의학박사 학위논문

The Effect of Toll-like Receptor 4 Deficiency on the Development of Emphysema among Smokers

흡연자에서 톨 유사 수용체4의 발현 감소가 폐기종의 발생에 미치는 영향

2012년 8월

서울대학교 대학원

의학과 내과학 전공

이 세 원

흡연자에서 톨 유사 수용체4의 발현 감소가 폐기종의 발생에 미치는 영향

지도교수 이 춘 택

이 논문을 의학박사 학위논문으로 제출함

2012년 8월

서울대학교 대학원

의학과 내과학 전공

이 세 원

이세원의 의학박사 학위논문을 인준함

2012년 8월

위원장 _____

부위원장 _____

위 원 _____

위 원 _____

위 원 _____

The Effect of Toll-like Receptor 4 Deficiency on the Development of Emphysema among Smokers

by

Sei Won Lee, M.D.

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Medicine at the Seoul National University

August 2012

Approved by thesis committee:

Professor ____________________ **Chairman**

Professor ____________________ **Vice Chairman**

Professor ____________________

Professor ____________________

Professor ____________________

학위논문 원문제공 서비스에 대한 동의서

본인의 학위논문에 대하여 서울대학교가 아래와 같이 학위논문 저작물을 제공하는 것에 동의합니다.

1. 동의사항
   ① 본인의 논문을 보존이나 인터넷 등을 통한 온라인 서비스 목적으로 복제할 경우 저작물의 내용을 변경하지 않는 범위 내에서의 복제를 허용합니다.
   ② 본인의 논문을 디지털화하여 인터넷 등 정보통신망을 통한 논문의 일부 또는 전부의 복제.배포 및 전송 시 무료로 제공하는 것에 동의합니다.
2. 개인(저작자)의 의무
   본 논문의 저작권을 타인에게 양도하거나 또는 출판을 허락하는 등 동의 내용을 변경하고자 할 때는 소속대학(원)에 공개의 유보 또는 해지를 즉시 통보하겠습니다.
3. 서울대학교의 의무
   ①서울대학교는 본 논문을 외부에 제공할 경우 저작권 보호장치(DRM)를 사용하여야 합니다.
   ②서울대학교는 본 논문에 대한 공개의 유보나 해지 신청 시 즉시 처리해야 합니다.

논문제목 :

학위구분 : **석사 □.박사 ■**
학 과 : 의학과
학 번 : 2009-30530
연 락 처 :
저 작 자 : 이세원 (인)

제 출 일 : 2012 년 8 월 1 일

서울대학교총장 귀하

Abstract

Background: Toll-like receptor 4 (TLR4) deficiency in the lung can cause emphysema in animals, but has not been investigated in humans. We analyzed the association between TLR4 expression in the lung and the development of emphysema in smokers.

Methods: We enrolled patients with a smoking history and who had undergone a lung resection. We measured TLR4 expression in lung lysates and classified patients as high, intermediate, and low expressers. The severity of emphysema was evaluated on computed tomography using the Goddard classification. TLR4 expression was also evaluated immunohistochemically. We compared TLR4 expression between inbred rats with and without emphysema to examine the effect of emphysema development on TLR4 expression.

Results: In total, 53 patients were enrolled. Sixteen subjects were grouped as high expressers; 25, as intermediate; and 12, as low. Age, gender, smoking amount, and tuberculosis history did not differ among the groups. As TLR4 expression decreased, emphysema severity by the Goddard classification increased significantly (P = 0.02), and forced expiratory capacity in one second per forced vital capacity decreased significantly (P = 0.007). The difference in TLR4 expression based on immunohistochemistry was most prominent in bronchial and alveolar epithelial cells on IHC staining. The proportion of severe emphysema cases was highest in the low expresser group (P = 0.006). No difference in TLR4 expression was observed between rats with and without emphysema.

Conclusion: Even with the same amount of smoking, subjects with

TLR4 deficiency in the bronchial and alveolar epithelia appeared to be more susceptible to emphysema.

***Keywords**:* Smoking, Emphysema, COPD, Toll-like receptor

Student number: 2009-30530

Contents

List of Table

List of Figures

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major global health problem, with a prevalence of 5–25% in adults.(1) COPD is projected to be the fourth leading cause of death by 2020.(2, 3) Smoking is the most important cause of COPD, accounting for 90% of the cumulative risk.(4) Nevertheless, not all smokers will develop COPD. In fact, only a small portion (about 15%) will develop clinically relevant disease.(5, 6) Compared with non-smokers, smokers have a steeper lung function decline. However, this is only true of susceptible smokers, as non-susceptible smokers can have the same lung function decline as never-smokers.(7) This implies that additional susceptibility factors are involved in lung obstruction and the development of emphysema.

Several candidate genes and biomarkers have been suggested to explain “susceptibility” in smokers.(8) Candidate genes regulate proteases and antiproteases, modulate the metabolism of toxic substances in cigarette smoke, participate in mucocillary clearance, or influence inflammatory mediators.(9) Microsatellite DNA instability has been proposed as a useful genetic screening marker for “susceptible” smokers.(10) However, unlike other diseases, COPD lacks established markers that can be applied to track disease progression.(11)

Some studies have reported an association between innate immunity and COPD pathogenesis. The main components of innate immunity are phagocytes of inflammatory cells, which discriminate between pathogens and self cells by utilizing signals from Toll-like receptors (TLRs). TLRs may be important in COPD because they participate in defense against viral and bacterial infections, and infections in the airway worsen the disease process in the lungs of patients with

COPD.(12) Among the TLRs, TLR4 appears to play a pivotal role in lung homeostasis by contributing to the defense of endothelial cells against oxidants.(13, 14) Moreover, TLR4 deficiency in the lung leads to spontaneous emphysema in animals, which is associated with an oxidant/antioxidant imbalance.(15) Thus, differences in TLR4 expression have been associated with COPD development in some animals, but this is not evident in humans.

In the present study, we hypothesized that TLR4 expression differs among people and that this can be associated with the “susceptible smoker.” To test this hypothesis, we analyzed the association between TLR4 expression in lung tissue and emphysema.

MATERIALS and METHODS

Study Patients

We retrospectively recruited patients with a smoking history ≥10 pack-years, who were ≥40 years old, and whose lung had been resected for lung cancer from 2008 to 2010 at Seoul National University Bundang Hospital. We enrolled the patients whose normal lung tissues were available from a certified tissue bank. The lung tissues were dissected by pathologists to discriminate normal tissue from cancer and then preserved at −70ºC until the experiment. This study was reviewed and approved by the Institutional Review Board of Seoul National University Bundang Hospital (B1008/110-006).

Western Blot Assay

TLR4 expression was assessed by Western blot analysis. Normal lung tissues were homogenized (IKA T10 Basic) in lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl , 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na_3VO_4, 1 μg/ml leupeptin, 1 mM PMSF). Protein concentrations were quantified using a Bio-Rad Protein Assay Reagent (Hercules, CA, USA) according to the manufacturer's instructions. Thirty micrograms of protein were used for SDS-PAGE, and the separated proteins were transferred to nitrocellulose membrane. The membranes were blocked in 1× TBST with 5% milk for 1 h at room temperature and then incubated with anti-TLR4 antibody (H-80; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a concentration of 0.2 μg/ml overnight at 4°C. Goat anti-rabbit IgG (1:5,000; Santa Cruz Biotechnology) was applied

for 1 h at room temperature. The signal was detected and quantified using Scion Image v7.0 (Scion Corp., Frederick, MD, USA). All samples were normalized to actin signals. The results were used to categorize patients into three groups: high (TLR4/actin > 0.5), intermediate (0.15 ≤ TLR4/actin < 0.5), and low expressers (TLR4/actin < 0.15, Figure 1).

Radiological Features and Grading of Emphysema Severity

Computed tomography (CT) images of all enrolled subjects were reviewed by an experienced chest radiologist (T.J.K.) and pulmonologist (S.W.L.) who were blinded to patient information. After jointly scoring 10 patients, who were not enrolled in this study, and adjusting their eye levels, the two readers independently reviewed the images. Any disagreements were discussed by the two readers, and another experienced pulmonologist (C.T.L.) helped in reaching an agreement. Preoperative CT images available within 1 month before the resection were reviewed, and emphysema severity was scored by the Goddard classification.(16) Briefly, six CT images were selected for each patient: right and left fields of the upper lung (1 cm above the superior margin of the aortic arch), of the middle lung (1 cm below the carina), and of the lower lung (3 cm above top of the diaphragm). We scored each image from 0 to 3 according to the proportion of vascular disruption and the areas of low attenuation. A score of 0 meant no abnormality; 1, 25% involvement; 2, 25–50%; 3, 50–75%; and 4, 75–100% involvement. Emphysema was graded based on the total score of the six images: 0–7, mild; 8–15, moderate; and 16–18, severe emphysema.

CT scanning was performed during full inspiration using various CT scanners, including a Brilliance-64, MX-8000 IDT, and iCT 256 (Philips Medical Systems, Cleveland, OH, USA). Scanning was conducted from the thoracic inlet to the upper portion of the kidneys. Images were obtained using a window level of 600 Hounsfield units (HU), a window width of 1500 HU (lung window), a level of 30 HU, and a width of 400 HU (mediastinal window). Conventional CT images were obtained from the thoracic inlet to the lung base using a 3-mm section thickness section thickness.

Pulmonary Function Test

All patients underwent a pulmonary function test within 1 month before resection. We used the results that contained the largest forced expiratory volume in one second (FEV_1). Spirometry was conducted by trained pulmonary technicians, according to the 2005 American Thoracic Society/European Respiratory Society recommendations (17) using a V_{max}229 spirometer (Sensor-Medics, Yorba Linda, CA, USA).

Exposure of Rats to Cigarette Smoke

The Institutional Animal Care and Use Committee of Asan Medical Center approved this study. Eight-week-old inbred female Lewis rats (Orient Bio, Seongnam, Korea) were exposed to mainstream smoke from 20 filtered commercial cigarettes, each containing 8.5 mg tar and 0.9 mg nicotine (Eighty-Eight Lights, Seoul, South Korea), 5 days per week for 6 months according to the protocol of Cavarra et al. (18) with modifications. Ten to 12 rats were placed in an inhalation box (50 × 40

× 30 cm) connected to a pump and were exposed to mainstream cigarette smoke generated simultaneously from 10 cigarettes for 10 min. The rats remained in the box for an additional 10 min after the cigarettes had burned. The box was then ventilated to remove the cigarette smoke, and the rats breathed normal room air for 5 min. A second exposure was performed in the same manner. After exposure to cigarette smoke, the rats were returned to their cages. Control animals inhaled only clean room air in their cages.

Immunohistochemistry (IHC)

To analyze the major cells that resulted in a difference in TLR4 expression, immunohistochemical staining for TLR4 was performed in three randomly selected patients with emphysema who were low TLR4 expressers and three randomly selected patients without emphysema who were high TLR4 expressers. For the IHC analysis, 4-μm-thick sections were cut from the patient's lung tissue blocks, deparaffinized in xylene, and rehydrated in a graded alcohol series. Antigen retrieval was performed in pH 6.0 citrate-phosphate buffer using a microwave oven for 15 min. The sections were incubated with anti-TLR4 antibody (1:50; Santa Cruz Biotechnology) for 1 h at room temperature. To detect the signals, an Envision kit (Dako, Glostrup, Denmark) was used according to the manufacturer's instruction. An experienced pathologist (J.H.P.), who was blinded to the patient information, read the IHC findings. TLR4 expression was graded from 0 to 3 (0, no or very faint staining; 1, positive staining; 2, strong positive staining; 3, very strong positive staining).

Statistical Analysis

Emphysema severity and lung function were compared between groups using Spearman correlation analysis for continuous variables and the χ^2 test for categorical variables. Statistical significance was assessed at a two-tailed *P*-value of 0.05. Agreements in the emphysema scores between the two readers were determined using the κ statistic.(19) All data are presented as means ± standard deviations. All statistical analyses were conducted using PASW software ver. 18.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Demographic Characteristics

Fifty-three patients were enrolled. The median age was 67 years (range, 37–78 years), and 51 patients (96.2%) were male. Mean smoking history was 31.3 ± 21.2 pack-years. A pilot study showed that TLR4 expression was lower in patients with emphysema than in those without emphysema. We categorized the patients into three groups according to TLR4 expression. Twelve patients were low expressers, 25 patients were intermediate expressers, and 16 patients were high expressers. No differences in age, gender, tuberculosis, or smoking history were observed among the groups.

Figure1. The results of pilot study. Healthy smokers without emphysema showed higher TLR4 expression than smokers without emphysema. Study patients were categorized according to their expression levels of TLR4 in lung.

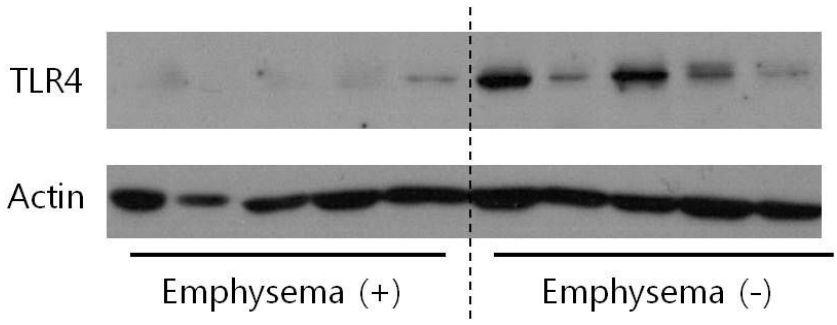


Table1. Comparison of demographic characteristics between groups

TLR4 expression	High Expressers (TLR4/actin ≥ 0.5) N = 16	Intermediate Expresser (0.15≤TLR4/actin<0.5) N = 25	Low Expressers (TLR4/actin < 0.15) N = 12	*P* value
Age, years	67.4 ± 7.8	64.0 ± 9.2	65.3 ± 8.3	0.49
Male Sex, n (%)	15 (93.8)	24 (96.0)	12 (100)	0.69
Smoking, pack years	38.8 ± 22.3	35.6 ±17.8	38.8 ± 27.2	0.87
TB history present, n (%)	3 (18.7)	6 (24.0)	2 (16.7)	0.85
ICS users*, n (%)	1 (6.3)	2 (8.0)	2 (16.7)	0.61

TLR4, toll-like receptor 4; ICS, inhaled corticosteroid

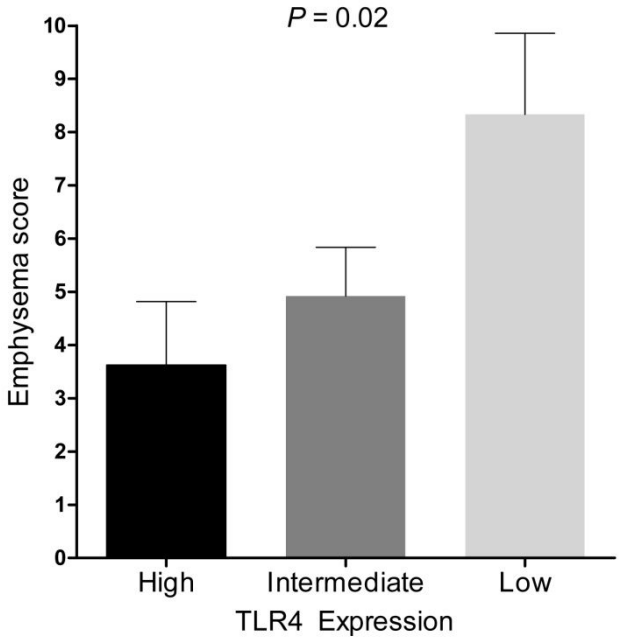
Emphysema Severity and Lung Function According to TLR4 Expression

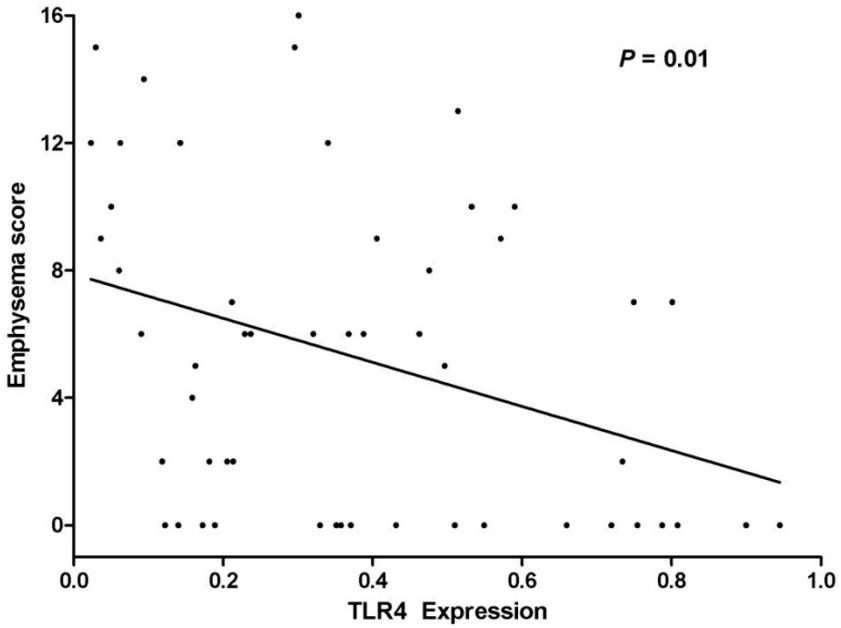
The mean emphysema score increased as TLR4 expression decreased; the mean emphysema scores of the high, intermediate, and low expressers were 3.63 ± 4.77, 4.92 ± 4.60, and 8.33 ± 5.26, respectively (P = 0.02, Figure 2A). The TLR4 low expresser group included a higher number of severe-stage emphysema cases; the proportions of severe compared with mild emphysema in the high, intermediate, and low expressers were 43.8% (7/16), 72.0% (18/25), and 83.4% (10/12) (P = 0.007, Figure 2B). Airflow limitation also became more severe with lower levels of TLR4 expression; the mean FEV_1 per forced vital capacity (FVC) values in the high, intermediate, and low expressers were 0.69 ± 0.07, 0.67 ± 0.11, and 0.58 ± 0.11 (P = 0.008, Figure 2C). Although the diffusing capacity (DL_{CO}) had a decreasing tendency and FVC had an increasing tendency as TLR4 expression decreased, no significant difference was observed among the groups for FEV_1, FVC, or DL_{CO}, except FVC per its predicted value. (Table 2)

Agreement between the two readers (T.J.K. and S.W.L.) for emphysema grades was almost perfect (κ = 0.82, $P < 0.001$). The differences in emphysema scores between the two readers were 0 for 30 (56.6%) patients, 1 for 12 (22.6%) patients, 2 for nine (17.0%) patients, and ≥3 for two (3.8%) patients.

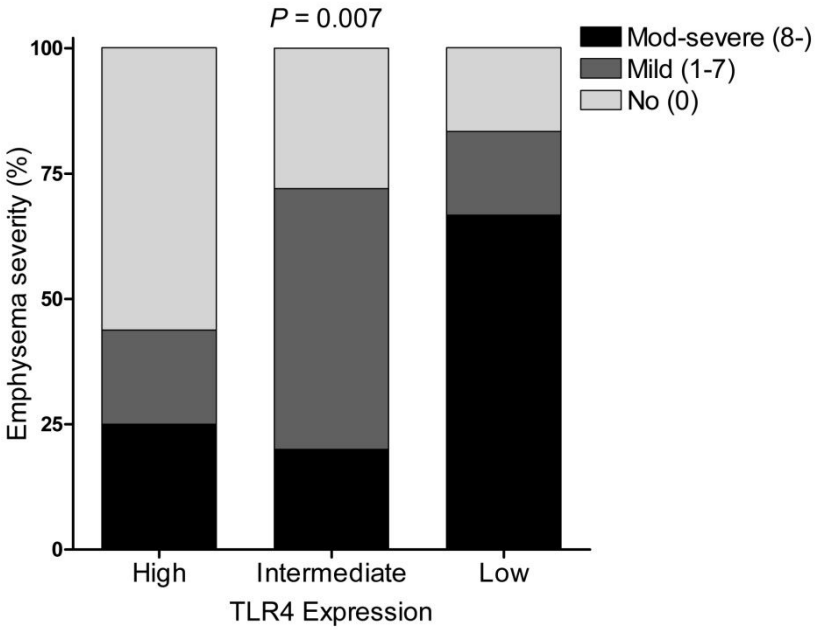
Figure2. Emphysema severity and lung function according to TLR4 Expression.

(A) The mean emphysema scores increases as the TLR4 expression decreases. It was consistent in continuous variable.

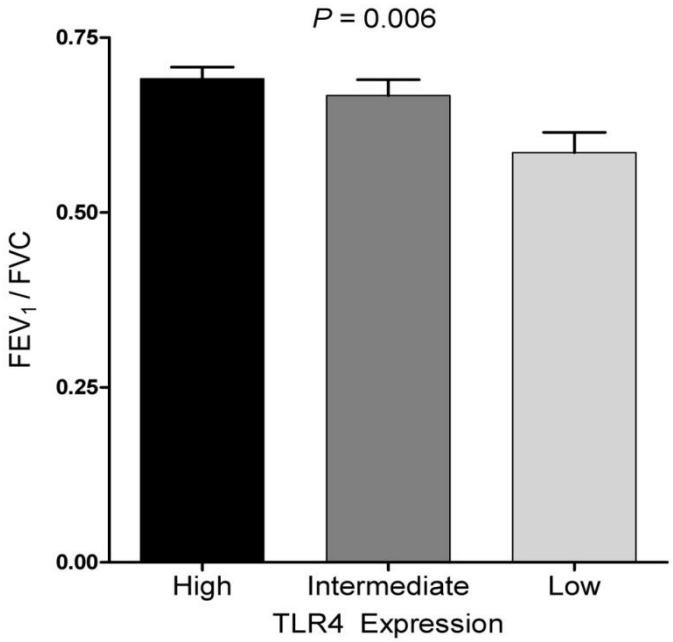


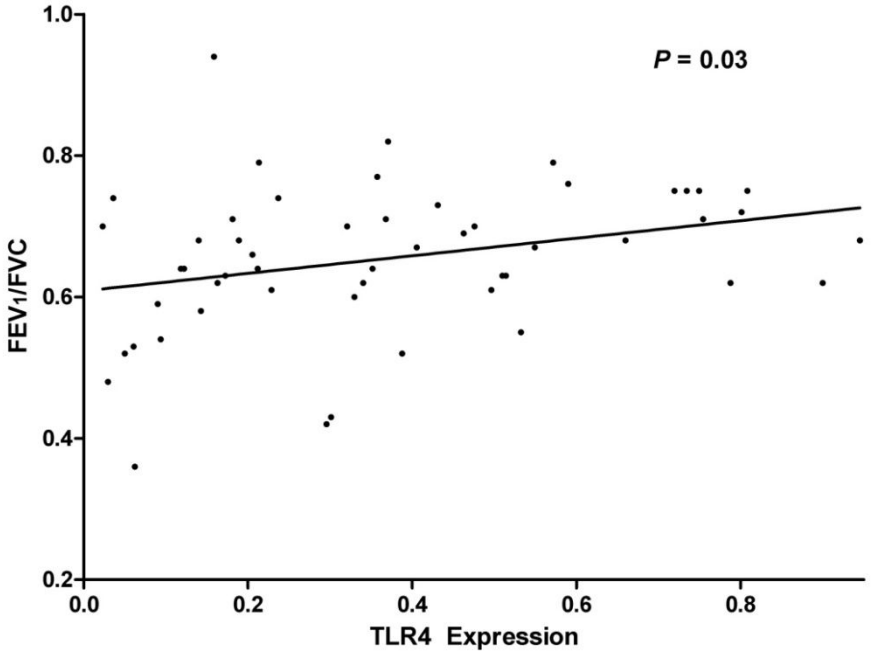


(B) Patients with severe stage of emphysema were included as TLR4 expression decreased.



(C) Airflow limitation became severer as the TLR4 expression weakened. It was consistent in continuous variable.





Major Cells Resulting in TLR4 Expression Differences in Lung Tissue

Immunohistochemical staining for TLR4 showed that bronchial and alveolar epithelial cells were the major cells exhibiting differences in TLR4 expression. Alveolar macrophages showed high TLR4 expression in all patients and thus did not contribute to the difference in expression among groups.

Table2. Comparison of lung functions between groups.

TLR4 expression	High Expressers (TLR4/actin ≥ 0.5) N = 16	Intermediate Expresser (0.15≤TLR4/actin<0.5) N = 25	Low Expresser (TLR4/actin<0.15) N = 12	*P* value
FEV_1/FVC	0.69 ± 0.07	0.67 ± 0.11	0.58 ± 0.11	0.006
FEV_1 (L)	2.40 ± 0.60	2.54 ± 0.57	2.31 ± 0.25	0.72
FEV_1 (% per predicted value)	90.8 ± 18.0	93.2 ± 17.3	86.2 ± 11.0	0.52
FVC (L)	3.46 ± 0.69	3.62 ± 0.83	3.99 ± 0.38	0.059
FVC (% per predicted value)	90.6 ± 14.1	96.0 ± 18.0	104.0 ± 13.4	0.03
DL_{CO} (mL/mmHg/min/L)	17.96 ± 4.63	17.77 ± 4.98	17.20 ± 3.97	0.68
DL_{CO} (% per predicted value)	105.2 ± 25.9	103.1 ± 25.9	97.4 ± 22.2	0.40
DL_{CO}/VA (mL/mmHg/min/L)	3.56 ± 0.57	3.45 ± 0.76	3.24 ± 0.83	0.26
DL_{CO}/VA (% per predicted value)	99.9 ± 18.5	91.9 ± 17.8	88.5 ± 20.0	0.10

TLR4, toll-like receptor 4; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; DL_{CO}, diffusing capacity

Figure3. IHC stain of TLR4 in lung tissues for each patient. (A) Patients without emphysema in high TLR4 expresser. (B) Patients with emphysema in low TLR4 expressers. The IHC stains showed bronchial and alveolar epithelial cells made major difference in TLR4 expression (x200).

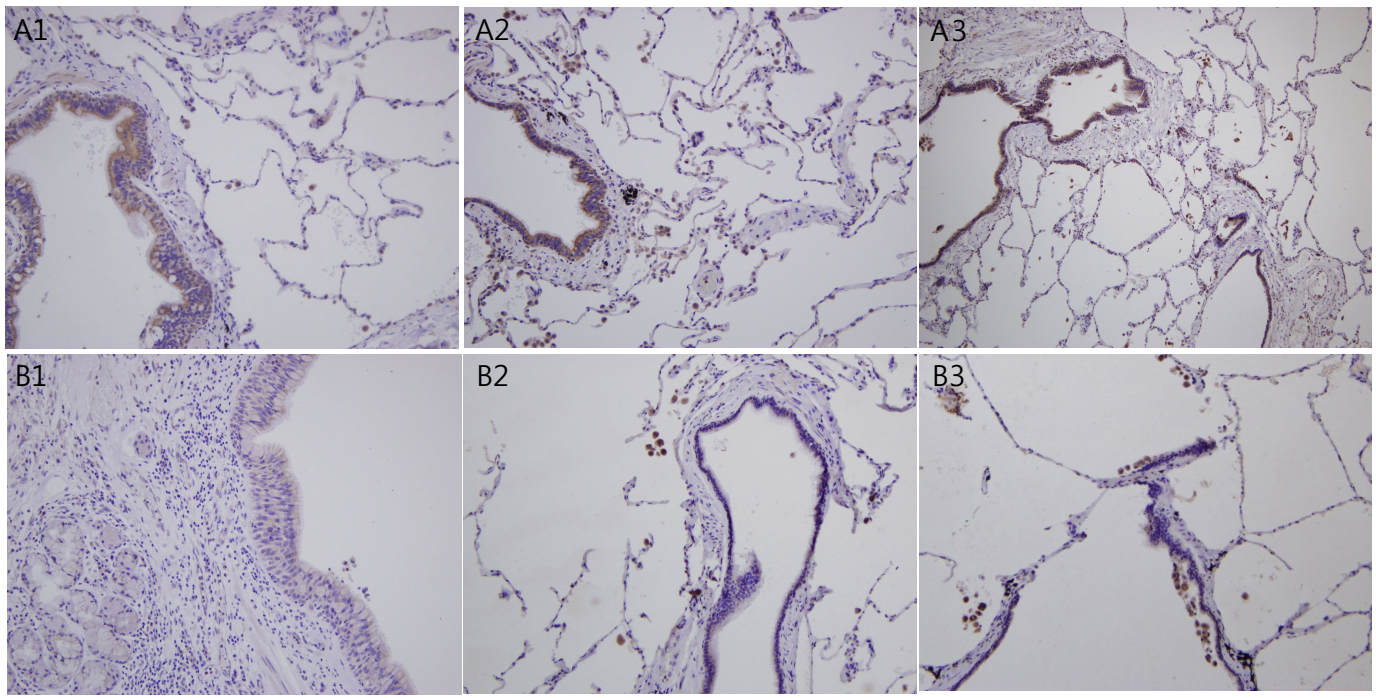


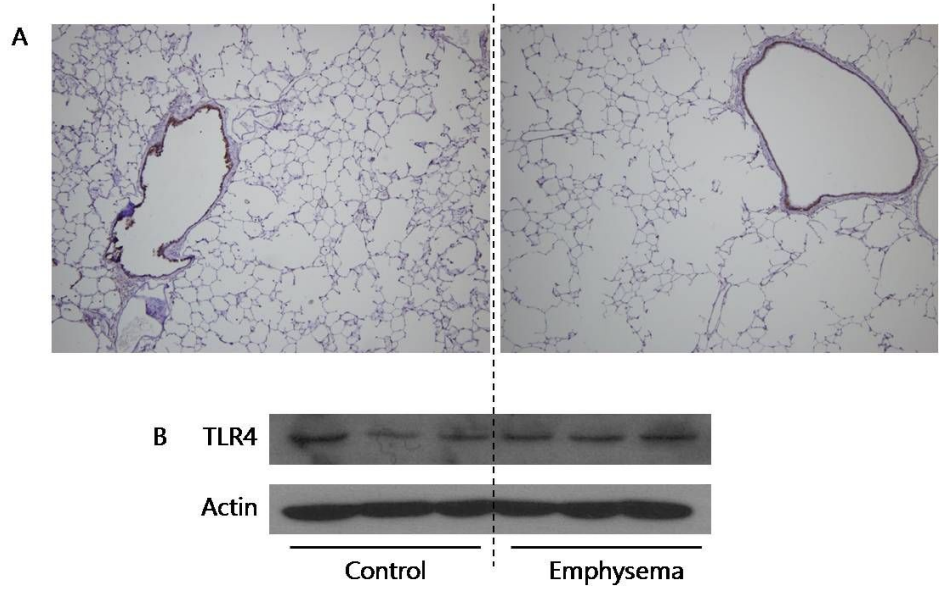
Table3. TLR4 expression of various cells in lung by IHC stain

	Patient	Bronchial epithelial cells	Alveolar epithelial cells	Macrophages
High expresser Emphysema (-)	1	3	2	3
	2	3	2	3
	3	3	1	3
Low expresser Emphysema (+)	4	2	1	3
	5	1	0	3
	6	1	0	3

TLR4 Expression among Inbred Rats According to Acquired Emphysema Development

We compared TLR4 expression between inbred rats with and without emphysema to exclude the possibility that emphysema development itself caused by smoking can affect TLR4 expression. We confirmed emphysema development after 6 months of smoke exposure. However, no difference in TLR4 expression was observed between rats with and without emphysema (Figure 4).

Figure4. TLR4 expression of inbred rat with and without emphysema. (A) The IHC stain of TLR4 showed no difference between rats with and without emphysema (x40). (B) TLR4 expression in lung was not also different between smoking and control rats in Western blot.



DISCUSSION

We showed that a TLR4 deficiency in the lung can be a factor related to emphysema susceptibility in smokers, as patients with TLR4 deficiency had a greater probability of developing emphysema and limited airflow. Differences in TLR4 expression were attributable mainly to expression levels in bronchial and alveolar epithelial cells, and smoke-induced emphysema itself did not appear to alter TLR4 expression in animals. The ability to categorize "susceptible smokers" is desirable, but it is extremely difficult based on current scientific knowledge. Genetic differences are the most likely factors determining this phenomenon, although the only well-established genetic risk factor is alpha-1-antitrypsin.(20, 21) Microsatellite DNA instability has also been suggested as a factor. Our findings make an important contribution because they show that TLR4 deficiency in the lung may be related to emphysema development in humans, as in animals.

Several studies have shown that defects in innate TLR4-mediated immunity can cause emphysema. TLR4 knockout causes spontaneous emphysema in animals.(15) The presence of the TLR4-T339I polymorphism is associated with a 2.4-fold increased risk for developing COPD, highlighting the relationship between impaired innate immunity and COPD development.(22) The Gly299 allele is present at a decreased frequency among patients with COPD and may be absent from COPD patients who have never smoked.(23)

An association between TLR4 expression and COPD has also been noted previously. Most studies have suggested that TLR4 expression is decreased in the blood of patients with COPD. The peripheral blood T_H1 cell response to lipopolysaccharide (LPS) is impaired in patients with COPD compared with the response in never-smokers, and TLR4

overexpression via transfection restores this impairment.(24) Compared with less severe disease, severe COPD is associated with reduced TLR4 expression in the nasal epithelium.(25) In contrast to some positive results in blood, cells in a sputum analysis failed to show this relationship. TLR4 expression on sputum neutrophils was not different in COPD patients,(26) and TLR4 mRNA in induced sputum did not differ significantly between COPD patients and healthy controls.(27) Epithelial cells from endobronchial biopsy showed no significant difference in TLR4 expression between COPD and normal subjects, but several factors such as smoking history, age and sex were not controlled and small number (total patients = 13) subjects limited the power of test.(28)

Theoretically, TLR4 deficiency may partially protect against smoke-induced emphysema, because TLR4 deficiency partially prevents smoke-induced influx of dendritic cells, lymphocytes, and neutrophils.(14) Clear correlations have been found between the numbers of these inflammatory cells and the severity of COPD.(29, 30) However, previous studies and our study have shown that TLR4 deficiency does not protect against the development of emphysema. To the contrary, it appears to induce emphysema, which can be explained by the role of TLR4 in the respiratory system.

Several TLR4-dependent mechanisms are likely to be involved in cigarette smoke-induced pulmonary inflammation. Smoke may activate TLR4 signaling on pulmonary epithelial cells and transmigrated resident cells such as macrophages, which act as the first line of defense against external threats.(14, 31) Innate immunity mediated by TLR4 also triggers the first-line host defense response to Gram-negative bacterial infections and is crucial for initiating subsequent T

cell-mediated adaptive immune responses.(31) TLR4 expression on the respiratory epithelium allows for rapid activation of host defenses against outside stimuli such as smoke and bacteria, resulting in the induction of inflammatory mediators and antimicrobial peptides. The increased risk for bacterial infection in patients with COPD may be caused by an inability to effectively clear bacteria and by misguided inflammatory responses. Emphysema develops due to chronic inflammation and impaired matrix and cellular repair. Thus, an impaired defense owing to TLR4 deficiency combined with repeated inflammation may result in the development of emphysema. However, it is unclear whether the reduced TLR4 expression in patients with COPD is an adaptive response to increased exposure to external threats such as Gram-negative bacteria or smoke, as part of the phenomenon of endotoxin tolerance.

Several limitations of this study should be noted. First, the causal relationship between TLR4 deficiency and emphysema is still ambiguous. To make a firm conclusion, smokers should be followed for several decades after obtaining lung tissues or respiratory cells; however, such studies are not practical and present ethical issues. Our data showed that smoke-induced emphysema itself did not affect TLR4 expression, providing more evidence that TLR4 deficiency results in emphysema. Previous reports showing that TLR4 knockdown induces spontaneous emphysema also support this idea.(15) Second, TLR4 expression can be induced or decreased by LPS stimulation.(24, 32) Thus, TLR4 expression levels can vary with different clinical conditions such as Gram-negative bacterial infections. In our study, lung tissues were taken during elective lung resection, when patients were clinically stable without evidence of pulmonary infection or exacerbation. Third, we could not find a causable gene that could

explain the differential TLR4 expression. Although we examined several single-nucleotide polymorphisms in the *TLR4* gene, including minor alleles with frequencies >5% in the HAP map of Asians, no significant results were identified (data not shown). Epigenetic factors may be associated with the differential expression of TLR4. Finally, functional aspects by TLR4 deficiency, such as cytokine secretion, could not be evaluated because of the retrospective nature of this study.

In conclusion, TLR4 deficiency in lung tissues was a component of emphysema susceptibility in smokers. This report will broaden our understanding of COPD pathogenesis, although further studies are required to clarify a causal relationship between TLR4 deficiency and emphysema.

REFERENCES

1. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet. 2007;370(9589):765-73. Epub 2007/09/04.

2. Murray CJ, Lopez AD, Black R, Mathers CD, Shibuya K, Ezzati M, et al. Global burden of disease 2005: call for collaborators. Lancet. 2007;370(9582):109-10. Epub 2007/07/17.

3. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS medicine. 2006;3(11):e442. Epub 2006/11/30.

4. Cigarette smoking and health. American Thoracic Society. American journal of respiratory and critical care medicine. 1996;153(2):861-5. Epub 1996/02/01.

5. Davis RM, Novotny TE. The epidemiology of cigarette smoking and its impact on chronic obstructive pulmonary disease. The American review of respiratory disease. 1989;140(3 Pt 2):S82-4. Epub 1989/09/01.

6. Burrows B, Knudson RJ, Cline MG, Lebowitz MD. Quantitative relationships between cigarette smoking and ventilatory function. The American review of respiratory disease. 1977;115(2):195-205. Epub 1977/02/01.

7. Fletcher C, Peto R. The natural history of chronic airflow obstruction. British medical journal. 1977;1(6077):1645-8. Epub 1977/06/25.

8. Tzortzaki EG, Tsoumakidou M, Makris D, Siafakas NM. Laboratory markers for COPD in "susceptible" smokers. Clinica chimica acta; international journal of clinical chemistry. 2006;364(1-2):124-38. Epub 2005/09/06.

9. Ouellette DR. The answer is fifteen percent: what is the question? Chest. 2004;125(1):3-5. Epub 2004/01/14.

10. Siafakas NM, Tzortzaki EG, Sourvinos G, Bouros D, Tzanakis N, Kafatos A, et al. Microsatellite DNA instability in COPD. Chest. 1999;116(1):47-51. Epub 1999/07/29.

11. Weiss ST, DeMeo DL, Postma DS. COPD: problems in diagnosis

and measurement. The European respiratory journal Supplement. 2003;41:4s-12s. Epub 2003/06/11.

12. Sarir H, Henricks PA, van Houwelingen AH, Nijkamp FP, Folkerts G. Cells, mediators and Toll-like receptors in COPD. European journal of pharmacology. 2008;585(2-3):346-53. Epub 2008/04/16.

13. Johnson GB, Brunn GJ, Kodaira Y, Platt JL. Receptor-mediated monitoring of tissue well-being via detection of soluble heparan sulfate by Toll-like receptor 4. J Immunol. 2002;168(10):5233-9. Epub 2002/05/08.

14. Maes T, Bracke KR, Vermaelen KY, Demedts IK, Joos GF, Pauwels RA, et al. Murine TLR4 is implicated in cigarette smoke-induced pulmonary inflammation. International archives of allergy and immunology. 2006;141(4):354-68. Epub 2006/08/31.

15. Zhang X, Shan P, Jiang G, Cohn L, Lee PJ. Toll-like receptor 4 deficiency causes pulmonary emphysema. J Clin Invest. 2006;116(11):3050-9. Epub 2006/10/21.

16. Goddard PR, Nicholson EM, Laszlo G, Watt I. Computed tomography in pulmonary emphysema. Clinical radiology. 1982;33(4):379-87. Epub 1982/07/01.

17. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38. Epub 2005/08/02.

18. Cavarra E, Bartalesi B, Lucattelli M, Fineschi S, Lunghi B, Gambelli F, et al. Effects of cigarette smoke in mice with different levels of alpha(1)-proteinase inhibitor and sensitivity to oxidants. Am J Respir Crit Care Med. 2001;164(5):886-90. Epub 2001/09/11.

19. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159-74. Epub 1977/03/01.

20. Black LF, Kueppers F. alpha1-Antitrypsin deficiency in nonsmokers. The American review of respiratory disease. 1978;117(3):421-8. Epub 1978/03/01.

21. Janus ED, Phillips NT, Carrell RW. Smoking, lung function, and alpha 1-antitrypsin deficiency. Lancet. 1985;1(8421):152-4. Epub 1985/01/19.

22. Speletas M, Merentiti V, Kostikas K, Liadaki K, Minas M, Gourgoulianis K, et al. Association of TLR4-T399I polymorphism with chronic obstructive pulmonary disease in smokers. Clin Dev Immunol. 2009;2009:260286. Epub 2010/02/20.

23. Rohde G, Klein W, Arinir U, Hagedorn M, Duerig N, T TB, et al. Association of the ASP299GLY TLR4 polymorphism with COPD. Respiratory medicine. 2006;100(5):892-6. Epub 2005/10/13.

24. Knobloch J, Schild K, Jungck D, Urban K, Muller K, Schweda EK, et al. The T-helper cell type 1 immune response to gram-negative bacterial infections is impaired in COPD. American journal of respiratory and critical care medicine. 2011;183(2):204-14. Epub 2010/08/17.

25. MacRedmond RE, Greene CM, Dorscheid DR, McElvaney NG, O'Neill SJ. Epithelial expression of TLR4 is modulated in COPD and by steroids, salmeterol and cigarette smoke. Respir Res. 2007;8:84. Epub 2007/11/24.

26. von Scheele I, Larsson K, Dahlen B, Billing B, Skedinger M, Lantz AS, et al. Toll-like receptor expression in smokers with and without COPD. Respiratory medicine. 2011;105(8):1222-30. Epub 2011/03/29.

27. Baines KJ, Simpson JL, Gibson PG. Innate immune responses are increased in chronic obstructive pulmonary disease. PloS one. 2011;6(3):e18426. Epub 2011/04/13.

28. Nadigel J, Prefontaine D, Baglole CJ, Maltais F, Bourbeau J, Eidelman DH, et al. Cigarette smoke increases TLR4 and TLR9 expression and induces cytokine production from CD8(+) T cells in chronic obstructive pulmonary disease. Respir Res. 2011;12:149. Epub 2011/11/11.

29. Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. Eur Respir J. 2003;22(4):672-88. Epub 2003/10/30.

30. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. The New England journal of medicine. 2004;350(26):2645-53. Epub 2004/06/25.

31. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nature immunology. 2004;5(10):987-95. Epub 2004/09/30.
32. Nomura F, Akashi S, Sakao Y, Sato S, Kawai T, Matsumoto M, et al. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. J Immunol. 2000;164(7):3476-9. Epub 2000/03/22.

Korean Abstract

목적: 폐에서 선천면역에 관여하는 Toll-like receptor 4 (TLR4)가 결핍되면 폐기종이 발생하는 것으로 동물에서는 알려졌으나 사람에서는 잘 증명되어 있지 않다. 이에 흡연자의 폐에서 TLR4의 발현에 따라 폐기종의 발생과 연관이 있는지에 대해 분석하였다.

방법: 10 갑년 이상의 흡연력이 있는 사람들 중 2008 년에서 2010 년까지 분당서울대학교병원에서 폐절제술을 시행받은 사람 중 정상 폐조직이 있는 사람들을 대상으로 하였다. TLR4 의 폐에서의 발현을 Western blot 방법으로 분석하여, 고발현, 중발현, 저발현군으로 분류하였고, 폐기종의 중증도는 Goddard classification 에 따라 분류하였다. TLR4 의 발현이 어떤 세포에서 두드러지는가를 확인하기 위해 면역화학염색을 시행하였다. 폐기종의 발생 자체가 TLR4 의 발현에 영향을 주는지 확인하기 위해서, 유전적으로 동일한 쥐에서 폐기종이 유무에 따른 TLR4 의 발현도 확인하였다.

결과: 총 53명의 환자가 연구에 등록되었고, 16명이 고발현군, 25명이 중발현군, 12명이 저발현군으로 분류되었다. 이들 그룹간에 연령, 성별, 흡연력, 결핵 병력 등에는 차이가 없었다. 폐기종의 정도는 TLR4의 발현이 떨어질수록 심해졌고 (P=0.02), 중증 폐기종의 비율 또한 저발현군에서 가장 높았다 (P=0.007). 1초간 강제호기환기량

(FEV_1)과 강제호기폐환기량 (FVC)의 비(FEV_1/FVC)도 TLR4의 발현이 떨어지면서 감소하였다 (*P*=0.006). 유전적으로 동일한 쥐에서 폐기종의 유무에 따라 TLR4의 발현 차이는 없었다. 면역화학염색에서 주로 TLR4의 발현에 차이를 주는 세포는 기관지상피세포와 폐포상피세포인 것으로 확인되었다.

결론: 같은 양의 흡연을 하였더라도 기관지상피세포와 폐포상피세포에서 TLR4가 결핍되었을 경우 폐기종에 취약한 것으로 보인다.

주요어: 흡연, 폐기종, 만성폐쇄성폐질환, 톨유사수용체

Acknowledgments

The study was supported by grant no. 02-2010-040 from the SNUBH Research Fund. The funding sources had no role in study design, data collection or analysis, manuscript preparation, or the decision to submit the manuscript for publication.

I would like to express my gratitude to all those who gave me the possibility to complete this thesis. I am deeply indebted to my mentor, Professor Choon-Taek Lee who have taught me not only knowledge, but also proper attitude as a doctor, a researcher and an educator. I am also grateful to Chul-Gyu Yoo, who taught bench work for the first time and inspired me to continuous effort in laboratory.

My colleagues Jin-Ho Paik, Tae Jung Kim, Jin-Haeng Chung, Jae-Ho Lee and Sanghoon Jheon supported me in my research work. I want to thank them for all their help, interest and valuable hints. They took their time to help me without hesitation despite of their busy schedule. Especially, I am grateful to Dal Rae Kim, my laboratory assistant for her technical support. Jin Won Huh helped animal experiments and this work could not have finished without her help. I also appreciate my precious colleagues, Hyo-Jeong Lim, Jong Sun Park, Young Jae Cho, Ho Il Yoon, Yeon-Mok Oh and Sang-Do Lee for their perpetual trust when I encountered difficulties.

I would like to give my special thanks to my wife whose love enabled me to complete this work. I want to extend my heartfelt gratitude to parents, sister and parents-in-law for their love and dedicated support. Especially, I want to thank my mother for their great help in difficult times. I would like to give all this achievement to my lovely daughters, Na-Hyeon and Soo-Hyeon, and I hope them to be aware of their father's effort someday when they are grown-up.

Above all, I thank God for his unspeakable miracle