Th17 immune responses in Brazilian dyslipidemic patients with atherosclerosis

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**ABSTRACT**

Lymphocytes, macrophages, and immunological mediators are critical in atherogenesis, but Th17 cells are still little studied. We investigated blood lymphocyte profiles, cytometric changes in blood Th17 cells, and production of Th17-associated cytokines by peripheral blood mononuclear cells (PBMCs) stimulated with phytohemagglutinin (PHA) in dyslipidemic patients. Forty-nine Brazilian dyslipidemic patients, including 14 with negative coronary angiograms (CAs) and 35 with positive CAs (atherosclerosis), were classified into groups G1 and G2, respectively, and 27 healthy individuals without dyslipidemia were included in the control group (HI).

In all participants, lipid profiles, CVD risk factors, atherogenic risk indexes, cytometry of blood T lymphocytes (CD4+ T, CD8+ T, and Th17 cells), and Th17 cytokine production were determined. Production of interleukin (IL)-17A, IL-17F, IL-21, and IL-22 was measured in culture supernatants of PHA-stimulated PBMCs. Patients with atherosclerosis exhibited low levels of high-density lipoprotein cholesterol and apolipoprotein A and showed increased atherogenic risk indexes and a higher frequency of prior acute myocardial infarction (AMI). CD8+ T-cell counts were lower in patients with previous AMI and higher in dyslipidemic obese individuals. Th17 cell frequency was increased in dyslipidemic women with atherosclerosis. PBMCs from patients with atherosclerosis produced less IL-17A and IL-21, but more IL-22. IL-17F production was unaltered. In summary, cytometric changes in CD8+ T and Th17 lymphocytes and altered production of Th17-associated cytokines were observed in these individuals and were related to endocrine factors, obesity, and AMI. The practical implications of these findings need to be investigated in future studies.

**1. Introduction**

Approximately 17.5 million people died in 2015 due to cardiovascular diseases (CVDs), accounting for 31% of deaths worldwide. These clinical entities are mainly associated with atherosclerosis, a leading cause of morbidity that has been widely studied, but is still incompletely understood [1, 2]. Atherosclerosis is a chronic disease associated with dyslipidemia and inflammation. Inflammation occurs in response to accumulation of atherogenic lipoproteins in the vessel wall and involves both innate and adaptive immune responses. Atherosclerosis is a progressive disease characterized by immune responses, which result in atheroma. Macrophages and subsets of T lymphocytes are critical components of atherosclerosis. They secrete cytokines and chemokines that play distinct roles in the pathogenesis of atherosclerosis. Additionally, these immune cells may represent up to 50% of the cells present in advanced lesions, suggesting their relevance in inflammation and atheroma instability [3–6].

Among the CD4+ T-helper lymphocytes, Th17 cells have been extensively studied in clinical studies or autoimmune and inflammatory diseases. In atherogenesis, there is a delicate balance between inflammatory and anti-inflammatory immune responses, and loss of the anti-inflammatory response favors atheroma rupture and clinical complications. In this context, Th17 cells and their associated cytokines have recently been investigated in atherosclerosis; however, the results of such studies are still inconclusive. Some studies in animals have shown that exacerbation of atherosclerotic lesions correlates with the increase in Th17 cells. This finding has been confirmed in human studies, which have revealed changes in the proportions of Th17 cells in
the circulation and the levels of their associated cytokines in patients with coronary artery disease, in whom there is a relationship between the severity and progression of atherosclerosis [7–10].

In addition to their inflammatory nature, cardiovascular diseases associated with atherosclerosis are influenced by risk factors, genetic background, ethnicity, and environment [11,12]. Thus, studies that consider such factors will enable significant advances in our understanding of CVD immunopathogenesis, contributing to a globalized vision of these clinical entities. Accordingly, in this study, we investigated the peripheral blood distribution of T lymphocytes in dyslipidemic patients living in Brazil northeast (Bahia), with a focus on the Th17 subpopulation and cytokines associated with these cells.

2. Materials and methods

2.1. Patients and healthy controls

Individuals of both sexes who attended the Hemodynamics Service of the Ana Nery Hospital/Bahia Heart Institute were invited to participate in the study. To be included, all patients had a laboratory diagnosis of dyslipidemia by the V Brazilian Guidelines for Dyslipidemia and Prevention of Atherosclerosis [13]. Thus, four types of dyslipidemia were investigated: isolated hypercholesterolemia (low-density lipoprotein cholesterol (LDL-C) ≥ 160 mg/dL), isolated hypertriglyceridemia (triglyceride (TG) ≥ 150 mg/dL), mixed hyperlipidemia (LDL-C ≥ 160 mg/dL and TG ≥ 150 mg/dL), and low-density lipoprotein cholesterol (HDL-C) (≤ < 50 mg/dL), isolated or associated with increased LDL-C or TG. The presence of atherosclerosis was identified by the observation of any degree of coronary stenosis in the coronary angiogram. The occurrence of a prior CVD, prior acute myocardial infarction or stroke was documented in patient medical records. Hypertension was diagnosed by a sustained high blood pressure (systolic pressure ≥ 140 mm Hg and/or diastolic pressure > 90 mm Hg). At enrollment, all participants were examined for blood pressure and asked for the use of antihypertensive medication. Obesity was defined by a body mass index (BMI) of 30 kg/m² or more. The presence of other risk factors for CVD, including a sedentary lifestyle, smoking, and family history of CVD, was registered during patient interviews.

The control group included 27 healthy volunteers of both sexes from the same local population. Individuals in this category volunteered after invitation and exclusion of dyslipidemia by laboratory exams using the same criteria above described [13]. During enrolment, their blood pressure and BMI were determined, and they were asked about the presence of CVD risk factors and medications.

The criteria for noninclusion used in this study for patients and healthy controls were as follows: the presence of neoplasia, tuberculosis, viral hepatitis B and C, human immunodeficiency virus infection, or Chagas’ disease. Additionally, individuals with inflammatory and autoimmune diseases were not included.

All participants signed written informed consent for inclusion in the study, and the study was approved by the Ethics Committee of the Nursing School of the Bahia Federal University.

2.2. Biological samples

Fasting blood samples were obtained by phlebotomy and used for blood cytometry and biochemical and serologic exams. Blood collected with ethylenediaminetetraacetic acid was used for hematologic analysis with a Cell Dyn-Ruby apparatus (Abbott Diagnostics, IL, USA). Immunophenotyping of T lymphocytes was performed by flow cytometry using heparinized blood and a FACSCanto II cytometer (BD Biosciences, USA).

2.3. Laboratory analyses

Serum levels of total cholesterol (TC), high-density lipoprotein (HDL-C), triglycerides (TGs), and glucose were determined by automated biochemical tests performed on a BT3000 PLUS analyzer (Wiener Lab Group, Buenos Aires, Argentina). Low-density lipoprotein (LDL-C) levels were calculated by Friedewald’s formula (LDL-C = TC – [HDL-C – TG / 5]), for TG values below 400 mg/dL [14]. Reference values for lipid profiles were those recommended for Brazilian individuals ≥ 20 years old, as follows: CT < 200 mg/dL, LDL-C < 130 mg/dL, HDL-C > 60 mg/dL, non-HDL cholesterol (N-HDL-C) < 160 mg/dL, and TGs < 150 mg/dL [13]. Serum levels of apolipoproteins A1 and B (ApoA and ApoB, respectively) and C-reactive protein (CRP) were measured using an IMMAGE apparatus (Beckman Coulter, USA). Reference values were as follows: ApoA, 90–170 and 107–214 mg/dL for women and men, respectively; Apo-B, 56–162 and 51–171 mg/dL for women and men, respectively. CRP levels of < 3 mg/L were considered normal [15].

2.4. Atherogenic indexes of CVD risk

To determine CVD risk, the following atherogenic indexes were determined: ApoB:ApoA ratio (low risk, 0.30–0.59; average risk, 0.60–0.79; and high risk, 0.80–1.00) and TG:HDL-C ratio (high risk > 3.0). The neutrophil/lymphocyte ratio (N/L) was also calculated using the blood counts of these cells (high risk > 3.0) [16–18].

2.5. Blood T-cell profiles

Whole heparinized blood samples were diluted 1:2 with RPMI 1640 complete medium (Cultilab, Brazil) containing 10% bovine fetal serum and gentamicin (10 mg/mL, Sigma-Aldrich, USA). Subsequently, an equal volume of activating medium containing phorbol 12-myristate 13-acetate (PMA, 10 ng/mL), ionomycin (0.4 mg/mL), and brefeldin A (5 mg/mL, Sigma-Aldrich) was added. Cell activation was carried out by incubation at 37 °C with 5% CO2 for 4 h [19]. After incubation, the samples were centrifuged for 5 min at 2000 rpm (room temperature), and the cell sediment was suspended in saline solution (0.85% NaCl). Subsequently, the cells were labeled with monoclonal antibodies for the cytometric analysis of the frequency of Th17 lymphocytes. Monoclonal antibodies used in flow cytometry were as follows: anti-CD3 PerCP-Cy 5.5, fluorescein isothiocyanate (FITC) anti-CD4, anti-CD8 phycoerythrin (PE)-Cy5 (eBioscience, USA), allophycocyanin (APC) anti-CD161, and anti-interleukin (IL)-17PE (BD Biosciences).

Phenotypic characterization of Th17 cells was performed by surface labeling with FITC mouse anti-human CD4 and APC mouse anti-human CD161 antibodies. Intracellular labeling was performed with PerCP-Cy 5.5 mouse anti-human CD3 and PE mouse anti-human IL-17 antibodies (BD Biosciences). PE rat IgG1 (BD Biosciences) was used as an isotype control of fluorescence overlapping. Unlabeled cells were negative controls of auto-fluorescence.

The cells were separated using a FACS Canto II cytometer (BD Biosciences). To determine cell frequency, we examined 10,000 unlabeled lymphocyte counts (negative control), 10,000 isotype control counts, 50,000 CD4+ CD8+ lymphocyte counts, and 100,000 Th17 lymphocyte counts. Diva FACS software from BD Biosciences was used to analyze the results.

2.6. Cytokine production

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll-Paque Premium (GE Healthcare, Sweden). Cells were adjusted to 1.0 × 10⁶ cells/mL in RPMI 1640 medium (Cultilab) containing 10% bovine fetal serum, 2 mM glutamine, 25 mM HEPES (pH 7.2), and 10 mg/mL gentamicin (Sigma-Aldrich). One hundred microliters of the cell suspension (1.0 × 10⁶ cells/mL) was added to the wells of a 96-well culture plate (KASVI, China). Cells were stimulated with 10 μL of
phytohemagglutinin solution (PHA; 5 μg/mL; Sigma). Negative control cells were stimulated only with 10 μL PHA diluent. Cells were incubated at 37 °C in an atmosphere containing 5% CO2 for 72 h. Culture supernatants were then collected by centrifuging the plates at 4 °C (1900 rpm, 10 min) and stored at −80 °C until analysis of cytokine levels.

2.7. Determination of cytokine levels
Quantification of IL-17A in culture supernatants was performed using a Cytometric Bead Array (BD Biosciences) with a FACs Canto II flow cytometer (BD Biosciences); a standard curve ranging from 20 to 5000 pg/mL. IL-17A was generated. Additionally, the levels of IL-17F, IL-21, and IL-22 in cell culture supernatants were determined by capture enzyme-linked immunosorbent assays (eBioscience, BenderMed Systems, Austria). The following reference curves with known amounts of cytokines were used in these immunoassays: IL-17F (15.6–1000 pg/mL); IL-21 (78–5000 pg/mL); IL-22 (31.3–2000 pg/mL). The production of a cytokine by PHA-stimulated PBMCs was calculated by subtracting production by unstimulated cells. In statistical analysis, outliers and cytokine levels below the minimum detection limit of the immunoassays (IL-17A, 0.3 pg/mL; IL-17F, 3.3 pg/mL; IL-21, 100 pg/mL; and IL-22, 8.0 pg/mL) were excluded.

2.8. Statistical analysis
Distributions of continuous variables were analyzed with D’Agostino and Pearson tests. Results were expressed as means ± standard deviations (SDs), medians and interquartile ranges (IQRs; Q1–Q3; 25th percentile to 75th percentile), or proportions (%). Medians of two groups were compared with Mann-Whitney U tests, whereas those of three or more groups were determined by Kruskal-Wallis tests followed by Dunn post-tests. The association between certain groups was analyzed using Fisher’s exact test or Chi-square tests. Differences with p values of < .05 were considered statistically significant. The statistical software Prism version 6.0 (GraphPad Software Inc., USA) was used in these analyses.

3. Results

3.1. Demographic and clinical findings
Dyslipidemic patients were classified into two groups in accordance with their coronary angiogram (CA) results. The group G1 without atherosclerosis included 14 individuals (56 ± 9 years of age), whereas the group G2 had 35 patients with atherosclerosis (59 ± 8 years of age). Patients with atherosclerosis exhibited a coronary heart disease (median stenosis 75% [IQR = 50–90%– in Cas]). Based on the clinical data, these groups included individuals presenting with CVD risk factors, such as hypertension, diabetes, obesity, smoking, prior acute myocardial infarction, and angina. Seven of 14 patients in group G1 and 28 of 35 patients in group G1 used daily statins. The control group was represented by 27 individuals with a mean age of 49 ± 9 years. All healthy individuals had an unaltered lipid profile, did not use any continuous medications, including statins, and did not have a diagnosis of hypertension, diabetes, or a prior AMI. In this group, risk factors included a family history of CVD, sedentary lifestyle and obesity (Table 1).

3.2. Laboratory evaluation

3.2.1. Lipid profiles and atherogenic risk indexes
There were differences in the levels of HDL-C and ApoA; both were decreased in dyslipidemic groups G1 and G2 in comparison with those in the healthy control group. The remaining lipid components showed similar levels in the three groups. Atherogenic risk indexes ApoA:ApoB and TG:HDL-C were higher in group G2 when compared with those in the G1 and control groups (Table 2).

CRP serum levels in dyslipidemic groups were twice as high as those in the control group. However, significant differences were only observed when comparing the G2 group with the control group (p = .017). Dyslipidemic patients presented similar neutrophil:lymphocyte ratios; however, this atherogenic index was higher in atherosclerotic patients than in healthy individuals (Fig. 1).

3.2.2. Peripheral blood T lymphocytes
The frequency and absolute counts of blood CD4+ T and CD8+ T lymphocytes were similar in the three groups (Table 3). Similar findings were observed for Th17 cells; although there was a higher frequency of these lymphocytes in dyslipidemic groups, these increases were not statistically significant (Table 3). However, female patients with atherosclerosis had a higher frequency of blood Th17 cells than healthy control women, but the Th17 frequency of dyslipidemic women without atherosclerosis was like those of HI and G2 women (Table 4).
The results are expressed as medians and IQRs. Groups were compared with Kruskal-Wallis tests with Dunn post-tests.

### Table 3

<table>
<thead>
<tr>
<th>T cell</th>
<th>HI (n = 27)</th>
<th>G1 (n = 14)</th>
<th>G2 (n = 35)</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>CD4⁺ (%)</td>
<td>52 (47-61)</td>
<td>52 (47-56)</td>
<td>55 (48-64)</td>
<td>.4781</td>
</tr>
<tr>
<td>CD4⁺ (cells/µL)</td>
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<td>1097 (893-1431)</td>
<td>1099 (834-1477)</td>
<td>.9347</td>
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<tr>
<td>CD8⁺ (%)</td>
<td>37 (29-44)</td>
<td>40 (33-44)</td>
<td>34 (26-42)</td>
<td>.3300</td>
</tr>
<tr>
<td>CD8⁺ (cells/µL)</td>
<td>687 (536-978)</td>
<td>765 (632-1107)</td>
<td>631 (508-778)</td>
<td>.1783</td>
</tr>
<tr>
<td>Th17 (%)</td>
<td>2.5 (1.8-3.5)</td>
<td>2.9 (2.3-5.8)</td>
<td>3.0 (2.2-5.3)</td>
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</tr>
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<td>Th17 (cells/µL)</td>
<td>49 (41-84)</td>
<td>80 (57-133)</td>
<td>63 (37-115)</td>
<td>.1870</td>
</tr>
</tbody>
</table>

Data are shown as medians and interquartile ranges. Groups were compared using Kruskal-Wallis tests and Dunn’s post-hoc test.

Th17:CD4⁺ T cell ratio was higher in atherosclerotic female patients in comparison to that of HI women (0.067 vs. 0.038, p = .0318), whereas the two groups of dyslipidemic women had similar Th17: CD4⁺ T cell ratio (G1 = 0.063, and G2 = 0.067; p = .7182).

Dyslipidemic patients with a prior myocardial infarction had lower absolute CD8⁺ T lymphocytes than dyslipidemic patients without this cardiovascular event. In contrast, dyslipidemic patients had more circulating CD8⁺ T cells than patients with a healthy BMI (Fig. 2).

No correlation was found between the frequency of Th17 cells and stenosis scores, non-HDL-C levels, and atherogenic indexes (ApoB:ApoA and TG:HDL-C indexes) or between the proportion of these cells and CRP levels.

#### 3.2.3. Levels of IL-17A, IL-17F, IL-21, and IL-22

Cytokines associated with the Th17 profile were measured in cell culture supernatants of PHA-stimulated cells. The production of IL-17A by PBMCs was decreased in patients with atherosclerosis. In contrast, IL-17F production was similar in the three groups. Importantly, differences in IL-21 and IL-22 levels were also observed. Few patients and healthy controls produced IL-21, and IL-21 production was decreased in patients with atherosclerosis compared with those in the healthy control group. However, IL-22 production was higher in the atherosclerosis group (Fig. 3).

The production of IL-17A and IL-22 cytokines by PBMCs from dyslipidemic patients with or without a prior AMI was also analyzed. In this analysis, IL-17A production was found to be similar in these two groups (negative AMI, n = 26, 76 pg/mL; positive AMI, n = 17, 71 pg/mL, p = .1494). Moreover, PBMCs from men and women with or without prior AMI produced similar amounts of IL-17A (data not shown). IL-22 production by PBMCs was similar in patients with or without a prior AMI (without AMI, n = 17, 169 pg/mL; prior AMI, n = 18, 256 pg/mL, p = .1756). However, there were differences between male and female patients. In men previously infarcted, there was a clear decrease in the production of this cytokine by PBMCs, although this decrease was not statistically significant. In contrast, PBMCs from women having a prior AMI exhibited increased production of IL-22 (Fig. 4).

### 4. Discussion

In this study, we investigated cytokine changes in blood T cells...
lymphocytes in dyslipidemic individuals living in Bahia, a tropical state; in this region, the population is predominantly of African descent, has distinct dietary habits, including the use of palm oil for cooking, eats high-fat foods, and has high alcohol intake. Nevertheless, the CVD risk factors found in this study were the same as those described in other populations from Brazil and abroad, highlighting the presence of dyslipidemia, hypertension, CVD family history, and sedentary lifestyle [20, 21].

Dyslipidemic patients with and without atherosclerosis differed in their levels of HDL-C and ApoA; however, no differences were observed in the levels of non-HDL cholesterol. In contrast, the atherogenic risk indexes represented by ApoB:ApoA and TG:HDL-C ratios were significantly increased in patients with atherosclerosis, predisposing them to a higher risk of CVD. These patients also showed high levels of CRP and an increased neutrophil:lymphocyte ratio, supporting their increased predisposition to CVDs, as demonstrated by their high frequency of prior AMI [15–18].

T lymphocytes, macrophages, and immune mediators (cytokines and chemokines) secreted from these cells are critical in atheroma development. However, we did not find cytometric alterations in the T-lymphocyte profile (CD4+ T, CD8+ T, and Th17 cells) of the dyslipidemic patients investigated in this study. The findings of CD4+ T cell frequency in patients with atherosclerosis are controversial. Although a lower rate of CD4+ T cells has been reported in dyslipidemic individuals, a higher proportion of these cells has been described in patients with AMI and unstable angina, with a decrease observed after treatment [22, 23]. Although the percentage of CD8+ T lymphocytes was unaltered in dyslipidemic groups, as previously reported [22], low

Fig. 2. CD8+ T cell counts in dyslipidemic patients presenting with or without a prior acute myocardial infarction (AMI) and in dyslipidemic patients with a normal body mass index (BMI) and obesity. Groups were compared with Mann-Whitney tests.

Fig. 3. Production of Th17-associated cytokines IL-17A, IL-17F, IL-21, and IL-22 by PBMCs from healthy individuals (HI) and dyslipidemic patients with or without atherosclerosis (G2 and G1, respectively). Groups were compared with Kruskal-Wallis tests with Dunn post-tests.
counts of these cells were observed in dyslipidemic patients reporting a prior AMI, consistent with another study [23]. We found higher absolute numbers of CD8+ T lymphocytes in obese patients, confirming the increased proliferation of these cells in the context of obesity [24].

Some studies have suggested that Th17 cells actively participate in the development of atherosclerosis, as demonstrated by a high proportion of Th17 cells in patients with severe coronary atherosclerosis and acute coronary syndromes (e.g., AMI and unstable angina) [25–27]. Herein, the frequency of Th17 cells was unaltered in dyslipidemic groups, and a higher proportion of these lymphocytes was only observed comparing women with atherosclerosis with healthy control women. This finding suggested that atherosclerosis may enhance the differentiation of Th17 cells in female patients with dyslipidemia. Nevertheless, male and female atherosclerotic patients showed similar Th17 cell frequencies, demonstrating a lack of sex bias in the rate of these lymphocytes during atherosclerosis. Despite these findings, we cannot exclude that the imbalance between Th17/regulatory T cells described in patients with acute coronary syndromes [28–30] could be observed earlier in female patients than in male patients with atherosclerosis. Additionally, atherosclerotic women may be less sensitive than men to the effects of statin on the proliferation of regulatory CD4+ CD25+ Foxp3+ T cells [31, 32]. High correlations among the frequencies of Th17 cells and some inflammation biomarkers were reported in patients with acute ischemic stroke [28]. However, we did not find any correlation between the frequency of Th17 cells and the degree of coronary stenosis, atherogenic risk indexes (ApoB:ApoA, TG:HDL-C, and neutrophil/lymphocyte ratios), or serum CRP levels.

Low production of IL-17A by PBMCs from atherosclerotic patients was observed in this study, in contrast to previous reports showing no differences in IL-17A levels between patients with coronary artery disease and patients without atherosclerosis or increased levels of IL-17A in patients with AMI [33,34]. However, low levels of IL-17A were recently described and found to be associated with poor prognosis in patients with AMI, suggesting that this cytokine may play a protective role in coronary disease and that patients with low levels of IL-17A and high levels of soluble vascular cell adhesion molecule-1 would have an increased risk of recurrent AMI and death [35]. Taking this study as a reference, we suggest that atherosclerotic patients with lower levels of IL-17A may have a poor CVD prognosis since this group includes patients with very high coronary stenosis and elevated atherogenic indexes (ApoB:ApoA, TG:HDL, and neutrophil/lymphocyte ratios). Additionally, 57% of these individuals had documentation of a prior AMI. Although IL-17A and IL-17F share similar inflammatory activities [36], IL-17F production did not differ among the groups examined in our study, suggesting that this cytokine was not involved in atherogenesis or that its synthesis was more sensitive to the anti-inflammatory effects of statins than IL-17A.

The production of IL-21 by PBMCs was restricted to few healthy controls and patients, in contrast to the results for IL-17A and IL-22. Nevertheless, decreased production of this cytokine was verified in patients with atherosclerosis. IL-21 is a cytokine that regulates innate and adaptive immune responses and participates in antitumor and antiviral defenses. However, IL-21 can worsen inflammatory and autoimmune diseases. The involvement of IL-21 in atherosclerosis has not been thoroughly studied, and increased plasma levels of this cytokine have been reported in patients with peripheral arterial disease [37,38].

In the present study, PBMCs from patients with atherosclerosis produced more IL-22, consistent with a previous report demonstrating increased IL-22 plasma levels in patients with AMI and unstable angina [39]. IL-22 contributes to atheroma development by promoting the migration of smooth muscle cells to the vessel intima and participates in the pathogenesis of different inflammatory diseases inducing the mRNA expression of acute-phase proteins [40]. PBMCs from atherosclerotic women produced more IL-22 than those from atherosclerotic men, demonstrating the influence of endocrine factors on the synthesis of this cytokine and suggesting a higher CVD risk in female patients and higher resistance to the anti-inflammatory effects of statins. These observations were consistent with our previous findings demonstrating elevated serum levels of atherogenic cytokines (IL-8 and tumor necrosis factor-α) and low levels of anti-atherogenic IL-10 in women with a prior AMI history despite the use of statins. Moreover, all atherosclerotic female patients were postmenopausal women and were therefore more susceptible to atherosclerosis [41,42].

Parallel changes were not observed between Th17 counts, and the production of their associated cytokines by PBMC stimulated with PHA. Such findings suggested the involvement of other cell sources of these immune mediators in dyslipidemia and atherosclerosis like CD8+ T cells, natural killer (NK) cells, γδ T cells, invariant NKT cells, lymphoid tissue inducer-like cells and activated monocytes.

In conclusion, our results demonstrated that atherosclerosis in Brazilian dyslipidemic patients was associated with risk factors and laboratory changes in lipid profiles, as has been described in dyslipidemic individuals living in different countries. In these individuals, atherosclerosis was not related to cytometric changes in circulating T lymphocytes, except in women who exhibited an increased frequency of Th17 cells. However, alterations in the production of Th17-associated cytokines by PHA-stimulated PBMCs from patients with atherosclerosis were verified for IL-17A, IL-21, and IL-22. Thus, hormones and cardiovascular events, such as AMI, influenced the Th17 immune response in atherosclerosis. Such findings deserve more studies to know their practical implications in the treatment of cardiovascular diseases.
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Conflict of interest

The authors declare that they have no conflicts of interest associated with this study.

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