High sensitivity cytokine detection in acute coronary syndrome reveals up-regulation of Interferon Gamma and Interleukin-10 post Myocardial Infarction

K.D. Patel [a],*, S.P. Duggan [a], C.A. Currid [b], W.M. Gallagher [b], R. McManus [a], D. Kelleher [a], R.T. Murphy [c], A.W. Ryan [a]

[a] *Department of Clinical Medicine, Trinity College Dublin and Institute of Molecular Medicine, Trinity Centre For Health Sciences, St James's Hospital, Dublin, Ireland*
[b] *UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Ireland*
[c] *Institute of Cardiovascular Science, St James's Hospital, Dublin, Ireland*

Abstract   Inflammation is an important element in the development and destabilization of atherosclerotic plaque. Using a high sensitivity multiplex assay, previously untested in the context of atherosclerotic disease, we determined serum concentrations of GM-CSF, IFNγ, IL-1β, IL-2, IL-10, IL-12p70, TNF α, IL-6, and IL-8 in 48 Myocardial Infarction (MI) patients, 14 Unstable Angina (UA) patients and 12 healthy controls. IFNγ levels were significantly higher in MI compared to UA ($p=0.0091$) and Control groups ($p=0.0014$). IL-10 also showed higher expression levels between MI, UA groups and Controls ($p=0.0299$). This up-regulation may reflect the extent of plaque instability and/or rupture in MI patients. Our observations provide evidence that IFNγ and IL-10 merit further investigation in atherosclerotic disease states as potential markers of disease and therapeutic targets.
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Introduction

The role of inflammation in atherosclerosis is well recognised. T-lymphocytes have been detected in unstable atherosclerotic plaques, suggesting that T-cell activation is important in the initiation and destabilization of these plaques, with unstable plaque previously being shown to have a 10-fold increase in T-cell content by quantitative PCR [1]. The progression of plaque to a chronic state and its potential to rupture is also characterized by an elaborate pathology associated with cytokine activation, endothelial and smooth muscle cell (SMC) activation and lipid oxidation [2]. This activation thus makes atherosclerotic plaque vulnerable to rupture, contributing to a local and systemic inflammatory reaction in Acute Coronary Syndromes (ACS) [3,4].

Being involved in plaque development, cytokine activation may be a key player in plaque susceptibility to rupture. Currently, the inflammatory marker, C-reactive protein (CRP) has been established as a prognostic indicator for those diagnosed under the umbrella of ACS, and various cytokines, such as Interleukin 6 (IL-6) have been shown to be elevated, and are associated with an adverse prognosis [5–7]. This

* Corresponding author. Fax: +353 1 454 2043.
  E-mail address: kpatel@tcd.ie (K.D. Patel).

indicates that cytokines involved in plaque pathology are a future therapeutic target in ACS [8,9].

Recent studies have suggested a correlation between Il-17 and IFNγ, whereby IL-17 may promote IFNγ production in an atherosclerotic disease state [10]. We have previously demonstrated that IL-17 is not present at reliable levels in patients diagnosed with ACS, even in the presence of elevated hsCRP, a recognised atherosclerotic biomarker [11]. Several studies have focused on cytokine levels in ACS, in particular on TNF alpha, IL-6, and IL-8 [12–15]. However, there are few data relating to GM-CSF, IL-1β, IL-10, and IL-12p70 in patient based models. In addition, most studies looking at Il-17, IFNγ and other cytokines commonly related to atherosclerosis have used conventional ELISA kits. Results published are often at detection levels below the stated manufacturer's sensitivity levels.

We aimed to investigate the release of a panel of IFNγ and related cytokines in ACS, using a novel multiplex platform. These analyses are carried out to a greater degree of sensitivity than hitherto possible, using the Mesoscale Discovery (MSD) platform. This technology uses a combination of electrochemiluminescence (ECL) detection and patterned arrays of antibodies, offering greater sensitivity than ELISA based assays, which have traditionally been used in studies of cytokines in ACS.

Methods

Patient selection

The study group comprised of 74 subjects, 48 patients with a Myocardial Infarction (MI), 14 patients diagnosed with Unstable Angina (UA) and 12 healthy controls with no history of cardiovascular disease. The study population was consecutively selected, all showing clinical and electrocardiographic signs of acute coronary syndrome with indications for coronary angiography. All patients were being treated with Aspirin, a statin and a beta-blocker. Myocardial Infarction was defined by a rise in troponin >0.01 ng/ml, and Unstable Angina as an acute coronary syndrome without an enzyme rise. Exclusion criteria included recent infection, chronic inflammatory/auto-immune disease, renal failure or haemodialysis, immunosuppressive therapy and history of malignancy.

Venous blood samples were obtained on admission prior to angiography. Informed consent was obtained from all subjects. Clinical referral forms and the study protocol were approved by the St James's Hospital ethics committee.

Serum isolation and cytokine analysis

Blood samples were immediately centrifuged at 1500 rpm for 10 min. Serum was separated and stored at $-80\ °C$ until further analysis.

Cytokine serum concentrations were determined via a multi-array, sandwich immunoassay kit (Meso Scale Discovery MSD®, Maryland, USA) according to the manufacturer's instructions. Detection lower limits of IL-10, IL12p70, IL1-beta, IL-2, IL-6, IL-8, GM-CSF, TNFα and IFNγ were 0.292, 2.12, 0.199, 0.314, 0.595, 0.114, 0.317, 0.644 and 0.225 pg/ml respectively.

As a substudy, hsCRP was measured in 40 consecutive subjects including the control population (24 MI, 11 UA, 5 Controls).

Statistical analysis

Comparison of study populations was performed using non-parametric Wilcoxon/Kruskal–Wallis rank sum tests. Regression analysis was done to correct for age and sex. The JMP statistical package (SAS software, North Carolina, USA) was used. A p value<0.05 was regarded as significant.

Results

Clinical data

There was no difference in age or sex ratio or other risk factors between patients with MI or UA (Table 1). The time since diagnosis in the patient groups was comparable with a mean length of time of 6 ($\pm$0.97) days for the UA group, as opposed to 5.9 ($\pm$0.42) days for the MI group (Table 1). Atorvastatin was the predominant statin that was prescribed to both patient groups.

Cytokine concentration in serum

Significant differences in serum concentration between the three study groups were noted for measures of IFNγ (p=0.0009) and IL-10 (p=0.0114) (Table 2). Regression analysis, correcting for the effects of age and sex, supported the effect of IFNγ (p=0.0358) and IL-10 (p=0.0269). No statistically significant differences were noted in other cytokine levels, in particular IL-6, IL-8 and TNFα.

IFNγ expression vs study group

Comparing the three study groups, Controls, Unstable Angina and Myocardial Infarction, significant differences in IFNγ expression were noted between the MI and Control groups

Table 1 Baseline characteristics of study patients.

	Controls (n=12)	Unstable Angina (n=14)	Myocardial Infarction (n=48)
Age, mean$\pm$S.E.M	48$\pm$2.03	64$\pm$2.61	63$\pm$1.82
Sex (M/F)	8/4	10/4	36/12
Days since diagnosis, mean$\pm$S.E.M	N/A	6$\pm$0.97	5.9$\pm$0.42
Diabetes mellitus	Nil	4 (29%)	5 (36%)
Active smoker	Nil	5 (36%)	15 (31%)
Ex smoker	Nil	6 (43%)	21 (44%)
Non smoker	Nil	3 (21%)	12 (25%)
BMI, mean$\pm$S.E.M	26.51$\pm$1.99	28.45$\pm$1.46	27.86$\pm$0.76
Recorded statin on admission			
Atorvastatin	Nil	11	39
Pravastatin	Nil	0	2
Rosuvastatin	Nil	2	4
Simvastatin	Nil	1	3

Values are n (%) unless otherwise indicated. S.E.M. indicates Standard Error Mean; BMI, Body Mass Index.