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Circulating T-Cell Subsets, Monocytes, and Natural Killer Cells in Peripartum Cardiomyopathy: Results From the Multicenter IPAC Study

CHARLES F. MCTIERNAN, PhD¹, PENELOPE MOREL, MD², LESLIE T. COOPER, MD³, NAVIN RAJAGOPALAN, MD⁴, VINAY THOHAN, MD⁴, MARK ZUCKER, MD⁵, JOHN BOEHMER, MD⁶, BIYKEM BOZKURT, MD⁷, PAUL MATHER, MD⁸, JOHN THORNTON, MD⁹, JALAL K. GHALI, MD¹⁰, KAREN HANLEY-YANEZ, MD¹, JAMES FETT, MD¹, INDRANI HALDER, PhD¹, and DENNIS M. MCNAMARA, MD¹ FOR THE IPAC INVESTIGATORS

¹Heart, Lung, Blood and Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, Pennsylvania

²Department of Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania

³Mayo Clinic, Jacksonville, Florida

⁴Gill Heart Institute, University of Kentucky, Lexington, Kentucky

⁵Cardiac Transplant Center, Beth Israel Newark Medical Center, New Jersey

⁶Division of Cardiology, Penn State Hershey Medical Center, Hershey, Pennsylvania

⁷Winters Center for Heart Failure Research, Baylor College of Medicine, Houston, Texas

⁸Cardiovascular Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

⁹Division of Cardiology, Georgia Regents University in Augusta, Augusta, Georgia

¹⁰Division of Cardiology, Mercer University School of Medicine, Macon, Georgia

Abstract

Objective—The aim of this work was to evaluate the hypothesis that the distribution of circulating immune cell subsets, or their activation state, is significantly different between peripartum cardiomyopathy (PPCM) and healthy postpartum (HP) women.

Background—PPCM is a major cause of maternal morbidity and mortality, and an immune-mediated etiology has been hypothesized. Cellular immunity, altered in pregnancy and the peripartum period, has been proposed to play a role in PPCM pathogenesis.

Methods—The Investigation of Pregnancy-Associated Cardiomyopathy (IPAC) study enrolled 100 women presenting with a left ventricular ejection fraction of <0.45 within 2 months of

Reprint requests: Charles F. McTiernan, PhD, Heart, Lung, Blood, and Vascular Medicine Institute, Department of Medicine, University of Pittsburgh, 3550 Terrace St, Scaife 631a, Pittsburgh, PA 15213. Tel: +412 648 3016; Fax: +412 383 8857. mctiernanc@upmc.edu.

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Supplementary Data

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delivery. Peripheral T-cell subsets, natural killer (NK) cells, and cellular activation markers were assessed by flow cytometry in PPCM women early (<6 wk), 2 months, and 6 months postpartum and compared with those of HP women and women with non-pregnancy-associated recent-onset cardiomyopathy (ROCM).

Results—Entry NK cell levels (CD3–CD56+CD16+; reported as % of CD3– cells) were significantly ($P < .0003$) reduced in PPCM ($6.6 \pm 4.9\%$ of CD3– cells) compared to HP ($11.9 \pm 5\%$). Of T-cell subtypes, CD3+CD4–CD8–CD38+ cells differed significantly ($P < .004$) between PPCM ($24.5 \pm 12.5\%$ of CD3+CD4–CD8– cells) and HP ($12.5 \pm 6.4\%$). PPCM patients demonstrated a rapid recovery of NK and CD3+CD4–CD8–CD38+ cell levels. However, black women had a delayed recovery of NK cells. A similar reduction of NK cells was observed in women with ROCM.

Conclusions—Compared with HP control women, early postpartum PPCM women show significantly reduced NK cells, and higher CD3+CD4–CD8–CD38+ cells, which both normalize over time postpartum. The mechanistic role of NK cells and “double negative” (CD4–CD8–) T regulatory cells in PPCM requires further investigation.

Keywords

Cardiomyopathies; peripartum period; cellular immunity; flow cytometry

Peripartum cardiomyopathy (PPCM), which complicates ~1:3000 live births in the US, remains a major cause of maternal morbidity and mortality.¹ The etiology remains unknown. Although recent studies support a pathophysiologic role of genetic background² and vascular mediators,³ myocardial inflammation has long been postulated to play a significant role. Endomyocardial biopsies demonstrate myocarditis in 9%–76% of women with PPCM,⁴ which closely parallels the findings in other forms of primary nonischemic cardiomyopathy of recent onset.⁵ A similar prevalence of cardiac viral nucleic acid sequences in PPCM and idiopathic cardiomyopathies suggests a role for viral agents (and immune responses) in both diseases.⁶ However, evidence for pathophysiologic cell-mediated immune processes in PPCM is limited.^{7,8}

The timing of presentation of PPCM, typically in the early postpartum period, has fueled the speculation of an immune-mediated process. Adaptive immunity is suppressed during pregnancy to allow fetal tolerance, whereas innate immunity is enhanced.⁹ The number of natural killer (NK) cells decline during pregnancy and postpartum, whereas monocytes and granulocytes increase.¹⁰ T cell numbers may also be lowered during pregnancy, accompanied by an alteration in T cell cytokine production. Regulatory T cell numbers are increased and are required for maintenance of pregnancy.¹¹ Immune-mediated diseases can be variably influenced by pregnancy. Pregnancy often ameliorates rheumatoid arthritis disease severity and reduces the incidence of multiple sclerosis disease flares, both of which can rebound postpartum,^{12,13} whereas thyroiditis shows an increased incidence postpartum.¹⁴ Prolactin, the major lactogenic hormone, which shows elevated expression in late pregnancy and postpartum, has immune-modulating properties and may influence autoimmune disease severity.¹⁵ Interestingly, suppression of prolactin secretion with the use

of bromocriptine has been proposed as a targeted therapy for PPCM because of an observed toxicity on cardiac capillary endothelium by prolactin-cleavage products.³

Because some autoimmune diseases (lupus erythematosus, scleroderma, type 1 diabetes, multiple sclerosis) show differences in racial prevalence,¹⁶ it is of interest to note differences in the racial prevalence of PPCM. PPCM is endemic in Haiti and parts of Africa, and in North America it is more prevalent in black women compared with their white counterparts.¹⁷ In the recent multicenter Investigation of Pregnancy-Associated Cardiomyopathy (IPAC), compared with their white counterparts, black women with PPCM presented with more severe left ventricular dysfunction with a lower left ventricular ejection fraction (LVEF) at 6 and 12 months postpartum.¹⁸ Acknowledging the mixed genetic heritage of North American blacks,¹⁹ this observation suggests that biogeographic ancestry associated with African genomic heritage may be a risk factor for PPCM.²⁰

These observations provided rationale for the hypothesis of a central role for a cellular immune-mediated pathophysiologic process in PPCM. To test this hypothesis, we undertook a multicenter flow cytometry-based analysis of circulating immune cells in patients with PPCM and compared results with those obtained from a control group of healthy postpartum (HP) women. We also examined the same immune cell subsets in normal nonpostpartum (NP) women and recent-onset cardiomyopathic (ROCM) patients to test the additional hypothesis that PPCM and ROCM patients would display a different subset of immune cells, reflecting differing pathophysiologic processes. Finally, because North American black women appear to be overrepresented among PPCM cases and show worse outcomes than North American white PPCM patients, we hypothesized that any alterations in immune cell subsets that exist between PPCM and HP women would be exacerbated in black PPCM patients.

Methods

Cohort

Women with newly diagnosed PPCM (n = 100) were enrolled at 30 centers (Appendix) from December 2009 to September 2012.¹⁸ All women were 18 years old and had no previous history of cardiac disease, estimated clinical LVEF ≥ 0.45 , and an evaluation consistent with nonischemic cardiomyopathy. For exclusion criteria, see the Supplemental Methods. The study protocol was approved by the Institutional Review Boards at all of the participating centers, with informed consents obtained from all of the subjects. The control group consisted of 10 HP women with normal echocardiography at entry. The second control group (NP; n = 13) consisted of women with no history of cardiovascular disease and who were not postpartum when enrolled. A third control group (ROCM; n = 5) consisted of women presenting with the recent onset (<6 mo) of nonischemic cardiomyopathy not associated with pregnancy.

At enrollment, demographic information, including self-designated race, previous clinical evaluation, and current medical therapy, were recorded. Women were followed until 1 year postpartum. All hospitalizations and major cardiac events, including death, cardiac transplantation, or implantation of a left ventricular assist device (LVAD), were recorded. All

subjects in the PPCM group underwent echocardiography to assess LVEF at the time of enrollment and at 6 and 12 months postpartum. In addition, women with PPCM enrolled within 6 weeks postpartum ($n = 67$) had an additional assessment of left ventricular (LV) function at 2 months. For the control subjects, HP, NP, and ROCM women underwent echocardiography at entry. LV volumes and LVEF were assessed in a core laboratory (University of Pittsburgh) with the use of the biplane Simpson rule and manual tracing of digital images.

Flow Cytometry

PPCM patients had blood collected at early (16.6 ± 10.6 days postpartum, range 0–42; $n = 67$), 2-month (62.1 ± 11.5 days postpartum; range 42–84; $n = 73$), and 6-month (179.2 ± 33.2 days postpartum; range 85–240; $n = 77$) time points. The PPCM subjects ($n = 67$) enrolled early (during the 1st 6 weeks postpartum) had immunophenotyping repeated at 2 and 6 months postpartum. The remaining 34 subjects, enrolled at 2 months postpartum, had immunophenotyping performed at 2 and 6 months postpartum. Forty-two patients had bloods collected at all 3 time points. HP control subjects had blood collected at 2 months (48 ± 12 days, range 28–65) and 6 months (96.3 ± 13.5 days, range 77–115) postpartum. NP and ROCM control subjects had 1 blood draw at entry.

Immunophenotyping of circulating cells was performed on whole blood collected and stabilized in Cyto-Chex BCT tubes ~3 days before multicolor flow cytometry. See Supplemental Methods for additional details. Antibodies against CD3, CD4, CD8, CD16, and CD56 were used for determination of cellular subsets: overall T cells (CD3+), T-helper cell subset (CD3+CD4+), cytotoxic T cells (CD3+CD8+), “double negative” or DN T cells (CD3+CD4–CD8–), monocytes (CD14+CD16–), macrophages (CD14–CD16+), and NK cells (CD3–CD56+CD16+, CD3–CD56–CD16–). Cell “activation” status was assessed by expression of CD25, CD38, or HLA-DR. Panels for multicolor flow cytometry are presented in Supplemental Table S1. Flow cytometry data was acquired with the use of a BD FACS ARIA 1 and analyzed with the use of FACSDiva v6.1.3 software. For gating example, see Supplemental Data Fig. S1. Data are presented as the percentage of all events within a particular immunophenotyping “gate.”

Statistical Analysis

Analyses were performed with the use of SPSS v 24 (IBM Corp). Continuous variables were tested for normal distribution. Given the smaller sample size ($n < 30$) and nonnormal distribution of circulating immune cells, the nonparametric Mann-Whitney *U* test was used to compare groups. The percentage of each cell type was used as a continuous variable and compared between HP and NP, PPCM and HP, and NP and ROCM. Given the large number of comparisons, we used a false discovery rate method and computed *Q* values to control for multiple tests.²¹ Cell subgroups consisting of related immune cell types were included in the same group, and multiple test corrections were applied to cell types within subgroups. Tables report asymptotic significance (2-sided *P* value) from the Mann-Whitney *U* test. We then highlighted the subgroup specific *Q* values that remained significant after using false discovery rate for multiple test comparisons, and used those comparisons for interpreting data, reporting significance, and making conclusions (see Expanded Statistical Analyses,

Supplemental Table S2). When analyzing time-specific changes in cell types that were significantly elevated or lowered in PPCM patients at entry, we first used the Friedman test to examine patients for whom we had data at all time points (early and 6 and 12 months). Post hoc pairwise Wilcoxon signed rank tests were used to identify the group(s) that differed significantly from the others. The whole PPCM cohort and racially defined subgroups were similarly analyzed. A 2nd approach compared cellular data from all black versus white PPCM subjects at early and 6- and 12-month periods in time point-specific analyses with the use of Mann-Whitney *U* tests. Multiple test correction was applied within cell subgroups. Data are reported as mean $\pm$ SD, with $P < .05$, or an appropriate multiple-test false discovery rate *Q* value, considered to be significant.

Results

Cohorts

The overall IPAC cohort of 100 women with peripartum cardiomyopathy was 65% white, 30% black, and 5% other race, age 30 ± 6 years, gravida 2.8 ± 1.9 , para 2.2 ± 1.4 , and LVEF 0.34 ± 0.10 . At entry 88% of subjects were on beta-blockers and 81% on angiotensin-converting enzyme inhibitors, with a distribution of New York Heart Association functional class I/II/III/IV of 12%/47%/24%/17%. PPCM subjects were enrolled postpartum at a median of 24 days (range 0–95, mean 31 ± 25). The first control group consisted of 10 HP women (8 white, 2 black), age 33 ± 5 years, gravida 2.4 ± 1.7 , para 1.5 ± 0.7 , and normal (LVEF 0.60 ± 0.03) echocardiography at entry (median 49 days postpartum, mean 48 ± 12 , range 28–65). The second control group (NP) consisted of 13 women (12 white, 1 black), age 36 ± 7 years, gravida 1.0 ± 1.3 , para 1.0 ± 1.3 , normal LVEF (0.61 ± 0.04), and no history of cardiovascular disease, who were not postpartum at the time of enrollment. The third control group (ROCM; $n = 5$) consisted of women presenting with recent-onset (<6 mo) nonischemic cardiomyopathy (2 white, 3 black, age 34 ± 11 years, gravida 2.6 ± 1.5 , para 2.0 ± 1.2 , and LVEF 0.31 ± 0.08).

Differences in HP and NP Circulating Immune Cells

To identify appropriate control groups, we first compared the circulating cellular immunophenotypes between HP and NP control subjects. Results presented in Table 1 and corrected for multiple test comparisons revealed that HP and NP significantly differed in the percentages of T cells (CD3+CD8+HLA-DR+, CD3+CD4+8-HLA-DR+), monocytes (CD14+CD16-, CD14+CD16-HLA-DR+), macrophages (CD14+CD16+, CD14+CD16+HLA-DR+), and NK cell (CD3-CD56+CD16-) subsets. The marked differences in immune cell subgroups confirmed the appropriate use of HP control subjects for the PPCM population.

Differences in HP and PPCM Circulating Immune Cells

When early PPCM samples (enrolled <6 wk postpartum; $n = 67$) were compared with the HP control group, and corrected for multiple subgroup comparisons, 2 immune cell subsets were significantly different. CD3-CD56+CD16+ (NK) cells were significantly lower in PPCM ($6.6 \pm 0.4\%$) compared to HP women ($11.9 \pm 5\%$; $P < .001$) (Table 2). In addition, CD3+CD4-CD8- double negative (DN) CD38+ cells were more abundant in PPCM

subjects ($24.5 \pm 12.4\%$; HP $12.6 \pm 6.4\%$, $P < .001$). No other significant differences were evident in the percentage of circulating cell subsets, or activated subsets.

The postpartum dates of collection for early PPCM samples were significantly earlier than the postpartum date of collection for the HP samples (PPCM 16.6 ± 10.6 days, HP 48.8 ± 11.2 days). To address this possible confounder, we compared a cohort ($n = 52$) of PPCM samples matched for days postpartum (49.6 ± 10.6 days) to the HP samples ($n = 10$; 48.8 ± 11.2 days; Fig. 1). In this analysis the percentage of CD3–CD56+CD16+ NK cells remained significantly lower in the PPCM group ($8.41 \pm 5.14\%$) compared with the HP group ($11.90 \pm 5.00\%$; $P = .04$), and the percentage of CD38+ DN cell levels remained significantly elevated ($P = .002$) in PPCM ($22.48 \pm 10.5\%$) compared with HP (12.46 ± 6.4) samples.

Changes in NK Cell Subsets Over Time

Given differences in the percentage of circulating CD3–CD56+CD16+ NK cells between PPCM subjects presenting early and HP control subjects, we also evaluated the change in the percentage of NK cells over time postpartum, overall and within the white and black subsets. For this analysis we considered the 42 PPCM women (35 white, 5 black, 2 other) for which we had CD3–CD56+CD16+ NK cell determinations for all 3 time points (early, 2 mo, and 6 mo). In this set of PPCM women, we found a significant effect of time on CD3–CD56+CD16+ NK cell levels (Fig. 2A; Friedman test: $P = .003$). Post hoc pairwise tests (Wilcoxon signed rank tests) showed that early NK levels were significantly lower than either the 2-month ($P = .001$) or 6-month ($P = .002$) levels. However, 2-month and 6-month NK cell levels were not significantly different ($P = .583$).

When examined separately in whites and blacks, we observed similar results in whites ($n = 35$) but not in blacks ($n = 5$). Friedman test results were significant in whites ($P = .007$), signifying a significant effect of time on NK levels. Post hoc pairwise tests again showed that in whites, early NK cell levels were significantly lower than either 2-month ($Z = -3.112$; $P = .002$) or 6-month ($Z = -3.61$; $P = .002$) levels. However, the 2-month and 6-month levels did not differ ($Z = -0.69$; $P = .51$; Fig. 2B).

In blacks, we had limited power to detect differences across time (Friedman test comparing the effect of time overall: $P = .25$). In post hoc analyses, the early group did not differ significantly from 2-month NK cell levels ($Z = -0.41$; $P = .69$). However the early levels differed significantly from the 6-month levels ($Z = -2.023$; $P = .04$; Fig. 2C). Given the low numbers of black PPCM women for whom we had data across all 3 time points, we also examined whether the mean levels of NK cells in PPCM women varied between races at each time point, using data from all patients available at each time point. At the early postpartum time point there were no differences by race in either NK (white [$n = 50$] $6.92 \pm 4.2\%$; black [$n = 14$] $6.05 \pm 3.5\%$) or CD3+CD4–CD8–CD38+ DN cell levels (white $23.97 \pm 11.7\%$, black 28.33 ± 16.3 ; Mann Whitney U test: $P = .4$). However, blacks did have significantly lower levels of NK cells at 2 months compared with whites (Fig. 2D).

Changes in CD3+CD4–CD8–CD38+ Subsets Over Time

We similarly evaluated the change in the percentage of CD38+ DN cells over time for a set of 41 PPCM subjects (34 white, 5 black, 2 other) and within the white and black subsets for which we had CD38+ DN cell determinations for all 3 time points (early, 2 mo, and 6 mo).

In the 41 PPCM women, we found a significant effect of time on CD38+ DN cell levels (Fig. 3A; Friedman test: $P = .02$), suggesting that 1 CD38+ DN cell level differed significantly from others. However, post hoc pair wise tests showed only a trend ($P = .097$) for differences between early and 6-month time points, with no significant differences observed between early and 2 months ($P = .257$) or 2 months versus 6 months ($P = .31$). Similar results were observed for the analyses of the white PPCM subset (Friedman test: $P = .028$) but with no significant post hoc pairwise comparisons ($P = .114-.422$; Fig. 3B). Although a numeric pattern suggesting decreased CD38+ DN cell levels with increasing time postpartum was observed in the black subset, similar to that observed in the white subset, there was no significant effect of time on these values (Friedman test: $P = .439$; Fig. 3C).

Given the low numbers of black PPCM women for whom we had data across all 3 time points, we examined whether the mean levels of CD38+ DN cells in PPCM women varied between races at each time point (so each time point has different sample numbers). This analysis included data from all patients available at each time point. Although we observed that black women displayed an average numeric percentage of CD38+ DN cells higher than white women, no significant differences were observed at any time point (Fig. 3D).

Comparison of PPCM and ROCM Immune Cell Subsets

To assess whether the immune cell profiles observed in PPCM patients were unique from other forms of heart failure, we similarly analyzed cohorts of normal NP women ($n = 13$) and female patients with ROCM not associated with pregnancy ($n = 5$). Similarly to PPCM patients, ROCM patients had significantly lower levels of CD3–CD56+CD16+ NK cells ($4.4 \pm 2\%$) compared with NP women ($9.3 \pm 4.1\%$; $P = .01$). CD38+ DN cells were numerically, but not significantly, higher in ROCM patients than in NP women. Significant differences were also observed between ROCM and NP women for additional cell subsets (CD3+CD4–8– and CD14+CD16–HLA-DR+; Table 3). The cell subtypes significantly different between ROCM and NP women showed a nonsignificant but directionally similar difference between PPCM and HP women (Tables 2 and 3).

Discussion

Pregnancy occurs with complex progressive modulations in maternal immune function necessary to accommodate the fetal paternal antigens, support fetal development, and maintain immune defenses. Depending on the stage of pregnancy (early, middle, or late trimester; postpartum) and end point used to assess immune status (circulating cytokine levels, stimulated cytokine production by immune cells, immune cell profiles), pregnancy may be considered to be a state of activated innate immunity, proinflammatory, antiinflammatory, or tolerance, with further changes postpartum.^{7,22} Specific pregnancy-

associated changes in immune modulation depend on whether one is considering the fetal-maternal interface, circulating cells and factors that constitute maternal immunity against infectious disease, or the active/quiescent state of concurrent maternal autoimmune diseases. Classically, pregnancy was thought of as an immunosuppressed state to tolerate paternal antigens of the fetus.²³ Contemporary research suggests that early pregnancy requires a local inflammatory response for the blastocyst to penetrate the epithelium of the uterine lining and establish an appropriate blood supply, and the decidua is rich in macrophages, uterine NK, dendritic, and T cells, which are required for successful establishment of pregnancy.²² Progressive pregnancy appears to show decreased expression of T_H1 cytokines, and unchanged T_H2 cytokine production by stimulated maternal NK or CD4 T cells.¹⁰ In addition, parturition is initiated with infiltration of the myometrium by neutrophils and macrophages, and an increase in cytokine levels in the maternal circulation, again suggesting an enhanced inflammatory process.²⁴ Relative to the postpartum state, in the maternal circulation, phagocytic cells (monocytes/granulocytes) and dendritic cells are elevated during pregnancy, CD3+ T cells are decreased, and NK cells show a progressive decline during pregnancy that rebounds postpartum.¹⁰

Considering these dynamic and complex changes in maternal immunity during pregnancy and postpartum, the present study focused on the hypothesis that alterations in cellular immune-mediated processes occur in PPCM and are detectable in the postpartum interval. We analyzed peripheral blood samples collected from PPCM women enrolled in the multicenter IPAC study and compared them with a control group of HP women. From the early postpartum samples, PPCM patients displayed a significant alteration in only 2 classes of cells: decreased levels of NK cells (CD3–CD56+CD16+) and an increased level of a T-cell population of DN cells expressing the activation marker CD38 (CD3+CD4–CD8–CD38+). Both NK cell and CD38+ DN cell percentages normalized over time and were not different from those observed in HP women by 2 and 6 months postpartum. Although we observed no baseline differences between white and black women, black women showed a slower recovery of NK (but not CD38+ DN) cells to HP levels.

Interestingly, the reduction in NK cell subsets in PPCM compared with HP women resembled the numeric difference observed between ROCM and NP women. Although this is the first study to examine NK cells in a large population of women with PPCM, the findings of a decrease in NK cells is consistent with past reports that observed decreased levels of NK cells in heart failure patients.²⁵ Although a small number of ROCM subjects were examined, we confirmed a decrease in NK cell levels compared with appropriate NP control subjects. These findings suggest that decreased NK cell levels are not unique to PPCM but reflect changes in immune cell subsets common in heart failure arising from multiple etiologies.

NK Cells in Heart Failure

NK cells are bone marrow–derived lymphocytes that play a central role in the innate immune system. NK cells recognize and kill virally infected and neoplastic cells through cytotoxic activity; they also serve to secrete cytokines that enhance T-cell responses and cytotoxicity. During normal pregnancy and continuing for several postpartum months,

circulating NK cell numbers and activity are decreased.¹⁰ In autoimmune/inflammatory diseases, peripheral NK cell numbers can decrease along with cytotoxic activity and may infiltrate the affected organ, but their role in limiting or exacerbating disease severity varies with different diseases.²⁶

NK cells are important in limiting cardiac viral infection and reduce cardiac eosinophilic infiltration.²⁷ Interestingly, increased cardiac eosinophilic infiltration has been reported in PPCM cardiac tissues.²⁸ One could hypothesize that a naturally occurring pregnancy-associated decrease of peripheral NK cell number allows for exacerbated eosinophilic infiltration in response to an idiopathic inflammatory or autoimmune process occurring in the peripartum interval, precipitating the development of PPCM. Furthermore, transfer of NK cells to NK cell-deficient mice shortly after their infection with coxsackie B virus limited cardiac damage and improved systolic function.²⁹ Because NK cells are being explored as immunotherapeutic agents,³⁰ there may be rationale for investigating their role as a therapeutic for PPCM.

Role of Double Negative Cells

As far less is known regarding the nature of DN cells or the functional consequence of enhanced expression of CD38 antigen, the importance or functional role of CD38+ DN cells in PPCM is unclear. DN cells have T-cell regulatory properties and participate in autoimmune, infectious, inflammatory, and neoplastic conditions.³¹ Understanding of the CD38 antigen is also evolving. First identified as an “activation marker” in leukocytes, CD38 is expressed in T, B, myeloid, and NK cells, among others. CD38 protein is both a receptor for CD31 (PECAM-1) and a complex ectoenzyme that directs the synthesis of cyclic adenosine diphosphate (ADP)-ribose (cADPR), and hydrolysis of cADPR to ADPR. Enhanced expression of CD38 antigen is observed in T-cells of HIV-infected adults, various forms of leukemia, and multiple myeloma.³² Our observations regarding differences in the abundance of CD38+ DN cells between PPCM and HP women appears to be the first analysis of these cells in any form of heart failure.

Study Limitations

For this report we performed one of the few studies using a multicenter sample collection and single-site flow cytometry analyses of immune cell subtypes in any heart failure etiology, and it is the largest study reported for PPCM patients. However, several limitations of the current study are acknowledged. First, the low level of adverse outcomes in the IPAC cohort limited the analysis of the relationship between immune cells and outcomes. The reduced number of patients for which blood samples were acquired at all 3 preselected time points reduced power for analyses of temporal changes, particularly for the black patients. We also had only a small group of ROCM patients to compare with PPCM patients. Nonetheless, we could confirm reports of decreased NK cell levels in heart failure patients. Results are limited to the panels of cell markers used (eg, no data were gathered on B cells). In addition the absence of cardiac tissue from these patients precludes analysis of changes that may occur in infiltrating cell type (eg, NK cells or eosinophils). Finally, this was an observational study, and the relationship of slower recovery of NK cell number to the poorer

outcomes and myocardial recovery in black women with PPCM is intriguing but of uncertain significance to pathophysiologic mechanisms.

Conclusion

In summary, this multicenter study on circulating immune cell subsets in women with PPCM observed significant differences in the presence of NK cells and CD38+ DN cells compared with HP women. In a cohort that largely recovers normal cardiac function, the values progress to those observed in healthy women during a 6-month postpartum interval.

Clinical Perspectives

PPCM patients display discrete changes in circulating immune cell profiles that are not unique to PPCM but resemble those observed in other forms of cardiomyopathy. Black PPCM patients, who have worse outcomes compared with white PPCM patients, appeared to have a slower postpartum normalization of NK cell levels.

Translational Outlook

It will be important to determine if the alterations in the presence of NK and CD38+ DN cells are reflective of diminished LV function or are causative contributors to cardiac dysfunction. The utility of PPCM immune cell profiles as outcome biomarkers will require a larger study cohort to relate rare adverse events to immune cell profiles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

IPAC Investigators

(Number of patients per center in parentheses) *University of Pittsburgh Medical Center (10)*: Dennis M McNamara, MD; James D. Fett, MD; Jessica Pisarcik, RN, BSN; Charles McTiernan, PhD; Karen Hanley-Yanez, BS; John Gorcsan III, MD; Erik Schelbert, MD. *Intermountain Medical Center (8)*: Rami Alharethi, MD; Kismet Rasmusson, CRNP; Kim Brunisholz; Amy Butler, BS, CCRP; Deborah Budge; A.G. Kfoury, MD; Benjamin Horne, PhD; Joe Tuinei; Heather Brown. *Vanderbilt University (7)*: Julie Damp, MD; Allen J. Naftilan, MD; Jill Russell, RN, MSN; Darla Freehardt, LPN, BS, CCRP. *Cleveland Clinic (7)*: Eileen Hsieh, MD; Cynthia Oblak, CCRC. *Washington University, St. Louis (6)*: Greg Ewald, MD; Donna Whitehead, RN; Jean Flanagan, RN; Anne Platts. *University of Southern California (6)*: Uri Elkayam, MD; Jorge Caro, MPH; Stephanie Mullin, RN. *Brigham and Women's Hospital (5)*: Michael M. Givertz, MD; M. Susan Anello, RN, BS.

University of Kentucky (5): Navin Rajagopalan, MD; David Booth, MD; Tiffany Sandlin, RN; Wendy Wijesiri, RN. *Mayo Clinic (4):* Leslie T. Cooper, MD; Lori A. Blauwet, MD; Joann Brunner, RN; Mary Phelps; Ruth Kempf. *Louisiana State University (4):* Kalgi Modi, MD; Tracy Norwood. *University of Illinois (4):* Joan Briller, MD; Decebal Sorin Griza, MD. *Duke University (4):* G. Michael Felker, MD; Robb Kociol, MD; Patricia Adams, RN. *Wake Forest (4):* Gretchen Wells, MD; Vinay Thohan, MD; Deborah Wesley-Farrington, RN, BSN, CCRC, CCA; Sandra Soots, RN, CCRC. *Jewish General (3):* Richard Sheppard, MD; Caroline Michel, MD; Nathalie Lapointe, RN, PhD; Heather Nathaniel. *University of Calgary (3):* Angela Kealey, MD. *Massachusetts General (2):* Marc Semigran, MD; Maureen Daher, RN. *Penn State Milton S. Hershey Medical Center (2):* John Boehmer, MD; David Silber, MD; Eric Popjes, MD; Patricia Frey, RN; Todd Nicklas, RN. *University of Rochester (2):* Jeffrey Alexis, MD; Lori Caufield, RN, BSN, CCRC. *Georgia Health Sciences University (2):* John W. Thornton III, MD; Mindy Gentry, MD; Vincent J.B. Robinson, MBBS; Gyanendra K. Sharma, MD; Joan Holloway, BS; Maria Powell, LPN, CCRC. *University of Texas, Southwestern (2):* David Markham, MD; Mark Drazner, MD; Lynn Fernandez, RN. *Newark Beth Israel Medical Center (2):* Mark Zucker, MD; David A. Baran, MD; Martin L. Gimovsky, MD; Natalia Hochbaum, MD; Bharati Patel, RN, CCRC; Laura Adams, RN, BSN. *University of Maryland (2):* Gautam Ramani, MD; Stephen Gottlieb, MD; Shawn Robinson, MD; Stacy Fisher, MD; Joanne Marshall, BSN, MS. *Columbia University (2):* Jennifer Haythe, MD; Donna Mancini, MD; Rachel Bijou, MD; Maryjane Farr, MD; Marybeth Marks, Henry Arango. *Baylor College (2):* Biykem Bozkurt, MD, PhD, FACC, FAHA; Mariana Bolos. *Thomas Jefferson (1):* Paul Mather, MD; Sharon Rubin, MD; Raphael Bonita, MD; Susan Eberwine, RN. *Stony Brook University Medical Center (1):* Hal Skopicki, MD, PhD; Kathleen Stergiopoulos, MD; Ellen McCathy-Santoro, MD; Jennifer Intravaia, RN, CCRCII; Elizabeth Maas. *Morristown Hospital (0):* Jordan Safirstein, MD; Audrey Kleet, RN, MS, ACNP-BC, CCRN, CCTC; Nancy Martinez, RN; Christine Corpoin, RN; Donna Hesari, RN. *University of Miami (0):* Sandra Chaparro, MD; Laura J. Hudson, MA, MPH, CCRC. *Harper University Hospital (0):* Jalal K. Ghali, MD; Zora Injic, RN, BSc, MSA. *Johns Hopkins Hospital (0):* Ilan S. Wittstein, MD.

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Fig. 1.

CD3-CD56+CD16+ and CD3+CD4-CD8-CD38+ cell presence in peripartum cardiomyopathy (PPCM) and healthy postpartum (HP) samples matched by postpartum day. Flow cytometry determination of the percentage of (A) Natural killer (NK; CD3-CD56+CD16+) and (B) CD38+ “double negative” (DN; CD3+CD4-CD8-CD38+) cells in HP (white; n = 10) and PPCM (black; n = 52) samples matched by postpartum day (PPCM 49.6 ± 10.6 days; HP 48.8 ± 11.2 days) and presented as box plot. *Significant by Mann-Whitney *U* test.

Fig. 2.

Changes in NK (%CD3-CD56+CD16+) cell subsets over time, comparison by race. (A) Percentage of NK cells in PPCM patients who had samples collected at each time point (n = 42; 35 white, 5 black, 2 other). (B) Percentage of NK cells in white PPCM patients with samples collected at each time point (n = 42). (C) Percentage of NK cells in black PPCM patients with samples collected at each time point (n = 5). (D) Percentage of NK cells in all available patients at each time point (early, 2 mo, 6 mo, respectively: white n = 50, 48, and 53; black n = 14, 22, and 21). Abbreviations as in Fig. 1. * $P < .05$ vs 2 months; † $P < .05$ vs 6 months; ‡ $P < .05$ black vs white, same time point.

Fig. 3.

Changes in CD38+ DN (%CD3+CD4-CD8-CD38+) cell subsets over time, comparison by race. (A) Percentage of CD38+ DN cells in PPCM patients who had samples collected at each time point (n = 41; 34 white, 5 black, 2 other). (B) Percentage of CD38+ DN cells in white PPCM patients with samples collected at each time point (n = 41). (C) Percentage of CD38+ DN cells in black PPCM patients with samples collected at each time point (n = 5). (D) Percentage of CD38+ DN cells in all available patients at each time point (early, 2 mo, 6 mo, respectively: white n = 50, 47, and 53; black n = 13, 22, and 21). Abbreviations as in Fig. 1.

Table 1

Flow Cytometry Analysis of HP and NP Circulating Cells

Cell Subset	HP (n = 10)	NP (n = 13)	Asymptotic Significance
T cells			
CD3+	57.5 ± 5.2	51.4 ± 5.2	0.11
CD3+CD4+	60.2 ± 8.6	56.9 ± 10.2	0.54
CD3+CD4+HLA-DR+	2.7 ± 1.2	3.6 ± 2.0	0.20
CD3+CD4+CD38+	39.8 ± 5.7	43.6 ± 11.9	0.09
CD3+CD4+CD25+	3.2 ± 1.4	5.3 ± 3.4	0.26
CD3+CD8+	28.9 ± 5.6	27.8 ± 10.2	0.69
CD3+CD8+HLA-DR+	3.4 ± 2.2	6.2 ± 3.3	0.008 [*]
CD3+CD8+CD38+	23.2 ± 10.4	24.2 ± 9.9	0.24
CD3+CD8+CD25+	0.2 ± 0.2	0.4 ± 0.5	0.83
CD3+CD4+CD8+	2.2 ± 0.8	1.4 ± 1.4	0.95
CD3+CD4-CD8-	5.3 ± 3.4	3.6 ± 4.0	0.09
CD3+CD4-8-HLA-DR+	2.0 ± 1.7	6.1 ± 2.5	0.001 [*]
CD3+CD4-8-CD38+	12.5 ± 6.4	17.5 ± 10.0	0.90
CD3+CD4-8-CD25+	0.2 ± 0.2	0.8 ± 1.7	0.26
CD3+CD56+	2.1 ± 1.4	3.4 ± 5.1	0.95
CD3+CD56+CD8+	1.3 ± 1.3	1.9 ± 2.5	1.000
Monocytes/macrophages			
CD14+	14.3 ± 3.4	14.2 ± 3.9	0.78
CD14+CD16-	91.3 ± 1.9	77.2 ± 18.1	0.002 [*]
CD14+CD16-HLA-DR+	53.2 ± 19.4	82.6 ± 11.1	0.001 [*]
CD14+CD16-CD38+	89.2 ± 12.7	84.0 ± 33.0	0.17
CD14+CD16+	8.9 ± 2.0	15.9 ± 5.7	0.002 [*]
CD14+CD16+HLA-DR+	68.2 ± 14.0	85.7 ± 9.0	0.006 [*]
CD14+CD16+CD38+	77.6 ± 11.7	86.1 ± 11.2	0.08
NK cells			
CD3-CD56+CD16+	11.9 ± 5.0	9.3 ± 4.1	0.15
CD3-CD56+CD16+HLA-DR+	4.7 ± 6.8	4.2 ± 2.9	0.40
CD3-CD56+CD16+CD38+	92.6 ± 4.0	87.5 ± 10.6	0.38
CD3-CD56+CD16-	2.8 ± 1.0	1.7 ± 0.8	0.01 [*]

Asymptotic significance calculated by means of Mann-Whitney *U* and Wilcoxon *W* tests. HP, healthy postpartum; NP, normal nonpostpartum.

^{*} Significant after using *Q* value false discovery rate correction within cell subgroups.

Table 2

Cytometry Analysis of HP Versus PPCM

Cell subset	HP (n = 10)	PPCM (n = 67)	Asymptotic Significance
T cells			
CD3+	57.5 ± 5.2	50.7 ± 14.9	0.32
CD3+CD4+	60.2 ± 8.7	58.3 ± 10.8	0.74
CD3+CD4+HLA-DR+	2.7 ± 1.2	2.6 ± 1.9	0.34
CD3+CD4+CD38+	39.8 ± 5.7	44.9 ± 11.9	0.09
CD3+CD4+CD25+	3.24 ± 1.4	3.9 ± 2.9	0.75
CD3+CD8+	29.0 ± 5.6	30.6 ± 7.5	0.69
CD3+CD8+HLA-DR+	3.4 ± 2.2	6.5 ± 7.3	0.13
CD3+CD8+CD38+	23.2 ± 10.4	31.6 ± 13.2	0.06
CD3+CD8+CD25+	0.2 ± 0.2	0.3 ± 0.5	0.66
CD3+CD4+CD8+	2.2 ± 0.8	2.1 ± 0.8	0.70
CD3+CD4-CD8-	5.3 ± 3.4	7.8 ± 9.1	0.65
CD3+CD4-8-HLA-DR+	2.0 ± 1.7	4.4 ± 5.3	0.04
CD3+CD4-8-CD38+	12.5 ± 6.4	24.5 ± 12.5	0.002*
CD3+CD4-8-CD25+	0.2 ± 0.2	0.5 ± 0.7	0.74
CD3+CD56+	2.1 ± 1.5	2.6 ± 4.4	0.64
CD3+CD56+CD8+	1.3 ± 1.3	1.3 ± 1.9	0.53
Monocytes/macrophages			
CD14+	14.3 ± 3.4	15.3 ± 7.7	0.84
CD14+CD16-	91.3 ± 1.9	87.0 ± 7.4	0.05
CD14+CD16-HLA-DR+	53.2 ± 19.4	46.2 ± 19.4	0.39
CD14+CD16-CD38+	89.2 ± 12.7	92.0 ± 14.6	0.16
CD14+CD16+	8.9 ± 2.0	12.8 ± 5.9	0.04
CD14+CD16+HLA-DR+	68.2 ± 14.0	59.5 ± 19.5	0.18
CD14+CD16+CD38+	77.6 ± 11.7	80.3 ± 16.7	0.18
NK cells			
CD3-CD56+CD16+	11.9 ± 5.0	6.6 ± 4.9	0.002*
CD3-CD56+CD16+HLA-DR+	4.7 ± 6.8	7.7 ± 6.8	0.03
CD3-CD56+CD16+CD38+	92.6 ± 4.0	93.5 ± 12.3	0.08
CD3-CD56+CD16-	2.78 ± 1.0	2.3 ± 1.4	0.15

Asymptotic significance calculated by means of Mann-Whitney *U* and Wilcoxon *W* tests. HP, healthy postpartum; PPCM, peripartum cardiomyopathy.

* Significant after using *Q* value false discovery rate correction within cell subgroups.

Table 3

Flow Cytometry Analysis of NP Versus ROCM

Cell subset	NP (n = 13)	ROCM (n = 5)	Asymptotic Significance
T cells			
CD3+	51.4 ± 10.7	47.4 ± 14.1	0.59
CD3+CD4+	56.9 ± 10.2	56.3 ± 9.6	0.84
CD3+CD4+HLA-DR+	3.6 ± 2.0	2.9 ± 1.4	0.69
CD3+CD4+CD38+	43.6 ± 11.9	37.0 ± 15.9	0.28
CD3+CD4+CD25+	5.3 ± 3.4	5.1 ± 2.9	0.73
CD3+CD8+	27.8 ± 10.2	31.2 ± 10.1	0.49
CD3+CD8+HLA-DR+	6.2 ± 3.3	6.3 ± 3.8	0.96
CD3+CD8+CD38+	24.2 ± 9.9	26.3 ± 13.3	0.66
CD3+CD8+CD25+	0.4 ± 0.5	0.3 ± 0.3	0.95
CD3+CD4+CD8+	2.4 ± 1.4	2.0 ± 1	0.62
CD3+CD4-CD8-	3.6 ± 4.0	10.5 ± 2.4	0.01 *
CD3+CD4-8-HLA-DR+	6.1 ± 2.5	3.2 ± 2.1	0.04
CD3+CD4-8-CD38+	17.5 ± 10.0	22.9 ± 20.1	0.59
CD3+CD4-8-CD25+	0.8 ± 1.7	0.2 ± 0.05	0.92
CD3+CD56+	3.4 ± 5.1	2.3 ± 1.3	0.80
CD3+CD56+CD8+	1.9 ± 2.5	1.3 ± 0.8	0.92
Monocytes/macrophages			
CD14+	14.2 ± 4.0	16.0 ± 6.7	0.73
CD14+CD16-	77.2 ± 18.1	86.1 ± 8.4	0.38
CD14+CD16-HLA-DR+	82.6 ± 11.1	49.2 ± 9.4	0.002 *
CD14+CD16-CD38+	84.0 ± 33.0	90.9 ± 10.3	0.49
CD14+CD16+	15.9 ± 5.7	14.2 ± 8.1	0.66
CD14+CD16+HLA-DR+	85.7 ± 9.0	66.5 ± 14.9	0.02
CD14+CD16+CD38+	86.1 ± 11.2	76.8 ± 16.7	0.26
NK cells			
CD3-CD56+CD16+	9.3 ± 4.1	4.4 ± 2.0	0.01 *
CD3-CD56+CD16+HLA-DR+	4.2 ± 2.9	11.1 ± 9.4	0.04
CD3-CD56+CD16+CD38+	87.5 ± 10.6	92.2 ± 4.3	0.62
CD3-CD56+CD16-	1.7 ± 0.8	1.6 ± 0.4	0.81

Asymptotic significance calculated by means of Mann-Whitney *U* and Wilcoxon *W* tests. HP, healthy postpartum; ROCM, recent-onset cardiomyopathy.

* Significant after using *Q* value false discovery rate correction within cell subgroups.