

중이 진주종조직에서 ICAM-1, VCAM-1, ELAM-1의 발현에 관한 연구

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Expression of Adhesion Molecules(ICAM-1, VCAM-1, ELAM-1) in Middle Ear Cholesteatoma Tissues

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—ABSTRACT—

Background and Objectives : Cholesteatoma is associated with an intense inflammatory reaction with resultant destruction of tissue and bone. To evaluate the potential role of several adhesion molecules in the pathogenesis of cholesteatoma and its bone destruction, the authors studied mRNA expression of three adhesion molecules in the cholesteatoma tissue. **Materials and Methods** : We investigated mRNA expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and endothelial derived leukocyte adhesion molecule-1 (ELAM-1) in 20 cholesteatoma tissues as well as in the normal postaural skins using reverse transcription-polymerase chain reaction (RT-PCR). To evaluate the relationship between degree of bone destruction and adhesion molecules, we collected different portions of cholesteatoma according to the destructive status of middle ear bony structure in 5 cases and examined the expression of the mentioned factors to be compared. **Results** : Of 20 cholesteatoma, eighteen (90%) showed ELAM-1 and VCAM-1 gene expression and 8 (40%) showed ELAM-1 mRNA. There were much lower gene expressions in the normal postaural skin than the cholesteatoma. **Conclusion** : These results suggest that ICAM-1 and VCAM-1 play a central role in the regulation of the inflammatory disorders observed in cholesteatoma including migration, adhesion and proliferation of inflammatory cells. (J Clinical Otolaryngol 2002;13:65-72)

KEY WORDS : Cholesteatoma · Adhesion molecule.

서 론

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1 primer OLIGO . Ge-
 nomic DNA cDNA , elongation 70 1 , cycle
 intron exon primer 35 . 4
 . Southern hybridization probe (target sequence) 1.2% agarose
 primer intron total RNA
 exon 가 50~60% - actin agarose
 (Table 2). OLIGO southern hybridization
 primer probe
 primer
 RT-PCR product Fig. 1

ICAM - 1, VCAM - 1, ELAM - 1
 Southern hybridization
 - actin
 1.2% agarose TBE (50 mM
 Tris - borate, 1 mM EDTA pH 8,0) 3.3 V/cm
 1 , ultraviolet illuminator
 (UVP Inc, USA)
 depurination buffer(0.25 M HCl)
 10 , de-
 naturation buffer(1.5 M NaCl, 0.5 N NaOH)
 45 DNA 가 ,
 Hybond - N+ membrane(Amersham, England)
 (TransVac - TE80 ; Hoefer Scientific Instru-
 ment Inc. USA) southern transfer .
 12.5 cmHg , 1
 transfer 1,500 Jule 5
 DNA membrane crosslinking .

First strand cDNA의 합성

2 µg total RNA oligo d(T)₁₅₋₁₈ 2 µg
 70 10 1 mM dNTP(Ta-
 kara, Japan), 200 unit Moloney murine leukemia
 virus(MMLV) (promega, USA) 1x
 (50 mM Tris - HCl, pH 8.3, 75 mM KCl, 3 mM
 MgCl₂, 10 mM DTT) 25 µl가 42
 1 .
 95 5 .

중합효소 연쇄반응

Premix PCR kit(,
 Korea) . DNA polymerase 2.5 unit, 1
 mM dNTP가 cDNA 2 µl pri-
 mer 100 pM 가 가 20
 µl가 . Perkin Elmer
 2400(Perkin - Elmer corp., USA)
 table 1 cytokine annealing Tm
 . denaturation 96 30

Table 2. Oligonucleotide probe sequence used in southern hybridization

Cytokine	Oligonucleotide probe
-actin	5' GGC CCC CCT GAA CCC CAA GGC CAA 3'
ICAM-1	5' GAC CGT GTT TGT GCT CTC CCC CCG 3'
VCAM-1	5' GTT GAG ATC TCC CT GGA CCC CGG 3'
ELAM-1	5' AAA TGT TCA AGC CTG GCA GTT CCG 3'

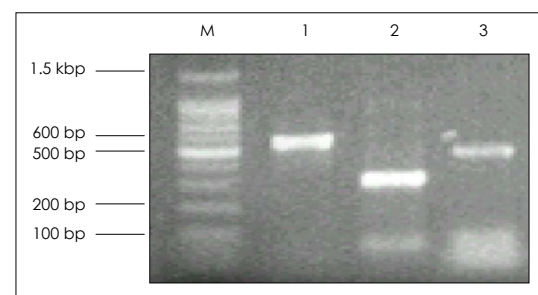


Fig. 1. Representative data for RT-PCR products of various adhesion molecules in cholesteatoma (M : 100 bp ladder DNA marker, Lane 1 : intercellular adhesion molecule-1, Lane 2 : vascular cell adhesion molecule-1, Lane 3 : endothelial derived leukocyte adhesion molecule-1).

Oligonucleotide probe end labelling
 Hybridization probe DNA
 5'-end labelling
 . 100 pM probe DNA 10 unit polynucleotide kinase(Promega, USA) 50 µCi [-³²P] ATP 37 1 5'
³²P probe
 G50 spin column

Group A
 (cholesteatoma only group)
 Group B(bone erosion group)
 Group C(bone destruction group)
 가

Southern hybridization
 DNA가 Hybond - N+ membrane prehybridization (6X SSC, 0.01 M sodium phosphate, pH 6.8, 1 mM EDTA pH 6.8, 0.5 % SDS, 100 µg/ml salmon sperm DNA) hybridization chamber 68 2

5 mRNA
 primer ICAM - 1 VCAM - 1
 1 가
 total mRNA가

blocking . ³²P
 가 probe 1 × 10⁶ cpm/ml
 62 18 hybridization membrane 3
 . 1 2X SSC, 0.1%
 SDS 5 2 , 2 35 ,
 3 45 5 Geiger
 counter background noise noise가
 membrane Kodak
 X - AR(Kodak) - 70 18

결 과
진주종 조직에서의 cytokine mRNA의 발현
 - actin 20 20
 2 ++
 +++ ++++
 adhesion molecule ICAM - 1
 20 18 (90%) ,
 16 (80%) ,
 (55%) . VCAM - 1 18
 (90%) , 15 (75%)
 가 12 (60%) . ELAM - 1 8

mRNA band image analysis software
 - actin band
 (density) . - actin mRNA
 100% ' - ' ; 10%
 ' +/- ' ; 10~30% ' + ' ; 30~50% ' ++ ' ;
 50~80% ' +++ ' ; 80% ' ++++'

환자의 임상상

Table 3. Expression of Adhesion molecules in cholesteatomatous tissue and retroauricular skin

	ICAM-1		VCAM-1		ELAM-1	
	S	C	S	C	S	C
-	4	2	5	2	5	12
+/-	0	0	0	0	0	1
+	6	5	3	4	13	3
++	4	3	5	4	1	2
+++	4	6	5	4	0	1
++++	2	4	2	6	1	1

S : retroauricular skin, C : cholesteatoma

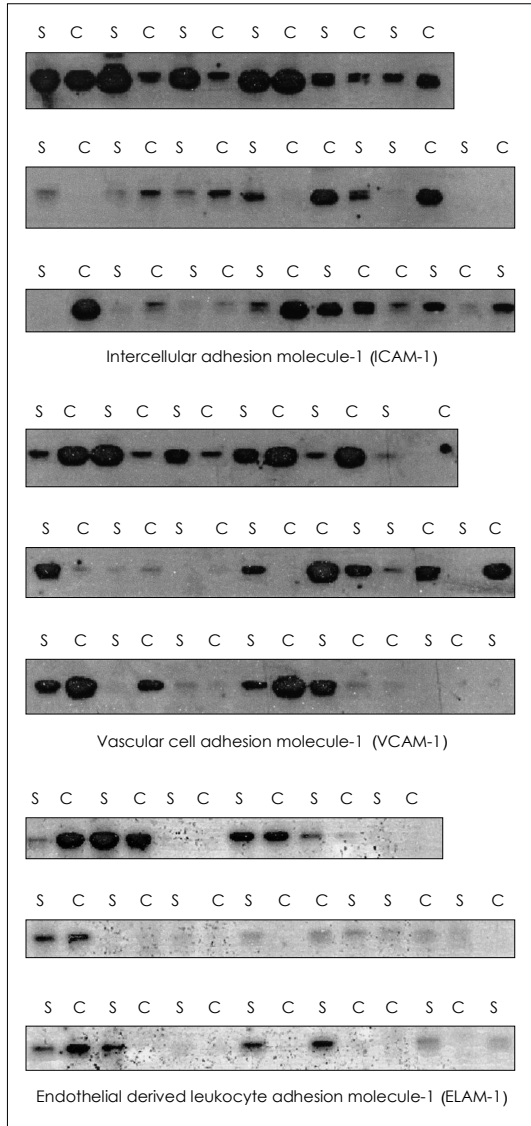


Fig. 2. Expression of mRNA of adhesion molecules (ICAM-1, VCAM-1, ELAM-1) from cholesteatoma (C) and retroauricular skin (S) (southern blot).

(40%) , 15 (75%)
 가 2 (10%) (Table 3, Fig. 2).

중이 진주종의 골파괴와 Cytokine의 발현양상간의 관계

A, B, C
 cytokine A (N=8) mRNA

Table 4. Expression of adhesion molecules relation to bone destruction

	ICAM-1	VCAM-1	ELAM-1
-	2	1	5
+/-	0	0	0
Group A (n=8)			
+	0	1	1
++	1	0	2
+++	3	2	0
++++	2	4	0
-	0	0	5
+/-	0	0	1
Group B (n=9)			
+	4	3	1
++	2	4	0
+++	2	0	1
++++	1	2	1
-	0	1	2
+/-	0	0	0
Group C (n=3)			
+	1	0	1
++	0	0	0
+++	1	2	0
++++	1	0	0

group A : cholesteatoma only group
 group B : bone erosion group
 group C : bone destruction group

VCAM - 1 7 (88%), ICAM - 1 6 (75%), ELAM - 1 3 (38%) . B (N=9)
 ICAM - 1, VCAM - 1 (100%), ELAM - 1 4 (44%) . C (N=3)
 ICAM - 1 3 (100%), VCAM - 1 2 (67%), ELAM - 1 1 (33%) (Table 4).

동일환자에서 진주종 골파괴가 심한 부위와 경미한 부위에서의 발현양상

가
 5 1
 4 ICAM - 1 VCAM - 1 mRNA
 가 가
 가 (Fig. 3).

고 찰

가

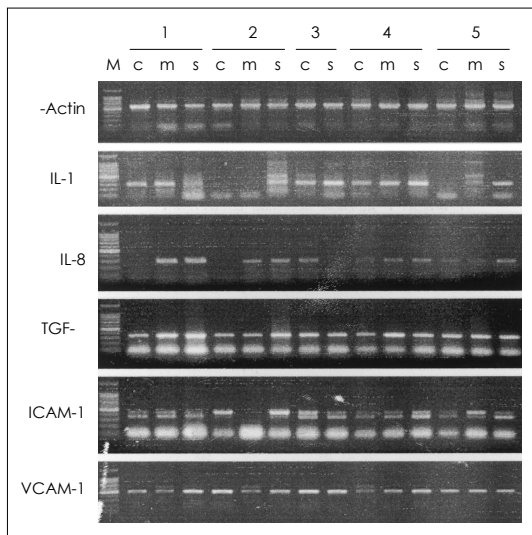


Fig. 3. Electrophoresis of RT-PCR products of β -actin, ICAM-1, and VCAM-1 from control retroauricular skin (c), cholesteatoma with mild bone destruction (m), cholesteatoma with severe bone destruction (s). The number means individual patient. M : 100 bp ladder DNA marker.

(migration)
 가 , immuno-
 competence migration
 trafficking homing
 가 가 tr-
 afficking
 .
 antigen presenting
 cell T - T -
 cytokine
 cytokine
 가 cytokine
 가
 3) 가
 가

가 가
 selectin, immunoglobulin
 superfamily, integrin
 ICAM - 1, VCAM - 1, ELAM -
 1 ICAM - 1
 IL -
 1, TNF - , LPS, IFN -
 4) cytokine
 ICAM - 1 T -
 T - 5)
 ICAM - 1
 ,
 6)
 ICAM - 1 Bujia 7) monoclonal anti-
 body
 ,
 Shinoda
 8)
 ICAM - 1
 18 (90%)
 16 (80%)
 ICAM - 1 mRNA
 ,
 11 (55%) ICAM - 1
 가 ICAM - 1
 ,
 ICAM - 1 가
 9) 가

가
가
VCAM - 1 ELAM - 1
migration
VCAM - 1
IL - 1,
TNF - , LPS, IL - 4 가
ELAM - 1 E - selectin
, TNF - , IL - 1, LPS
memory T - cell
ELAM - 1
T - 가
ELAM - 1
Ottaviani
perimatrix
80% ELAM - 1
18 (90%) 15
(75%) VCAM - 1 mRNA
가 12 가
(60%) ELAM - 1 8 (40%)
2 (10%)

ICAM - 1, VCAM - 1, ELAM - 1
T - , cytokine di-
fferential expression single cell PCR
PCR in situ 가 ,
cytokine , 가
결 론
ICAM - 1, VCAM - 1
cytokine

중심 단어 :

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가
가
VCAM - 1 mRNA 가
가
ELAM - 1
가
가
quantitative PCR

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