

Acute Coronary Syndromes

Enhanced T-Helper-1 Lymphocyte Activation Patterns in Acute Coronary Syndromes

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OBJECTIVES	We sought to determine whether different stages of coronary artery disease (CAD) are associated with distinct differentiation patterns of activated T cells.
BACKGROUND	Atherosclerosis is an inflammatory disease. However, little is known about specific inflammatory cell activation in atherosclerosis, for example, the T-helper (Th)1/Th2-balance.
METHODS	We studied 18 patients with stable angina (SA), 28 patients with acute coronary syndrome (ACS) (16 with unstable angina pectoris and 12 with ST-segment elevation myocardial infarction), 19 patients with unheralded myocardial infarction (UH), and 16 control patients. Cytokine patterns and transcription factor signaling pathways of circulating T cells were characterized using reverse transcription polymerase chain reaction and flow cytometry.
RESULTS	Although interferon (IFN)- γ^+ /CD3 $^+$ T cells were ~2-fold greater in patients with SA or UH than in control subjects, there was a massive expansion of Th1 cells in patients with ACS ($p < 0.001$). This increase was paralleled by significantly increased mRNA transcript levels for signal-transducer-and-activator-4 (ACS 1.17 ± 0.14 relative units [RU]; control patients 0.44 ± 0.09 RU; SA 0.67 ± 0.12 RU; UH 0.61 ± 0.17 RU), interleukin-2 (ACS 1.55 ± 0.51 RU; control patients 0.21 ± 0.09 RU; SA 0.54 ± 0.18 RU; UH 0.45 ± 0.16 RU), and IFN- γ in ACS (1.27 ± 0.39 RU; control patients 0.35 ± 0.09 RU; SA 0.58 ± 0.11 RU; UH 0.53 ± 0.24 RU; $p < 0.002$). Th2 and Th0 cells were not different across patient subsets. The burden of CAD was identical between SA (1.4 ± 0.2 diseased vessels, $68 \pm 13\%$ diameter stenosis) and ACS (1.4 ± 0.2 diseased vessels, $64 \pm 17\%$ diameter stenosis) but significantly greater in patients with UH (2.5 ± 0.5 diseased vessels, $95 \pm 7\%$ diameter stenosis; $p < 0.05$).
CONCLUSIONS	Patients with UH have a greater burden of obstructive CAD than SA but no greater T-cell activation. Patients with ACS have the same extent of CAD than SA but significantly greater activation of Th1 cells that may contribute to the increasing instability. Differences in circulating Th1 cells might indicate different pathogenic components, leading to ACS and UH. (J Am Coll Cardiol 2005;45:1939–45) © 2005 by the American College of Cardiology Foundation

Coronary artery disease (CAD) can be considered as an interplay between luminal obstruction and intramural inflammation (1). The former may lead to diminished fractional flow reserve, supply-demand mismatch, myocardial ischemia, and infarction. Inflammation can induce a cascade of events that amplify immune cell infiltration, platelet activation and adhesion, plaque rupture, and intermittent arterial occlusion, leading to unstable angina pectoris (UA) or myocardial infarction (2–6). T cells in particular exert proatherogenic and plaque-destabilizing influences and are present in human atheroma (7,8). However, the activation of inflammatory pathways in CAD is not confined to the coronary lesions but includes stimulation of circulating monocytes and lymphocytes (6,9–11). On activation, T lymphocytes differentiate into T-helper

(Th)1 and Th2 subsets, characterized by differences in cytokine repertoire, adhesion mechanisms, effector function profile, and local immune response. Th1 cells express a distinct cytokine profile, including interferon (IFN)- γ , tumor necrosis factor- β , and interleukin (IL)-2, whereas Th2 cells primarily secrete IL-4 and IL-10 (12–14). Factors in the signal-transducer-and-activator-of-transcription (STAT) protein family regulate Th1 and Th2 cell differentiation. It has been hypothesized that STAT4 controls IL-12-mediated Th1 differentiation, whereas STAT6 controls IL-4-initiated Th2 development (15–18).

Lesions in patients across the spectrum of clinical coronary syndromes possess distinct patterns of occlusion, inflammatory cell infiltration, and the presence of immune mediators. However, the distinction among patients symptomatic with stable angina pectoris (SA), acute coronary syndrome (ACS), those who progress to myocardial infarctions (i.e., ST-segment elevation myocardial infarction [STEMI]), and those whose infarctions are entirely asymptomatic and unheralded (UH) remains unclear. Previous reports have demonstrated different immune activation patterns between patients with ACS and those with UH (2,10,19), although differences in T-cell activation patterns

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Abbreviations and Acronyms

ACS	= acute coronary syndrome
CAD	= coronary artery disease
CK	= creatine kinase
CRP	= C-reactive protein
FACS	= fluorescence-activated cell sorter
IFN	= interferon
IL	= interleukin
PBS	= phosphate-buffered saline
SA	= stable angina pectoris
STAT	= signal transducer and activator of transcription
STEMI	= ST-segment elevation myocardial infarction
Th	= T-helper
UA	= unstable angina pectoris
UH	= unheralded myocardial infarction

between these two entities have not been demonstrated. Therefore, we determined activation patterns of circulating T cells in patients with SA and ACS and compared them with circulating cells in patients with UH and control patients without evidence of CAD.

METHODS

Study patients. This investigation was performed with approval by the ethics committee of the Ludwig-Maximilians-University, Munich, Germany. Informed consent was obtained from all subjects. Sixty-five of 119 consecutive patients admitted to our hospital with a diag-

nosis of CAD were included into the study. Blood samples were obtained from all patients in the recumbent position with a 21-gauge needle via antecubital venepuncture as soon as possible after admission. Concentrations of C-reactive protein (CRP), white blood cell count, cholesterol, glucose, troponin-I, and creatine kinase (CK) were measured according to routine protocols.

Patients were grouped by presentation (Table 1). A total of 18 patients had clinical evidence of Canadian Cardiovascular Society class II and III SA (group I) and at least one coronary artery stenosis detected at angiography (>50% diameter stenosis). A total of 28 patients were admitted to the coronary care unit of the University Hospital Grosshadern, Munich, Germany, with a diagnosis of ACS and onset of ischemic chest pain at rest within the preceding 6 h. A total of 16 of these patients (group II) fell into Braunwald class II or III, that is, exhibiting transient ST-segment depression and/or T-wave inversion but no evidence of myocardial infarction by ST-segment elevation or rise in CK or CK-myocardial band (34 U/l; range, 16 to 53 U/l). Troponin I was detected in five of the patients with UA, albeit at levels <10.5 ng/ml (6.9; range, 0.9 to 10.5 ng/ml). The remaining 12 patients with ACS had STEMI (group III), with elevated levels of CK (394 U/l; range, 297 to 521 U/l), CK-myocardial band mass and troponin I (87 ng/ml; range, 34 to 123 ng/ml). A total of 19 patients who presented within 6 h of the onset of the first prolonged chest pain with ST-segment elevation and increases in CK (392

Table 1. Demographic Characteristics and Biologic Parameters of Patients and Control Subjects

	Control (n = 16)	SA (n = 18)	UA (n = 16)	STEMI (n = 12)	UH (n = 19)
Age, yrs	56 ± 11	66 ± 13	72 ± 10	65 ± 14	62 ± 13
Men	8	9	9	6	10
Risk factors					
Hypertension	—	12 (67)	10 (63)	8 (67)	12 (63)
Smoking	—	8 (44)	8 (50)	5 (42)	8 (42)
Cholesterol, mg/dl	181 ± 27	179 ± 22	183 ± 27	192 ± 23	194 ± 36
LDL-cholesterol, mg/dl	120 ± 30	132 ± 36	127 ± 29	129 ± 34	142 ± 24
HDL-cholesterol, mg/dl	45 ± 3	43 ± 8	41 ± 8	40 ± 9	37 ± 7
Lp _a , mg/dl	30 ± 25	24 ± 38	35 ± 46	35 ± 29	37 ± 31
Glucose, mg/dl	109 ± 9	114 ± 8	113 ± 12	118 ± 19	116 ± 13
Laboratory parameters on admission					
CRP, mg/dl	0.1 (0–0.3)	0.2 (0–0.5)	1.0 (0.3–3.6)	0.9 (0.1–4.5)	0.7 (0.1–2.5)
White blood count, U/I	7.1 ± 0.3	7.2 ± 0.5	7.4 ± 1.0	7.3 ± 2.2	7.5 ± 3.8
Lymphocytes, %	32 ± 3.5	38 ± 2.5	33 ± 4.0	33 ± 1.9	35 ± 1.5
CK, U/I	35 (14–51)	33 (20–43)	34 (16–53)	394 (297–521)*	392 (213–629)*
Troponin I, ng/ml	n.d.	n.d.	6.9 (0.9–10.5)	87 (34–123)†	79 (14–187)†
Extension of coronary artery disease					
Number of diseased vessels	—	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	2.5 ± 0.5*
% diameter stenosis	—	68 ± 13	66 ± 16	62 ± 19	95 ± 7‡
Medication on admission					
Beta-blockers	—	12 (67)	11 (69)	9 (75)	14 (74)
Aspirin	—	16 (89)	12 (75)	10 (83)	15 (79)
ACE inhibitors	—	8 (44)	8 (50)	7 (58)	10 (53)
Lipid-lowering drugs	—	14 (78)	12 (75)	9 (75)	14 (74)

Values are expressed as mean ± SD, median (range), or number (percentage). *p < 0.01; †p < 0.05 UA vs. STEMI and UH; ‡p < 0.05.

ACE = angiotensin-converting enzyme; CK = creatine kinase; CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; Lp_a = lipoprotein (a); n.d. = not detectable; SA = stable angina pectoris; STEMI = ST-segment elevation myocardial infarction; UA = unstable angina pectoris; UH = unheralded myocardial infarction.

U/l; range, 213 to 629 U/l) and troponin I (79 ng/ml; range, 14 to 187 ng/ml) with no history of ischemic heart disease or chronic SA and no acute events or worsening of symptoms in the last six months were identified as group IV (patients with UH). Identification of STEMI and UH followed the recent consensus document of the American Heart Association and the American College of Cardiology (20). All patients underwent coronary angiography during hospitalization.

Exclusion criteria for this study included previous myocardial infarction within six months (15 patients), admission more than 6 h from onset of symptoms (18 patients), inflammatory conditions likely to be associated with an acute-phase response (8 patients), autoimmune disease (4 patients), and neoplastic disease (9 patients). None of the included patients had advanced liver disease, renal failure, or valvular heart disease. Group V included 16 healthy volunteers with no clinical signs of CAD and who were without coronary risk factors. All of these control patients had normal electrocardiography and echocardiograms and no evidence of atherosclerosis by carotid artery sonography.

Isolation of CD4⁺-lymphocytes, mRNA isolation, and semiquantitative reverse transcription polymerase chain reaction (RT-PCR). Isolation of mononuclear cells from heparinized blood was performed by Ficoll density gradient centrifugation (Becton Dickinson, Heidelberg, Germany). After isolation, mononuclear cells were washed twice in phosphate-buffered saline (PBS) and re-suspended at 10⁷ cells/ml in PBS. CD4⁺ T lymphocytes were isolated using MACS-CD4⁺ beads following the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). Dynabeads Oligo(dT)₂₅ were used to isolate mRNA (Dyna, Oslo, Norway). First-strand cDNA synthesis was performed using oligo(dT)₁₂₋₁₈ and a superscript preamplification kit (Invitrogen, Karlsruhe, Germany). Primers for PCR were designed with MacVector 5.0 (Oxford Molecular Scientific, Sunnyvale, California), and logarithmic amplification ranges established as described previously (21). For each individual primer pair, specific annealing temperature and cycle number were optimized by serial annealing studies, PCR cycle studies, and cDNA dilution studies (Table 2). Duplicate samples were amplified with AmpliTaq Gold DNA polymerase (Applied Biosystems, Darmstadt, Germany). After electrophoresis, agarose gels were incubated for 20 min at room temperature with SYBRGold (Molecular Probes, Eugene, Oregon). Fluorescence was induced by UV transillumination (300 nm) and samples quantified using a BioRad FluorS-Multiimager and Quantity-One Software (BioRad, Munich, Germany) for densitometric analysis. A negative control and an internal standard were run on each gel. Transcript levels were normalized to glyceraldehyde-3-phosphate dehydrogenase and reported as mean ± SD.

Intracellular staining and flow cytometry. Heparinized whole blood was diluted (1:1) with RPMI1640 and stimulated with 1 μg/ml phorbol 12-myristate 13-acetate and

Table 2. Primer Sequences, Sequence Accession Numbers, Annealing Temperatures, and Cycle Numbers

GAPDH (M33197), 68°C/25 cycles	
Sense	AAGGTCGGAGTCAACGGATTTGG
Antisense	AGGCATTGCTGATGATCTTGAGGC
IFN-γ (M29383), 59°C/33 cycles	
Sense	CATCCAAGTGATGGCTGAACTGTGC
Antisense	AAGCACTGGCTCAGATTGCAGGC
IL-2 (V00564), 62°C/36 cycles	
Sense	GCATTGCACTAAGTCTTGCACTTG
Antisense	AGCACTTCCTCCAGAGGTTTGAG
IL-4 (M13982), 66°C/34 cycles	
Sense	CGTAACAGACATCTTTGCTGCCTCC
Antisense	TCTTTGCTGCCTCAAGAACAAC
IL-10 (M57627), 68°C/32 cycles	
Sense	AACCAAGACCCAGACATCAAGGCG
Antisense	AAGCACTTCTTCACCTGCTCCACG
STAT4 (L78440), 66°C/31 cycles	
Sense	GTGGTTGAGCGACAGCCATGTATG
Antisense	TGGATGCCAAGCATTAGGTAAGT
STAT6 (U16031), 66°C/28 cycles	
Sense	GCAGTTCAACAAGGAGATCCTGCTG
Antisense	TTTCCACGGTCATCTTGATGGTAGC

GAPDH = glyceraldehyde-3-phosphate dehydrogenase; IFN = interferon; IL = interleukin; STAT = signal transducer and activator of transcription.

50 μg/ml ionomycin in 500 μg/ml of Brefeldin A (all Sigma-Aldrich, Poole, United Kingdom). Samples were incubated for 4 h at 37°C/5% CO₂, which are conditions that maximally activate T cells and accumulate intracellular cytokine (22). Stimulated and unstimulated (Brefeldin A alone) samples were then stained with CD3-PerCP antibody (mouse IgG1 anti-human, clone SK7, Pharmingen, San Jose, California) at room temperature for 15 min. Erythrocytes were lysed with fluorescence-activated cell sorter (FACS) lysis buffer (Becton Dickinson Immunocytometry Systems [BDIS], San Jose, California). Lymphocytes were washed in PBS 0.5% bovine serum albumin and 0.1% sodium azide and then incubated in FACS permeabilization solution (BDIS) for 10 min. Permeabilized cells were washed twice and stained with IFN-γ-fluorescein isothiocyanate (mouse IgG_{2b} anti-human, clone 25723.11) and IL-4-phycoerythrin (mouse IgG₁ anti-human, clone 3010.211) antibodies (or IgG_{2b} fluorescein isothiocyanate/IgG₁ phycoerythrin isotype-control, all obtained from Pharmingen) for 30 min at room temperature. Finally, samples were washed once and then resuspended in 1% paraformaldehyde/PBS before acquisition on a FACScan (BDIS). At a threshold set to exclude non-CD3⁺ cells, 10,000 events were collected by Cellquest software (BDIS). The population of CD3⁺ T lymphocytes was gated by forward and side-scatter characteristics. Isotype controls enabled correct compensation and confirmed antibody specificity. Unstimulated samples set the quadrants for two-dimensional dot plot analysis. Results were expressed as percentage of all CD3⁺ T-cell subtypes staining positive for the specific cytokine. Intra-assay variability was assessed by independently activating, staining, and analyzing two aliquots of the same blood sample.

Statistical analysis. Statistical analysis was performed with JMP statistical software (SAS Institute Inc., Cary, North Carolina). The Shapiro-Wilk test was used to determine whether values followed a normal distribution. Because CRP, low-density lipoprotein cholesterol, troponin I, and CK values did not follow a normal distribution, comparisons between groups were conducted using the Wilcoxon test for two and the Kruskal-Wallis test for more than two groups. The remaining continuous variables were compared using the Student *t* tests for two and one-way analysis of variance for more than two groups. Categorical variables were compared by the chi-square test. Significant values were not adjusted for multiple comparisons. C-reactive protein, low-density lipoprotein cholesterol, troponin I, and CK values are expressed as median and range, the remaining variables are expressed as mean \pm standard deviation or number (%). A Spearman correlation determined relations between mRNA transcript levels and frequencies of IFN- γ -producing T cells. A p value of <0.05 was considered statistically significant.

RESULTS

Patient characteristics. There were no significant differences in age, gender, lipid or blood glucose, frequency of smoking, or use of medications in patients with SA, ACS, or UH. Patients with ACS or UH were treated with standard antianginal medications, intravenous heparin, aspirin, and nitroglycerin. Patients with UH had a greater extent of CAD (2.5 ± 0.5 diseased vessels; $p < 0.01$) and higher degree of diameter stenosis ($95 \pm 7\%$; $p < 0.05$) than those with SA (1.4 ± 0.2 diseased vessels; $68 \pm 13\%$), UA (1.3 ± 0.2 diseased vessels; $66 \pm 16\%$), or STEMI (1.4 ± 0.2 diseased vessels; $62 \pm 19\%$) (Table 1, Fig. 1).

Assessment of frequencies of IFN- γ - and IL-4-producing T cells. Patients with SA and UH showed raised levels of activated T cells compared with control patients; 1.9- and 1.7-fold greater than control values (Figs. 2 and 3), although only the patients with SA reached statistical significance ($p < 0.01$). Clinical instability of

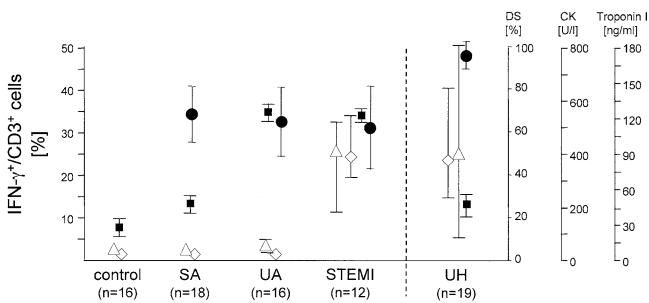


Figure 1. Frequency of interferon (IFN)- γ /CD3⁺ T cells in relationship to diameter of stenosis and to markers of myocardial damage. Results are shown as mean values \pm SD or as median and range. **Closed squares** = IFN- γ /CD3⁺ cells; **closed circles** = diameter stenosis (DS); **open diamonds** = creatine kinase (CK); **open triangles** = troponin I. SA = stable angina pectoris; STEMI = ST-segment elevation myocardial infarction; UA = unstable angina pectoris; UH = unheralded myocardial infarction.

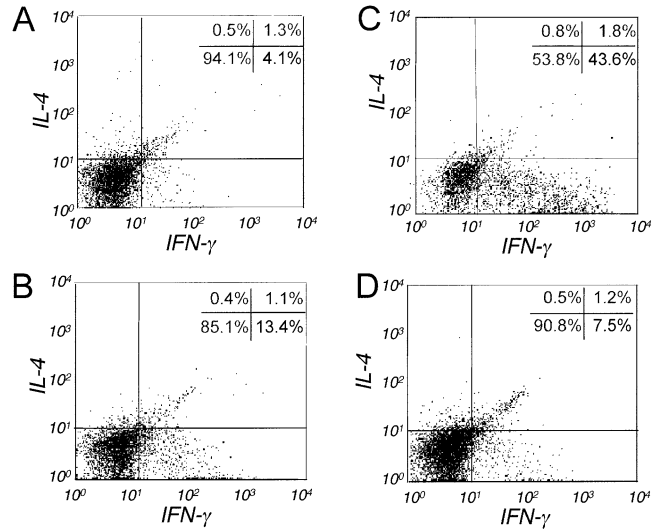


Figure 2. Representative plots from a control patient (A) and a patient with stable angina pectoris (B), acute coronary syndrome (C), and unheralded myocardial infarction (D). Ten thousand CD3⁺ cells were gated and analyzed for expression of interferon (IFN)- γ or interleukin (IL)-4. Each dot represents a CD3⁺ T cell. Numbers represent the percentage of cells in the quadrants, respectively.

coronary syndromes was associated with increased expansion of circulating IFN- γ ⁺/CD3⁺ lymphocytes in patients presenting with ACS (4.6-fold increase to controls, 2.4-fold to SA, and 2.7-fold to UH; $p < 0.0002$ vs. controls, $p < 0.001$ vs. SA and UH) (Figs. 2 and 3). There were no significant differences between patients with UA and patients with STEMI ($p = 0.68$) (Fig. 3) or between patients with high and low troponin levels ($p = 0.53$). In contrast, there were no significant differences in the frequencies of peripheral circulating Th2 cells (IL4⁺/CD3⁺ population) or Th0 cells (IL4⁺/IFN- γ ⁺/CD3⁺ population) between the groups (Fig. 2, Table 3).

Th0, Th1, and Th2 cytokine and STAT4 and STAT6 transcript levels. mRNA expression by RT-PCR followed protein detection by FACS analysis. Transcript levels for the Th1 signal transducer STAT4 were significantly higher in patients with UA (1.14 ± 0.11 relative units [RU]; $p <$

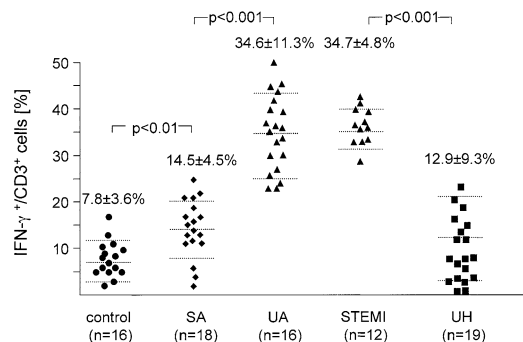


Figure 3. Peripheral blood mononuclear cells from control patients and from patients with stable angina pectoris (SA), unstable angina pectoris (UA), ST-segment elevation myocardial infarction (STEMI), and unheralded myocardial infarction (UH) were immunostained with antibodies against CD3 and interferon (IFN)- γ and analyzed by flow cytometry. **Symbols** = individual data; **dashed lines** = mean values \pm SD.

Table 3. No Expansion of Th2 or Th0 Cells in Patients With Different Stages of Atherosclerosis

	Control (n = 16)	SA (n = 18)	UA (n = 16)	STEMI (n = 12)	UH (n = 19)
Th2 cells (IL-4 ⁺ /CD3 ⁺)	0.9 ± 0.8%	1.7 ± 1.9%	0.8 ± 1.2%	1.1 ± 0.9%	1.1 ± 1.5%
Th0 cells (IL-4 ⁺ /IFN-γ ⁺ /CD3 ⁺)	0.9 ± 0.7%	1.2 ± 1.2%	1.0 ± 1.0%	0.9 ± 0.8%	1.4 ± 1.9%

Data are presented as mean percentage ± SD.

IFN = interferon; IL = interleukin; SA = stable angina pectoris; STEMI = ST-segment elevation myocardial infarction; Th = T-helper; UA = unstable angina pectoris; UH = unheralded myocardial infarction.

0.002) and STEMI (1.2 ± 0.18 RU; $p < 0.002$) than in all other groups (control, 0.44 ± 0.09 RU; SA, 0.67 ± 0.12 RU; UH, 0.61 ± 0.17 RU). Patients with SA and UH had increased mRNA levels of STAT4 compared with controls, but only patients with SA reached statistical significance ($p < 0.05$) (Fig. 4). Similar results were observed for the Th1 marker cytokines IL-2 and IFN-γ, with increased mRNA transcript levels in the ACS group compared with SA, UH, and control patients ($p < 0.002$). Patients with SA and UH showed increased IL-2 and IFN-γ levels compared with control patients, although only patients with SA reached statistical significance ($p < 0.05$) (Fig. 4). There were no significant differences in relative transcript levels for the Th2 signal transducer STAT6 or for the Th2 marker cytokines IL-4 and IL-10 in any of the patient groups (Fig. 4). A strong correlation was noted between the frequency of peripheral circulating IFN-γ⁺/CD3⁺ lymphocytes and mRNA transcript levels of IFN-γ ($r = 0.68$; $p < 0.002$) and STAT4 ($r = 0.88$; $p < 0.001$) across all patient groups (Fig. 5).

DISCUSSION

Evidence is accumulating that the destabilization of atherosclerotic disease is associated with a systemic immune activation (3–6,10). However, factors responsible for the transition from stable to unstable coronary disease remain incompletely understood. Coronary lesions infiltrated with immune cells, including macrophages and T lymphocytes, are thought to be most unstable. However, it is unclear whether inflammation precedes and perhaps triggers plaque rupture, platelet activation, coronary occlusion, and resultant myocyte ischemia/necrosis or is a side effect of these pathologic processes. In this regard, the current investigation suggests two important novel observations. First, although patients with stable CAD and ACS have the same extent of CAD, patients with ACS exhibit an expansion of peripheral circulating activated CD3⁺ cells, phenotypically characterized as Th1 lymphocytes. More importantly, increased activation of Th1 cells in these patients is observed before myocardial damage and remains elevated in patients with STEMI. Second, patients presenting with entirely UH have a greater burden of atherosclerotic disease and a high grade of coronary artery occlusion, but their T-cell activation levels comparable with patients with SA.

The results of our study are consistent with previous findings that an inflammatory stimulus, in addition to the macroscopic obstructive disease, may be present throughout

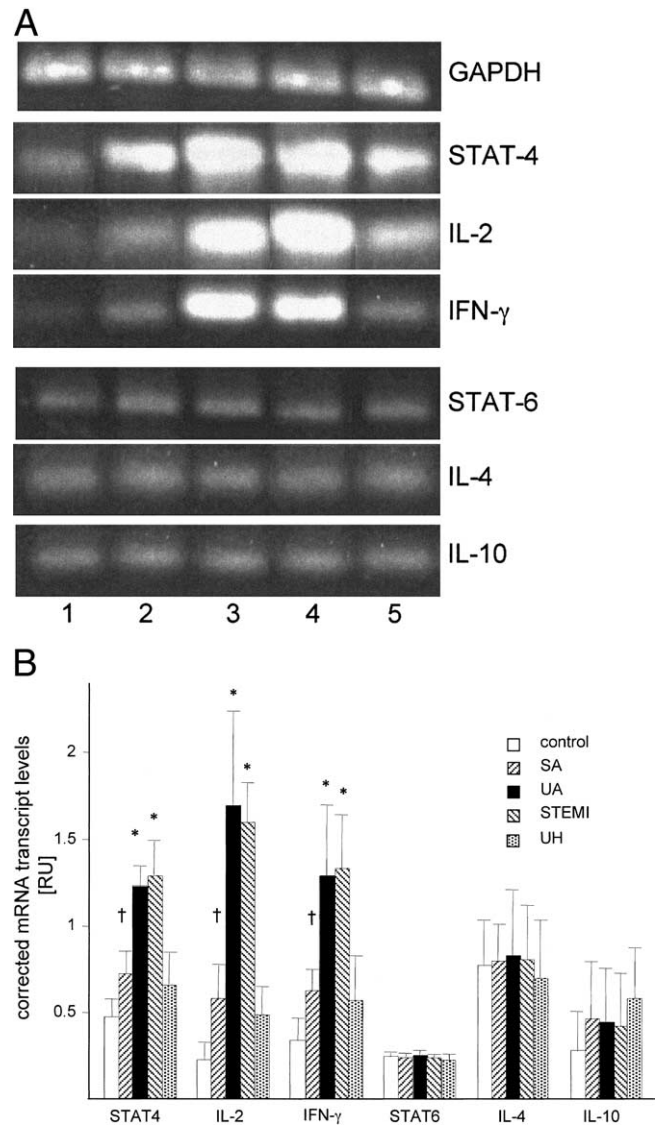


Figure 4. (A) Representative examples of reverse transcription polymerase chain reaction amplification products of each group. 1, control; 2, stable angina; 3, unstable angina; 4, ST-segment elevation myocardial infarction; 5, unheralded myocardial infarction. (B) Reverse transcription polymerase chain reaction amplification was normalized against glyceraldehyde-3-phosphate dehydrogenase and is presented as mean corrected transcript levels ± SD in relative units (RU) of duplicate analysis of all patients in each group. * $p < 0.002$ vs. control, SA, and UH; † $p < 0.05$ vs. control patients. GAPDH = glyceraldehyde-3-phosphate dehydrogenase; IFN = interferon; IL = interleukin; SA = stable angina pectoris; STAT = signal transducer and activator of transcription; STEMI = ST-segment elevation myocardial infarction; UA = unstable angina pectoris; UH = unheralded myocardial infarction.

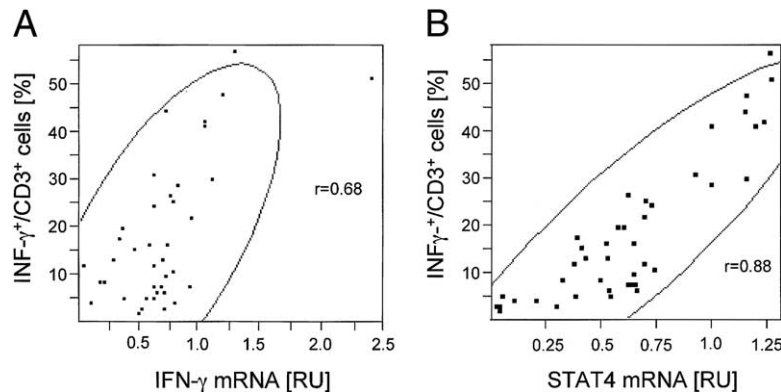


Figure 5. Spearman correlation of frequencies of circulating interferon (INF)- γ ⁺/CD3⁺ T cells and mRNA transcript levels of INF- γ (A) and signal transducer and activator of transcription 4 (B). Area of the density ellipse represents the 95% confidence interval. RU = relative units; STAT = signal transducer and activator of transcription.

the atherosclerotic disease process (23) and that a time-dependent activation of the immune system can be found in patients with UA (2,10). The existence of an acute inflammatory state in patients with UA is supported by other clinical studies demonstrating elevated levels of unspecific markers of inflammation, ILs and INF- γ , as well as activation of circulating T lymphocytes and monocytes (3–5,10,24). Several *in vivo* animal studies have stressed the importance of a Th1-driven immune response in atherosclerosis. The transfer of CD4⁺ T cells from atherosclerotic donors to immunodeficient mice aggravates the atherosclerotic process. In turn, T-cell transfer is accompanied by an elevation of systemic INF- γ levels (25). Furthermore, lesion development in mice prone to atherosclerosis can be inhibited by the pharmacologic blockade of Th1 or through IL-10 stimulation of Th2 differentiation, which counteracts Th1 differentiation (26,27). Moreover, data derived from patients with coronary heart disease have revealed that peripheral circulating T cells appear to change around the time of clinically manifest atherosclerotic disease (7,11,28,29) and that patients with spastic angina showed a preference toward a Th1 response (3,30).

To our knowledge, there are no reports on the relation between the signaling factors of the two T-cell subset programs (STAT4 and STAT6) and different stages of CAD. Our findings could be further proof that Th1 cells are activated throughout the evolution of CAD. It was previously shown that targeted deletion of the transcription factor STAT4 resulted in attenuated cardiac allograft vasculopathy in transgenic mice (31). Here, we show for the first time that SA and ACS are associated with the up-regulation of STAT4 transcript levels in peripheral circulating CD4⁺ T cells.

Notably, the extent of Th1 cell activation was significantly lower in UH patients with myocardial infarction without antecedent instability than in patients with ACS. Patients with UH were more likely to have multivessel disease and more significant stenoses of the infarct-related artery than patients with ACS. A missing correlation between extent of coronary artery stenosis and frequency of INF- γ ⁺/CD3⁺ T cells already

has been demonstrated (30), and the number of T cells present in human plaques relates to the overall plaque morphology, *i.e.*, low in more stable fibrous plaques but high in rupture prone lesions. T cells accumulate at the shoulder region of lesions where the cap is often thinnest, most heavily infiltrated with inflammatory cells, and most likely to rupture (32). Hence, T-cell activation may not be the driving force in patients with a substantial burden of fixed obstructive disease but is all the more essential in destabilization of vulnerable and susceptible metabolically active plaques. Characteristic histomorphologic features of vulnerable plaques include a high lipid content, increased numbers of inflammatory cells, and extensive neovascularization. T cells facilitate the propagation of the immune response via attraction and activation of leukocytes and macrophages, reduce smooth muscle cell population, and contribute to endothelial cell damage, partly because of an INF- γ -induced dysregulation of nitric oxide production (33–35). Activated T cells also inhibit matrix synthesis through the production of INF- γ and promote apoptotic processes within vulnerable plaques (33,36,37). Thus, plaques producing non-flow-limiting stenosis likely account for more plaque rupture and thrombosis than more severe stenosis (38,39).

Increases in inflammatory markers in patients with UA when compared with UH have been previously reported (2,10,19). Unlike patients with UH, those with UA exhibit higher levels of tissue factor expression than patients with SA (9). Furthermore, patients with UA showed higher levels of CRP, serum amyloid A protein, and IL-6 when compared with patients with UH (19,40).

Study limitations. This study was performed with a small number of patients and did not accumulate data sequentially with time. Moreover, the critical question remains as to whether Th1 cells are activated by the processes involved in plaque destabilization or whether they cause the destabilization to occur in the first place. Further studies might examine the kinetics of T-cell activation to underline the notion of transitory phenomenon and to determine whether the patients with UH, in particular, will demonstrate late T-cell activation.

Conclusions. The temporal relationship of Th1 expansion to indexes of myocardial damage supports the idea that activation of cellular immune responses and up-regulation of immune mediators contribute to plaque instability and are not simply a consequence of myocardial injury. Our observation of T-cell activation in patients with SA brings further evidence to the hypothesis that a triggering inflammatory stimulus is present throughout the atherosclerotic disease process (23). Therefore, STAT4-mediated immune processes and Th1 subtypes likely affect the evolution of ACS. In line with other studies, our findings suggest that the two clinical presentations of UA preinfarction and UH might be associated with different pathogenetic components of acute coronary occlusion.

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