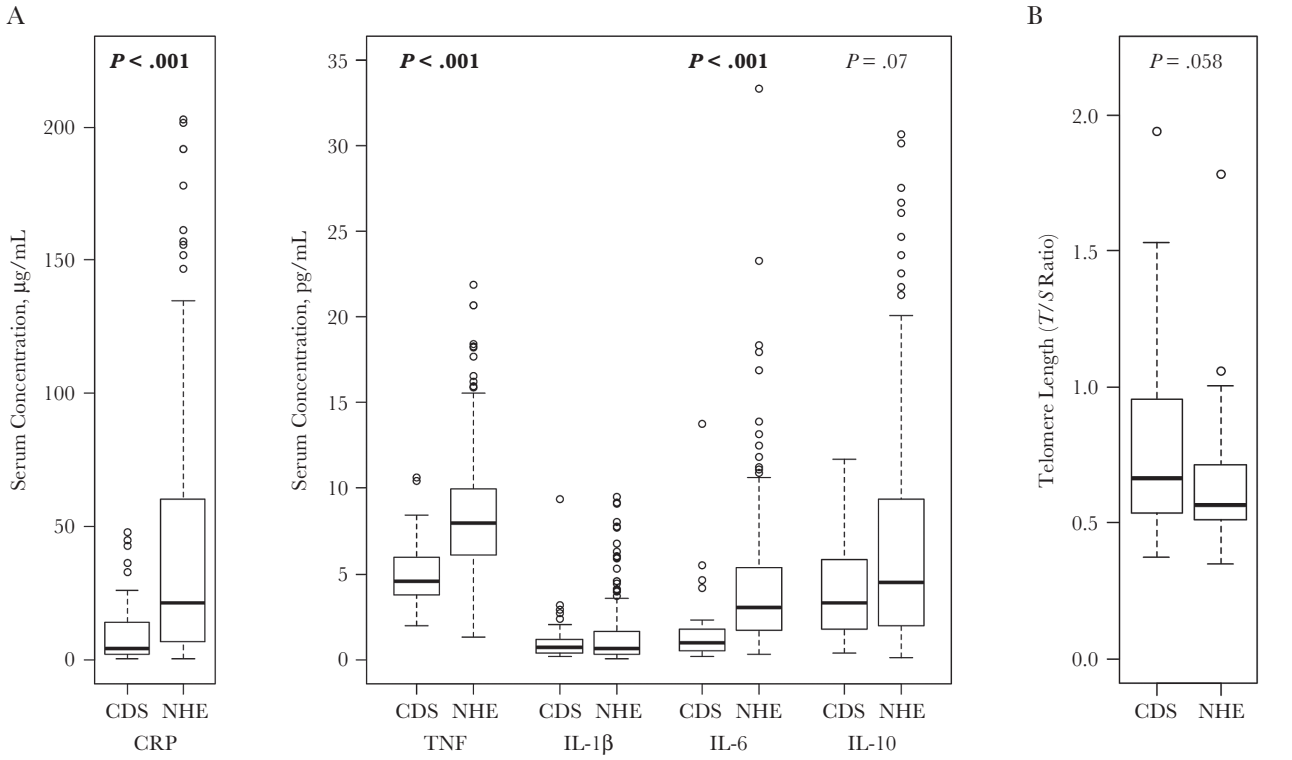

Figure 1. Serum levels of inflammatory mediators are significantly higher in elderly nursing home residents. *A*, Serum C-reactive protein (CRP), tumor necrosis factor (TNF), and interleukin 1β, 6, and 10 (IL-1β, IL-6, and IL-10) were measured in the nursing home elderly (NHE; n = 187) and community-dwelling senior (CDS; n = 50) cohorts. *B*, Telomere length was measured in leukocytes from a subset of NHE (n = 35) and CDS (n = 39) donors. Significance was determined by means of Wilcoxon rank sum test, and only results with *P* values <.10 are shown.

mean [standard error (SE)], 2.24 μg/mL [0.13] vs 3.13 μg/mL [0.12]; $P = .002$), those with hemiplegia had significantly lower levels of TNF (1.85 [0.04] vs 2.06 [0.04]; $P = .045$), and those with peripheral vascular disease had significantly higher levels of TNF (2.12 [0.05] vs 1.90 [0.06]; $P = .004$). No associations were observed between the levels of serum cytokines/CRP and frailty or comorbidity score, or between telomere length and disease status, frailty, or comorbidity score.

Association of CRP and Disease Status With VZV Vaccine Response in Elderly but Not Senior Participants

Lelic et al [10] have shown elsewhere that the immunogenicity of the VZV vaccine does not differ between the elderly nursing home residents and community-dwelling seniors (fold change in elderly participants, median [interquartile range], 1.8 [1.2–3.2]; fold change in seniors, 1.5 [1.2–2.3]); however, the variance in this response was significantly greater in the elderly (F test, $P < .001$), indicating the presence of underlying contributing factors. To ascertain whether serum cytokine and CRP levels were associated with the $\log_2$ VZV vaccine response, we performed multiple linear regression, adjusting for age, sex, and $\log_2$ VZV baseline response. Adjusting for the baseline response was critical, given that it is strongly correlated with the follow-up response ($\rho = 0.7$; $P < .001$). Of all molecules tested, only CRP was found to predict the VZV vaccine response; an inverse relationship was observed in the elderly nursing home residents ($\beta = -.14$ [SE, .06], $P = .01$; Figure 2A), but not in community-dwelling seniors (Figure 2B). No associations with telomere length were observed.

We also sought to determine whether disease- status was a significant predictor of the VZV vaccine response. Elderly participants with CHF were at a significantly lower odds of having a ≥2-fold VZV vaccine response (odds ratio [95% confidence interval], 0.08 [.019–.27]; $P < .001$), whereas those with dementia had significantly higher odds (2.2 [1.1–4.5]; $P = .04$) (Figure 3). Both of these associations remained significant when CRP was also adjusting for, as did the above association with CRP with adjustment for disease status.

Increased T-Cell Markers of Immunosenescence in Elderly Residents With CHF

Immunosenescence of the T-cell compartment plays a prominent role in the response of older individuals to vaccination [22]. Hence, to further investigate the mechanism of CHF on VZV vaccine responsiveness, we analyzed a large cohort of elderly nursing home residents (median age [range], 86 [65–102] years), comparing the frequency of peripheral blood $CD4^+$ and $CD8^+$ T-cell subsets (naive, effector and central memory, terminally differentiated, and exhausted) between individuals with (n = 148) or without (n = 924) CHF (Table 1). Adjusting for age, sex, and nursing home residence, we found that participants with CHF had a significantly greater frequency of senescent $CD4^+$ T cells (mean [SE], 4.5% [0.2%] vs 3.4% [0.2%]; $P = .02$), and a lower frequency of naive (1.2% [0.03%] vs 1.4% [0.03%]; $P = .03$), effector memory (6.8% [0.2%] vs 8.0% [0.2%]; $P = .008$) and central memory (0.51% [0.01%] vs 0.70% [0.02%]; $P < .001$) $CD8^+$ T cells.

DISCUSSION

Most of our knowledge on the factors that influence vaccine responsiveness in older adults come from studies of influenza vaccination. Interestingly, though there is overwhelming

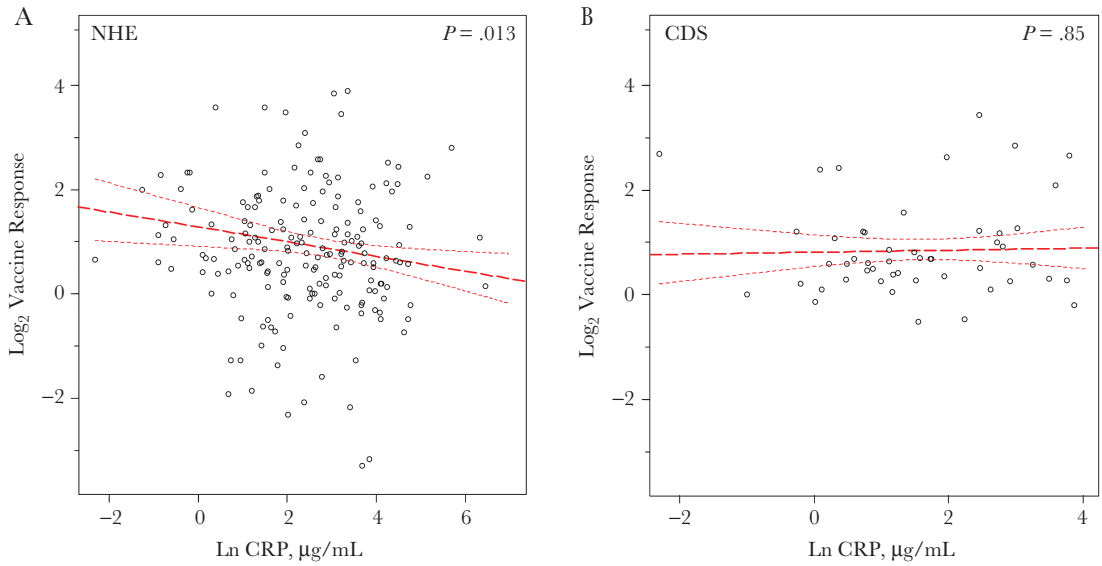

Figure 2. Circulating C-reactive protein (CRP) levels predict the response to varicella-zoster virus (VZV) vaccination in the nursing home elderly (NHE) but not the community-dwelling senior (CDS) cohort. The $\log_2$ fold-change response to VZV vaccination at 6-week follow-up was correlated with natural-log-transformed (Ln) CRP levels in NHE (n = 187) (*A*) and CDS (n = 50) (*B*) donor groups. Slope, 95% confidence intervals (upper and lower dashed lines), and significance were determined using multiple linear regression, with adjustments for age, sex, and $\log_2$ baseline vaccine response, in separate models for NHE and CDS participants.

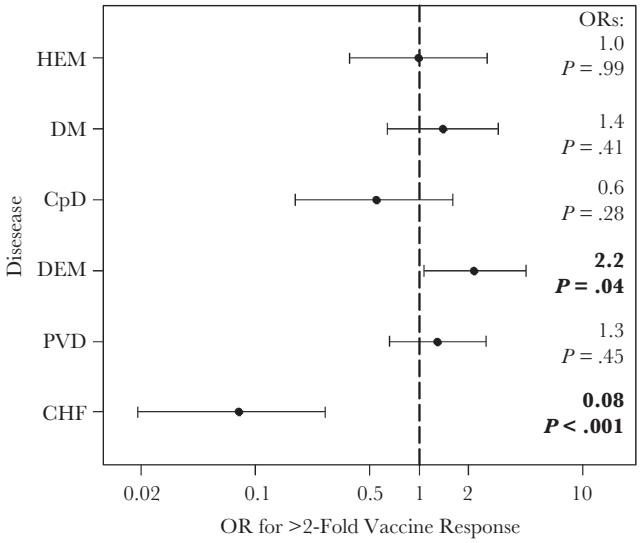

Figure 3. Elderly individuals (n = 187) with congestive heart failure (CHF) are likely to have a poor varicella-zoster virus (VZV) vaccine response. The odds of achieving a ≥2-fold VZV vaccine response was determined by multiple logistic regression, with adjustments for age, sex, and $\log_2$ baseline VZV response, for the following diseases: CHF, peripheral vascular disease (PVD), dementia (DEM), chronic pulmonary disease (CPD), diabetes mellitus (DM), and hemiplegia (HEM). Odds ratios (ORs) and *P* values are listed at right.

evidence indicating that the response to vaccination is reduced in older adults compared with younger persons [23], it is not clear whether responses of elderly nursing home residents

Table 1. Association of CHF With Elevated T-Cell Immunosenescence in Elderly Nursing Home Residents[a]

T Cells	T-Cell Frequency, Mean (SE), %		β Coefficient (SE)	*P* Value[b]
	CHF (n = 148)	No CHF (n = 924)		
CD4+				
Naive	15.7 ± 1.0	15.9 ± 0.4	−0.04 ± 0.08	.645
Effector memory	32.7 ± 1.0	32.0 ± 0.4	0.03 ± 0.04	.433
Central memory	11.8 ± 0.5	13.2 ± 0.2	−0.11 ± 0.06	.069
Terminally differentiated	12.4 ± 0.8	10.6 ± 0.3	0.11 ± 0.07	.113
Senescent	4.5 ± 0.5	3.4 ± 0.2	0.29 ± 0.13	.024[c]
CD8+				
Naive	1.2 ± 0.08	1.4 ± 0.04	−0.14 ± 0.06	.027[c]
Effector memory	6.8 ± 0.4	8.0 ± 0.2	−0.18 ± 0.07	.008[c]
Central memory	0.51 ± 0.04	0.70 ± 0.02	−0.22 ± 0.06	<.001[c]
Terminally differentiated	12.0 ± 0.7	11.1 ± 0.3	0.05 ± 0.07	.471
Senescent	9.1 ± 0.6	8.1 ± 0.3	0.07 ± 0.1	.488

Abbreviations: CHF, congestive heart failure; SE, standard error.

[a]The frequencies of naive, effector and central memory, terminally differentiated and senescent CD4+ and CD8+ T cells were measured in the peripheral blood of individuals with or without CHF.

[b]*P* values represent significance based on linear mixed-model regression analyses natural-log-transformed T-cell frequencies, with adjustment for age, sex, and nursing home residence.

[c]Significant at $P < .05$.

are further compromised [24–26]. That being said, numerous factors have been reported that significantly influence influenza vaccine immunogenicity in that population, in particular, malnutrition [27–29], vaccine dose [30], and disease status [26, 29]. We have shown that elderly long-term care residents exhibit VZV vaccine responses similar to those in older adults, responses correlated with cytomegalovirus-reactive CD4+ T-cell and regulatory T-cell frequency [10]; unfortunately, little else is known. Identifying factors related vaccine immunogenicity in elderly nursing home residents will allow us to discriminate those at risk of vaccine failure and target interventions accordingly—for example, pharmacologically, using mammalian target of rapamycin (mTOR) inhibitors [31], nutritionally, by complex, fortified supplements [32], or through exercise [33].

A major finding of the current study was that serum levels of CRP, but not TNF, IL-1β, IL-6, or IL-10, were inversely related to vaccine responsiveness in the elderly nursing home population; no associations with serum CRP or cytokines were observed for community-dwelling seniors. As a canonical biomarker often used to estimate systemic inflammatory status, this finding was not surprising. Chronic, low-grade inflammation is widely considered to have a negative impact on vaccine responsiveness [34], supported by associations reported for circulating proinflammatory molecules, such as endotoxin [35], interleukin 12 [36], and neopterin [37]. Although the mechanism of this phenomenon is not completely understood, it may be related to an inflammation-dependent expansion of immunosuppressive regulatory T cells [38]. Lelic et al [10] previously showed that regulatory T-cell frequency at baseline was also inversely correlated to VZV vaccine responsiveness in our elderly cohort, but we cannot comment on whether expansion occurs after vaccination and whether circulating inflammatory levels modify this process.

We also found a significant effect of disease status in determining vaccine responsiveness in nursing home residents; in particular, having CHF greatly reduced the odds (odds ratio, 0.08) of having a ≥2-fold vaccine response, whereas having dementia actually improved the odds (2.2). The association between heart failure and vaccine immunogenicity is well documented, and, interestingly, the effect is bidirectional. On the one hand, influenza vaccination has been shown to reduce rates of hospitalization [39, 40] and death related to heart failure [41], the mechanism of which is thought to function by reducing or completely preventing the proatherogenic responses related to influenza infection (ie, proinflammatory cytokine secretion and leukocyte recruitment to the vascular intima) [42]. On the other hand, similar to our findings, individuals with acute or CHF exhibit reduced cell-mediated immunity [43, 44] and seroconversion [43, 45] in response to influenza vaccination.

Although the mechanism of this effect is less well known, findings in mice [46] and humans [47] suggest that heart failure induces an immense expansion of T cells, which contributes

to an overall reduction of the naive T-cell pool and, therefore, a greater degree of immunosenescence. We further investigated this mechanism by examining T-cell immunosenescence markers in a separate cohort of elderly nursing home residents. Specifically, we compared the frequency of $CD4^+$ and $CD8^+$ naive, senescent, terminally differentiated and effector and central memory T cells in individuals with or without CHF. Indeed, the differences observed were consistent with T-cell immunosenescence; $CD4^+$ senescent cells ($CD28^-CD57^+$) were elevated, whereas $CD8^+$ naive ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), and effector memory ($CD45RA^-CCR7^-$) were reduced in individuals with CHF. Although these results and those of others would suggest that CHF accompanies T-cell immunosenescence in older adults, thereby correlating with impaired vaccine responses, further investigation is required to establish causality in these relationships.

In summary, we have shown that circulating CRP and CHF are significant predictors of the VZV vaccine response in elderly nursing home residents. Furthermore, CHF in this population seems to be related to T-cell immunosenescence, which would explain the observed reduction in vaccine immunogenicity. Our findings add to a body of literature suggesting that targeted interventions aiming to boost vaccine immunogenicity could be a viable approach to protect those at risk of vaccine failure. This would be particularly relevant for residents of long-term care, given their enhanced susceptibility to infection and related comorbid conditions.

Notes

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