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Circulating immune cell phenotypes are associated with inflammatory biomarkers in dementia-free participants from the Framingham Heart Study Offspring cohort

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Abstract

Inflammatory responses are characterized by the activation of immune cells, while inflammatory biomarkers intricately interact with the immune system. While experimental studies have provided important mechanistic insights, large community-based investigations jointly profiling immune cell phenotypes and inflammatory biomarkers remain limited. This study aims to investigate the association between circulating immune cell phenotypes and inflammatory protein biomarkers in the Framingham Heart Study Offspring cohort. A sample of 873 dementia-free participants (52% female, mean age 61) had extensive profiling of peripheral blood mononuclear cells and inflammatory plasma protein biomarkers (OLINK Proteomics) collected at Offspring Exam 7 (the seventh examination cycle of the cohort, 1998 to 2001). Among cross-sectional pairwise associations between 77 immune cell phenotypes and 68 inflammatory biomarkers, CD8 naïve T cells showed negative associations with multiple inflammatory proteins, including CD40, CD5, CXCL9, CXCL10, IL8, OPG, TGF-alpha, TNF, TNFRSF9, and 4E-BP1. Higher levels of CD8 Cytotoxic T cells, CD8+CD27-, CD8 effector T cells, and interferon gamma-producing CD8 T cells (Tc1) were all associated with higher levels of soluble CD8 alpha chain (CD8A). In contrast, CD4/CD8 T cell ratio, Immunoglobulin D (IgD)-expressing B cells and naïve Immunoglobulin D and Immunoglobulin M double-positive B cells (IgD+IgM+B cells) were associated with lower CD8A. Stratified analyses revealed significant associations primarily in males and participants over 60. These findings provide a comprehensive population-level characterization of the relationships between circulating immune cell phenotypes and inflammatory biomarkers in a well-defined community-based cohort, offering insight into immune cell-inflammatory profiles associated with aging.

Keywords

Immune cell, peripheral inflammation, protein biomarkers, cognitive aging

1. Introduction

Inflammation is triggered by the immune system's response to various harmful conditions including pathogens, damaged tissues, injuries, foreign compounds, or abnormal environments [1, 2], which induces acute inflammation that starts the healing process[2]. However, unresolved acute inflammation will become a state of low-grade chronic inflammation that increases susceptibility to chronic diseases such as cardiovascular diseases (CVD), cancer, type 2 diabetes, and neurodegenerative disorders[3]. Inflammatory responses are characterized by the activation of immune cells[3], through mechanisms that detect harmful stimuli using cell surface pattern receptors, initiation of inflammatory pathways, release of inflammatory biomarkers, and involvement of inflammatory cells[2]. Circulating inflammatory biomarkers, which often include cytokines, chemokines, growth factors, and soluble receptors or other immune-regulatory proteins, engage in intricate interactions with the immune system [2, 4]. This complexity arises as immune cells stimulate the production of inflammatory biomarkers, while the inflammatory biomarkers in turn direct interactions among cells and contribute to cell activation and differentiation. Various immune cell subtypes are of relevance to different biomarkers[4]. For example, Th1, Th2, and Th17 cells are characterized by their production of interferon gamma (IFN-gamma), IL4, and IL17, respectively. However, they are not the only cells that produce these proteins.

Previous experimental and human studies have provided important insights into immune-inflammatory relationships [2, 4, 5]. In particular, several population-based studies[6, 7], especially in the context of aging, have linked specific immune cell subsets to systemic inflammatory markers in older adults. However, these studies have often focused on selected immune phenotypes or a limited set of inflammatory biomarkers, involved modest sample sizes, or did not comprehensively profile immune cell phenotypes and inflammatory proteins within the same community-based

cohort. As a result, large-scale studies that systematically characterize associations between detailed immune cell phenotypes and a broad inflammatory proteomic panel in humans remain relatively limited. Here we report on the relationship between circulating immune cell phenotypes and inflammatory biomarkers in dementia-free participants in the community-based Framingham Heart Study (FHS) Offspring cohort. Using extensive immunophenotyping of peripheral blood mononuclear cells (PBMCs) and high-throughput inflammatory protein profiling, we aimed to characterize population-level relationships between immune cell phenotypes and inflammatory biomarkers. We hypothesized that several immune cell phenotypes would be associated with protein biomarkers cross-sectionally. Given the age/sex associations with circulating immune cell phenotypes[8], we also examined the association by sex and age strata.

2. Materials and Methods

2.1.Study sample

The Framingham Offspring Study began in 1971 and recruited 5214 offspring of the Framingham Heart Study (FHS) original cohort and their spouses, a community-based sample from Framingham, Massachusetts and surrounding communities[9]. The Offspring cohort has been examined every 4-8 years since enrollment. The first examination with existing stored PBMCs took place at FHS Offspring Exam 7, where a total of 3539 participants attended from 1998 to 2001. For this current study, we utilized immune cell profiling data collected from ~1,000 Offspring participants who were selected based on availability of 2 or more vials of PBMC samples, who were dementia-free and aged 40 years and older at Exam 7[8]. Among them, 879 participants also had a stored plasma sample for inflammatory biomarker profiling and passed the quality control procedures. We excluded participants with extensive missing immune phenotype data (n=4)

and those with incomplete inflammatory biomarker profiles (n=2), yielding a final analytic sample of 873 participants (**Figure S1**). All participants provided written informed consent before each examination attendance. The Institutional Review Board at Boston University Medical Center reviewed and approved the study protocol and examinations. All research was performed in accordance with the Declaration of Helsinki and relevant regulations.

2.2. Immune cell phenotyping

Immune cell phenotyping was previously performed and published [8], following protocols similar to those previously used in other large population studies [10, 11]. PBMCs were collected at FHS Offspring Exam 7 and cryopreserved at -135°C for long-term storage. For this study, cryopreserved PBMC vials were retrieved from storage and thawed for flow cytometry-based immunophenotyping in 2021. Briefly, PBMCs were thawed, diluted, and filtered, then divided into five assay panels: (1) CD4/CD8 naïve/memory panel, (2) CD4/CD8 regulatory panel, (3) T-cell, B-cell and NK-cell panel, (4) CD4/CD8 stimulation panel for intracellular staining of Th1/2/17 and Tc1/2/17, (5) monocyte subset panel. The complete flow cytometry procedures were described previously [8]. We measured a broad panel of innate and adaptive immune cell phenotypes and reported 116 immune cell phenotypes, including T cells, B cells, NK cells, and monocytes[8]. Marker□based definitions anchored differentiation states across T cells, B cells, and monocytes in this study. T-cell subsets are defined by combinations of surface markers, with CD27 and CD28 levels discriminating undifferentiated cells from progressively differentiated cells[12]. B cells (CD19+) were partitioned by CD27/CD43 and IgD/IgM into B-1 cells (CD43+CD27+) and memory B cells (Bmem; CD43-CD27+), each resolved into IgD+, IgM+, and IgD+IgM+ subsets[13, 14]. Monocytes were classified along the CD14/CD16 continuum into classical (CD14++CD16-), intermediate (CD14+CD16+), and non-classical (CD14dimCD16+); within each subset, markers

including HLA-DR, TREM2, CD87, CCR2, Slan, CD33, and CD163 provided activation annotations[15]. For downstream analyses, we selected 71 primary immune cell subtypes and 6 composite ratios of cell types. These 71 primary subtypes were represented as proportions relative to their respective parent populations (e.g., T-cell and B-cell subsets as proportions of live lymphocytes, and monocyte subtypes as proportions of gated monocytes), and **Table S1** provides the complete list of phenotypes with corresponding defining markers. The complete flow cytometry gating strategies underlying these marker-based definitions have been previously published and validated in the Framingham Heart Study Offspring cohort [8], where major immune cell populations are shown in Figure 1A-1E and detailed gating strategies are provided in Supplementary Figures 3-7 of that paper. The six composite ratio measures [16] were calculated using immune cell phenotypes profiled in our study, in which three are age-related immune phenotype (ARIP) measures and the other three are acute disease-related measures. ARIP is characterized by a reduced T-cell repertoire, fewer naïve T cells (Tn), and an accumulation of memory (Tm) and effector T (Teff) cells[17]. This reflects the natural differentiation trajectory in which Tn progressively give rise to central memory (Tcm), effector memory (Tem), and effector (Teff) subsets. The three ARIP measures included the CD4/CD8 T cell ratio, and the naïve-to-memory ratios between Tn and Tm for both CD4 T cells and CD8 T cells, all of which typically decline with age. The latter ratio, denoted as Tn/Tm, was calculated by Tn/Tm = Tn / (Teff + Tem + Tcm) using Tn cells, Teff cells, Tem cells, and Tcm cells of CD4 and CD8 separately [17]. The three acute disease-related measures were the ratios of CD4 Th17/Treg[18, 19], CD8 Tc17/Treg[20] (Th17 and Tc17 cells: IL-17-secreting CD4 and CD8 T cells, respectively), and Granzyme B+ CD8+/Granzyme B+ CD4+ [21, 22]; higher values of these ratios generally indicate a more inflammatory immune environment.

2.3.OLINK inflammation panel

The OLINK Inflammation panel was applied to stored fasting EDTA (ethylenediaminetetraacetic acid) plasma samples collected from FHS Offspring participants at Exam 7 (1998–2001). Samples were stored at -80°C until assay. A total of 92 inflammatory protein biomarkers were quantified in 2021 using the OLINK Inflammation panel. The measurement relied on the high-throughput and multiplexing Proximity Extension Assay (PEA) technology, where the protein expression levels were quantified in a relative measure on a logarithmic scale (log_2) named Normalized Protein eXpression (NPX) units, with one NPX difference corresponding to a doubling of protein concentrations [23]. More information is available at https://olink.com/. Samples were randomly distributed among 11 plates and processed together in a single batch, with the coefficient of variation for all proteins across the plates less than 5%, indicating the absence of any plate-related effects [24]. The full list of protein biomarkers included in the OLINK inflammation panel and the corresponding percentage of samples missing or with values below limit of detection (LOD) are provided in **Table S2**. For outcomes of interest, we focused on the 68 proteins for which fewer than 50% of participant values were below the LOD, as suggested by OLINK and other literature [25-27]. For the 68 proteins analyzed, we used the actual reported value when it fell below LOD for the protein subset that fell below LOD [28].

2.4. Selected covariates and clinical definitions

This section focuses on selected covariates that require explicit definition. Cytomegalovirus (CMV) is a common pathogen that has an impact on the T cell subsets of individuals who are infected with it. The CMV IgG (immunoglobulin G antibody) was measured in units of U/ml from an existing plasma sample from Offspring Exam 7[8], with levels above the LOD (CMV > 800 U/ml) imputed

as 850 U/ml. CMV serostatus was defined as CMV-positive if CMV IgG > 15 U/mL and CMV-non-positive otherwise.

Prevalent cardiovascular disease (CVD) was determined by occurrences before Exam 7 of coronary heart disease (myocardial infarction, angina pectoris, coronary insufficiency), transient ischemic attack, intermittent claudication, and congestive heart failure, reviewed and confirmed by a panel of physicians based on established criteria[29]. Diabetes status was determined based on the presence of any of the following conditions: the use of antidiabetic medications, a fasting blood glucose level of ≥126 mg/dL, or a random blood glucose level of ≥198 mg/dL.

2.5.Statistical analyses

We first performed descriptive correlation analyses between immune cell proportions and inflammatory biomarker levels using Spearman's rank correlation. Correlations were computed using pairwise complete observations for each immune cell-biomarker pair. For each analysis (full sample and each stratum), we calculated p-values for all pairs of correlations and controlled for multiple testing using FDR ≤0.05 as statistically significant. Significant pairs were highlighted in the correlation heatmaps. Correlation analyses were conducted in the full sample and stratified by sex, age group (≤ 60 and over 60), and CMV serostatus (CMV-positive and CMV-non-positive). Pairwise associations were investigated between the 77 immune cell phenotypes (predictors) and the 68 protein biomarker outcomes. Rank-based inverse normal transformation was applied to immune cell phenotypes and inflammatory biomarkers to reduce skewness and map variables to an approximately standard normal scale (mean 0, SD 1), thereby enabling comparison of effect sizes across phenotypes with different marginal distributions. Full sample analyses, sex-stratified analyses (male and female), and age-stratified analyses (≤ 60 and over 60) were conducted for each pair of associations. Two statistical models were considered for two sets of covariates. The

primary model (Model 1) included covariates of sex, age, and CMV levels measured at exam 7. A secondary model (Model 2) included all the covariates in Model 1 and further accounted for prevalent CVD, kidney function, and CVD risk factors to study the robustness of the associations. Specifically, the additional covariates in Model 2 were: 1) indicators for prevalent CVD, prevalent stroke, and prevalent atrial fibrillation (AF) at Exam 7, 2) a measure of kidney function (estimated glomerular filtration rate), and 3) CVD risk factors including systolic and diastolic blood pressures (SBP, DBP, mmHg), diabetes status, treatment for hypertension, body-mass index (BMI, kg/m2), current smoking status, total cholesterol level (TC, mg/dL), high-density lipoprotein cholesterol levels (HDL, measured in mg/dL), and use of lipid-lowering agents at Exam 7.

Stratified analyses within each sex or age stratum were conducted separately using the Model 1 and Model 2 covariates. For the sex-stratified analyses, all covariates were included except sex. In the age-stratified analyses ($\leq$ 60 and over 60), the covariate age was included in each subgroup to adjust for age effects within the older and younger age groups.

Linear mixed-effects models were utilized to establish the associations between each of the 77 immune cell phenotypes with each of the 68 inflammatory protein biomarkers (outcome), accommodating familial relationships via the kinship coefficient matrix. Effect sizes and 95% confidence intervals (CIs) of the immune cell phenotypes were presented and interpreted as deviations in standard deviation units, as standardization was applied to both predictors and outcomes. The false discovery rate (FDR) [30] was used to control the risk of erroneously rejecting true null hypotheses in these correlated pairwise association tests, with FDR $\leq$0.05 set to declare significant associations. FDR control procedures were conducted in each stratum separately. All statistical analyses were conducted using R-4.2.1 software [31], and the linear mixed-effect models were implemented via the *lmekin* function within the *coxme* package [32].

2.6.Network analysis

In addition to exploring marginal pairwise associations between immune cell phenotypes and inflammatory biomarkers one pair at a time, we further developed a relevance network in the full sample. This network takes into account all variables included in the two datasets: immune cells and inflammatory biomarkers, with edges representing the correlations between these variables. The Sparse Partial Least Squares (sPLS) method was employed to construct the network[33], which generated latent variables from each of the two datasets that maximized the covariance between them and adjusted for sex, age, and CMV levels. Subsequently, all variables in both datasets were projected based on orthogonal latent variables, and correlations between variables in the two datasets were calculated. The sPLS was conducted by the function *spls* in *mixOmics* package [34]. Overall, the relevance network provides a holistic perspective by capturing the relationships among multiple immune cells and inflammatory biomarkers simultaneously instead of isolated pairwise associations. The network may highlight the key immune cells or inflammatory biomarkers that play central roles in the system and allow for pathway analyses based on the intercorrelations observed.

3. Results

3.1.Participants characteristics

Table 1 presents the demographic characteristics of the participants in the study sample. The average age of the participants was 61 years, approximately 52% of the participants were female. The proportion of CMV-positive (CMV > 15 U/ml) was 50.4%. The demographic characteristics were comparable between females and males. The list of the 77 immune cell phenotypes of interest, quantified as proportions relative to their respective parent populations, and their summary

statistics (mean and standard deviation/SD for each cell phenotype) stratified by age group (≤ 60 and over 60), by sex, and by CMV serostatus are presented in **Supplementary File Tab 1. Table S3** provides the mean and SD of inflammatory biomarkers in the full sample and across age and sex strata. To further contextualize potential CMV- and sex-related differences in inflammatory profiles, we additionally summarize mean inflammatory biomarker levels (after inverse normal transformation) across the four sex-by-CMV strata (female CMV-non-positive, female CMV-positive, male CMV-non-positive, and male CMV-positive) in **Figure S2**.

3.2. Association results

To provide a global overview of immune cell-protein relationships, we first examined Spearman correlations between immune cell proportions and inflammatory biomarkers in the full sample and across strata defined by sex, age (≤60 vs >60 years), and CMV serostatus (CMV-positive vs CMV-non-positive; **Figure S3a-g**). Overall, correlations were modest in magnitude but exhibited clear and structured patterns across immune subsets and biomarkers. Notably, CD8 Tn and CD8 Tn/Tm, displayed the most consistent correlation patterns across strata. These co-variation patterns tended to be more pronounced among CMV-positive participants and in older individuals, whereas correlations were generally attenuated in CMV-non-positive and younger groups. Sex-stratified analyses showed similar overall correlation structures, with differences primarily in magnitude. Together, these correlation analyses provide a descriptive, high-level overview that complements and motivates the detailed regression-based association results presented below.

Figures 1 provide the forest plots of significant associations between the immune cell phenotypes and the inflammatory proteins in the full sample using Model 1. Sex/age-stratified analyses for Model 1 were presented in **Figure S4-S5**. **Figures S6-S7** show the same associations and any newly discovered significant associations from Model 2 after adjusting for CVD, kidney function,

and CVD risk factor covariates. Complete results for all the association analyses including both Model 1 and Model 2 in the full sample and stratified analyses by sex, age, and CMV serostatus are provided in **Supplementary File Tab 2**.

As shown in the full sample analysis using Model 1 (**Figure 1**), a number of immune cell phenotypes were associated with protein biomarkers, especially among the CD8 T cell subtypes and B cell subtypes. **Figure 1** highlights that higher levels of CD3 T cells were significantly associated with lower TNFRSF9, while higher CD4 T helper cells were associated with lower Flt3L and TNFRSF9. Higher CD4 Tcm was associated with lower TNFB. Among CD8 phenotypes, higher levels of CD8 Cytotoxic T cells, CD8+CD27-, and CD8 Teff were all significantly associated with higher CD8A, whereas a higher CD4/CD8 T cell ratio was associated with lower CD8A. Additionally, CD8 Tn/Tm was negatively associated with TNF, and CD4+CD25+ was negatively associated with TWEAK. It is noteworthy that CD8 Tn was significantly associated with 10 proteins: 4E-BP1, CD40, CD5, CXCL10, CXCL9, IL8, OPG, TGF-alpha, TNF, and TNFRSF9, with negative effect sizes ranging from -0.17 to -0.13 SD units per SD unit increase in CD8 Tn levels. **Figure 1** also shows significant associations among B-cell and CD3 T-cell subsets. Higher CD38+ T cells were significantly associated with lower OSM. Higher Immunoglobulin D-expressing B cells (IgD+B cells) and naïve Immunoglobulin D and Immunoglobulin M double-positive B cells (IgD+IgM+B cells) were both associated with lower CD8A, while higher CD43+ B cells were associated with higher TNFRSF9. Among CD27+ B cells, higher levels were associated with lower CXCL10, CXCL11, CXCL9, and IL-12B. In addition, memory B cells (CD43-CD27+ Bmem) showed negative associations with CXCL10, CXCL11, CXCL9, IL-12B, and TNFB. **Figure 1** shows that higher levels of Tc1 cells were associated with higher CD8A. In

monocyte phenotypes, higher CD14 total, classical monocytes (CM), and CD87+ CM were positively associated with HGF, and higher CD14 total was also associated with higher OSM.

More significant associations were observed in age-stratified analyses compared to sex-stratified analyses. As observed in **Figure S4a,b,c**, the stratum aged over 60 years old showed significance for a large proportion of associations regarding CD4 T cell subtypes, CD8 Tn, and B cell subtypes that were observed in the full sample. There were also several associations significant only in the older group such as those with CD8 Tn (CD6, HGF, IL-15RA, IL6, MMP-1, MMP-10) and CD8 Tn/Tm (CD5, CD6, SLAMF1). Moreover, IgD+Bmem cells, IgM+Bmem cells, and IgD+IgM+Bmem cells were all negatively associated with CD40 specifically in this stratum. Only three significant associations were observed in the stratum ≤ 60 years old (**Figure S4a,c,d**): higher CD8 Cytotoxic T cells with higher CD8A, higher memory B cells with lower IL-12B, and higher Tc1 with lower TNFB. Notably, the association between memory B cells and IL-12B was also significant in the older group, whereas the associations of CD8 Cytotoxic T cells with CD8A and Tc1 with TNFB were not observed in the older group. **Figure S5** presented the sex-stratified results, and significant associations only appeared in the male group. Many of these significant associations were also detected in the full sample analysis (male and female combined), while several were unique to males. Specifically, **Figure S5a** shows that CD4 Tn and CD4+CD27- were both negatively associated with AXIN1 and STAMBP, CD4 Tcm cells were negatively associated with IL-10RB, CD4 Teff cells were negatively associated with LAP TGF-beta-1 and TNFB, and CD8 Tn cells were negatively associated with IL-15RA. **Figure S5b** demonstrates that B cells, IgD+B cells, IgM+B cells, and IgD+IgM+B cells were all negatively associated with IL-18. Memory B cells were negatively associated with CD5, IL-10RB, PD-L1, and TNFRSF9. IgD+Bmem cells were negatively associated with CD5 and PD-L1, while IgM+Bmem cells were

negatively associated with CD40, CD5, CXCL9, IL-12B, MCP-2, and PD-L1. In addition, IgD+IgM+Bmem cells were negatively associated with CD5 and PD-L1. **Figure S5c** further illustrates that TH17 cells were negatively associated with CD5, Tc17 cells were negatively associated with TNFSF14, OSM, and VEGFA, Granzyme B+CD4 cells were negatively associated with STAMBP, and NCM and HLADR+NCM were positively associated with CXCL10. No significant associations were observed in the female group. We additionally refer readers to **Figure S8a-e**, which summarize the regression-based association effects using heatmaps to provide a global overview of positive and negative associations across immune cell phenotypes and inflammatory biomarkers for Model 1.

After accounting for kidney function and CVD risk factors (Model 2), a large proportion of the significant associations observed using Model 1 covariates remained significant, but with attenuated effect sizes. While several effects were no longer significant, a few new associations were observed (see **Figure S6-S7**). As in Model 1, no significant associations were observed in the female participant stratum (**Figure S7a,b,c**). However, in the stratum ≤ 60 years old, new positive associations emerged between Tc1 and CD8A and between IgM+B cells and IgD+IgM+B cells with IL-18R1, while the previously observed negative association of Tc1 with TNFB and the positive association of CD8 Cytotoxic T cells with CD8A remained (**Figure S6a,c,d**). In the full sample, additional associations were identified, including positive associations of CD8+CD28-CD27- and Granzyme B+CD8 cells with CD8A, as well as a negative association of CD3 T cells with TWEAK (**Figure S6a,d**). These served as evidence indicating the involvement of additional biological pathways in these associations.

3.3Network representation

Figure 2 shows the top correlated pairs between immune cell phenotypes and inflammatory biomarkers (absolute similarity measure > 0.12) in the full sample. The immune cells are marked in blue boxes and proteins are in pink circles. CD8A played the role of a central node in a hub connected to 8 immune cell phenotypes, with most of them positively correlated. The observations of positive marginal associations between CD8 Cytotoxic T cells, CD8 Teff, Tc1, and CD8+CD27- with CD8A, and negative associations between CD4/CD8 with CD8A in **Figure 1** were consistently presented in the network. Similarly, CD8 Tn cells were negatively correlated with CXCL9, CXCL10, and TNF, which were also suggested by significant marginal associations in **Figure 1**. Besides, CM and CD87+ CM were both positively correlated with HGF, which was consistent with the marginal associations identified in **Figure 1**. The network also highlighted B cell subtypes-protein relationships, including negative correlations of memory B cells (CD43-CD27+ Bmem), IgD+Bmem cells, IgM+Bmem cells, and IgD+IgM+Bmem cells with CD40, as well as negative associations of memory B cells with CXCL9, CXCL11, and IL-12B, reflecting the robustness of marginal associations detected in **Figure S4c**. New associations observed in Model 2 were also reflected in this network such as CD8+CD28-CD27- with CD8A (**Figure S6a**). Several new connections were identified by this network approach, including CD8+ Tem and Granzyme B+ CD8+/Granzyme B+ CD4+ with CD8A, CD8 Tn and CD8 Tn/TM with CXCL11, memory B cells, IgD+Bmem cells, IgM+Bmem cells, and IgD+IgM+Bmem cells with CD5, etc. Different from the marginal associations that consider one pair of variables at a time, the network approach integratively incorporates all immune cells and proteins altogether to examine their intercorrelations. Hence, this network, which integrates all the phenotypes together, provided evidence supporting the significant marginal associations previously identified while also

presenting additional insights, including newly identified correlated pairs, to better visualize and establish potential pathways between immune cells and proteins.

4. Discussion

Among a dementia-free study sample from the community-based FHS Offspring cohort, we examined the cross-sectional association of innate and adaptive immune cell phenotypes and inflammatory biomarkers and observed many significant associations between 77 peripheral immune cell phenotypes and 68 inflammatory biomarkers. First, CD8 Tn, the immune cell with the highest number of significant associations, were associated with several inflammatory proteins in the full sample, including 4E-BP1, CD40, CD5, CXCL10, CXCL9, IL8, OPG, TGF-alpha, TNF, and TNFRSF9, all in negative direction. This inverse relationship suggests that lower circulating CD8 Tn proportions may serve as a systemic proxy for active immune mobilization, aligning with the naïve-to-effector transition. In support of our findings, an *in vitro* study reported that 4E-BP1 was crucial in optimal CD8 Teff cells differentiation[35]. Additionally, CD40 Ligand (CD40L) reportedly engages in the proliferation of CD8+ T cells through CD40 expression on activated CD8+ T cells and is instrumental in the development of CD8 Tm cells in an *in vitro* study, where the complicated mechanisms in the CD40-CD40L interactions were involved in the cross-talk between CD4+ and CD8+ T cells [36]. Our observation that CXCL9 and CXCL10 were inversely associated with CD8 Tn is consistent with a report by Nolz et al that showed when CD8 T cells become activated and transform into CD8 Teff cells, they express chemokine receptors that interact with CXCL9 and CXCL10 secreted by tissues undergoing inflammation[37]. IL8 is expressed at low levels in naive CD4+ and CD8+ T cells, with its production and IL8R surface expression increasing during T cell activation [38] , which may explain the negative association

between IL8 and CD8 Tn observed in our study, while the absence of association with CD4 Tn remains unclear. Mice studies suggested that TNF promotes the activation and proliferation of both CD4 and CD8 Tn and Teff cells, ultimately contributing to the removal of infected cells and playing its protective role against pathogens [39]. Furthermore, several associations with CD8 Tn were significant only in the older group. For example, CD6, a glycoprotein implicated in T cell development and activation in mouse models[40], is associated with CD8 Tn and CD8 Tn/Tm only in individuals aged 60 and above. In mice, IL6 drives the differentiation of a subset of CD8 Tn cells expressing IL6R into a specific type of CD8 Teff cells that promotes isotype switching in B cells [41], which corresponds to our observations of negative association between CD8 Tn and IL6 levels in individuals aged 60 and above.

Second, CD8A protein, the soluble form of the T-cell surface glycoprotein CD8 alpha chain, was the inflammatory biomarker with the highest number of significant associations, where 7 immune cell phenotypes were associated with it in the full sample: CD8 Cytotoxic T cells, CD8+CD27-, CD8 Teff, and Tc1 in positive directions, whereas CD4/CD8, IgD+B cells, and IgD+IgM+B cells were associated in negative direction. These findings are consistent with CD8A reflecting CD8 lineage activation and effector differentiation[42, 43], as activation of CD8 T cells increases circulating soluble CD8 and late differentiated cytotoxic CD8 subsets (characterized by loss of CD27/CD28 and higher Granzyme B) expand. This data is further substantiated in mouse models that virally-challenged cells demonstrated a loss in the number of CD8 molecules per cell (as observed by lower mean fluorescent intensity), despite little change in the number of CD8 cells or the CD8 mRNA levels, indicating that CD8 is still being produced but is not remaining on the cellular surface [44]. Because B cells do not express CD8A, the negative associations with IgD+and IgD+IgM+B cell subsets likely reflect B cell crosstalk[45] where the expansion of the cytotoxic

compartment occurs alongside the contraction of the naïve B-cell reservoir during chronic or systemic inflammation.

Third, B cell subtypes and monocyte subtypes showed multiple significant associations with inflammatory proteins in the full sample. Specifically, CD27+ B cells and memory B cells were consistently negatively associated with CXCL9, CXCL10, CXCL11, and IL-12B, providing a clear example of chemokine-mediated tissue trafficking. Rather than a decrease in production, the CXCR3 ligands (CXCL9/10/11) likely reflect the recruitment of CXCR3+ memory B-cells out of the peripheral circulation and into inflamed tissues where these chemokines are being expressed [46], whereas the IL-12B acts to shift naïve B cells to long-lasting memory cells [47]. In addition, higher CD14 total, CM, and CD87+ CM were positively associated with HGF, consistent with CD14+ CM expressing the HGF receptor, and CD87 marks a more migratory CM subset, so a more activated CM compartment aligns with higher HGF[48, 49]. CD14 total and CD38+ T cells were associated with OSM, as might be expected as it is expressed mainly by activated T cells, monocytes, and dendritic cells during active inflammation [50].

Fourth, sex-stratified analyses identified significant associations only in the males, while in age-stratified analyses the majority of significant associations were among those over age 60, with only the CD8A - CD8 Cytotoxic T cells, the IL-12B - memory B cells, and the TNFB - Tc1 associations significant in those $\leq$ 60 in Model 1. Upon further adjustment for prevalent CVD, kidney function, and CVD risk factors (Model 2), effect sizes for a large proportion of significant associations were attenuated, while some new associations emerged, suggesting additional biological pathways relevant to CVD. This trend, particularly in older men, reflects general "inflammaging" where chronic low-grade inflammation drives T-cell exhaustion and CVD risk, exhibiting as higher innate and pro-inflammatory activity and lower adaptive activity [51].

We identified TNFB to be associated with CD4 Teff and CD4 Tcm in the full sample from the two models, and with Tc1 in the younger age group (<=60). The association suggests that TNFB is a key player in both the active defense (Teff) and the long-term immune "memory" (Tcm) that the body maintains and in younger populations [52], TNFB more strongly promotes a cytotoxic (killer) response [53]. CD8 Tn, CD3 T cells, and CD4 T helper cells were negatively associated with TNFRSF9. CD137 (TNFRSF9) is an inducible co-stimulatory receptor expressed upon T-cell activation[54], so negative associations with naïve or broad T-cell pools (CD8 Tn, CD3 T cells, and CD4 T helper cells) are consistent with its role in driving differentiation into effector subsets. Likewise, FLT3 ligand has been shown to expand dendritic cells and promote CD4+ and CD8+ T-cell activation[55], and IL-12B is central for Th1 polarization and effector differentiation[56]; both would be expected to correlate with differentiated rather than naïve populations. These examples illustrate that while cross-sectional associations cannot capture the full dynamics of activation, several of our observations align with established mechanisms of naïve-to-effector transition.

In our previous work[8], most immune cell phenotypes were not strongly associated with traditional CVD risk factors, apart from limited relationships such as with smoking status. Nevertheless, these risk factors are strong predictors of inflammatory protein levels. Including them in Model 2 enabled us to evaluate the robustness of the immune cell-protein associations after taking into account cardiovascular influences on circulating proteins. The attenuation observed for several associations suggests that part of the immune cell-protein relationships may be intertwined with broader cardiovascular pathways. New positive associations emerged between IgM+B cells and IgD+IgM+B cells with IL-18R1 in the stratum ≤ 60 years old. Prior work has shown that B cells themselves can express IL-18 and its receptor[57], providing a plausible biological basis for these associations.

Our study has several strengths. First, we leveraged the community-based FHS Offspring cohort that underwent extensive phenotyping for a comprehensive array of peripheral immune cells and inflammatory biomarkers, as well as characterized for CVD risk factors. Second, the stratified analyses by sex and age have revealed the differential associations by strata. Third, the immune cell-protein biomarker associations provide a comprehensive population-level characterization of the relationships between circulating immune cell phenotypes and inflammatory biomarkers, considering both marginal associations and also integrative network analysis. There are some limitations of this study. First, the limited sample size of our study restricted the power to detect significant associations. The significant associations detected in this study were more represented by males and the older people, posing challenges for interpretation. Second, the immune cell and inflammatory biomarkers were all measured at Exam 7 with all participants being dementia-free. The cross-sectional nature of our analyses may not capture associations during the progression of inflammation, where dynamic activation and differentiation processes cannot be fully captured by proportions of cell subsets at a single time point. The establishment of causality and directional causation is not possible: the cross-sectional design did not allow for determining whether the cells secret cytokines or the cytokines activate cell differentiation, etc. Third, when blood samples were collected, participants' vaccination or recent infection status was not recorded, so we could not account for these potential modifying factors in our analyses. Fourth, our study measured immune cell phenotypes and protein levels in blood samples, which were easy to obtain but may not reflect the precise associations in the brain. Fifth, we assessed circulating inflammatory proteins using the Olink platform, which was not directly benchmarked against other proteomic platforms (e.g., SomaScan, mass spectrometry). Prior large-scale studies have reported only weak-to-moderate correlations across platforms[58, 59], with concordance varying by protein and assay characteristics,

underscoring the need for further investigation of platform-specific findings. Sixth, PBMCs and plasma were collected at FHS Offspring Exam 7 and assayed years later using long-term stored specimens; while standardized storage conditions and assay quality control procedures were applied, we cannot fully exclude the possibility that long-term storage may have modestly influenced some biomarkers. Lastly, the FHS Offspring cohort participants are primarily white, well-educated, and located in New England. To validate our findings, replication in ethnically and geographically diverse independent samples is essential.

In conclusion, several peripheral immune cell phenotypes demonstrated cross-sectional associations with inflammatory biomarkers in a community-based sample free of dementia. Stratified analyses indicated that these associations were predominately observed in males and older individuals. Notably, CD8 Tn cells exhibited associations with numerous inflammatory biomarkers, all suggestive of negative relationships. Significant proteins identified in this study overlap with those in our prior study (IL6, IL8, TGF-alpha, HGF, OSM, VEGFA, TNF, etc.) which were associated with cognitive domain scores and incident Alzheimer's Disease and related dementias [24], supporting inflammatory pathways in cognitive aging. Future work should test these immune–inflammation–cognitive aging pathways longitudinally and replicate findings in independent cohorts.

AUTHOR CONTRIBUTIONS

JC performed data curation, formal analysis, investigation, and visualization, wrote the original draft and wrote for revisions and editing. MFD, KLL, and JMM performed conceptualization, funding acquisition, data curation, investigation, and visualization, wrote the original draft, and

wrote revisions and editing. YC, SI, and AAYR performed the investigation and wrote for review and editing.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Framingham Heart Study Offspring data are available for request via NHLBI Biological Specimen and Data Repositories Information Coordinating Center (BioLINCC) https://biolincc.nhlbi.nih.gov/studies/framoffspring/.

SUPPORTING INFORMATION

Additional supporting information may be found online in Supporting Information Section.

References

1. Medzhitov, R. Inflammation 2010: new adventures of an old flame. *Cell* **140**, 771–776 (2010).

2. Chen, L. *et al*. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* **9**, 7204–7218 (2017).

3. Furman, D. *et al*. Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* **25**, 1822–1832 (2019).

4. Murphy, K. & Weaver, C. in *Janeway's immunobiology* (Garland Science/Taylor & Francis Group, LLC, New York, NY, 2016).

5. Ramesh, G., MacLean, A. G. & Philipp, M. T. Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. *Mediators Inflamm.* **2013**, 480739 (2013).

6. Bachstetter, A. D. *et al*. A blunted TH17 cytokine signature in women with mild cognitive impairment: insights from inflammatory profiling of a community-based cohort of older adults. *Brain Communications* **5**, fcad259 (2023).

7. Zhou, L. *et al*. Centenarians alleviate inflammaging by changing the ratio and secretory phenotypes of T helper 17 and regulatory T cells. *Frontiers in Pharmacology* **13**, 877709 (2022).

8. Fang, Y. *et al*. Circulating immune cell phenotypes are associated with age, sex, CMV, and smoking status in the Framingham Heart Study offspring participants. *Aging (Albany, NY.)* **15**, 3939–3966 (2023).

9. Feinleib, M., Kannel, W. B., Garrison, R. J., McNamara, P. M. & Castelli, W. P. The framingham offspring study. Design and preliminary data. *Preventive Medicine* **4**, 518–525 (1975).

10. Olson, N. C. *et al*. Innate and adaptive immune cell subsets as risk factors for coronary heart disease in two population-based cohorts. *Atherosclerosis* **300**, 47–53 (2020).

11. Bailin, S. S. *et al*. T Lymphocyte Subsets Associated With Prevalent Diabetes in Veterans With and Without Human Immunodeficiency Virus. *The Journal of infectious diseases* **222**, 252–262 (2020).

12. Akbar, A. N. & Henson, S. M. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? *Nature Reviews Immunology* **11**, 289–295 (2011).

13. Budeus, B., Kibler, A. & Küppers, R. Human IgM–expressing memory B cells. *Frontiers in Immunology* **14**, 1308378 (2023).

14. Griffin, D. O., Holodick, N. E. & Rothstein, T. L. Human B1 cells in umbilical cord and adult peripheral blood express the novel phenotype CD20 CD27 CD43 CD70−. *J. Exp. Med.* **208**, 67–80 (2011).

15. Ziegler-Heitbrock, L. Blood monocytes and their subsets: established features and open questions. *Frontiers in immunology* **6**, 423 (2015).

16. Ragab, A. A. Y. *et al*. Immune cell phenotypes and mortality in the Framingham Heart Study. *Immun. Ageing* **21**, 37–6 (2024).

17. Ramasubramanian, R. *et al*. Evaluation of T-cell aging-related immune phenotypes in the context of biological aging and multimorbidity in the Health and Retirement Study. *Immunity & ageing* **19**, 1–33 (2022).

18. Littman, D. R. & Rudensky, A. Y. Th17 and regulatory T cells in mediating and restraining inflammation. *Cell* **140**, 845–858 (2010).

19. Zhang, S. *et al*. The Alterations in and the Role of the Th17/Treg Balance in Metabolic Diseases. *Front. Immunol.* **12**, 678355 (2021).

20. Hipp, A. V., Bengsch, B. & Globig, A. Friend or Foe - Tc17 cell generation and current evidence for their importance in human disease. *Discov. Immunol.* **2**, kyad010 (2023).

21. Lin, L. *et al*. Granzyme B secretion by human memory CD4 T cells is less strictly regulated compared to memory CD8 T cells. *BMC Immunol.* **15**, 36–1 (2014).

22. Santos-Zas, I. *et al*. Cytotoxic CD8(+) T cells promote granzyme B-dependent adverse post-ischemic cardiac remodeling. *Nat. Commun.* **12**, 1483–9 (2021).

23. Kuan, P. *et al*. Molecular linkage between post-traumatic stress disorder and cognitive impairment: a targeted proteomics study of World Trade Center responders. *Translational Psychiatry* **10**, 269 (2020).

24. Chen, J. *et al*. Peripheral inflammatory biomarkers are associated with cognitive function and dementia: Framingham Heart Study Offspring cohort. *Aging cell*, e13955 (2023).

25. Costi, S. *et al*. Peripheral immune cell reactivity and neural response to reward in patients with depression and anhedonia. *Translational Psychiatry* **11**, 565 (2021).

26. Drake, I. *et al*. Methodological considerations for identifying multiple plasma proteins associated with all-cause mortality in a population-based prospective cohort. *Scientific Reports* **11**, 6734 (2021).

27. Harlid, S. *et al*. A two-tiered targeted proteomics approach to identify pre-diagnostic biomarkers of colorectal cancer risk. *Scientific Reports* **11** (2021).

620 28. Maglinger, B. *et al*. Proteomic changes in intracranial blood during human ischemic stroke.
621 *Journal of Neurointerventional Surgery* **13**, 395 (2021).

622 29. Lloyd-Jones, D. *et al*. Prediction of lifetime risk for cardiovascular disease by risk factor
623 burden at 50 years of age. *Circulation* **113** (2006).

624 30. Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: a Practical and
625 Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society.Series B* **57**,
626 289–300 (1995).

627 31. R Core Team, R. R: A language and environment for statistical computing. (2013).

628 32. Therneau, T. M. & Therneau, M. T. M. Package 'coxme'. *R package version* **2** (2015).

629 33. Le Cao, K., Rossouw, D., Robert-Granie, C. & Besse, P. A sparse PLS for variable selection
630 when integrating omics data. *Stat. Appl. Genet. Mol. Biol.* **7**, Article 35–6115.1390. Epub 2008
631 Nov 18 (2008).

632 34. Rohart, F., Gautier, B., Singh, A. & Le Cao, K. mixOmics: An R package for 'omics feature
633 selection and multiple data integration. *PLoS Comput. Biol.* **13**, e1005752 (2017).

634 35. Dimitriou, I. D. *et al*. Translational Control by 4E-BP1/2 Suppressor Proteins Regulates
635 Mitochondrial Biosynthesis and Function during CD8(+) T Cell Proliferation. *J. Immunol.* **208**,
636 2702–2712 (2022).

637 36. Choi, H., Lee, H., Sohn, H. & Kim, T. CD40 ligand stimulation affects the number and
638 memory phenotypes of human peripheral CD8(+) T cells. *BMC Immunol.* **24**, 15–2 (2023).

639 37. Nolz, J. C., Starbeck-Miller, G. R. & Harty, J. T. Naive, effector and memory CD8 T-cell
640 trafficking: parallels and distinctions. *Immunotherapy* **3**, 1223–1233 (2011).

641 38. Gesser, B. *et al*. Interleukin-8 induces its own production in CD4+ T lymphocytes: a process
642 regulated by interleukin 10. *Biochem. Biophys. Res. Commun.* **210**, 660–669 (1995).

643 39. Mehta, A. K., Gracias, D. T. & Croft, M. TNF activity and T cells. *Cytokine* **101**, 14–18
644 (2018).

645 40. Orta-Mascaro, M. *et al*. CD6 modulates thymocyte selection and peripheral T cell
646 homeostasis. *J. Exp. Med.* **213**, 1387–1397 (2016).

647 41. Yang, R. *et al*. IL-6 promotes the differentiation of a subset of naive CD8+ T cells into IL-
648 21-producing B helper CD8+ T cells. *J. Exp. Med.* **213**, 2281–2291 (2016).

649 42. Nishanian, P. *et al*. Serum soluble CD8 molecule is a marker of CD8 T-cell activation in
650 HIV-1 disease. *AIDS* **5**, 805–812 (1991).

43. Siemiątkowska, A. *et al*. Low on-treatment levels of serum soluble CD8 (sCD8) predict better outcomes in advanced non-small cell lung cancer patients treated with atezolizumab. *Cancer Immunology, Immunotherapy* **72**, 1853–1863 (2023).

44. Xiao, Z., Mescher, M. F. & Jameson, S. C. Detuning CD8 T cells: down-regulation of CD8 expression, tetramer binding, and response during CTL activation. *J. Exp. Med.* **204**, 2667–2677 (2007).

45. Srinivasan, S., Zhu, C. & McShan, A. C. Structure, function, and immunomodulation of the CD8 co-receptor. *Frontiers in Immunology* **15**, 1412513 (2024).

46. Muehlinghaus, G. *et al*. Regulation of CXCR3 and CXCR4 expression during terminal differentiation of memory B cells into plasma cells. *Blood* **105**, 3965–3971 (2005).

47. Kim, S. J. *et al*. Increased IL-12 inhibits B cells' differentiation to germinal center cells and promotes differentiation to short-lived plasmablasts. *J. Exp. Med.* **205**, 2437–2448 (2008).

48. Rutella, S. *et al*. Hepatocyte growth factor favors monocyte differentiation into regulatory interleukin (IL)-10 IL-12low/neg accessory cells with dendritic-cell features. *Blood* **108**, 218–227 (2006).

49. Baart, V. M. *et al*. Molecular imaging of the urokinase plasminogen activator receptor: opportunities beyond cancer. *EJNMMI research* **10**, 87 (2020).

50. Stawski, L. & Trojanowska, M. Oncostatin M and its role in fibrosis. *Connect. Tissue Res.* **60**, 40–49 (2019).

51. Márquez, E. J. *et al*. Sexual-dimorphism in human immune system aging. *Nature communications* **11**, 751 (2020).

52. Sabbagh, L., Snell, L. M. & Watts, T. H. TNF family ligands define niches for T cell memory. *Trends Immunol.* **28**, 333–339 (2007).

53. Macaulay, R., Akbar, A. N. & Henson, S. M. The role of the T cell in age-related inflammation. *Age* **35**, 563–572 (2013).

54. Jung, I. & Oh, G. T. The roles of CD137 signaling in atherosclerosis. *Korean Circulation Journal* **46**, 753–761 (2016).

55. Zhao, F. *et al*. FLT3LG modulates the infiltration of immune cells and enhances the efficacy of anti-PD-1 therapy in lung adenocarcinoma. *BMC Cancer* **25**, 831 (2025).

56. Stobie, L. *et al*. The role of antigen and IL-12 in sustaining Th1 memory cells in vivo: IL-12 is required to maintain memory/effector Th1 cells sufficient to mediate protection to an infectious parasite challenge. *Proceedings of the National Academy of Sciences* **97**, 8427–8432 (2000).

57. Airoldi, I. *et al*. Heterogeneous expression of interleukin-18 and its receptor in B-cell lymphoproliferative disorders deriving from naive, germinal center, and memory B lymphocytes. *Clinical cancer research* **10**, 144–154 (2004).

58. Haslam, D. E. *et al*. Stability and reproducibility of proteomic profiles in epidemiological studies: comparing the Olink and SOMAscan platforms. *Proteomics* **22**, 2100170 (2022).

59. Eldjarn, G. H. *et al*. Large-scale plasma proteomics comparisons through genetics and disease associations. *Nature* **622**, 348–358 (2023).

Table 1. Participant characteristics at time of blood draw used for immune phenotyping and protein profiling for the study sample

	Full Sample	Female	Male
Sample size, n	873	450	423
Female, n (%)	450 (51.5 %)	——	——
Age, mean(range)	61 (40, 88)	61 (41, 85)	62 (40, 88)
CMV positive, n (%)	440 (50.4%)	239 (53.1%)	201 (47.5%)
Current smoker, n (%)	115 (13.2%)	71 (15.8%)	44 (10.4%)
BMI Kg/m2, mean (sd)	28 (5)	27 (6)	28 (4)
SBP mmHg, mean (sd)	127 (18)	126 (20)	128 (17)
DBP mmHg, mean (sd)	74 (9)	72 (9)	75 (9)
Hypertension Rx, n (%)	301 (34.5%)	130 (29.0%)	171 (40.4%)
Total cholesterol mg/dL, mean (sd)	199 (37)	206 (37)	192 (36)
Triglycerides mg/dL, mean (sd)	137 (85)	132 (76)	143 (93)
Lipid Rx, n (%)	199 (22.8%)	81 (18.0%)	118 (27.9%)
Fasting blood glucose mg/dL, mean (sd)	105 (27)	101 (26)	109 (28)
Type 2 Diabetes Rx, n (%)	53 (6.1%)	24 (5.3%)	29 (6.9%)
Prevalent CVD, n (%)	127 (14.5%)	39 (8.7%)	88 (20.8%)
Prevalent stroke, n (%)	16 (1.8%)	6 (1.3%)	10 (2.4%)
Prevalent AF, n (%)	36 (4.1%)	14 (3.1%)	22 (5.2%)

Note: CMV = cytomegalovirus, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, sd = standard deviation, CVD=cardiovascular disease; AF= atrial fibrillation. Rx=treatment

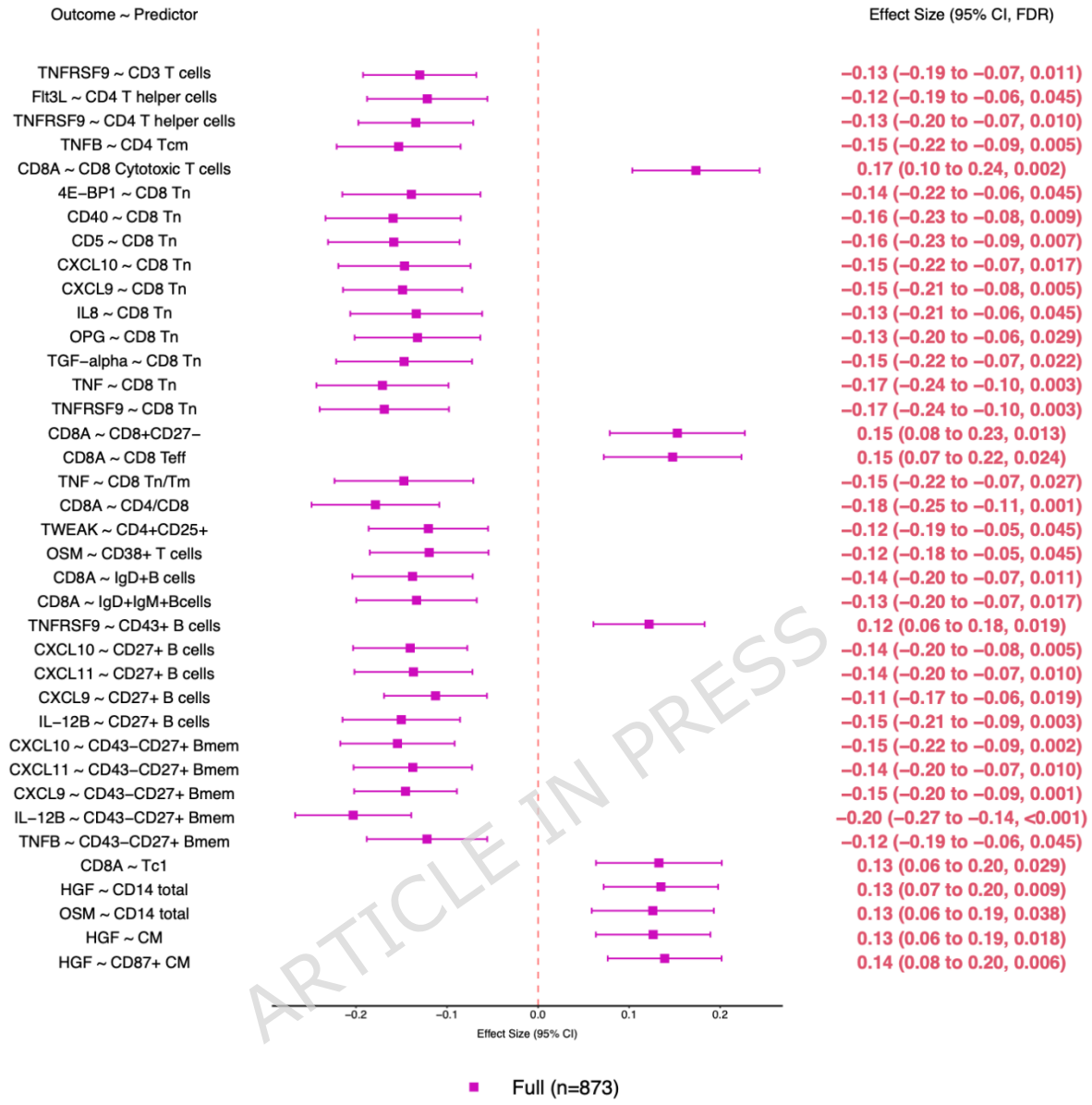

Figure 1. Forest plots of effect size for significant associations of immune cell phenotypes with the protein biomarker outcomes in the full sample using Model 1 (FDR ≤ 0.05).
Note: Model 1 covariates included: sex, age, and CMV levels. FDR = false discovery rate, CI = confidence interval, CMV = cytomegalovirus.

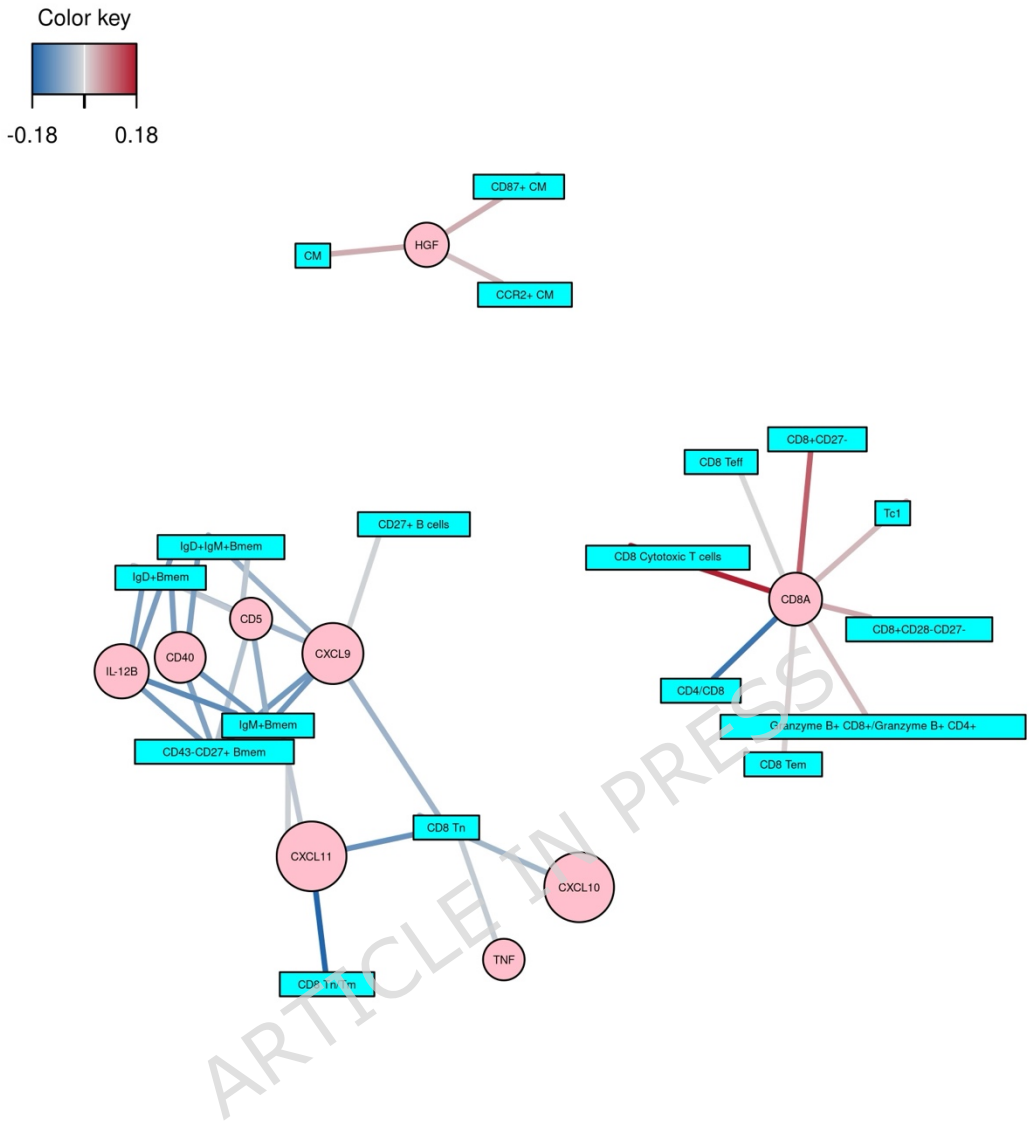

Figure 2. Network representation from the sparse partial least squares was performed on the immune cell phenotypes and inflammatory biomarkers, with edges linking immune cells to inflammatory biomarkers according to a similarity measure calculated from latent variables (see Color key for similarity measure size and direction; red edges indicate positive correlations, while blue edges indicate negative correlations).

Note: Tc1 cells, Cytotoxic T cells type 1; Tn, naïve T cells; Tm, memory T cells; Teff, T effector cells; Tem, T effector-memory cells; Bmem, memory B cells; CM, classical monocytes.