

협심증환자의 혈장 염증성 지표 및 T-림프구의 활성화에 관한 연구

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Plasma Inflammatory Markers and T-Lymphocytes Are Activated in Angina Pectoris

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ABSTRACT

Background and Objectives : Inflammation and activation of immune cells play important roles in the pathogenesis of atherosclerosis. We investigated the activation status of plasma inflammatory markers and immune cells in angina patients. **Methods :** We analyzed the plasma level of C-reactive protein (CRP) as a marker of inflammation in 24 patients with angina pectoris (12 unstable angina, 12 stable angina), and 12 normal subjects. The degree of activation of peripheral blood monocytes was assessed by Northern analysis of pro-atherogenic cytokines and the activation status of T-lymphocytes was measured by flow-cytometric analysis of HLA-DR expression on T-cells. **Results :** Plasma level of CRP was highest in unstable angina patients (1.63 ± 0.70 mg/l) and lowest in the control subjects (0.22 ± 0.08 mg/l) ($p = 0.03$). We also observed a high correlation between CRP level and the occurrence of minor and major coronary events during 6 months of follow-up. The percentage of HLA-DR positive T-lymphocyte was significantly increased in the unstable angina patients ($26.8 \pm 1.4\%$) compared with that in the control ($14.7 \pm 1.2\%$) ($p = 0.0053$). When baseline levels of cytokine mRNA were measured in monocytes, the percentages of the patients expressing higher than normal levels of IL-8, IL-1b, MCP-1, and TF mRNAs was 37.5, 29.2, 33.3, and 37.5%, respectively ($p = 0.0143, 0.0371, 0.0233, \text{ and } 0.0143$, respectively). Basal mRNA levels of interleukin (IL)-8, tissue factor (TF), IL-1b and monocyte chemoattractant protein-1 (MCP-1) showed a strong correlation with each other

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($p < 0.01$ in all combination) but not with tumor necrosis factor (TNF)- or transforming growth factor (TGF)- 1. **Conclusion** : We observed increase in plasma CRP levels and activation of T-lymphocytes in angina patients. These results may help further classification of angina patients according to the activation of inflammatory markers and understanding the prognosis of the disease. (**Korean Circulation J 2000;30(1):56-65**)

KEY WORDS : Angina · Monocytes · T-lymphocytes · Inflammation · C-reactive protein.

서론

T-가

T- very late activation antigen-1 (VLA-1) HLA-DR . Hansson

T-가 VLA-1 HLA-DR

T-²⁹⁾

가²⁾³⁾가가²⁹⁾

T- , HLA-DR

T-가

CD4+ T- CD8+ T-³⁰⁾

tissue factor(TF)가

4)5) TF가

가

IL-1⁶⁾ IL-6,⁷⁾ IL-8,⁸⁾ granulocyte/macrophage colony stimulating factors(G-CSF, M-CSF, and GM-CSF),⁹⁾¹⁰⁾ monocyte chemoattractant protein-1(MCP-1),¹¹⁾¹²⁾ tissue factor(TF),¹³⁻¹⁵⁾ TGF- β ¹⁶⁻¹⁸⁾

IL-8, MCP-1, TF, TGF- β 1 mRNA

T-

TNF- , IL-1 ,

대상 및 방법

19-23) 대 상

12

12

C-reactive protein(CRP), serum amyloid A IL-6가

(coronary events)

가²⁴⁻²⁷⁾가

3

6

soluble intercellular adhesion molecule-1(sICAM-1)가

28)가

MO,) 15
1200 g
heparin PBS
Table 1 . 10 ml RPMI1640 1 - 3 × 10⁷
50% 37 CO₂ 1
3 5 RNA
30 ml (mRNA)
1 mg/l lipopolysaccharide(LPS) 3
RNA
혈장에서의 CRP 및 사이토카인의 농도 측정
Heaprin Northern blot 분석
- 70 . CRP CRP - 7 mg RNA formaldehyde가
Latex(Denka Seiken Co., ,) 가 hybridization
Hitachi 747 - 100(Hitachi Co.,) transfer membrane(Du Pont - NEN, Boston, MA,
31) <0.1mg/l) 10% dextran sulfate, 1% SDS,
0.058 g/ml NaCl, H₂O 5 ml
bottle RNA - transferred nylon membrane
단구세포의 분리 100 mg/ml denatured salmon sperrm DNA
30 ml heparin 800 g 5 65 2 prehybridization .
30 ml ph - [- ³²P]dCTP labelling cDNA sephadex G -
osphate buffered saline(PBS) Hi - 50 column cDNA probe 90 100
stopaque 1077(Sigma Diagnostics, Inc., St. Louis, 5 가 ml 1 × 10⁶cpm probe

Table 1. Characteristics of the study subjects

Group	Unstable angina (n = 12)	Stable angina (n = 12)	Normal (n = 12)
Age (yrs)	58.3 ± 11.2	60.0 ± 8.9	57.0 ± 10.3
Sex (male/female)	9 / 3	11 / 1	10 / 2
Body mass index (kg/m ²)	25.4 ± 3.2	22.2 ± 3.5	24.6 ± 2.8
Total cholesterol (mg/dl)	188.6 ± 30.2	193.5 ± 25.0	215.1 ± 23.6
HDL (mg/dl)	42.0 ± 12.7	44.2 ± 13.5	50.0 ± 12.9
LDL (mg/dl)	110.0 ± 26.3	103.0 ± 14.3	116.0 ± 25.3
Smoking (current/ex/non-smoker)	6 / 3 / 3	4 / 6 / 2	3 / 2 / 7
Hypertension	6	5	0
Diabetes mellitus	3	1	0
Previous medications			
Acetylsalicylic acid	3	5	0
HMG-CoA reductase inhibitor	2	3	0
-blockers	3	9	0
Calcium antagonists	2	2	0
Nitrates	2	2	0
ACE inhibitor	3	3	0

Age, body mass index, total cholesterol, HDL and LDL levels are expressed as mean ± SD

prehybridization buffer 가 . 65 over -
 night hybridization 2 × SSC, 0.1% SDS
 0.1 × SSC, 0.1% SDS 가
 20 5 wash . - 70 film
 American Type Culture Collection (Ma -
 nassas, VA,) cDNA
 mRNA
 densitometer
 GAPDH
 Table Fig.
 1

활성화된 T-림프구의 측정

FACS - vantage (Bec -
 ton - Dickinson, Mountain View, CA,)
 . 50 ml 0.5 mg - HLA -
 DR FITC 0.5 mg - CD3 - PE (Phar -
 mingen, San Diego, CA,) 가 50 ml
 (0.5% BSA 0.1% NaN₃ PBS)
 20 32)
 50 ml Cal - Lysis
 (CALTAC lab., Burlingame, CA,) 가
 5 , 1.5 ml 가
 10 . 2% parafo -
 rmaldehyde PBS
 3
 CD3
 T - HLA - DR
 T -
 통계처리
 mRNA T - HLA -
 DR
 (nonparametric Kruskal - Wallis ANOVA, Dun -
 net's multiple comparison test)
 mRNA
 Spearmans
 mRNA 가
 chi - square test

LPS mRNA
 paired t - test
 p < 0.05 (2 tailed)

결 과

Fig. 1 2 , 2
 2 Northern
 LPS mRNA TGF - 1
 가 (p < 0.005) (Table
 2). LPS
 . LPS TGF - 1
 mRNA LPS

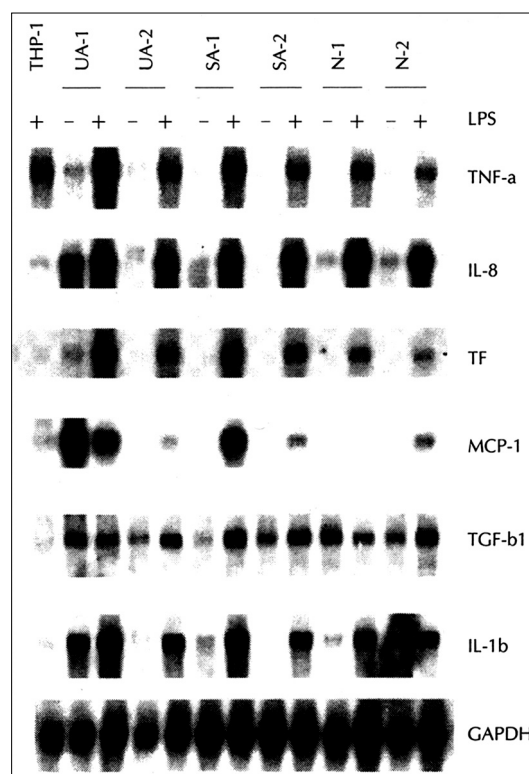


Fig. 1. Northern analysis of cytokine mRNA levels in peripheral blood monocytes isolated from LPS treated THP-1 cell line, two unstable angina patients (UA-1 and UA-2), two stable angina patients (SA-1 and SA-2), and two control subjects (N-1 and N-2) are shown. Total RNA was extracted from monocytes before and after LPS treatment for the analysis. One representative blot out of 6 blots with same arrangement is shown.

Table 2. Cytokine mRNA levels in monocytes isolated from angina patients and normal subjects

	Normal	Unstable angina	Stable angina
n	12	12	12
Basal level			
MCP-1	1.00 ± 0.17	7.00 ± 4.97	3.26 ± 1.38
TF	1.00 ± 0.24	1.94 ± 0.37	1.86 ± 0.43
IL-8	1.00 ± 0.12	1.09 ± 0.18	1.53 ± 0.30
IL-1	1.00 ± 0.13	1.33 ± 0.16	1.42 ± 0.19
TNF-	1.00 ± 0.21	1.27 ± 0.20	1.28 ± 0.23
TGF- 1	1.00 ± 0.12	0.94 ± 0.07	0.93 ± 0.07
After activation with LPS			
MCP-1	9.08 ± 1.79***	12.91 ± 2.78*	12.59 ± 3.10*
TF	11.86 ± 1.58***	13.49 ± 2.23***	12.13 ± 1.45***
IL-8	5.37 ± 0.55***	4.59 ± 0.54***	5.02 ± 0.50***
IL-1	3.07 ± 0.27***	2.77 ± 0.23*	2.95 ± 0.18**
TNF-	8.52 ± 1.17***	9.44 ± 1.99***	7.22 ± 0.70***

Data are expressed as mean ± S.E. of the cytokine signal/GAPDH ratio. Each values were adjusted to set the mean value of normal subjects (before activation) as 1

*p<0.005, **p<0.001, and ***p<0.0001 compared with the values before and after LPS stimulation

TGF- 1 mRNA 1 (0.22 ± 0.08 mg/l) (p
3 = 0.028 0.0226). CRP 가 0.3 mg/l
42%
IL - 8, IL - 1 , MCP - 1, TNF - (12 5) 85%(12 10)
TF mRNA (p = 0.035)
46%(12 6)
(Table 2). 가
IL - CRP 가
8, IL - 1 , MCP - 1 TF 37.5, 29.2, CRP 6
33.3 37.5% (p = 0.0143, 0.0371, 0.0233, . CRP 가 0.3 mg/dl
0.0143)(Fig. 2). MCP - 1, IL - 8, TF IL - 1 가 12 8
CRP
(p<0.01). TNF - TGF - 가
1 mRNA 가 (67% 0%, p=0.0012). 8
가 (Table 3, Fig. 3). 5
CD3 T - HLA - DR 1
(23.8 ± 1.4%) , 1
(14.3 ± 1.2%) (p= 1
0.0004). (20.2 ± 3.5%)
가
B -
(Fig. 4). IL - 1 , IL - 8, TNF -
CRP (1.63 MCP - 1
± 0.70 mg/l) (0.93 mg/l ± 0.31)

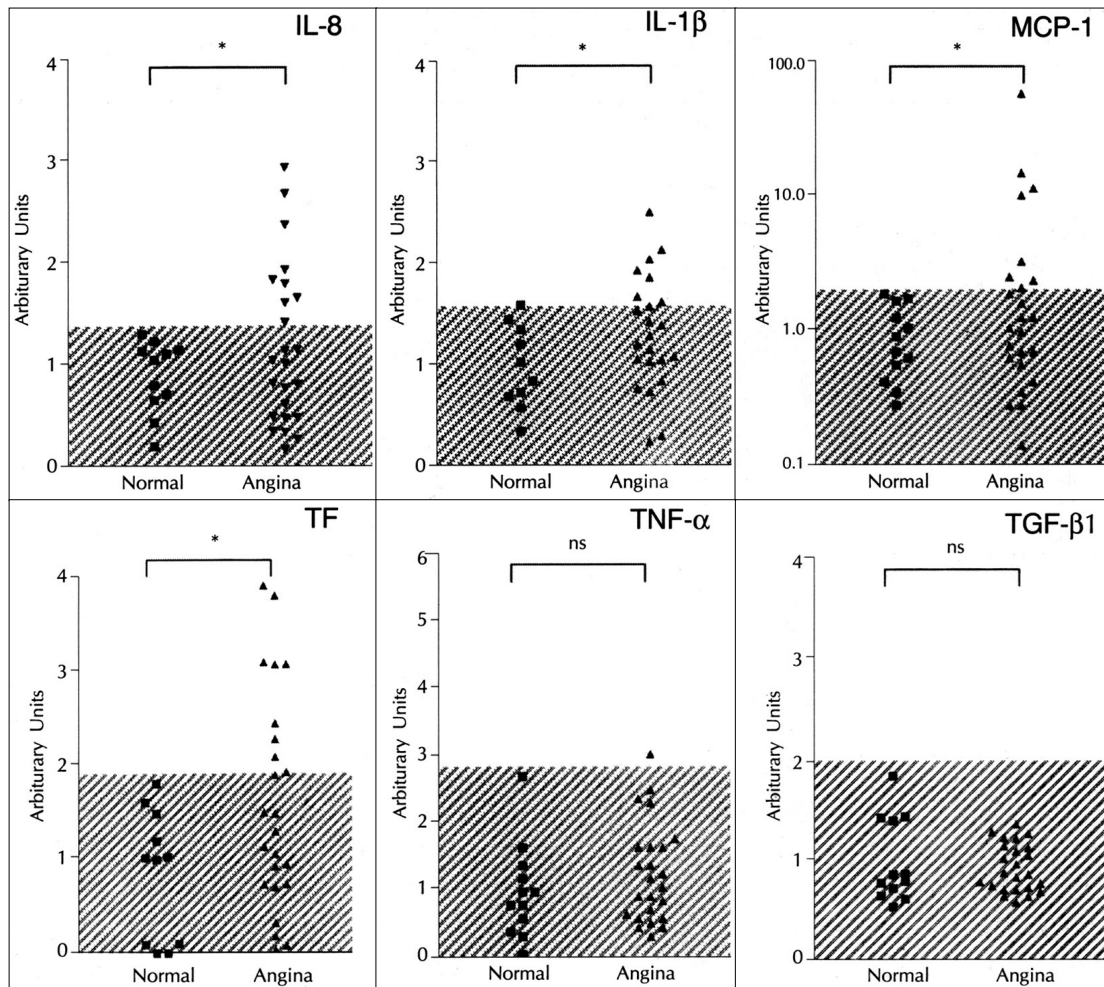


Fig. 2. Comparison of basal level of cytokines mRNAs between normal subjects and angina patients. Individual points represent the cytokine/GAPDH signal ratio. Shaded areas indicate normal range. *p<0.05.

Table 3. The correlation among the basal cytokines levels. Calculation was based on Spearman correlation analysis. Numbers represent Spearman correlation coefficient. Numbers in the parentheses represent p values

	IL-8	MCP-1	TF	IL-1	TNF-	TGF- 1
n	36	36	36	33	36	36
IL-8	-	0.68634 (<0.0001)	0.7119 (<0.0001)	0.73188 (<0.0001)	0.22457 (0.1879)	0.04989 (0.7726)
MCP-1		-	0.4249 (0.0098)	0.450177 (0.0086)	0.16557 (0.3345)	0.21709 (0.2034)
TF			-	0.68140 (<0.0001)	0.32768 (0.0511)	0.05510 (0.7496)
IL-1				-	0.22568 (0.2067)	0.34921 (0.0464)
TNF-					-	0.02578 (0.8813)
TGF- 1						-

CRP

T -

CRP

결 론 :

T -

가

요 약

연구목적 :

중심 단어 :

(C98 - 004)
(1998 - 021 - F00268)

가

대상 및 방법 :

12

12

12

C - reactive protein(CRP)

Northern

T -

HLA - DR

T -

결 과 :

C - reactive protein(CRP)

가 (1.63±0.70 mg/l)

가 (0.22±0.08 mg/l ; p=0.03).

CRP 6

T -

(HLA - DR

가

(26.8±1.4%) 가 (14.7
±1.2% ; p=0.0053).

mRNA

interleukin(IL) - 8, IL - 1 ,

monocyte chemoattractant protein - 1(MCP - 1), ti -
ssue factor(TF) 37.5, 29.2, 33.3,

37.5% 가 가
(p=0.0143, 0.0371, 0.0233, 0.0143). IL - 8,

TF, IL - 1 MCP - 1 mRNA
(

p<0.01) tumor necrosis factor(TNF) - tran -
sforming growth factor(TGF) - 1

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