Serum levels of interleukin-18 in patients with stable and unstable angina pectoris

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Received 16 April 2003; received in revised form 28 July 2003; accepted 11 August 2003
Available online 23 December 2004

Abstract

Recent evidence suggests that atherosclerosis is an inflammatory disorder in which cytokines appear to play an important role. Special attention centered over the possible contribution of cytokines to the destabilization of the plaque.

IL-18 is a proinflammatory cytokine of the IL-1 family, recognized for its ability to promote IFN-γ secretion. It has recently been detected in human plaques and its administration was associated with increased atherosclerosis in apolipoprotein E (apoE) mice concomitant with an increase in plaque infiltrating inflammatory cells.

In our study, we investigated whether patients with established atherosclerosis, with either stable or unstable angina, possessed high levels of IL-18.

Patients with stable angina (n = 48) were from the outpatient clinic whereas patients with unstable angina (n = 73) were recruited upon admission and prior to performance of coronary angiography. Control patients (n = 19) were healthy subjects with no evidence of coronary artery disease. Serum levels of IL-18 were assayed by ELISA.

Patients with stable and unstable angina exhibited higher serum levels of IL-18 (77.1 ± 7.2 and 61.5 ± 5.1 pg/ml, respectively) in comparison to control subjects (p = 0.002 and p = 0.02, respectively). However, levels of IL-18 did not differ significantly between patients with stable and unstable angina. No differences were evident in the serum concentrations of IL-18 in patients with unstable angina (n = 17) upon admission and 1–3 months later when the angina was already controlled.

Although IL-18 serum levels appear elevated in the presence of coronary atherosclerosis, there is no evidence to associate this progression towards plaque instability.

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Keywords: IL-8; Unstable angina; Inflammation; Atherosclerosis

1. Introduction

The idea that atherosclerosis is an inflammatory disorder has fueled intensive research in recent years, attempting to elucidate potential players that could eventually be exploited to modulate of this relentless process [1]. Subsequent to finding activated T cells in the vicinity of the atheroma [2], it was assumed that cytokines that are produced by activated lymphocytes would possibly be involved in the progression atherosclerosis. In this respect, the functional dichotomy of T cells based on their pro- versus anti-inflammatory phenotypes (T-helper 1 and T-helper 2, respectively; reviewed in Ref. [3]) appeared as an attractive modulator of the composition of the plaque. Indeed, partial support has been provided for this paradigm as follows [4]. In experimental models of atherosclerosis, interferon-α and IL-12 (produced Th1 cells) exhibit proatherogenic roles [5–7], whereas IL-10 displays antiatherogenic properties [8]. However, this issue remains unsettled as IL-4 produced by Th2 cells either promote [9], or do not influence [10], lesion progression in hyperlipidemic mice. In humans, IFN-γ secreting cells are shown to increase [11] whereas IL-10 serum levels are reduced [12] in patients with unstable angina, indicating a potential stabilizing/destabilizing effect of the Th cytokines in rendering the plaque vulnerable.
IL-18 is a member of the IL-1 cytokine family initially recognized as an IFN-γ inducing factor (reviewed in Ref. [13]). Active IL-18 is secreted in its active form upon cleavage with caspase-1 or other caspases. The IL-18 produced both by immune and non-immune cells is processed similar to IL-1α, and the receptor systems of both cytokines bear structural resemblance. Several properties of the former are relevant to its possible influence on atherosclerosis. IL-18 promotes the secretion of the proinflammatory and proatherogenic IFN-γ in concert with IL-12. IL-18 was recently identified in atherosclerotic human plaques [14], and overexpression of an endogenous IL-18 inhibitor (IL-18 related protein) was associated with a reduction in atherosclerosis progression in apolipoprotein E (apoE)-deficient mice [15]. Very recently, direct evidence was provided for the proatherogenic role of IL-18, by showing that administration of the cytokine to apoE mice increased plaque formation [16]. Interestingly, IL-18-treated mice exhibited increased plaque T lymphocyte and MHC-II expressing cell numbers and IFN-γ gene disruption abolished the proatherogenic effects of IL-18.

Collectively, the above observations suggest that IL-18 may have a role in accelerating atherosclerosis, and possibly act as a destabilizing agent contributing to the occurrence of acute coronary syndromes. In our study, we tested the hypothesis that IL-18 serum levels increase in patients with established atherosclerotic coronary heart disease and evaluated its potential association with unstable angina.

2. Materials and methods

2.1. Patients

Patients were selected with a severe unstable angina of recent onset (<3 days before admission). All patients had at least one episode of rest angina or an episode lasting more than 20 min, during the last 24 h, accompanied by transient ST-segment changes and with no rise in creatine kinase-MB levels or troponin T levels. Full medical therapy, including beta-adrenergic blocking agents and/or calcium antagonists, low-dose aspirin and continuous i.v. infusion of nitrates and heparin, was introduced on admission, and continuous electrocardiogram (ECG) telemetric monitoring was applied to all patients during their stay in our coronary care unit. Some of the patients were followed up for 1–3 months following discharge and sera obtained again for comparison with the initial sample. Patients with stable angina were recruited from the outpatient clinic. Patients with stable or unstable angina were included provided they had undergone coronary angiography in the past documenting the presence of coronary atherosclerosis. Control patients were free of risk factors of atherosclerosis or any evidence of ischemic heart disease.

Table 1

<table>
<thead>
<tr>
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<th>Stable angina (n = 48)</th>
<th>Unstable angina (n = 73)</th>
<th>Control (n = 19)</th>
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<tbody>
<tr>
<td>Men/women, n</td>
<td>33/15</td>
<td>47/26</td>
<td>10/9</td>
</tr>
<tr>
<td>Age, years</td>
<td>70.9 ± 11</td>
<td>68.0 ± 6.5</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>β-blockers, n</td>
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<td>69</td>
<td>0</td>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>Calcium-channel blockers, n</td>
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<td>23</td>
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</tr>
<tr>
<td>Lipid-lowering drugs, n</td>
<td>24</td>
<td>41</td>
<td>0</td>
</tr>
</tbody>
</table>

None of the parameters were statistically significant.

Fig. 1. (A) Sera from patients with stable and unstable angina in comparison with controls were evaluated for IL-18 levels by an ELISA. (B) Mean levels of IL-18 in 17 patients with unstable angina upon admission were compared with sera from the same patients 1–2 months later, upon complete resolution of their symptoms.

2.2. IL-18 serum determination

The assay was performed in accord with the manufacturer’s recommendation (R&D Systems). The Human IL-18 Kit measures human IL-18 by sandwich ELISA. The assay
uses two monoclonal antibodies against two different epitopes of human IL-18.

The concentration of human IL-18 is calibrated from a dose–response curve based on reference standards. The sensitivity of the assay is 12.5 pg/ml. The minimum detection limit estimated by serial dilution was 12.5 pg/ml since the mean ± 2 S.D. of the 12.5 pg/ml was lower than the mean − 2 S.D. of the 12.5 pg/ml.

2.3. Statistical analysis

All data are expressed as the mean value ± S.E.M. A combination of analysis of variance, based on ranks, and a multiple comparisons procedure (i.e. the Bonferroni/Dunn procedure) was used. Categorical variables were compared using the χ² or the Fisher’s exact test. A p value < 0.05 was considered significant.

3. Results

No statistically significant differences were evident between both groups with respect to the age or presence of risk factors for atherosclerosis (Table 1).

IL-18 serum levels were significantly elevated in patients with stable (mean level of 77.1 ± 7.2 pg/ml) and unstable angina (61.5 ± 5.1 pg/ml) as compared with control subjects (36.8 ± 3.7 pg/ml; p = 0.002 and p = 0.02, respectively) (Fig. 1A). No difference was evident between mean serum levels of IL-18 between patients with stable and unstable angina (p = 0.07).

Mean levels of IL-18 in patients with unstable angina that were successfully treated (either invasively or non-invasively) following 1–3 months (n = 17; 72.2 ± 7.9 pg/ml) did not differ from their baseline levels (n = 17; 79.5 ± 12.7 pg/ml; p = 0.63) obtained upon their hospital admission (Fig. 1B; Table 2).

4. Discussion

Recent evidence incriminated IL-18, the IFN-γ promoting cytokine in atherosclerosis [14–16]. Thus, IL-18 treatment of atherosclerosis prone mice increased [16] whereas administration of IL-18 related protein (IL-18 inhibitor) decreased [15] plaque formation in atherosclerosis prone apoE-deficient mice. Moreover, injection of IL-18 was associated with an increased number of plaque infiltrating T lymphocytes as well as MHC-II containing cells, suggesting the cytokine may contribute to plaque destabilization [16]. IL-18 has also identified in human plaques [15].

We wished to test whether patients with stable angina and documented coronary heart disease have increased levels of IL-18, and whether patients with unstable angina have a further increase in IL-18 that may reveal an additional potential mechanism leading to plaque disruption. We indeed found that patients with both stable and unstable angina who were confirmed as having coronary atherosclerosis show increased levels of IL-18 in comparison with control healthy subjects. However, IL-18 levels in patients with unstable angina did not significantly differ from those in patients with stable angina. Moreover, mean levels of IL-18 upon successful treatment of the patients with unstable angina failed to drop in comparison to the levels present upon admission. Recent studies [17,18] showed that serum levels of IL-18 were slightly but significantly increased in patients with acute coronary syndromes in comparison to those of patients diagnosed as suffering from stable angina. The differences in the results may result from different population and inclusion of patients with unstable angina of more recent onset in our study. Alternatively, the lower levels of IL-18 in patients with unstable angina in our study could reflect the binding of circulating IL-18 to specific receptors at plaque site.

The results of the current study point towards IL-18 as a cytokine that may potentially influence plaque development, yet it is questionable whether it is indeed associated with an increased inflammatory phenotype within the plaque that could result in its rupture.

References


