Interleukin-12 Is Associated With Arterial Stiffness in Healthy Individuals

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Background
Atherosclerotic cardiovascular disease (CVD) is a chronic inflammatory disease mediated by the proinflammatory cytokines interleukin-12 (IL-12) and interleukin-18 (IL-18). Evidence suggests that IL-12 is dominant in early atherosclerosis, while IL-18 is critical in advanced atherosclerosis. In this study, we explore the association between IL-12 and IL-18 and arterial stiffness in healthy individuals.

Methods
We performed a cross-sectional study examining pulse wave velocity (PWV), augmentation index (AIx), IL-12, and IL-18 in healthy individuals (N = 53) without CVD risk factors.

Results
In multivariate regression, age (P < 0.01), systolic blood pressure (P = 0.05), and IL-12 (P < 0.01) were positively associated with PWV, and high-density lipoprotein (P = 0.04) was negatively associated with PWV (model R² = 0.476, P < 0.01).

Conclusions
IL-12, but not IL-18, is associated with PWV in healthy individuals without clinical CVD, supporting a role for IL-12 in early atherosclerosis as suggested by animal studies.

Keywords: atherosclerosis; arterial stiffness; blood pressure; hypertension; inflammation; interleukin-12; interleukin-18.

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Atherosclerotic cardiovascular disease (CVD) is believed to be a chronic inflammatory disease characterized predominantly by a T-helper 1 (Th1) immune response.1 Clinical studies and animal models have clearly demonstrated a complex association between proinflammatory Th1 cytokines such as interleukin-12 (IL-12) and interleukin-18 (IL-18) produced by antigen-presenting cells, and interferon-γ (IFN-γ) produced by activated T cells in the pathogenesis of atherosclerosis.1 Furthermore, while animal studies may suggest that IL-12 has a more critical role during the early phases of atherosclerosis, studies in human atherosclerotic plaque have demonstrated a more important role for IL-18 in advanced atherosclerosis.2–3 Elevated serum levels of IL-12 and IL-18 have also been observed in several human disease states including metabolic syndrome, obesity, type 2 diabetes, hypertension, and systemic lupus erythematosus (SLE).4–7 Noninvasive measurements of arterial stiffness, including aortic pulse wave velocity (PWV) and augmentation index (AIx), are established surrogate markers of CVD-associated and all-cause mortality in the general population, and may have utility in CVD-risk stratification.8 IL-18 is independently associated with CVD-related outcomes and also PWV in prospective and cross-sectional studies limited to individuals with CVD risk factors.9–11 However, there is a lack of data regarding IL-12 and surrogate CVD markers or CVD-related outcomes.

In this study, we explore the association between IL-12 and IL-18 and arterial stiffness in healthy individuals to test the hypothesis that IL-12 is a marker of early subclinical risk for CVD rather than IL-18.

Materials and Methods
Study population
We performed a cross-sectional study examining arterial stiffness and inflammatory markers in healthy individuals without known CVD risk factors or previous CVD history. Study participants were recruited via local community advertisement by the research staff at the Departments of Renal Medicine and Hepatology at Sir Charles Gairdner Hospital, Western Australia, between 1 January 2009 and 31 December 2011. This study was approved by the local Human Research Ethics Committee and written informed consent was obtained from all participants.

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Study methods

All study visits occurred in the morning following an 8-hour fast. A mean of 3 blood pressure measurements were determined after the participants were rested in the supine position for 10 minutes. Baseline characteristics included age, sex, smoking status (yes or no), and body mass index (BMI; kg/m²).

Biochemical analysis

Fasting venous blood was collected for analysis of cardiometabolic risk factors including lipids and inflammatory markers. Serum IL-18 was measured using a commercial sandwich enzyme-linked immunoassay (ELISA) kit (MBL Co, Nagoya Japan) with detection threshold of 12.5 pg/mL. Serum IL-12p70 was measured with a commercial ELISA kit (BD Biosciences, San Jose, CA) with a detection threshold of 7.8 pg/mL. Intra- and interassay variabilities were 7% and 8%, respectively. High-sensitivity C-reactive protein (hs-CRP) was measured using BNII Systems (Siemens Healthcare Diagnostics, Newark, DE) with a detection threshold of 7.8 pg/mL. Intra- and interassay variabilities were 7% and 8%, respectively. Serum 25-hydroxy vitamin D (25(OH)D) was measured by the DiaSorin LIASON chemiluminescent immunoassay (Stillwater, MN).

Arterial stiffness

Arterial stiffness was measured noninvasively using SphygmoCor (North Ryde, Sydney, Australia) by a single operator with a coefficient of variation of <10%. Aortic PWV was measured as the carotid-femoral PWV using the foot-to-foot method and the carotid-femoral distance measured as the total distance between the two measured sites, i.e., distance from suprasternal notch to common carotid artery added to distance from suprasternal notch to common femoral artery. Pulse wave analysis (PWA) was measured from the radial artery and a validated transfer function used to derive a surrogate index of central arterial compliance corrected for heart rate known as augmentation index (AIx). The AIx is an indirect measure of arterial stiffness dependent not only upon speed of wave travel but other factors including reflected wave amplitude, point of reflectance, and left ventricular stroke volume. The average of three and two measurements was used for AIx and PWV, respectively.

Statistical analysis and sample size

Normally distributed variables were presented as mean ± SD and nonnormally distributed variables were log transformed (base e) and presented as geometric mean (95% confidence interval). Skewed variables were presented as median (interquartile range). Associations with PWV and AIx were examined using linear regression methods. Associations with IL-12 and IL-18 were examined using logistic regression models in which IL-12 and IL-18 were assessed as categorical variables either as above median or below median as they were significantly skewed in distribution. A P value <0.05 was considered statistically significant. All statistical analysis was carried out with SPSS version 18.0 for Windows.

Our power calculation to derive a sample size to demonstrate a significant correlation between PWV and IL-18 was based upon a previous study by Vlachopoulos et al., which showed a significant correlation between PWV (but not AIx) and IL-18 with a Pearson correlation coefficient of 0.37 in men without previous CVD. Using this Pearson correlation coefficient, for a two-tailed test with a level of 0.05 and 80% power, a minimum of 48 subjects would be required to show a significant correlation. Our sample size of 53 subjects would therefore be sufficiently powered to detect a significant correlation between PWV and IL-18.

RESULTS

Baseline characteristics

Fifty-three healthy participants were recruited, of whom 42% were male. Four study volunteers were current smokers (8%). The median age and BMI were 52 (44.0–57.5) years and 25.75 (23.3–27.0) kg/m² and mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) were 120 ± 15 mm Hg and 72 ± 10 mm Hg, respectively. The mean total cholesterol, low density lipoprotein (LDL), and high-density lipoprotein (HDL) levels were 5.2 ± 0.85 mmol/L, 3.2 ± 0.76 mmol/L, and 1.5 ± 0.36 mmol/L, respectively. The mean 25(OH)D and hs-CRP levels were 70 ± 29.5 nmol/L and 2.2 ± 4.1 mg/L, respectively.

The median IL-12 and IL-18 levels were 274 pg/mL (65.5–465.0 pg/mL) and 323 pg/mL (249.0–443.5 pg/mL), respectively. PWV and AIx values were 7.3 ± 1.3 m/s and 22.3% ± 11.7%, respectively.

Arterial stiffness

On univariate analysis, there was a significant positive correlation between age (P < 0.01), BMI (P = 0.02), SBP (P < 0.01), IL-12 (P < 0.01), and PWV, and an inverse correlation between HDL and PWV (P < 0.01). There was no significant correlation between BMI, 25(OH)D, hs-CRP, or IL-18 and PWV. On univariate analysis for AIx, there was significant correlation with age only (P < 0.01). There were no associations between BMI, SBP, cholesterol, 25(OH)D, hs-CRP, IL-12, IL-18, and AIx.

On multivariate analysis, only age (P < 0.01), SBP (P = 0.05), HDL (P = 0.04), and IL-12 (P < 0.01) were significantly associated with PWV. In comparison on multivariate analysis, only age (P < 0.01) and male sex (P < 0.01) were associated with AIx (Table 1).

IL-12 and IL-18

There was a significant inverse correlation between IL-12 and IL-18 (P = 0.033). In logistic regression, there was a significant inverse association between 25(OH)D level and IL-12 in univariate (P = 0.018) and multivariate (P = 0.016) analysis. There were no associations between either IL-12 or IL-18 and hs-CRP, age, blood pressure, or cholesterol.
Inflammation and Arterial Stiffness in Healthy Individuals

Table 1. Univariate and multivariate analysis of pulse wave velocity & augmentation index

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<tr>
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<th>PWV regression model</th>
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<tbody>
<tr>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B (SE)</td>
<td>P value</td>
<td>B (SE)</td>
</tr>
<tr>
<td>Age</td>
<td>0.04 (0.02)</td>
<td>0.01</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td>Male</td>
<td>0.99 (0.34)</td>
<td>&lt;0.01</td>
<td>0.34 (0.34)</td>
</tr>
<tr>
<td>SBP</td>
<td>0.04 (0.01)</td>
<td>&lt;0.01</td>
<td>0.02 (0.01)</td>
</tr>
<tr>
<td>HDL</td>
<td>−1.37 (0.48)</td>
<td>&lt;0.01</td>
<td>−0.85 (0.41)</td>
</tr>
<tr>
<td>IL-12 &gt; median</td>
<td>1.2 (0.31)</td>
<td>&lt;0.01</td>
<td>1.1 (0.28)</td>
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|                  | AIx regression model |                      |                      |
|                  | Univariate           | Multivariate         |                      |
|                  | B (SE) | P value | B (SE) | P value |
| Age              | 0.38 (0.14) | <0.01    | 0.48 (0.12) | <0.01   |
| Male             | −8.65 (3.07) | <0.01    | −12.68 (2.63) | <0.01   |
| IL-18            | −5.53 (3.07) | 0.078    | −1.98 (2.60) | 0.45    |

PWV model $R^2 = 0.477$, AIx model $R^2 = 0.390$.

Abbreviations: AIx, augmentation index; HDL, high-density lipoprotein; IL-12, interleukin 12; IL-18, interleukin 18; PWV, pulse wave velocity; SBP, systolic blood pressure.

DISCUSSION

To our knowledge, this is the first evidence that suggests an independent association between IL-12 and PWV in healthy individuals without CVD risk factors and supports a previous animal study in which IL-12 appeared more important during early atherosclerosis. Davenport et al. observed that IL-12 deficiency in compound ApoE/IL12 (−/−) mice resulted in a 52% reduction in atherosclerosis at 30 weeks of age. However, by 45 weeks of age, these lesions had increased significantly to a size similar to lesions in wild-type ApoE (−/−) mice, suggesting that IL-12 appeared to have a dominant role during the early phases of atherosclerosis but IL-12 deficiency did not affect either the progression of atherosclerosis or extent of disease.

The specific mechanisms for inflammation in the pathophysiology of atherosclerosis suggest that antigen-presenting cells, including dendritic cells (DCs) and macrophages, are involved during early atherosclerosis by presentation of plaque antigens such as oxidized LDL to naive T cells. Subsequently, IL-12 and IL-18 produced by these immune cells within atherosclerotic plaque stimulate activated T cells to produce IFN-γ. IFN-γ in turn promotes plaque progression and rupture through its ability to decrease collagen production and vascular smooth muscle cell proliferation, upregulate chemoattractant and adhesion molecules, and increase protease and matrix metalloproteinase (MMP) activity. Exogenous administration of IL-12, IL-18, or IFN-γ to hybrid ApoE knockout mice deficient in these cytokines results in increased size and number of atherosclerotic lesions, further highlighting the importance of these proinflammatory cytokines in the pathogenesis of atherosclerosis. Furthermore, IL-8 and the IL-8 receptor are present within a variety of cell types that constitute atherosclerotic plaque, including endothelial cells, smooth muscle cells, and macrophages, and are also thought to play key roles in plaque progression and eventual rupture.

Chronic inflammation is believed to be responsible at least in part for the increased CVD risk observed in autoimmune diseases such as psoriasis and SLE, whereby chronic DC stimulation by damaged epithelial cell antigens (psoriasis) or double stranded-DNA (SLE) would result in excess production of IL-12, a key cytokine responsible for T-cell activation and production of IFN-γ. Our finding of an association between IL-12 and PWV supports evidence that chronic inflammation, possibly mediated through IL-12 is associated with increased risk of CVD.

Clinical studies have demonstrated a significant association between adiponectin receptor expression, MMP levels, and PWV in patients with coronary heart disease. Adiponectin is an important regulator of inflammation which induces anti-inflammatory cytokines such as interleukin-10 while MMP produced by activated macrophages in response to IFN-γ is another inflammatory mediator thought to contribute to atherosclerotic plaque progressive through its effects upon collagen and elastin degradation. These observations by Ikonomidis et al. suggest a complex relationship between pro- and anti-inflammatory mediators in atherosclerosis and the potential utility of adiponectin as both potential therapeutic target and surrogate CVD risk marker.

There are very limited studies examining associations between vascular function and IL-12 and/or IL-18. An association between IL-12 and in vitro markers of endothelial dysfunction, including nitric oxide, has been reported in a cohort of 100 patients with type 2 diabetes. While IL-18 levels were not measured in this study, the findings are still in keeping with our study, as increased PWV or arterial stiffness is in part a consequence of reduced nitric oxide bioavailability and associated endothelial dysfunction. However, unlike our study, a recent study of 97 males with...
significant cardiac risk factors demonstrated a strong association between IL-18 and PWV (but not IL-12). In this study, a large proportion of subjects had CVD risk factors such as hypertension (52%), dyslipidemia (36%), diabetes (8%), and smoking (60%), which suggests that the association between IL-18 and PWV may relate to increased CVD burden compared with the healthy subjects in our study. This study still supports our hypothesis that IL-12 may be an early CVD risk marker, whereas IL-18 is associated with progressive or more established disease. Similar to the study in men with CVD risk factors by Vlachopoulos et al., there was no association between IL-12, IL-18, or hs-CRP with AIx in our study, and this finding may reflect the differential effects of inflammation on larger compared to smaller blood vessels.

We also found an inverse association between 25(OH)D level and IL-12, suggesting a potential role for vitamin D in early atherosclerosis. There is growing recognition of the potential role of vitamin D in CVD, and in vitro studies have demonstrated that co-culture of DCs and T cells in the presence of 1,25-hydroxyvitamin D inhibits IL-12 production by macrophages and DCs, while treatment of nonobese diabetic mice with 1,25-hydroxyvitamin D induces a shift from Th1 to Th2 immune responses. This association between vitamin D levels and IL-12 warrants further examination in larger prospective cohorts.

In conclusion, we have demonstrated the possibility of an independent association between IL-12 and PWV, a surrogate CVD risk marker, in healthy individuals without previous CVD. This would support previous evidence implicating IL-12 during early atherosclerosis development and requires further study to determine if IL-12 is a potentially modifiable novel CVD risk factor.

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DISCLOSURE

The authors declared no conflicts of interest.

REFERENCES


