SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

NOME E COGNOME: Loreta Pia Ciuffreda

UNIVERSITÀ: Seconda Università degli Studi di Napoli

DIPARTIMENTO (in caso di borsa per soggiorno all'estero specificare l'ente presso cui si è svolta la ricerca): Dipartimento di Medicina Sperimentale, Sez. Farmacologia

TUTOR (in caso di borsa per soggiorno all'estero specificare il tutor dell'ente presso cui si è svolta la ricerca): Liberato Berrino

TIPOLOGIA DI BORSA RICEVUTA: Borsa SIF-MSD

TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale): finale

TITOLO DELLA RELAZIONE: "Prognostic and predictive markers of acute atherothrombotic events"

Borsista

Dott.ssa Loreta Pia Ciuffreda

Tutor

Prof. Liberato Berrino

RELAZIONE:

Introduction

Atherosclerosis is a lipid-driven, chronic inflammatory disease of the vessel wall in which both innate and adaptive immune responses play a role (Hansson GK, 2002). Immune cells and their mediators directly cause the chronic arterial inflammation that is a hallmark of atherosclerosis. Macrophages, T lymphocytes and, to a lesser extent, mast cells contribute to the smoldering inflammatory response in the vessel wall (Kovanen PT, 1995).

It is now generally accepted that a specific T-cell response is crucial in determining not only the development and growth of the atherosclerotic lesion, but also its fate toward a stable or an unstable pattern of evolution (i.e., rupture or ulceration), eventually leading to the clinical occurrence of ACS (Andersson J, 2010). T-cells are able to produce a large array of pro-inflammatory cytokines. Cytokines are pleiotropic proteins that regulate the activity of many cells. The progression of plaques from a chronic state toward rupture is accompanied by activation of a complex inflammatory network, characterized by an intricate expression and secretion of many cytokines (Golino P, 2010). The effects of this complex network of inflammation on gene expression profiling within the coronary circulation is still under investigation. Thus, using gene induction and microarray technology, the aim of the present study was to evaluate the changes in gene expression profile in endothelial cells stimulated with plasma obtained from the coronary sinus (CS) and the aorta (Ao) of patients with ACS or stable angina (SA).

Materials and methods

Patient Population

Twelve patients, divided in 2 groups, entered the study. Group I included patients with chronic stable angina (SA, n=4) undergoing elective coronary angiography; group II included patients with ACS (n=8), undergoing urgent coronary angiography and eventually PTCA. All patients in group I had a history of effort chest pain with a stress test positive for inducible myocardial ischemia. Patients with previous myocardial infarction were excluded. ACS was defined as typical chest pain at rest occurring <48 hours from hospital admission with ECG changes suggesting myocardial ischemia, with or without increase in serum markers of myocardial damage. Within the diagnosis of ACS, normal or increased serum markers of myocardial necrosis defined unstable angina (UA) or non- ST segment elevation myocardial infarction (NSTEMI), respectively. Patients with ST-segment elevation myocardial infarction were excluded from the study. As per protocol design, in both groups, only patients with TIMI flow grade ≥2 were included, as well as patients with a single vessel disease and a culprit lesion located either in the left anterior descending coronary artery or in the circumflex coronary artery, both of which drain into the coronary sinus.

Experimental Protocol

After coronary angiography was performed, and before performing PTCA, a 6F multipurpose catheter was positioned into the coronary sinus (CS). Blood samples (4.5 ml) were simultaneously obtained from the CS and the ascending aorta (Ao) and immediately placed into empty pre-chilled Vacutainer® tubes for serum separation. Serum samples were immediately centrifuged at 1,000 x g at 4 °C for 20 minutes, and the sera were stored in aliquots at -80 °C.

Cell culture

Experiments were performed on human coronary artery endothelial cells (HCAECs, Cambrex Bio Science, USA). Cells were grown in EGM 2 medium (Cambrex Bio Science, USA) with endothelial cell growth supplement and 10% fetal serum. Cells were used at passages 2–5.

Microarray gene expression analysis

Agilent microarray analyses were done to evaluate the gene expression profile in ECs exposed to plasma obtained from CS and Ao in patients with ACS and SA. These experiments were performed using a one colour labeling microarray system. The quantity of each of the total RNA samples and determination of the A260/280 nm ratio was determined by spectrophotometry and the size distribution was assessed using an Agilent Bioanalyzer. Eight hundred ng of total RNA were converted into labelled cRNA with nucleotides coupled to a fluorescent dye (either Cy3) using the Quick Amp Kit (Agilent Technologies, Palo Alto, CA) following the manufacturer's protocol. The A260/280 nm ratio and yield of each of the cRNAs were determined using an Thermofisher Nanodrop. Eight hundred twenty-five ng of cRNA-labeled from SC and Ao were hybridized to Agilent Human Whole Genome 4 × 44k Microarrays. The hybridized array was washed and scanned and data were extracted from the scanned image using Feature Extraction version 10.2 (Agilent Technologies). The raw data and associated sample information were loaded and processed by Gene Spring® 11.5X (Agilent Technologies). For identification of genes significantly altered in patients with unstable angina, total detected entities were filtered by signal intensity value (upper cut-off 100th and lower cut-off 20th percentile) and flag to remove very low signal entities. Data were analyzed using Student's t test ($p < 0.05$) with a Bonferroni multiple test correction to minimize selection of false positives. Of the significantly differentially expressed RNA, only those with greater than 2-fold increase or 2-fold decrease as compared to controls were used for further analysis. Subsequently, hierarchical clustering (condition tree) was applied to the data files. In this way, the relationships between the different groups are shown. The condition tree was displayed as a heat map, based on expression levels of the probe sets. Functional and network analyses of statistically significant gene expression changes were performed using Ingenuity Pathways Analysis (IPA) 8.0 (Ingenuity® Systems, http://www.ingenuity.com). Analysis considered all genes from the data set that met the 2-fold (p-value < 0.05) change cut-off and that were associated with biological

functions in the Ingenuity Pathways Knowledge Base. The significance of the association between the data set and the canonical pathway was measured in 2 ways: (1) Ratio of the number of genes from the dataset that map to the pathway divided by the total number of genes that map to the canonical pathway is displayed; (2) Fisher's exact test was used to calculate a P value determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone.

Results

In my project I investigated the expression of ACS-related genes, that was determined by comparing gene expression in ECs exposed to plasma obtained from Ao and SC of patients with instable and stable angina.

The results obtained show that the plasma collected by the Ao does not determine significant changes in ECs gene expression of patients with ACS and SA. However, the plasma obtained from SC causes significant changes in the two groups of patients. Only genes with 2-fold increase or decrease were considered significant. Between these genes, 684 were up-regulated and 283 down-regulated in patients with ACS (Fig. 1).

Data analysis pointed out that many of the up-regulated and down-regulated genes were involved in the inflammatory process. In particular, selectin E (SELE) was the most up-regulated gene, with a 19.66 fold change. Selectin E is a glycoprotein involved in the adhesion process of leukocytes to endothelial walls and in their migration from the intravascular lumen to the injured tissue. Interestingly, chemokines (CXCL1, CXCL2, CXCL3, CCL2) genes were up-regulated as well. Chemokines expression is triggered by pro-inflammatory cytokines stimulation and it is involved in the recruitment of monocytes, granulocytes and T cells.

It is noteworthy the increase in expression of pro-inflammatory interleukins (IL3RA, IL7R, IL8, IL11), proteins secreted by the immune system cells and the up-regulation of NFKB related genes

(NFKBIA, NFKBIZ, NFKBIE). NF-kB ("nuclear factor kappa-light-chain-enhancer of activated B cells") is a transcription factor that is activated by pro-inflammatory stimuli such as stress, cytokines, free radicals, ultraviolet irradiation and bacterial or viral antigens. Accordingly, several genes involved in the inflammatory process were up-regulated, i.e. prostaglandin-endoperoxide synthase 2 (PTGS2), fibroblast growth factor 18 (FGF18), vascular endothelial growth factor A (VEGFA), intercellular adhesion molecule 1 (ICAM1) and platelet factor 4 (PF4).

Moreover, many of the down-regulated genes obtained through our microarray experiment were involved in the inflammatory process. In particular, STAT1 is a cytoplasmic transcription factor that is activated by several signals, such as cytokines, transcription factors (INF-α, INF-γ), hormones and T-bet, that represents the main transcription factor in TH1 cells. STAT1 mediates also IL-23 expression, related to TH17 cells inhibition (Kimura A, 2007; Stumhofer JS, 2006). Conversely, T-bet promotes IFN-γ and IL-2 synthesis (Kimura A, 2011; Cheng X, 2008). The reduced expression of these genes confirms the unsuccessful inhibition of TH17 cells, further confirming how plaques may be affected both by the inflammation and immune system responses.

T-bet and STAT1 act as suppressors on Th17 cells production. Having a down-regulation at this level may imply higher Th17 cells production and IL-17 and IL-6 expression. IL-17 and IL-6, in turn, arrest both Treg cells and IL-10 production (Tab. 1 a, b).

Among the up-regulated genes, the majority were associated with the pathways of IL-17 Signaling: Chemokine (C-C motif) ligand 2 (CCL2), Chemokine (C-X-C motif) ligand 1 (CXCL1), Interleukine 8 (IL8), Janus kinase 1 (JAK1), v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), muscle RAS oncogene homolog (MRAS), prostaglandin-endoperoxide synthase 2, prostaglandin G/H synthase and cyclooxygenase (PTGS2) (Tab. 3).

To confirm the results obtained with microarray experiments, Real Time PCR experiments were performed on IL17A, which is mainly produced by Th-17 cells, Phospholipase A2 (PLA2 IIa),

Phosphodiesterase 4A (PLA2 IVa), phospholipase C, beta 4 (PLCB4) and their product Prostaglandin E2 (PGE2), which determine the activation of TH-17 cells.

The quantitative analysis of mRNA levels measured by Real Time PCR amplification showed in ECs exposed to plasma obtained from CS of patients with ACS a significant increase in the IL-17A (mean of arbitrary units ± SEM, 176.7 ± 1.1%, n = 3 $p < 0.05$, Student's T Test), PLA2 IIa (mean of arbitrary units ± SEM, 8.8 ± 2.0%, n = 3 $p < 0.05$, Student's T Test) and PGE2 (mean of arbitrary units ± SEM, 38.3 ± 1.4%, n = 3 $p < 0.05$, Student's T Test) compared with ECs exposed to plasma obtained from CS of patients with SA. Instead, PLA2 IVa (mean of arbitrary units ± SEM, 0.6 ± 1.1%, n = 3 $p < 0.05$, Student's T Test) did not show a significant increase (Fig. 2).

Moreover, the increase of the expression of these genes suggesting that Th17 cells may play a specific role in promoting plaque instability.

Conclusions

The aim of the present study was to determine the gene expression patterns in endothelial cells exposed to sera collected at the level of the coronary sinus (CS) and aortic arch (AO) of 8 patients with ACS or 4 patients with stable angina (SA), in order to investigate the mechanisms involved in the pathophysiology of plaque rupture. Using this approach, we could directly document for the first time that these events are the result of local events limited to unstable plaques and not, as often argued, represent phenomena of systemic activation.

In fact, the data obtained during this year from the present study indicate that sera collected from the Ao, which represents the systemic circulation, did not determine significant changes in gene expression profiles when patients with ACS were compared to patients with SA. In contrast, sera collected from the CS of ACS patients, induced up-regulation of 684 genes and down-regulation of 283 genes, as compared to SA patients, thus confirming the presence of local factors able to affect gene expression profile in human coronary endothelial cells in vitro (Fig. 3 a, b). Many the up-

regulated genes were associated with IL-17 Signaling pathways: E-Selectin (SELE), CCL2, CXCL1, IL8, JAK1, KRAS, MRAS, PTGS2. Interestingly, SELE was the most up-regulated gene, showing a 19.66 fold-change. Moreover, we observed the up-regulation of several chemokines genes (i.e.: CXCL1, CXCL2, CXCL3, CCL2). Consistently, the pro-inflammatory interleukins IL3RA, IL7R, IL8, IL11, which are proteins secreted by immune system cells, and the NFKB related-genes (NFKBIA, NFKBIZ, NFKBIE) over-expression sustain the atherosclerosis process progression in the presence of the plaque rupture. Gene networks analysis showed that most of the up-regulated genes are selectively related to the inflammatory pathway, supporting the critical role held by inflammation in the atherosclerotic disease. Microarray data and biological pathways analysis point out that the involvement of such signaling pathways depend from the IL-17 up-regulation.

IL-17A is able to induce a proinflammatory (TNF-a, IL-6, MCP-1, CCL5), pro-thrombotic (tissue factor) and plaque destabilizing (MMP-1, MMP-9) response on various atherogenic cell types (macrophages, smooth muscle cells, monocytes, dendritic cells, endothelial cells) (Erbel C, 2009). Moreover, IL-17A expression was associated with plaques containing a complicated formation or a lipid-rich lesions (Erbel C, 2011).

Moreover, many of the down-regulated genes obtained through our microarray experiment were involved in the inflammatory process. In particular, STAT1 is a cytoplasmic transcription factor that is activated by several signals, such as cytokines, transcription factors (INF-α, INF-γ), hormones and T-bet, that represents the main transcription factor in TH1 cells. STAT1 mediates also IL-23 expression, related to TH17 cells inhibition (Kimura A, 2007; Stumhofer JS, 2006). Conversely, T-bet promotes IFN-γ and IL-2 synthesis (Laurence A, 2007; Kimura A, 2011). The reduced expression of these genes confirms the unsuccessful inhibition of TH17 cells, further confirming how plaques may be affected both by the inflammation and immune system responses.

T-bet and STAT1 act as a suppressor on Th17 cells production. If we have a down-regulation in this point it is possible to suppose a greater Th17 cells production and a more IL-17 and IL-6 expression. IL-17 and IL-6 arrest Treg cells and IL-10 production (Kimura A, 2011) (Fig. 4).

Finally, in order to validate our microarray results we performed Real Time PCR. We examined IL17A mainly produced by TH17 cells, PLA2 IIa, PLA2 IVa, PLCB4 and their product PGE2, which seem to play a key role in TH17 cells activation. Quantitative analysis of mRNA levels showed a significant increase in IL-17A, PLA2IIa, PLCB4 and PGE2 in endothelial cells exposed to plasma obtained from the SC of patients with ACS, compared to endothelial cells exposed to plasma obtained from the SC of patients with AS. Otherwise, PLA2 IVa did not show a significant increase.

Our data have been presented as a poster in several scientific congresses and recently submitted as an original paper to the Journal of Vascular Research. They support the evidence that in patients with ACS a significant intracoronary production of selected cytokines/chemokines occurs; this phenomenon leads to the up-regulation or the down-regulation of several genes involved in different inflammatory responses.

In fact, the inflammatory response is crucial for the growth and the development of the atherosclerotic plaque and it is associated with an increase in circulating IL- 17A, suggesting that Th17 cells may play a specific role in promoting plaque instability. This work shows that the IL-17 pathway seems to be a key factor in the development of atherosclerosis. Specifically, we suggest a novel pathophysiological mechanism involving Th-17 cells that lays the basis for the possible development of new biological markers capable to predict the course of ACS.

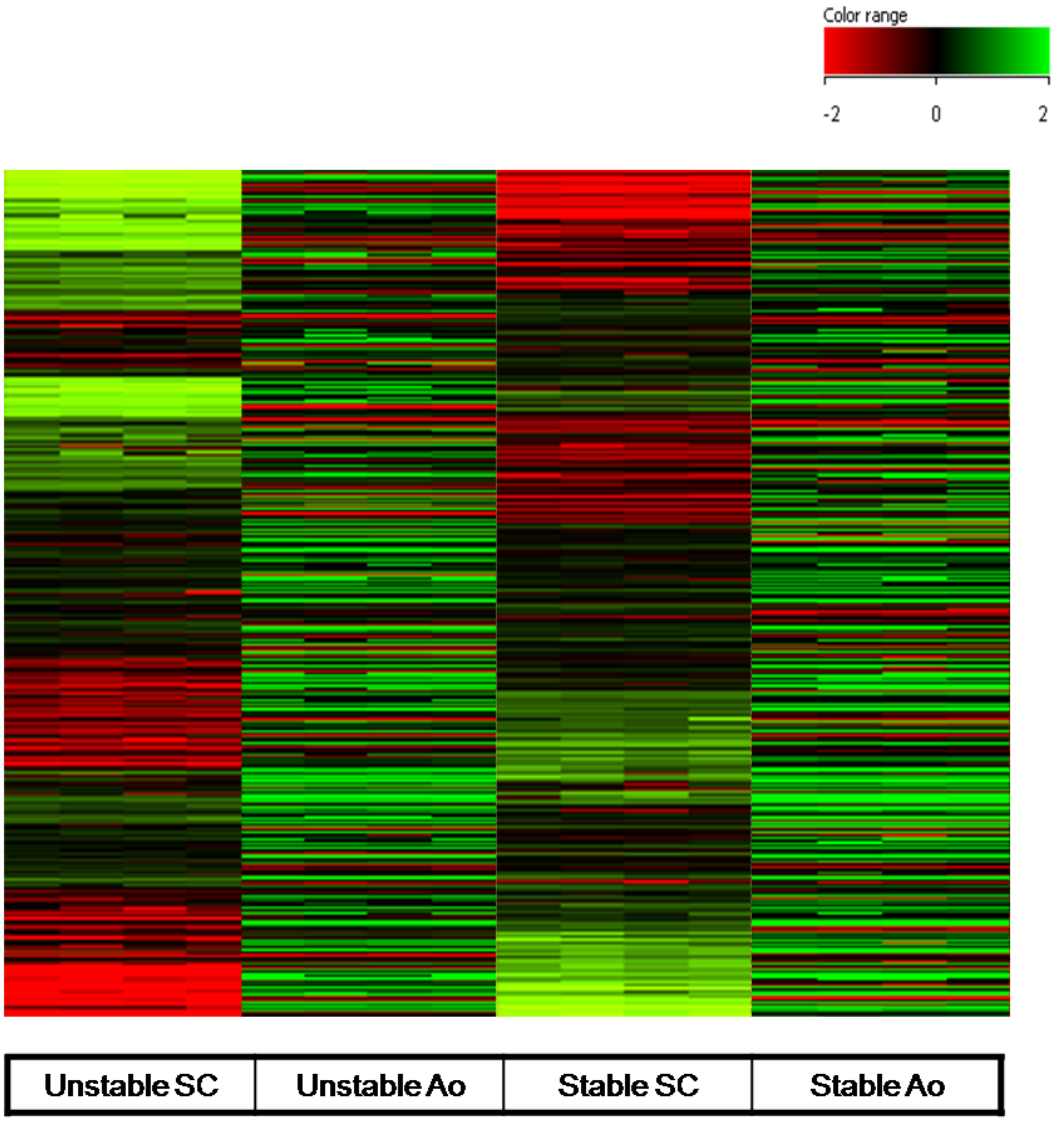

Figure 1. Different gene expression in Ao and CS of patients with ACS and SA. 684 up-regulated (green) and 283 down-regulated (red). ($P \leq 0.01$, and ≥2-fold, or ≤-2-fold difference).

Gene Symbol	Gene Name	p value	Fold change
SELE	selectin E	3.01E-10	19,661016
CXCL1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	1.05E-19	8,485691
CCL2	chemokine (C-C motif) ligand 2	2.75E-20	6,3248153
PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	3.53E-28	5,8133426
CD86	CD86 molecule	1.34E-06	4,9321218
CXCL2	chemokine (C-X-C motif) ligand 2	3,95E-25	6,6117725
RELB	v-rel reticuloendotheliosis viral oncogene homolog B	1.63E-20	4,7633815
FOSB	FBJ murine osteosarcoma viral oncogene homolog B	4,75E-15	4,325817
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	3.81E-17	4,0123706
ID1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	1.14E-15	3,679152
CXCL3	chemokine (C-X-C motif) ligand 3	2,48E-13	2,7130814
NFKBIZ	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	5.64E-23	3,3618975
FOS	FBJ murine osteosarcoma viral oncogene homolog	1.12E-16	3,2505574
FGF18	fibroblast growth factor 18	5.17E-12	3,2300966
IL7R	interleukin 7 receptor	8.03E-07	3,1095386
ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	4.85E-20	3,1251428
CD83	CD83 molecule	2.72E-12	2,8649588
IL8	interleukin 8	1.22E-17	2,8357644
IL11	interleukin 11	4.98E-11	2,8224506
HBEGF	heparin-binding EGF-like growth factor	1.68E-18	2,3418474
TRAF1	TNF receptor-associated factor 1	1.02E-09	2,4518566
NFKBIE	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	4.15E-24	2.3899362
VEGFA	vascular endothelial growth factor A	1.12E-14	2,3613784
FOSL2	FOS-like antigen 2	7.37E-21	2,5525434
MAP3K8	mitogen-activated protein kinase kinase kinase 8	2,69E-16	2,270313
IL3RA	interleukin 3 receptor, alpha (low affinity)	2,29E-10	2,2516959
IL6	interleukin 6	1.44E-08	2.20
VASP	vasodilator-stimulated phosphoprotein	7.61E-08	2,2024417
GPR68	G protein-coupled receptor 68	0.0041	2.16986
BMPR1B	bone morphogenetic protein receptor, type IB	5.94E-05	2.12
FBLN1	fibulin 1	3.95E-07	2,1075332
ICAM1	intercellular adhesion molecule 1	1.46E-20	2.6648185
PF4	platelet factor 4	0.0043	2,0621264

Gene Symbol	Gene Name	p value	Fold change
CXCR4	Chemokine (C-X-C Motif) Receptor 4	3.44E-26	- 4.4
IL10	Interleukin 10	2.63E-0.7	- 4.15
CCNG2	Cyclin G2	4.98E-12	- 3.5
CDKN1B	Cyclin-Dependent Kinase Inhibitor 1B	6.43E-24	- 2.95
CFH	Complement factor H	2.68E-13	- 2.59
IL33	Interleukin 33	2.39E-12	- 2.43
NAMPT	Nicotinamide phosphoribosyltransferase	6.88E-07	- 2.15
TBET	T-Cell-Specific T-Box Transcription Factor T-Bet	1.02 E-11	- 2.14
STAT1	Signal Transducer And Activator Of Transcription 1	1.36E-11	- 2.12
RAB5A	RAB5A, Member RAS Oncogene Family	5.37E-20	- 2.09
CCNG1	Cyclin G1	2.43E-11	- 2.04
SENP7	SUMO1/sentrin specific peptidase 7	1.30E-07	- 2.04
IL5	Interleukin 5	3.74E-08	- 2.03
VLDLR	Very Low Density Lipoprotein Receptor	1.18E-12	- 2.01
TNFSF10	Tumor Necrosis Factor (Ligand) Superfamily, Member 10	4.68E-15	- 2.00

Table 1. a) Up-regulated genes in unstable plaque. **b)** Down-regulated genes in unstable plaque

Gene Symbol	Gene Name	p value	Fold change
CCL2	Chemokine (C-C motif) ligand 2	2.75E-20	6.32
CXCL1	Chemokine (C-X-C motif) ligand 1	1.05E-19	8.49
IL8	Interleukin 8	1.22E-17	2.84
JUNB	Jun B proto -oncogene	1.07E-08	3.95
RASL10A	RAS-like, family 10, member A	2.60E-13	2.10
RASSF8	Ras association domain family	6.72E-06	2.34
PTGS2	Prostaglandin-endoperoxide synthase 2	3.53E-28	6.14

Table 2. IL17 signaling (p= 7,29E-4)

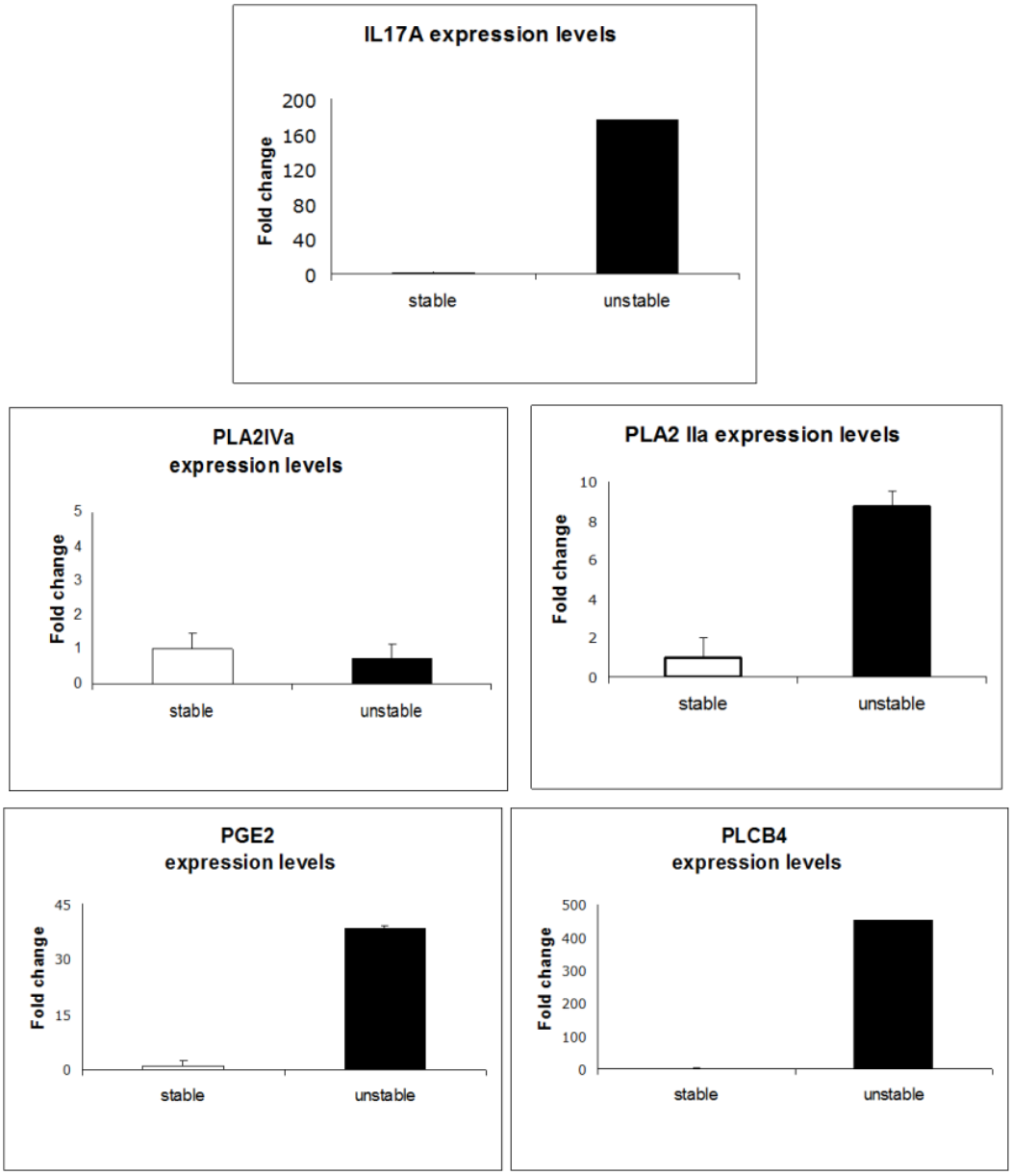

Figure 2. Gene expression in ECs stimulated in vitro for 12 hours with plasma obtained from the coronary sinus (CS) of patients with ACS, or patients with stable angina determined by real-time PCR analysis. Data are expressed as the mean ± SEM of 2 independent experiments. All samples were measured in triplicate.

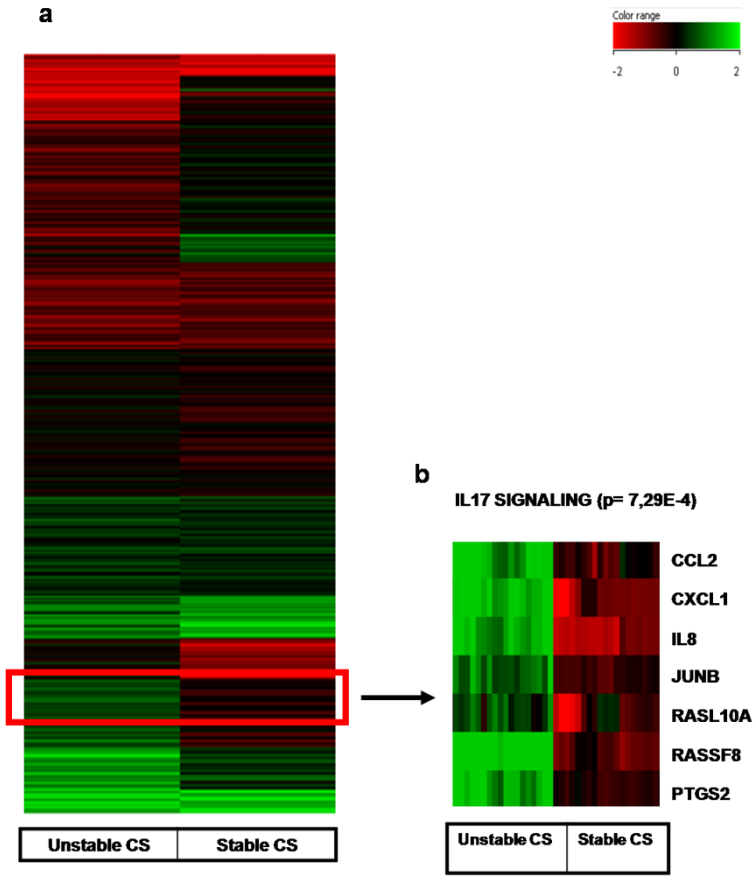

Figure 3. a) Heatmap obteined from ECs exposed to plasma obtained from CS of patients with unstable and stable angina normalized with ECs exposed to plasma obtained from Ao of the same patients; **b)** Heatmap of IL-17 pathway (p= 7,29E-4) obtained after IPA analysis.

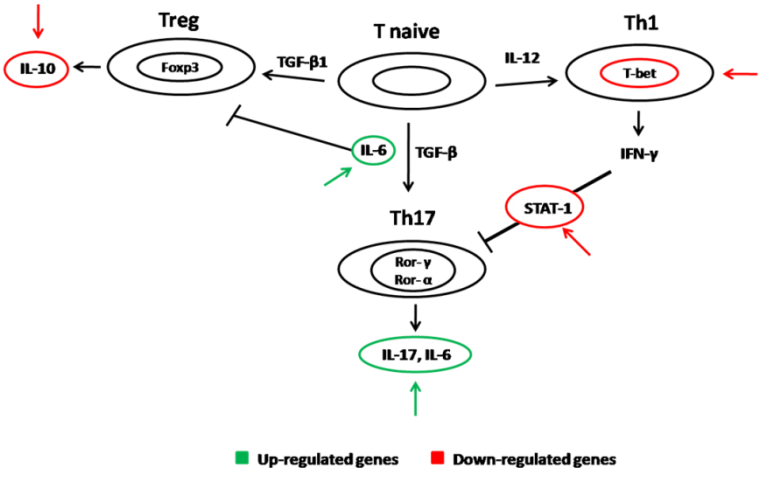

Figure 4. Th cell differentiation. Depending on the combination and level of cytokines, naive T cells develop into different lineages: Th1, Th2, Th17 and Treg. T-bet and STAT1 resulted down –regulated in our experiments, so they don't act as a suppressor on Th17 cells production. If we have a down-regulation in this point it is possible to suppose a greater Th17 cells production and a more IL-17 and IL-6 expression. IL-17 and IL-6 arrest Treg cells and IL-10 production (down-regulated in our microarray experiment).

References

1. Hansson GK, Libby P, Schönbeck U, et al. Innate and adaptive immunity in the pathogenesis of atherosclerosis. Circ Res. 2002 Aug 23;91(4):281-91.

2. Kovanen PT. Role of mast cells in atherosclerosis. Chem Immunol. 1995;62:132-70.

3. Andersson J, Libby P, Hansson GK. Adaptive immunity and atherosclerosis. Clin Immunol. 2010 Jan;134(1):33-46.

4. Golino P, De Palma R, D'Aiuto E, Cirillo G, et al. Cytokine and chemokine expression profile within the coronary circulation of patients with acute coronary syndromes. Journal of the American College of Cardiology. 2010;55:A106.E992-A106.E992.

5. Kimura A, Naka T, Kishimoto T. Il-6-dependent and -independent pathways in the development of interleukin 17-producing t helper cells. *Proc Natl Acad Sci U S A*. 2007;104:12099-12104.

6. Stumhofer JS, Laurence A, Wilson EH, Huang E, Tato CM, Johnson LM, Villarino AV, Huang Q, Yoshimura A, Sehy D, Saris CJ, O'Shea JJ, Hennighausen L, Ernst M, Hunter CA. Interleukin 27 negatively regulates the development of interleukin 17-producing t helper cells during chronic inflammation of the central nervous system. *Nat Immunol*. 2006;7:937-945.

7. Kimura A, Kishimoto T. Th17 cells in inflammation. *Int Immunopharmacol*. 2011;11:319-322.

8. Cheng X, Yu X, Ding YJ, Fu QQ, Xie JJ, Tang TT, Yao R, Chen Y, Liao YH. The th17/treg imbalance in patients with acute coronary syndrome. *Clin Immunol*. 2008;127:89-97.

9. Erbel C, Chen L, Bea F, Wangler S, Celik S, Lasitschka F, Wang Y, Böckler D, Katus HA, Dengler TJ. Inhibition of il-17a attenuates atherosclerotic lesion development in apoe-deficient mice. J Immunol. 2009;183:8167-8175

10. Erbel C, Dengler TJ, Wangler S, Lasitschka F, Bea F, Wambsganss N, Hakimi M, Böckler D, Katus HA, Gleissner CA. Expression of il-17a in human atherosclerotic lesions is associated with increased inflammation and plaque vulnerability. Basic Res Cardiol. 2011;106:125-134.

11. Kimura A, Naka T, Kishimoto T. Il-6-dependent and -independent pathways in the development of interleukin 17-producing t helper cells. Proc Natl Acad Sci U S A. 2007;104:12099-12104.

12. Stumhofer JS, Laurence A, Wilson EH, Huang E, Tato CM, Johnson LM, Villarino AV, Huang Q, Yoshimura A, Sehy D, Saris CJ, O'Shea JJ, Hennighausen L, Ernst M, Hunter CA. Interleukin 27 negatively regulates the development of interleukin 17-producing t helper cells during chronic inflammation of the central nervous system. Nat Immunol. 2006;7:937-945.

13. Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, Blank RB, Meylan F, Siegel R, Hennighausen L, Shevach EM, O'shea JJ. Interleukin-2 signaling via stat5 constrains t helper 17 cell generation. Immunity. 2007;26:371-381.

14. Kimura A, Kishimoto T. Th17 cells in inflammation. Int Immunopharmacol. 2011;11:319-322.

SOCIETÀ ITALIANA DI FARMACOLOGIA