Innate and adaptive immune cell subsets as risk factors for coronary heart disease in two population-based cohorts

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Abstract

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Author contributions

N.C.O. conceived the study design, interpreted data, and drafted the manuscript; C.M.S. performed statistical analyses and revised the manuscript; M.F.D. conceived the study design, generated the data, and revised the manuscript; S.A.H. conceived the study design and revised the manuscript; A.L.L conceived the study design and revised the manuscript; R.P.T. conceived the study design and interpreted data; B.M.P. conceived the study design, interpreted data, and revised the manuscript; J.A.D. conceived the study design, performed statistical analyses, interpreted data, and revised the manuscript. All authors approved the manuscript for publication.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Background and aims: Cell-mediated immunity is implicated in atherosclerosis. We evaluated whether innate and adaptive immune cell subsets in peripheral blood are risk factors for coronary heart disease.

Methods: A nested case-cohort study (n=2,155) was performed within the Multi-Ethnic Study of Atherosclerosis (MESA) and the Cardiovascular Health Study (CHS). Cases of incident myocardial infarction (MI) and incident angina (n=880 total cases) were compared with a cohort random sample (n=1,275). Immune cell phenotypes (n=34, including CD14$^+$ monocytes, natural killer cells, $\gamma\delta$ T cells, CD4$^+$, CD8$^+$ and CD19$^+$ lymphocyte subsets) were measured from cryopreserved cells by flow cytometry. Cox proportional hazards models with adjustment for cardiovascular disease risk factors were used to evaluate associations of cell phenotypes with incident MI and a composite phenotype of incident MI or incident angina (MI-angina) over a median 9.3 years of follow-up. Th1, Th2, Th17, T regulatory (CD4$^+$CD25$^+$CD127$^-$), naive (CD4$^+$CD45RA$^+$), memory (CD4$^+$CD45RO$^+$), and CD4$^+$CD28$^-$ cells were specified as primary hypotheses. In secondary analyses 27 additional cell phenotypes were investigated.

Results: After correction for multiple testing, there were no statistically significant associations of CD4$^+$ naive, memory, CD28$^-$, or T helper cell subsets with MI or MI-angina in MESA, CHS, or combined-cohort meta analyses. Null associations were also observed for monocyte subsets, natural killer cells, $\gamma\delta$ T cells, CD19$^+$ B cell and differentiated CD4$^+$ and CD8$^+$ cell subsets.

Conclusions: The proportions of peripheral blood monocyte and lymphocyte subsets are not strongly related to the future occurrence of MI or angina in adults free of autoimmune disease.

Graphical abstract
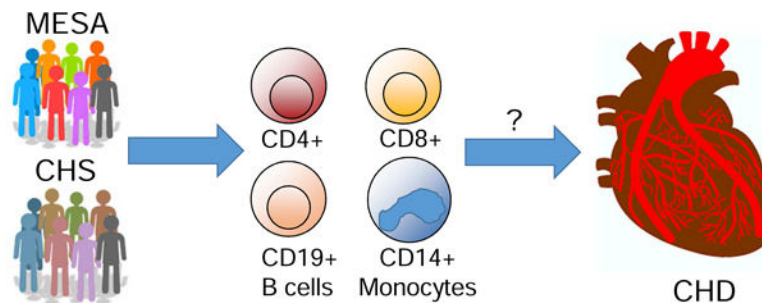
Keywords

Biomarkers; Cardiovascular Disease; Immunity; Inflammation; Risk Factors; T cells

Introduction

Coronary heart disease (CHD) remains a leading cause of death and disability worldwide. The major underlying cause of CHD is atherosclerosis, an inflammatory process initiated by cholesterol retention in the arterial wall. Arterial inflammation is orchestrated by cells of the innate (e.g., monocytes, macrophages) and adaptive (e.g., CD4$^+$ T helper cells and cytotoxic CD8$^+$ T cells) immune systems following their recruitment to the arterial intima [1].

The importance of cell-mediated immunity in atherosclerosis has been established in several experimental mouse models. Accumulation and modification of lipoprotein particles in the arterial intima (e.g., oxidized low-density lipoprotein (LDL)) leads to the recruitment of monocytes which differentiate into lipoprotein-scavenging macrophages [1, 2]. CD4$^+$ T helper cells are recruited to atherosclerotic lesions and play both atherogenic and atheroprotective roles [1]. CD4$^+$ cells include T helper type 1 (Th1), Th2, Th17, and T regulatory (Treg) cells. Interferon-γ (IFN- γ) producing Th1 cells contribute to inflammation and plaque destabilization, whereas IL-10 producing Tregs are anti-inflammatory and atheroprotective [1]. Th2 and Th17 cells have been implicated in atheroprotection and plaque stability, respectively, but their roles in experimental atherosclerosis remain unresolved [1, 3]. CD8$^+$ T cells and humoral immune B cells are suspected as important but their roles are still being clarified [1, 4]. Natural killer cells were shown to have no role in hypercholesterolemia-induced atherosclerosis, but exacerbated atherosclerosis under inflammatory conditions associated with viral infection [5].

Several immune cell populations, including natural killer, CD4$^+$ and CD8$^+$ subsets are present in human atherosclerotic lesions [6–8]. An immune cell atlas of human atherosclerotic plaques was recently generated using single-cell proteomic and transcriptomic analyses [9]. In this study CD4$^+$ and CD8$^+$ T cells isolated from plaques were chronically activated and differentiated, with the majority being effector-memory subsets. Cross-sectional analyses of peripheral blood samples from epidemiologic cohorts have shown associations of higher intermediate and non-classical monocyte subsets, CD19$^+$CD80$^+$ B cells, Th1, CD4$^+$ memory, CD4$^+$ effector-memory, and lower Th2 cells, with the severity of carotid artery intimal medial thickness (IMT) or coronary artery calcification (CAC) [10–15].

Although the involvement of cellular immunity has been established in murine models of atherosclerosis and indicated in cross-sectional human studies, it remains unclear whether these findings translate to risk of clinical cardiovascular disease (CVD) events [16]. Atherosclerotic plaques from patients with recent cerebrovascular events were characterized by an expansion of activated effector-memory CD4$^+$ T cells and gene expression signatures reflecting CD4$^+$ and CD8$^+$ T cell activation, differentiation and exhaustion [9]. Higher levels of intermediate monocytes, Th1, Th17, CD4$^+$CD28$^-$and CD8$^+$CD56$^+$ T cells, and lower Tregs, were reported in the circulating blood of subjects with coronary artery disease compared to those without disease [6, 7, 17]. The prospective Malmö Diet and Cancer Study (MDCS) has evaluated longitudinal relationships of circulating immune cells with risk of incident clinical CVD events [14, 15, 18–21]. In these studies, high levels of CD8$^+$ cells and low levels of Th2 and Tregs, were associated with an increased risk of incident coronary events [14, 18, 20].

To date, no prospective cohort studies have investigated the relationships of a broad panel of innate and adaptive immune cell phenotypes with the risk of incident CHD in a multi-ethnic population. In the present study, we measured 34 immune cell subsets using cryopreserved samples from participants of the population-based Multi-Ethnic Study of Atherosclerosis (MESA) and Cardiovascular Health Study (CHS). We evaluated their prospective relationships with incident myocardial infarction (MI) and incident angina over a follow-up

period of up to 15-years. Based on our prior cross-sectional results in MESA [11, 12], and findings from others [10, 14, 20, 22–24], we hypothesized that higher proportions of Th1, $CD4^+$ memory and $CD4^+CD28^-$ cells, and lower proportions of $CD4^+$ naive, Th2, Th17, and Treg cells, would be associated with increased risk of MI or a composite phenotype of incident MI or incident angina.

Materials and methods

Cohorts

The MESA is a prospective cohort study of subclinical cardiovascular disease [25]. At the baseline examination in 2000–2002, 6,814 men and women aged 45 to 84 years were recruited from six U.S. communities (Baltimore, MD, Chicago, IL, Los Angeles, CA, St. Paul, MN, New York, NY, and Forsyth County, NC). The cohort was 38% white, 28% African-American, 22% Hispanic, and 12% Chinese. Participants were excluded if they had clinical CVD or were undergoing active treatment for cancer. Follow-up examinations occurred in 2002–04, 2004–05, 2005–07, 2010–12, and 2016–18. During the examinations, participants went to the MESA Field Center clinics, underwent assessment for CVD risk factors, provided fasting blood samples, and answered standardized questionnaires [25].

The CHS is a prospective population-based cohort study of risk factors for CHD and stroke in older adults [26]. In 1989–1990, 5,201 men and women aged 65 and older were recruited from 4 U.S. field centers (Forsyth County, NC, Sacramento County, CA, Washington County, MD, and Pittsburgh, PA). An additional cohort of 687 primarily African-American participants were recruited in 1992–93. Participants answered standardized questionnaires assessing medical histories and CVD risk factors, and provided fasting blood samples. Between 1990 and 1999, semi-annual follow-up alternated between clinical examinations and telephone contacts. Since 1999, bi-annual follow-up occurred by telephone. CHS participants with prevalent CVD at the time of the 1998–99 blood draw were excluded from our study.

The current study is a case-cohort study nested within MESA and CHS. The study included 1,195 MESA and 1,197 CHS participants with available samples of cryopreserved cells. The study included all cases of incident MI and incident angina (n=485 MESA, n=492 CHS) and a cohort random sample (n=715 MESA, n=706 CHS). All participants provided written informed consent for participation in the study and all procedures were conducted under institutionally approved protocols for human subjects research.

Covariate data were from the same examination cryopreserved cells were collected: 2000–02 in MESA and 1998–99 in CHS (Year 11). Diabetes mellitus was defined as a fasting blood glucose 126 mg/dL or the use of insulin or oral hypoglycemic medications. Smoking was defined as never, former (no cigarettes within the past 30 days), or current. Education status was defined by less than college degree or college degree or above. IgG antibodies to cytomegalovirus (CMV) were measured in serum by enzyme immunoassay (Diamedix Corp., Miami Lakes, FL); the inter-assay coefficients of variation were 5.1%−6.8%.

Cellular phenotyping

Peripheral blood mononuclear cells (PBMCs) were isolated from 8-mL citrate CPT tubes during the MESA baseline examination (2000–02) and the Year 11 CHS examination (1998–99) and cryopreserved at −135°C. Cells were thawed at 37°C for 15 minutes and treated with benzonase (2500 U/mL in RPMI) for 20 minutes. Cells were slowly diluted 10-fold by the addition of RPMI supplemented with fetal bovine serum (10%), L-glutamine (2 mM), penicillin (100 IU/mL) and streptomycin (100 μg/mL) (fsRPMI) with mixing. Cells were pelleted by centrifugation (5 minutes at 200 x g) and treated with 250 U/mL benzonase in fsRPMI for 10 minutes. The pellet was washed and filtered through a 70 μM filter. Cells were placed in 96 well plates, centrifuged and resuspended in phosphate buffered saline for staining with a live/dead stain (Thermo Fisher Scientific, Waltham, MA) for 15 minutes at room temperature.

We performed a pilot study to assess viability of cryopreserved cells in MESA and CHS. We thawed 8 MESA PBMC samples cryopreserved in 2000–02 and 12 CHS samples cryopreserved in 1998–99. Phenotypes from cryopreserved cells were compared to data from the MESA-Inflammation study [11, 12] which evaluated cells isolated from fresh blood samples collected in 2005–07. Results are presented in Supplemental Table I and suggested cell phenotype data from fresh vs. cryopreserved samples were similar. For example, the mean value of $CD4^+CD45RO^+$ (memory) cells was 53.9% in fresh samples compared with 56.7% in cryopreserved samples. Mean Th1 ($CD4^+IFN-\gamma^+$) was 16.0% in fresh and 12.3% in cryopreserved samples.

Phenotyping assays for monocyte subsets, innate lymphocytes (i.e., natural killer, $\gamma\delta$ T), $CD19^+$ B cell subsets, and naive, memory, differentiated, and $CD45RA^+$ re-expressing effector memory (TEMRA) $CD4^+$ and $CD8^+$ T cell subsets were performed by cell-surface labeling. Cells were incubated with antibodies for 15 minutes in the dark at room temperature according to the manufacturer's recommendations. Cells were pelleted and fixed in 1% paraformaldehyde (Alfa Asaer, Tewksbury, MA). All antibodies were from Miltenyi Biotec (San Diego, CA) and used dilutions recommended by the manufacturer (Supplemental Material Table II).

In MESA, intracellular cytokine staining was used for Th1, Th2, Th17, $CD8^+$ T cytotoxic type 1 (Tc1), Tc2, and Tc17 phenotyping. These assays used phorbal myristic acetate, ionomycin, and Brefeldin A to activate cells and inhibit cytokine secretion as previously described [11]. Cells were subsequently incubated with APC-conjugated anti-CD4 or VioBrightFITC-conjugated anti-CD8 for 15 minutes at room temperature. Cells were washed with staining buffer and fixed with 2% paraformaldehyde for 10 minutes. Cells were then permeabilized using 0.1% saponin and incubated with PEVio770-anti-IFN-γ, PE-anti-interleukin-4 (IL-4), and APCVio770-anti-IL-17 for 15 minutes at room temperature. Cells were washed and fixed in 2% paraformaldehyde. In CHS, intracellular cytokine assays were not performed due to generally lower cell viability. Th1, Th2, and Th17 phenotypes were evaluated in CHS by cell-surface labeling of the chemokine receptors CXCR3 (VioBright FITC-CXCR3), CCR4 (PE-Vio770-CCR4), and CCR6 (APC-CCR6), as described above for CD4/CD8. Th1 cells were defined as $CD4^+CXCR3^+CCR4^-CCR6^-$. Th2 cells were defined as $CD4^+CXCR3^-CCR4^+CCR6^-$ and Th17 cells as $CD4^+CXCR3^-CCR4^+CCR6^+$ [27]. Tc1,

Tc2, Tc17, and CD14$^+$ monocyte subsets were not assayed in CHS. All markers used for immunophenotyping are presented in Supplementary Table III.

Cell phenotypes were evaluated using an MQ10 flow cytometry and MACS Quantify software (Miltenyi Biotec). Calibration beads were used for daily calibration. Compensation was set using single color compensation controls. Isotype-matched antibodies were used as controls to set negative gates for each assay. Cell phenotypes were expressed as proportions of larger "parent" populations. For example, T helper cell subsets were expressed as a percentage of CD4$^+$ cells (described in Supplementary Table III). Flow cytometry gating strategies are presented in Supplemental Figures I–VI.

Event ascertainment, definitions, and laboratory measurements

The primary clinical endpoints for this study were incident MI and a composite phenotype comprised of incident MI or incident angina (MI-angina). The MI outcome included non-fatal, fatal, and procedure-related MIs.

MESA participants or their proxies completed telephone interviews every 9–12 months providing information about potential cardiovascular events, including hospitalizations, diagnostic tests, procedures, and deaths [25]. MESA Field Center staff collected information from medical records, death certificates, autopsy reports, and interviews with physicians, participants, or their proxies. Information was reviewed and CVD events were adjudicated by the MESA Events Committee [25]. Follow-up for MESA participants in the present study was through December 31, 2015.

In CHS, events were reported at clinical examinations or during semiannual telephone contacts. Events ascertainment included hospital and out-patient medical records, including copies of ECGs, CT scans, MRI scans, death certificates, and autopsy and coroner reports. These were reviewed and adjudicated by the CHS Cardiac Events Committee [28]. In the current study, follow-up for CHS participants was through June 30, 2015.

Statistical analyses

Our analyses included participants with data for at least one of the immune cell assays (n=2155; MESA: 1113, CHS: 1042). The number of participants with data for each immune cell trait is presented in Supplemental Table IV. Missing cell data were due to poor sample quality and technical assay errors. These types of missing data were determined random, and were not considered to be related to any participant characteristics. We used multiple imputation (100 imputations) with chained equations to impute missing cell phenotype and covariate data from among these 2155 participants. To account for the case-cohort sampling, censoring indicators for MI and angina and time to event data were included in the multiple imputation model [29, 30].

Associations between immune cell traits and MI or composite MI-angina endpoints were evaluated by Cox proportional hazards models that used case-cohort weights and robust standard error (sandwich) estimates. Weights were the inverse sampling probabilities for inclusion in the study [31]. We analyzed results separately in each cohort. For cell assays that were comparable in both studies (e.g., CD4$^+$ naive, memory, and CD28$^-$ cells), MESA

and CHS results were combined using fixed effects inverse-variance-weighted meta-analysis. Cell phenotypes were analyzed per 1 standard deviation (SD) higher value and modeled singly to avoid collinearity. Demographic models included adjustment for age, sex, race/ethnicity, education, and clinical examination site. CHD risk factor models included these variables plus systolic blood pressure, use of anti-hypertension medications, LDL cholesterol, use of statins, diabetes, and smoking status. A sensitivity model included additional adjustment for log-transformed cytomegalovirus (CMV) antibody concentrations and season of blood draw due to known relationships of these variables with Th1 and Th2 cells in MESA, respectively [11].

Multiple hypothesis testing was controlled for using Bonferroni correction. Primary hypotheses included Th1, Th2, Th17, Treg, $CD4^+$ naive, $CD4^+$ memory, and $CD4^+CD28^-$ cells and were considered statistically significant if $p < 0.0071$. Secondary analyses evaluated n=27 additional immune cell subsets and used a significance threshold of $p < 0.0015$.

Results

In the combined MESA-CHS case-cohort there were 644 incident MIs and 880 cases of incident MI or incident angina over a median 9.3 years of follow-up (interquartile range (IQR) 4.3–14.0 years). By cohort, there were 296 incident MIs and 451 composite MI-angina events in MESA over a median 11.1 years of follow-up (IQR: 4.9–14.2). In CHS there were 348 incident MIs and 429 incident MI-angina events over a median 7.7 years of follow-up (IQR: 4.0–12.7). Table 1 shows the characteristics of the study population stratified by cohort and case status.

As primary hypotheses we evaluated associations of Th1 ($CD4^+IFN-\gamma^+$), Th2 ($CD4^+IL-4^+$), Th17 ($CD4^+IL-17A^+$), Tregs ($CD4^+CD25^+CD127^-$), naive ($CD4^+CD45RA^+$), memory ($CD4^+CD45RO^+$), and senescent ($CD4^+CD28^-$) $CD4^+$ T cells with incident MI and with incident MI-angina. As shown in Figure 1, there were no statistically significant associations among any of these cell populations with MI in MESA, CHS, or in a combined-cohort meta-analysis (all $p > 0.05$; analyzed per 1-SD higher proportion, adjusted for demographic variables and CHD risk factors). The associations of these cells with incident MI-angina were also not statistically significant (all $p > 0.05$; Figure 2).

In secondary analyses, we evaluated associations of 27 additional innate and adaptive immune cell phenotypes with incident MI and incident MI-angina. To correct for multiple hypothesis testing we defined statistical significance as $p < 0.0015$. Cell phenotypes included $CD14^+$ monocyte subsets, natural killer cells, $\gamma\delta$ T cells, $CD19^+$ B cell subsets, and differentiated and TEMRA $CD4^+$ and $CD8^+$ cell subsets (as described in Supplemental Table III). In models adjusted for CHD risk factors, none of the cells' associations with incident MI were statistically significant in either cohort individually or in combined-cohort analyses (all $p > 0.05$ Supplemental Table V). Associations of these cells with the composite endpoint of incident MI-angina were also null (all $p > 0.20$) (Figure 3).

Interpretation of the results was unchanged in models minimally adjusted for demographic variables or in CHD risk factor adjusted models that included season of blood draw and CMV antibody concentrations as additional covariates.

Discussion

In our study of American adults from two longitudinal population-based cohorts, we evaluated the prospective relationships of circulating monocyte and lymphocyte subsets with the risk of incident CHD. We observed no significant associations of $CD14^+$ monocyte, innate lymphocyte (natural killer or $\gamma\delta$), $CD19^+$ B cell, $CD4^+$ or $CD8^+$ T cell subsets with the future occurrence of MI or angina. Associations of each of the 34 immune cell phenotypes investigated were null in MESA and CHS separately, and in a combined-cohort meta-analysis that included 880 composite MI-angina events.

Experimental mouse models have demonstrated distinct roles of several lymphocyte populations in atherosclerosis [1]. Th1 cells promote atherosclerosis, whereas Tregs protect against it [1]. Although less firmly established, Th17, natural killer, $\gamma\delta$ T, and B2 B cells have been shown in some mouse models to be atherogenic [1, 7, 32] whereas Th2 and B1a B cells were protective [3, 33]. Overall, the translation of murine results to CVD risk in humans is uncertain.

In human atherosclerotic plaques, there are an abundance of $CD4^+$ and $CD8^+$ effector-memory T cells [9, 34]. Intermediate ($CD14^{++}CD16^+$) and non-classical ($CD14^+CD16^{++}$) monocytes, B cells ($CD19^+CD80^+$), Tc1 ($CD8^+CD56^-IFN\text{-}\gamma^+$), Th1, Th2, memory ($CD4^+CD45RO^+$), and effector-memory ($CD4^+CD45RA^-CD45RO^+CCR7^-$) T cells measured in peripheral blood are associated with the severity of atherosclerosis (evaluated by carotid stenosis, IMT, or CAC) [10–15, 18] in cross-sectional analyses, including studies in MESA [11, 12].

Variation in immune cells have also been reported in subjects with clinical CVD [6, 35]. Higher intermediate monocytes ($CD14^{++}CD16^+$), Th1, Th17, effector-memory ($CD4^+CD45RA^-CD45RO^+CCR7^-$), $CD4^+CD28^-$, and $CD8^+CD56^+$ T cells, and lower Tregs ($CD4^+CD25^+$ or $CD4^+CD25^+Foxp3^+$) were observed in the peripheral blood of patients with coronary artery disease (CAD) and acute coronary syndrome (ACS) [6, 10, 17, 22, 24, 36–42]. Single-cell analyses identified macrophage and T cell dysregulation in atherosclerotic plaques from patients with cerebrovascular events [9]. These collective findings implicate a role of cellular immunity in the development and progression of atherosclerotic CVD. However, as these studies analyzed cells collected from patients with prevalent CVD it is not possible to determine whether variation in immune cells occurred before or after disease onset [43].

To determine the importance of immune cell proportions in peripheral blood as risk factors for the future occurrence of clinical CHD events, we evaluated the prospective relationships of a broad panel of innate and adaptive immune cells with incident MI or incident angina in a population free of prevalent clinical CVD. Our results suggest that higher or lower

proportions of CD14^{+} monocyte, NK, $\gamma\delta$ T, CD4^{+} T helper, cytotoxic CD8^{+}, or CD19^{+} B cell subsets are not strongly related to the future onset of MI or angina.

To our knowledge, the MDCS and European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohorts are the only population-based studies to evaluate longitudinal relationships of immune cell subsets with incident CVD events [14, 15, 18–21, 44]. In these studies, higher peripheral blood CD8^{+} cells and lower Th2 and Treg (CD4^{+}FoxP3^{+}) cells were associated with increased risk of incident coronary events (defined as fatal or nonfatal MI or death attributed to ischemic heart disease) [14, 18, 20]. Higher classical monocytes (CD14^{++}CD16^{−}) were associated with incident CVD events (defined as MI, CHD death, or stroke) [15]. Apparent differences between these results and those of our study may be explained by the significant association of Th2 cells with MI in women but not men [14], the phenotyping markers used [20], how phenotypes were expressed (i.e., as percentages of parent populations, cell numbers or ratios) [14, 15, 18, 44], or differences in demographic and cultural factors related to CVD risk.

In support of the present null findings, the role of T cell subsets, such as Tregs and CD4^{+}CD28^{−} cells, in CVD prediction has been recently challenged [21, 45]. In the MDCS, lower CD4^{+}FoxP3^{+} Treg cells were associated with increased risk of coronary events [20], whereas in the EPIC-Heidelberg study a lower ratio of FoxP3^{+} Treg to CD3^{+} T cells was not related to incident MI [44]. In a case-control study by Ammirati et al. [46], peripheral blood Tregs (CD3^{+}CD4^{dim}CD25^{high}) were higher in patients with acute ST-elevation MI (STEMI), and lower among those with non-STEMI ACS, compared with CAD-free controls. Among all patients with CAD, however, Treg levels were similar to controls [46].

Tomas et al. recently reported conflicting CVD risk estimates for CD4^{+}CD28^{−} T cells. Higher CD4^{+}CD28^{−} cells were associated with decreased CVD risk in a population without prevalent CVD at baseline, but were associated with increased risk in patients with advanced atherosclerotic disease [21]. Tregs and CD4^{+}CD28^{−} cells were not associated with carotid IMT in these studies [20, 21, 46].

Taken together, these null results and others [14, 15, 18–21, 46] are consistent with the current study and suggest limited use of peripheral blood immune cells as CVD biomarkers.

Findings may suggest that cellular immunity is more strongly related to atherosclerosis than clinically manifest CVD. For example, while Th1, Th2, naïve (CD4^{+}CD45RA^{+}) and memory (CD4^{+}CD45RO^{+}) CD4^{+} cells were associated with subclinical atherosclerosis in prior cross-sectional studies in MESA [11, 12], they were not associated with incident MI or angina in the current prospective study. In the MDCS, intermediate (CD14^{++}CD16^{+}) and non-classical (CD14^{+}CD16^{++}) monocytes, T_C1 cells (CD8^{+}CD56^{−}INF-γ^{+}), and CD19^{+}CD86^{+} B cells were associated with IMT or stenosis in cross-sectional analyses but not with incident coronary events [15, 18, 19]. The magnitude of the associations reported for cell phenotypes and atherosclerosis are typically small. Evidence overall suggests that variation in circulating immune cell levels may explain only a small portion of the risk for atherosclerosis or atherosclerotic CVD. Findings from cross-sectional studies also raise the possibility of reverse causality.

The current study has several limitations. Some cell phenotypes were measured in MESA but not CHS and there was a high degree of missing phenotype data overall. Despite the broad panel of phenotypes investigated, important T cell markers such HLA-DR and CCR7 [10, 47] were not included. The consequences of cryopreservation on PBMC phenotyping results are not well established but some cell surface markers may be affected [48]. A recent study, however, demonstrated several immune cell proportions were not significantly different when measured in cryopreserved samples compared with fresh blood [49]. Evaluation of cell proportions but not their cytokine profiles or antigen specificities is another limitation as changes in cell function may be important in CVD pathogenesis. Similarly, cells measured in peripheral blood may not reflect the populations present in atherosclerotic lesions. For example, immunophenotyping from paired human blood and atherosclerotic plaques showed distinct cell distributions [9]. In particular, macrophage, $CD8^+$, $CD4^+$ & $CD8^+$ effector-memory, and $CD4^-CD8^-$ T cells were enriched in plaques compared with blood. Finally, it is possible that immune cell frequencies reflect an acute risk of plaque instability [50] and are less strongly related to long-term CVD events [45].

Strengths of our study include the prospective case-cohort design comprised of participants free of prevalent clinical CVD from two well-established independent population-based cohorts. The simultaneous evaluation of a large panel of innate and adaptive immune cell phenotypes is another strength.

In summary, while cell-mediated immunity may play roles in human atherosclerosis, our results suggest peripheral blood monocytes and lymphocytes have limited utility as CHD biomarkers. These findings, however, do not exclude the hypothesis that cellular immunity plays a role in coronary disease. Prospective cohort studies evaluating the relationships of immune cells with the progression of atherosclerosis, as well as a confirmation of our findings in other populations, remain important.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations:

CHD	coronary heart disease
CHS	Cardiovascular Health Study
CMV	cytomegalovirus
CVD	cardiovascular disease
MDCS	Malmö Diet and Cancer Study
MESA	Multi-Ethnic Study of Atherosclerosis
MI-angina	incident myocardial infarction or incident angina
Tc	cytotoxic T cell
Th	cell T helper cell
Treg	T regulatory cell
TEMRA	CD45RA$^+$ re-expressing effector memory cell

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Highlights

- Cell-mediated immunity is established in animal models of atherosclerosis

- Unclear if immune cell levels in blood are risk factors for future coronary events

- CD4$^+$ and CD8$^+$ T cells were not related to incident myocardial infarction or angina

- Monocytes, innate lymphocytes, and B cells were not related to coronary risk

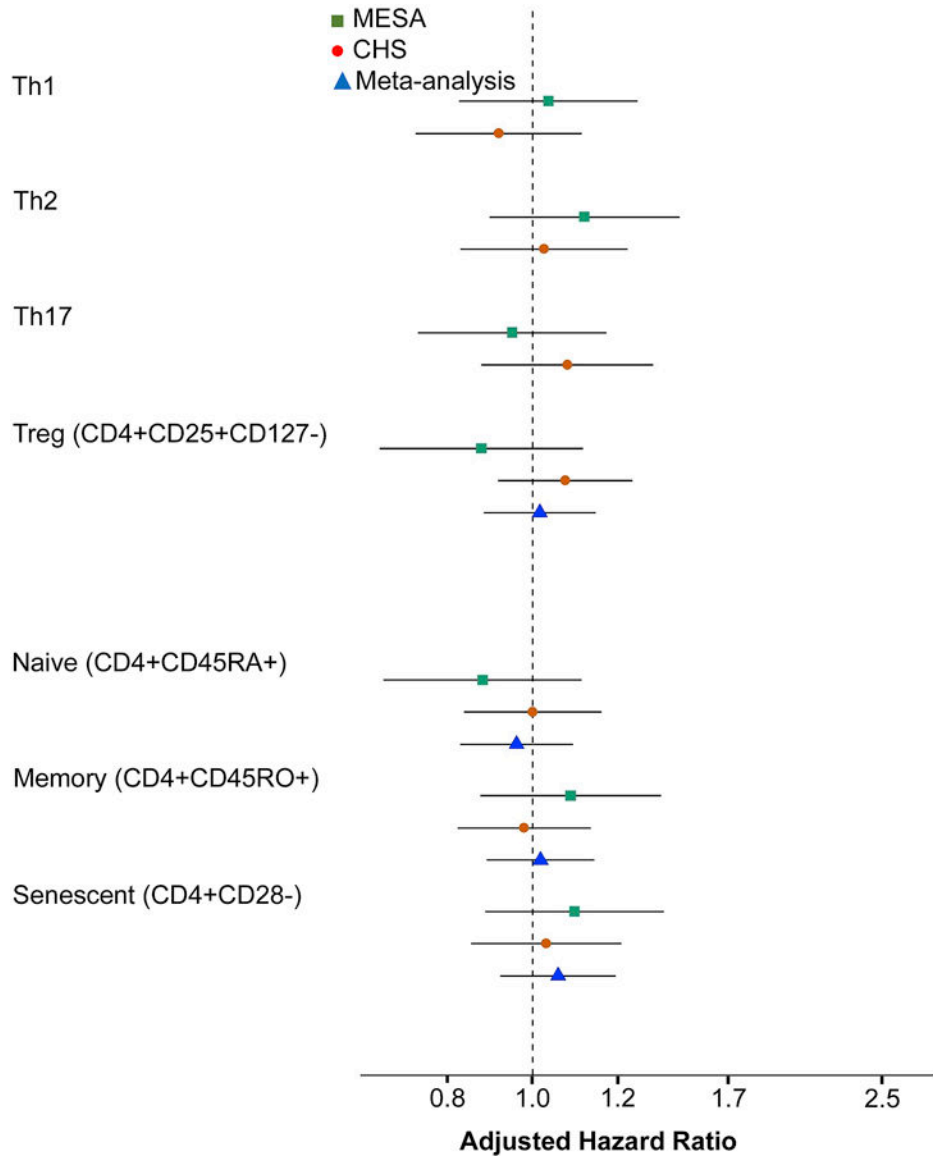- Blood lymphocytes and monocytes may have limited utility as CVD biomarkers

Figure 1. Associations of CD4$^+$ T helper cell subsets specified as primary hypotheses with incident myocardial infarction.

Cell subsets were analyzed per 1-SD higher values using Cox proportional hazards models with sampling weights. Confidence intervals (CIs) used robust (sandwich) standard error estimates and reflect the Bonferroni-adjusted significance level of p<0.0071. Th1, Th2, and Th17 cells were not meta-analyzed due to differences in phenotyping (as described in the Methods). Models were adjusted for age, sex, race/ethnicity, education, clinical site, systolic blood pressure, use of antihypertensive medications, low-density lipoprotein cholesterol, use of statins, smoking, and diabetes.
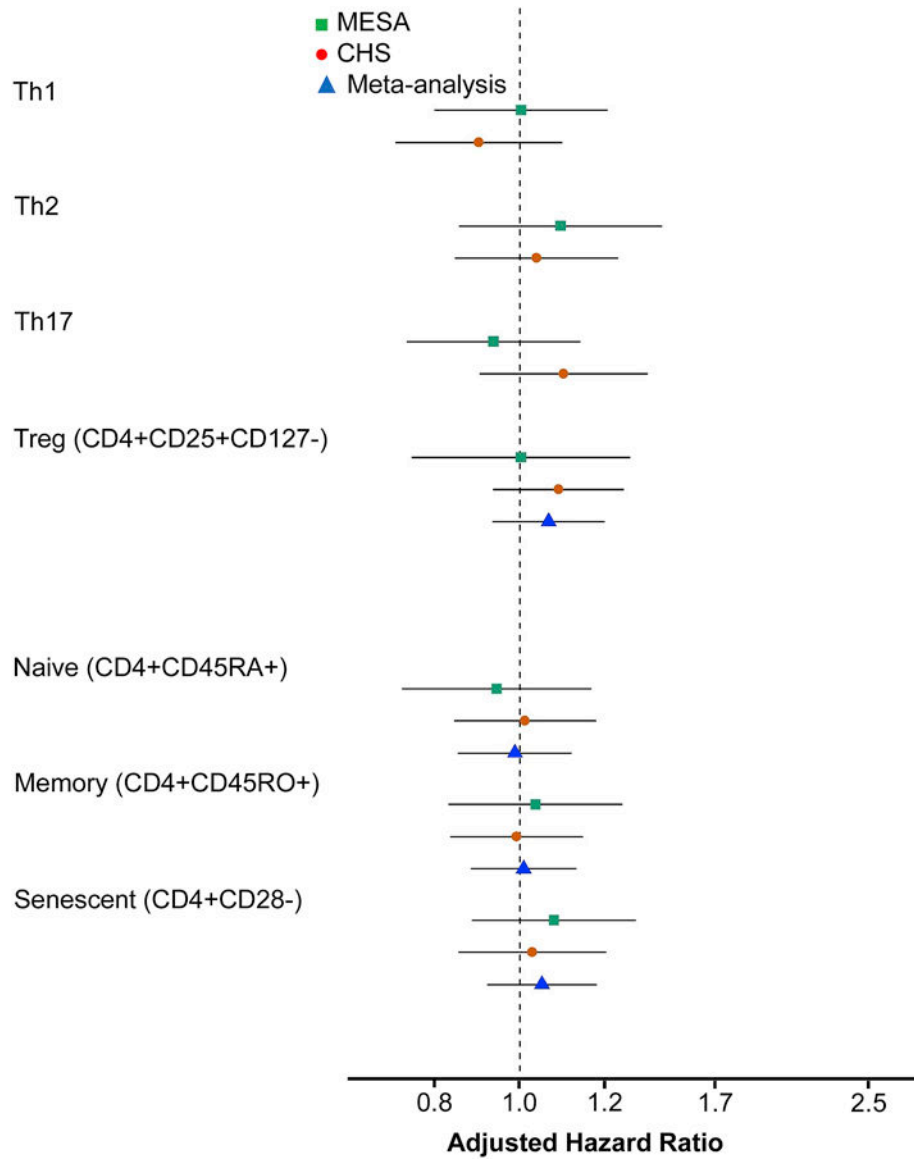
Figure 2. Associations of CD4$^+$ T helper cell subsets specified as primary hypotheses with incident myocardial infarction or incident angina.

Cell subsets were analyzed per 1-SD higher values using Cox proportional hazards models with sampling weights. Confidence intervals (CIs) used robust (sandwich) standard error estimates and reflect the Bonferroni-adjusted significance level of $p<0.0071$. Th1, Th2, and Th17 cells were not meta-analyzed due to differences in phenotyping (as described in the Methods). Models were adjusted for age, sex, race/ethnicity, education, clinical site, systolic blood pressure, use of antihypertensive medications, low-density lipoprotein cholesterol, use of statins, smoking, and diabetes.
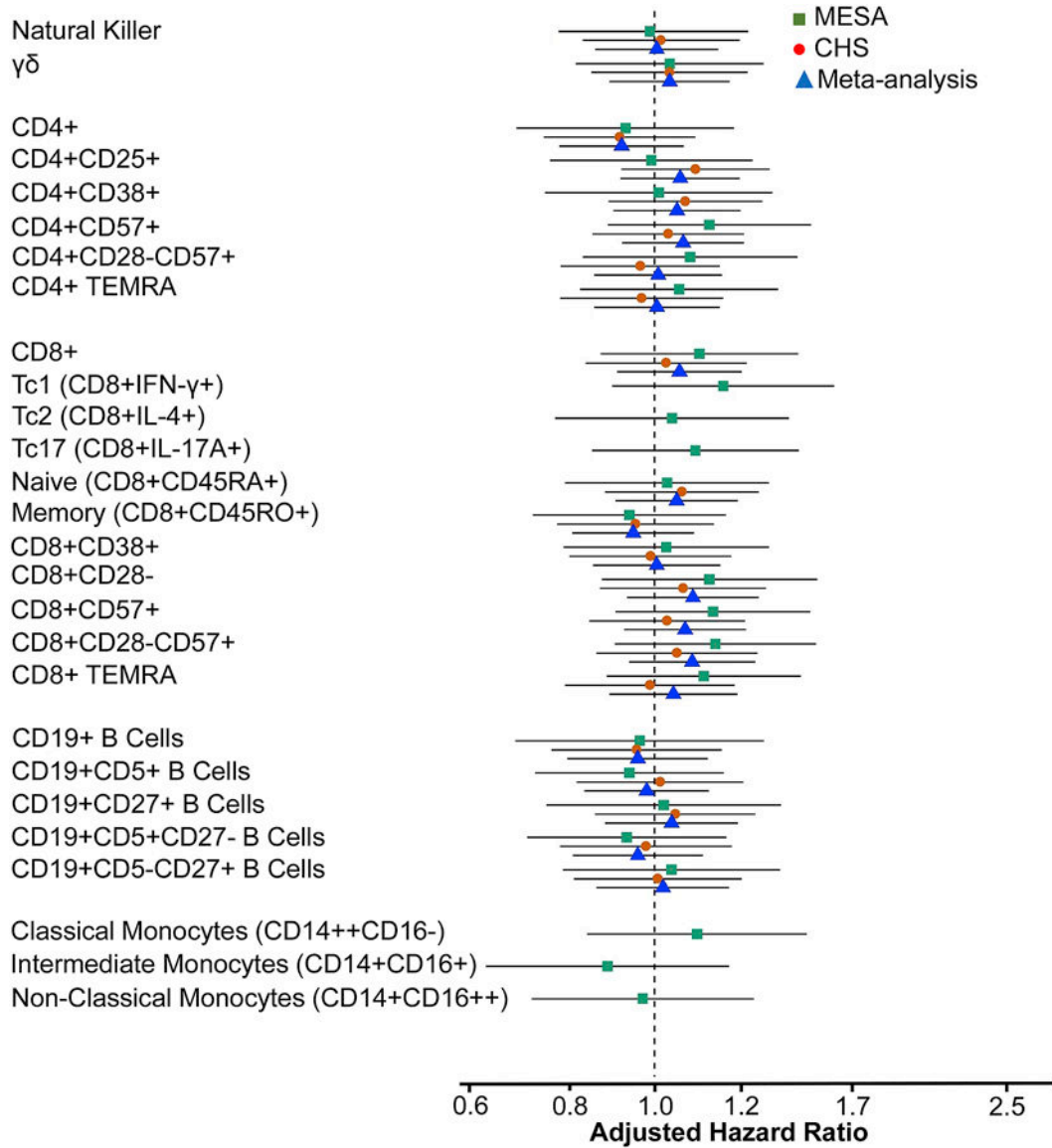
Figure 3. Associations of immune cell subsets included as secondary hypotheses with incident myocardial infarction or incident angina

Cell subsets were analyzed per 1-SD higher values using Cox proportional hazards models with sampling weights and robust (sandwich) standard error estimates. Confidence intervals reflect the Bonferroni-adjusted significance level of $p<0.0015$. Models were adjusted for age, sex, race/ethnicity, education, clinical site, systolic blood pressure, use of statins, low-density lipoprotein cholesterol, use of antihypertensive medications, smoking, and diabetes.

Table 1.

Characteristics of the study population stratified by cohort and case status

	MESA (*n*=1113)		CHS (*n*=1042)	
	No CHD (n=662)	CHD (n=451)	No CHD (n=613)	CHD (n=429)
Age, years (mean, SD)	62 (10)	67 (10)	80 (5)	80 (4)
Female sex (*n*, %)	296 (45%)	293 (65%)	408 (66%)	256 (60%)
Race/Ethnicity (*n*, %)				
White	229 (35%)	192 (43%)	503 (82%)	347 (82%)
African-American	209 (32%)	114 (25%)	109 (18%)	77 (18%)
Hispanic-American	132 (20%)	100 (22%)	-	-
Chinese-American	92 (13%)	45 (10%)	-	-
Bachelor's degree or above (*n*, %)	249 (38%)	142 (31%)	142 (23%)	102 (24%)
LDL-cholesterol, mg/dL (mean, SD)	117 (28)	119 (34)	201 (37)	201 (40)
Current smoker (*n*, %)	74 (11%)	74 (16%)	46 (7%)	32 (8%)
Systolic blood pressure, mm Hg (mean, SD)	127 (21)	134 (22)	135 (20)	138 (22)
Antihypertensive medication use (*n*, %)	242 (37%)	236 (52%)	318 (52%)	254 (60%)
Statin use (*n*, %)	97 (15%)	99 (22%)	68 (11%)	52 (12%)
Diabetes (*n*, %)	84 (13%)	103 (23%)	62 (10%)	73 (17%)
Cytomegalovirus antibodies, EU/mL (mean, SD)	235 (262)	242 (262)	301 (284)	262 (270)

Data are from MESA Exam 1 (2000–2002) and the Year 11 CHS examination (1998–1999). Coronary heart disease (CHD) is defined as incident myocardial infarction or incident angina. EU, ELISA units; LDL, low-density lipoprotein.