2013

Clinical approaches for understanding the expression levels of pattern recognition receptors in otitis media with effusion

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https://hdl.handle.net/2144/17154

Boston University
BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

CLINICAL APPROACHES FOR UNDERSTANDING THE EXPRESSION
LEVELS OF PATTERN RECOGNITION RECEPTORS IN OTITIS MEDIA WITH
EFFUSION

by

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Submitted in partial fulfillment of the
requirements for the degree of
Master of Arts
2013
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ACKNOWLEDGEMENTS

The invaluable services and tireless support of several individuals are gratefully acknowledged for their support and contributions:

    Dr. Hee-Young Park, I am grateful for all the guidance and support you have provided me as a graduate student and for refining my academic goals throughout the entire year.

    I would also express my sincere appreciation to Dr. Seung Geun Yeo, who offered me various research and internship opportunities at Department of Otorhinolaryngology-Head & Neck Surgery in Kyung Hee University Hospital in Seoul, South Korea.

    Dr. Theresa Davies, thank you for your insightful advice and kind feedback throughout the writing of the thesis.

    Thank you all for your fascinating professional strengths, insightful recommendations and wisdom. These are very valuable and great sources of encouragement that I will carry on with my future career.

    I would like to thank my family and friends for all their words of encouragement, advice and support during my research. I especially dedicate this thesis to my father who was and always will be my biggest support.

    Without all of these individuals, this research would not have been possible.
CLINICAL APPROACHES FOR UNDERSTANDING THE EXPRESSION LEVELS OF PATTERN RECOGNITION RECEPTORS IN OTITIS MEDIA WITH EFFUSION

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ABSTRACT

Objectives
Bacterial infections in the normally sterile environment of the middle ear cavity usually trigger host immune response, whereby the innate immune system plays a dominant role as the host’s first line of defense. In this study we evaluated the expression levels of Toll-like receptors (TLRs) -2, -4, -5, -9, and nucleotide-binding oligomerization domain-containing proteins (NODs) -1 and -2, all of which are related to bacterial infection in pediatric patients with otitis media with effusion (OME).

Methods
The study sample consisted of 46 pediatric patients with OME, all of whom had ventilation tubes inserted. The expression levels of TLR-2, -4, -5, -9, NOD-1 and -2 mRNA in middle ear effusion were assessed by polymerase chain reaction.
Results

All effusion fluid samples collected from patients with OME showed expression of TLR-2, -4, -5, -9, NOD-1, and -2 mRNA. However, we found no correlations among expression levels of pattern recognition receptors (PRRs) in relation to characteristics of exudates, presence of bacteria, or frequencies of ventilation tube insertion (p>0.05).

Conclusion

Our findings suggest that exudates of OME patients show PRR expressions that are related to the innate immune response regardless of the characteristics of effusion fluid, presence of bacteria in exudates, or frequency of ventilation tube insertion.
# TABLE OF CONTENTS

Title i

Reader's Approval Page ii

Acknowledgements iii

Abstract iv

Table of Contents vi

List of Tables viii

List of Figures ix

List of Abbreviations x

Introduction 1

Otitis Media with Effusion 1

Pathogen Recognition and Innate Immunity 8

Toll-like Receptors (TLRs) 10

Nucleotide-binding oligomerization domain-like receptos (NLRs) 15

OME and Innate Immunity 19

Materials and Methods 21

Results 25

Discussion 30

Study Limitations 34

Conclusion 36

List of Journal Abbreviations 37
# LIST OF TABLES

| Table | Title                                                  | Page |
|-------|--------------------------------------------------------|------|
| 1     | Risk Factors for Developmental Difficulties           | 6    |
| 2     | Toll-like Receptor (TLR) Recognition of Microbial     | 11   |
|       | Components                                            |      |
| 3     | Primers Used for Real-time RT-PCR                    | 23   |
| 4     | Expression of Pattern Recognition Receptors (PRRs)    | 25   |
|       | according to Characteristics of Exudates             |      |
| 5     | Culture Results from Middle Ear Effusion Fluid        | 26   |
| 6     | Expressions of PRRs According to Bacterial Culture    | 27   |
|       | Results in Effusion Fluid                            |      |
| 7     | Expressions of PRRs According to the Frequency of     | 28   |
|       | Ventilating Tube Insertion                            |      |
| 8     | Expressions of PRRs According to Culture Results and the | 29   |
|       | Frequency of Ventilating Tube Insertion               |      |
# LIST of FIGURES

| Table | Title                                                      | Page |
|-------|------------------------------------------------------------|------|
| 1     | Structure of the Ear                                       | 3    |
| 2     | Middle ear infection: Otitis Media with Effusion          | 4    |
| 3     | The Toll-like Receptor (TLR) Signaling Pathway            | 14   |
| 4     | Nucleotide-binding Oligomerization Domain-like Receptors   | 16   |
|       | (NLRs)                                                     |      |
| 5     | Cytoplasmic NLRs and Their Signaling                      | 18   |
### ABBREVIATIONS

| Abbreviation | Full Form |
|--------------|-----------|
| AOM          | Acute Otitis Media |
| ASC          | Apoptosis-associated speck-like protein containing a CARD |
| Bir          | Baculovirus inhibitor of apoptosis repeat |
| CARD         | Caspase activation and recruitment domain |
| CNS          | Coagulase-negative Staphylococcus |
| iE-DAP       | γ-D-glutamyl-meso-diaminopimelic acid |
| IL-1R        | Interleukin 1 receptor |
| IFN-β        | Interferon beta |
| IRAK         | IL-1R-associated kinase |
| LPS          | Lipopolysaccharide |
| LRR          | Leucin-rich-repeat |
| MAL          | MyD88-adaptor-like |
| MDP          | Muramyl dipeptide |
| MEEF         | Middle Ear Effusion Fluid |
| MRSA         | Methicillin-resistant Staphylococcus aureus |
| NACHT        | Naip, CIITA, HET-E and TP-1 |
| NEMO         | IKK-γ/NF-κβ essential modilator |
| NLRs         | Nucleotide-binding Oligomerization Domain -like Receptors |
| NOD          | Nucleotide-binding Oligomerization Domain |
| OME          | Otitis Media with Effusion |
| Acronym | Full Form |
|---------|-----------|
| PAMPs   | Pathogen-associated Molecular Patterns |
| PCR     | Polymerase Chain Reaction |
| PRRs    | Pattern Recognition Receptors |
| TAK1    | TGF-β activated kinase 1 |
| TICAM1  | TIR-domain-containing Molecule 1 |
| TIRAP   | TIR-associated Protein |
| TIRF    | TIR-domain-containing Adaptor Protein-inducing IFN-β |
| TLRs    | Toll-like Receptors |
| TRAF    | TNFR-associated factor |
| TRAM    | TRIF-related Adaptor Molecule |
INTRODUCTION

**Otitis media with effusion**

Otitis media with effusion (OME) is defined as the presence of fluid in the middle ear without signs or symptoms of acute ear infection, and is associated with bacterial infections of the upper respiratory tract and dysfunction of the Eustachian tubes (Figure 1) (1). The Eustachian tubes equalize the pressure between the middle ear cavity and the outside atmosphere and allow fluid and mucus to drain out of the middle ear cavity. In case of inflammation the tube closes causing the fluid to become trapped. Bacteria from the back of the nose travel through the Eustachian tube directly into the middle ear cavity and multiply in the fluid. The tympanic membrane is often cloudy with distinctly impaired mobility, and an air-fluid level or bubble may be visible in the middle ear (Figure 2). The development of more effective treatment is complicated by the fact the OME seems to be a multifactorial disease. Obstruction of the Eustachian tube with resultant negative pressure and exudation of serum seems to be a major factor (2). Bacterial infection via the Eustachian tube, perhaps facilitated by the presence of serum as a growth medium, is also well-recognized as a component in this complex etiology. OME is also strongly associated with prior upper respiratory viral infection (3), which increases the likelihood of subsequent bacterial middle ear infection for reasons that are unclear but could include increased bacterial adherence in the nasopharynx and reduced ciliary activity in
the Eustachian tube. Inflammatory reactions associated with immune response to bacterial antigens, bacterial polysaccharides, and disorders of inflammatory pathways, such as the cytokine network and the complement cascade, may also contribute to OME pathogenesis, especially in its chronic forms. Several other factors may also be involved in the development of OME, including the host immune system, environmental factors, family history, allergies, adenoid hypertrophy, chronic sinusitis, cleft palate, tumors, or even sharp changes in atmospheric pressure. Recently, it was reported that in many cases of OME, bacteria or viruses are found in middle ear effusion.
Figure 1. Structures of the Ear. Figure taken from Merriam-Webster, 2013 (51).
Figure 2. **Middle ear infection: Otitis media with effusion.** Figure taken from A.D.A.M. Inc., the American Accreditation HealthCare Commission (www.urac.org), 2013 (52).

OME can be detected by examining the ear with pneumatic otoscopy. Diagnosis can be confirmed only by directly looking in the ear and seeing how the eardrum responds to gentle pressure. In addition, two tests may be performed to give the doctor information that cannot be learned through
observation only. One of these tests is an audiogram, in which tones are sounded at various pitches. An audiogram is used to measure how much hearing loss has occurred. The other test, called a tympanogram, measures the air pressure in the middle ear; this indicates how well the Eustachian tube is functioning. In recurrent cases or when an acute case does not respond to treatment, it may be necessary to obtain a culture from the middle ear, through the eardrum. This is usually achieved by an otolaryngologist.

About 2.2 million diagnosed episodes of OME occur annually in the United States, yielding a combined direct and indirect annual cost estimate of $4.0 billion (4). OME is highly prevalent in young children. Studies show that about 90% of children (80% of individual ears) have OME at some time before school age, most often between ages 6 months and 4 years (5,6). In the first year of life, more than 50% of children will experience OME, increasing to more than 60% by age 2 years (7). The primary outcomes include hearing loss, effects on speech, language and learning, physiologic sequelae, health care utilization (medical, surgical), and quality of life (4,8). Therefore, it is important to distinguish children with OME who is at risk for speech, language, or learning problems from other children (Table 1.)
• Permanent hearing loss independent of otitis media with effusion
• Suspected or diagnosed speech and language delay or disorder
• Autism-spectrum disorder and other pervasive developmental disorders
• Syndromes (e.g. Down) or craniofacial disorders that include cognitive, speech and language delays
• Blindness or uncorrectable visual impairment
• Cleft palate, with or without associated syndrome
• Developmental Delay

Table 1. Risk factors for developmental difficulties*

*Sensory, physical cognitive, or behavioral factors that place children who have otitis media with effusion at increased risk for developmental difficulties (delay or disorder). Data taken from Rosenfeld et al, 2004 (#).

Treatment for OME is appropriate only if persistent and clinically significant benefits can be achieved beyond spontaneous resolution. Although statistically significant benefits have been demonstrated for some antibiotic medications, they are short term and relatively small in magnitude (1). Moreover, significant adverse events may occur with all medical therapies. Only for more serious, exacerbated OME is antibiotic therapy currently prescribed. Persistent, recurrent, and chronic forms of OME affect about 15% of children and represent
a more difficult problem. If a child becomes a surgical candidate, ventilation tubes can be effective in the treatment of chronic/recurrent OME, but they are not always effective and there is controversy regarding long-term benefit and the potential for late adverse outcomes such as tympanic membrane abnormalities. Surgical candidacy for OME depends largely on hearing status, associated symptoms, the child’s developmental risk, and the anticipated chance of timely spontaneous resolution of the effusion (1). Candidates for surgery include children with OME lasting 4 months or longer with persistent hearing loss or other signs and symptoms, recurrent or persistent OME in children at risk regardless of hearing status, and OME and structural damage to the tympanic membrane or middle ear (1). Ultimately the recommendation for medical and surgical care must be individualized, based on consensus between the primary care physician, otolaryngologist, and parent or caregiver that a particular child would benefit from intervention.

Since pediatric patients with OME often have previous histories of acute otitis media (AOM); OME is often regarded as the same disease as AOM (9). However, OME is considered distinct from acute otitis media (AOM), which is defined as a history of acute onset of signs and symptoms, the presence of middle-ear effusion, and signs and symptoms of middle-ear inflammation (1). AOM typically resolves swiftly, but patients suffering from chronic, recurrent otitis media may suffer long-term conductive hearing loss and permanent hearing loss due to scarring of the middle ear conductive apparatus and sensorineural
damage to the inner ear (10). Therefore, understanding the infections involved in AOM and following the immune response in the middle ear cavity are critical to determining the pathophysiological mechanism of OME.

**Pathogen Recognition and Innate Immunity**

The human body is constantly threatened by pathogens such as viruses, bacteria, fungi and parasites that are inhaled, swallowed, or inhabit our skin and mucous membranes. In cases of infection the immune system, an interactive network of lymphoid organism cells, humoral factors, and cytokines, play an essential function as the host defense mechanism to protect our body (11). Immunity is divided into two parts determined by the speed and specificity of the reaction. These are named the innate or adaptive immune system, or a cooperative interaction of both that release various immunological mediators.

The innate immune system is sometimes used to include physical, chemical, and microbiological barriers, but more usually encompasses the elements of the immune system (neutrophils, monocytes, macrophages, complement, cytokines, and acute phase proteins) (11). The innate immune system is considered the first line of defense during the host response to pathogens that is mediated by phagocytes including macrophages and dendritic cells, and the body discriminates infectious non-self from noninfectious self-based on the recognition of general patterns (12,13). Acquired immunity is involved in elimination of pathogens in the late phase of infection as well as the generation of
immunological memory. Acquired immunity is characterized by specificity and develops by clonal selection from a vast repertoire of lymphocytes bearing antigen-specific receptors that are generated via a mechanism generally known as gene rearrangement (12).

The innate immune system recognizes microorganisms via a limited number of germline-encoded pattern recognition receptors (PRRs). PRRs present common characteristics. First, PRRs recognize conserved molecular structures termed pathogen-associated molecular patterns (PAMPs) to sense the presence of microbial infections (14). Examples are lipopolysaccharide (LPS), lipotechoic acid, and mannans on gram negative, gram positive, and yeast cell walls, respectively. Second, PRRs are expressed constitutively in the host and detect the pathogens regardless of their life-cycle stage. Third, PRRs are germline encoded, nonclonal, expressed on all cells of a given type, and independent of immunologic memory (12). Furthermore, PRRs fall into three groups depending on function; those inducing endocytosis and thus enhancing antigen presentation; those initiating nuclear factor κβ transduction and cell activation (TLRs) and those, for example mannan binding lectin, which are secreted acting as opsonins (11).

Different PRRs react with specific PAMPs, show distinct expression patterns, activate specific signaling pathways, and lead to distinct antipathogen responses. The basic machineries underlying innate immune recognition are highly conserved among species, from plants and fruit flies to mammals. The
increasing knowledge of these recognition pathways highlights the close relation between the innate and specific response—PRRs recognize broad patterns on microbes and then presents the processed product to antigen-specific T cells (11).

**Toll-like receptors (TLRs)**

PRRs in humans include Toll-like receptors (TLRs) and cytoplasmic nucleotide-binding oligomerization domain-like receptors (NLRs). Toll, the founding member of the TLR family, was originally identified as a Drosophila gene required for ontogenesis and antimicrobial resistance (15). A human Toll homolog or human TLRs were then identified and found to induce cytokine production and expression of costimulatory molecules. To date, 12 members of the TLR family have been identified in mammals. TLRs are type 1 integral membrane glycoproteins characterized by the extracellular domains containing varying numbers of leucine-rich-repeat (LRR) motifs and a cytoplasmic signaling domain homologous to that of the interleukin 1 receptor (IL-1R) (16). Based on the primary sequences, TLRs can be further divided into several subfamilies, each of which recognizes related PAMPs: the subfamily of TLR1, TLR2, and TLR6 recognize lipids, whereas the highly related TLR7, TLR8, and TLR9 recognize nucleic acids (Table 1). However, the TLRs are unusual in that some can recognize several structurally unrelated ligands. For example, TLR 4
recognizes a very divergent collection of ligands such as LPS and heat-shock proteins (12).

Table 2. Toll-like receptor (TLR) recognition of Microbial Components.
Data taken from Akira, Uematsu, and Takeuchi, 2006 (12).

| Microbial Components | Species                          | TLR Usage |
|----------------------|---------------------------------|-----------|
| Bacteria             |                                 |           |
| LPS                  | Gram-negative bacteria          | TLR4      |
| Diacyl lipopeptides  | Mycoplasma                      | TLR6/TLR2 |
| Triacyl lipopeptides | Bacteria and mycobacteria       | TLR1/TLR2 |
| LTA                  | Group B Streptococcus           | TLR6/TLR2 |
| PG                   | Gram-positive bacteria           | TLR2      |
| Porins               | Neisseria                       | TLR2      |
| Lipoarabinomannan    | Mycobacteria                     | TLR2      |
| Flagellin            | Flagellated bacteria             | TLR5      |
| CpG-DNA              | Bacteria and mycobacteria       | TLR9      |
| ND                   | Uropathogenic bacteria           | TLR11     |
| Fungus               |                                 |           |
| Zymosan              | Saccharomyces cerevisiae         | TLR6/TLR2 |
| Phospholipomannan    | Candida albicans                | TLR2      |
| Mannan               | Candida albicans                | TLR4      |
| Glucuronoxylomannan  | Cryptococcus neoformans         | TLR2 and TLR4 |
| Parasites            |                                 |           |
| tGPI-mutin           | Trypanosoma                     | TLR2      |
| Glycoinositolphospholipids | Trypanosoma      | TLR4      |
| Hemozoin             | Plasmodium                      | TLR9      |
| Profilin-like molecule| Toxoplasma gondii              | TLR11     |
| Viruses              |                                 |           |
| DNA                  | Viruses                         | TLR9      |
| dsRNA                | Viruses                         | TLR3      |
| ssRNA                | RNA viruses                      | TLR7 and TLR8 |
| Envelope proteins    | Respiratory syncytial virus     | TLR4      |
| Hemagglutinin protein| Measles virus                    | TLR2      |
| Host                 |                                 |           |
| Heat-shock protein 60, 70 |                           | TLR4      |
| Fibrinogen           |                                 | TLR4      |
The engagement of TLRs by microbial components triggers the activation of signaling cascades, leading to the induction of genes involved in antimicrobial host defense. After ligand binding, TLRs dimerize and undergo conformational changes required for the recruitment of TIR-domain-containing adaptor molecules to the TIR domain of TLR. There are four adaptor molecules, namely MyD88, TIR-associated protein (TIRAP)/MyD88-adaptor-like (MAL), TIR-domain-containing adaptor protein-inducing IFN-β (TRIF)/TIR-domain-containing molecule 1 (TICAM1) (17,18), and TRIF-related adaptor molecule (TRAM). The differential responses mediated by distinct TLR ligands can be explained in part by the selective usage of these adaptor molecules. MyD88 and TRIF are responsible for the activation of distinct signaling pathways, leading to the production of proinflammatory cytokines and type 1 IFNs, respectively (12).

TLRs and IL-1R share common signaling pathways in general. Stimulation with their ligands recruits TIR-domain-containing adaptors including MYD88 and TIRAP to the receptor, and the subsequent formation of a complex of IL-1R-associated kinases (IRAKs), TNFR-associated factor 6 (TRAF6), and IRF-5 is induced. TRAF6 acts as an E3 ubiquitin ligase and catalyzes the K63-linked polyubiquitin chain on TRAF6 itself and IKK-γ/NF-κβ essential modulator (NEMO) with E2 ubiquitin ligase complex of UBC13 and UEV1A. This ubiquitination activates the TGF-β-activated kinase 1 (TAK1) complex, resulting in the phosphorylation of NEMO and activation of the IKK complex. Phosphorylated Ikβ undergoes K28-linked ubiquination and degradation by the
proteasome. Freed NF-κβ translocates into the nucleus and initiates the expression of proinflammatory cytokine genes. Simultaneously, TAK1 activates the MAP kinase cascades, leading to the activation of AP-1, which is also critical for the induction of cytokine genes. TLR4 triggers the MyD88 independent, TRIF-dependent signaling pathway via TRAM to induce type 1 IFNs. TRIF activates NF-κβ and IRF-3 resulting in the induction of proinflammatory cytokine genes and type 1 IFNs, TRAF6 and RIP1 induce NF-κβ activation and TBK1/IKK-I phosphorylate IRF-3, which induces the translocation of IRF-3 (Figure 3).
Figure 3. The Toll-like receptor (TLR) signaling pathway. Figure taken from Akira, Uematsu, and Takeuchi, 2006 (12).
Nucleotide-binding oligomerization domain-like receptors (NLRs)

Although the key function of TLRs in innate immunity is apparent and is supported by a rich and dense literature, many other observations have indicated the possibility that all features of the host response to pathogens cannot only be accounted for by TLRs alone, but also by NLRs as the cytoplasmic counterparts (19). The mammalian NLR family is composed of more than 20 members whose defining feature is a modular domain organization of a C-terminal leucine-rich repeat (LRR) domain, a central nucleotide-binding Naip, CIITA, HET-E and TP-1 (NACHT) domain and an N-terminal protein-protein-interaction domain composed of a caspase activation and recruitment domain (CARD), pyrin domain, or baculovirus ‘inhibitor of apoptosis’ repeat (Bir) domain (Figure 4). Studies have shown that several NLRs can detect bacterial molecules through mechanisms that remain poorly understood. Nevertheless, studies have indicated that many NLRs are necessary sensors of specific PAMPs, even though it is unclear if NLRs directly bind to the PAMPs they detect. The first NLRs reported to have a direct function as intracellular pattern recognition molecules were nucleotide-binding oligomerization domain (NOD) 1 and NOD2; both proteins detect distinct substructures from bacterial peptidoglycan (19). NOD2 detects muramyl dipeptide, the largest molecular motif common to Gram-negative and Gram-positive bacteria (20,21). In contrast, NOD1 senses peptidoglycan containing meso-diaminopimelic acid, which is more commonly found in Gram-negative bacteria (22,23).
Figure 4. Nucleotide-binding oligomerization domain-like receptors (NLRs).

The NLRs are characterized by three distinct domains: an N-terminal effector domain, which can be a pyrin domain (PYD), a CARD or a Bir domain; a central NACHT domain (NACHT stands for domain present in Naip, CIITA, HET-E (plant *het* product involved in vegetative incompatibility) and TP-1 (telomerase-associated protein1)) and a C-terminal LRR domain thought to constitute the microbe-sending portion. Figure taken from Fritz et al, 2006 (19).
Among the large number of NLR family members, the functions of NOD1 and NOD2 have been studied the most. Both NOD1 and NOD2 share the same characteristic where they contain N-terminal CARD domains. NOD1 and NOD2 detect γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP) and muramyl dipeptide (MDP), found in bacterial peptidoglycan, respectively (Figure 5). Consistently, macrophages lacking either NOD1 or NOD2 fail to produce cytokines in response to the corresponding ligands (24). Ligand binding to NOD1 and NOD2 causes their oligomerization and results in NF-κβ activation through the recruitment of RIP2/RICK, a serine/threonine kinase, to the NODs via their respective CARD domains by hemophilic interactions (12).

Infection with bacteria induces activation of caspase-1, which catalyzes the processing of pro-IL-1β to produce the mature cytokines. A complex of proteins responsible for these catalytic processes has been purified and designated the inflammasome (Figure 5). The inflammasome consists of caspase-1; caspase-5; apoptosis-associated speck-like protein containing a CARD (ASC); and members of the NALP family, which are pyrin-domain-containing proteins that also contain NLRs. ASC is an adaptor protein that contains a pyrin domain and a CARD. NALPs recruit ASC through a homotypic interaction between the pyrin domains, and ASC in turn recruits caspase-1 via its CARD, leading to the activation of IL-1β and IL-18 processing.
NLRs recognize bacterial proteins in the cytoplasm and trigger signaling pathways. NOD1 and NOD2 recognize iE-DAP and MDP, respectively, and activate NF-κβ via RIP2/RICK. MDP is also recognized by NALP3, which forms an inflammasome comprised of ASC, CARDINAL, and caspase-1. Activated caspase-1 cleaves pro-IL-1β for the maturation of IL-1β. Figure taken from Akira, Uematsu, and Takeuchi, 2006 (12).
OME and innate immunity

Resistance to infection of the middle ear is the basis for avoidance of OME and, if infection ensures, recovery from middle ear disease. As at other locations, such resistance entails a cooperative response involving innate and adaptive immunity. However, as very few lymphocytes are found in the healthy middle ear, innate immunity presumably mediates the initial host response to and defense against infection (2). This allows the middle ear to clear many infections rapidly, before significant cognate immune response has the opportunity to develop. This is especially important in the middle ear, as adaptive immunity appears to be poorly sensitized by antigen that is first presented at this site (2).

One of the most common isolated pathogens of otitis media, Haemophilus influenzae, contains molecular patterns that serve as ligands for several TLRs. Peptidoglycans and peptidoglycan-associated proteins, such as outer membrane protein P6, are ligands for TLR2 (25), which frequently forms heterodimers with TLR1 or TLR6. Lipooligosaccharide, closely related to LPS, activates cellular signals via TLR2 and TLR4 (26). Whereas middle ear responses to these TLR2 and TLR4 ligands have long been recognized, the role played by TLR receptors in infection-mediated OME pathogenesis has only recently been addressed.

NOD1 and NOD2 are primarily involved in mediating antibacterial defenses (27). These PRRs may play critical roles in antibacterial defenses because both NOD1 null and NOD2 null mice show increased susceptibility to
infection with certain gastrointestinal bacteria (28). These cytoplasmic receptors play an important role following infection of the middle ear by pathogens that avoid recognition by TLRs. In the absence of infection, most cytoplasmic receptors are present at low concentrations inside the cell, but they are induced by proinflammatory cytokines and pathogen invasion (28).

Recent studies on PRR expressions in pediatric patients with OME have reported that the decreased expression of PRRs may be associated with increased susceptibility to OME (28,29). Even though no mutations had been found in TLR-2 and -4, which were expressed in all isolated samples of middle ear fluids, levels of TLR-9 and NOD-1 mRNAs were significantly lower in individuals with recurrent OME (29). Based on this, we hypothesized that there may be a difference in expression level of PRRs dependent on the clinical states of OME. Thus, in the present study we focused on the innate immune response in OME patients according to the characteristics of effusion fluids, presence of bacteria in exudates, and the frequency of ventilation tube insertion. Specifically, in the innate immune response in OME, we addressed the expressions of TLR-2, -4, -5, -9, NOD-1, and -2, which may play important roles in bacterial infection.
MATERIALS AND METHODS

Subjects

The study sample consisted of 46 pediatric patients who visited the Department of Otorhinolaryngology at Kyung Hee University Hospital in Seoul, South Korea from January 2008 to April 2010 and underwent ventilation tube insertion for chronic OME. OME was diagnosed by the presence of an amber-colored tympanic membrane on otoscopic examination and by the presence of B-or C-type tympanograms as shown by impedance audiometry. Surgery was performed on chronic OME patients who did not show improvement after 2 weeks of antibiotic treatment and in patients who, after a 2–3 month follow up, showed progressive retraction of the eardrum or progression of hearing loss as shown by an increase in pure tone threshold.

Informed Consent

We received prior permission with written informed consent from the patients’ parents or guardians for using patient samples, and the purpose of the experiment was also explained to them. Children who were suspected of having acute otitis media, head and neck anomalies, systemic diseases, or congenital or acquired immunodeficiencies were excluded from participation.
Procedure

A tympanostomy tube was inserted after a radial shaped incision was made in the anterior inferior quadrant of the tympanic membrane. Prior to surgery, the external auditory canal was washed with potadine solution, and middle ear effusion fluid (MEEF) was collected using a collector (Xomed Treace Products, Jacksonville, FL, USA) by aseptic procedures, taking care to avoid bleeding. Each effusion fluid sample was collected using sterilized cotton swabs, added to Stuart transport medium, and used to inoculate blood agar medium and thioglycollate liquid medium. All cultures were incubated for at least 24 hours at 35°C, and the resultant bacteria were identified by Gram-staining and biochemical analyses.

Polymerase Chain Reaction

Total RNA was extracted from effusion using the RNA-Bee solution kit (Tel-Test, Friendswood, TX, USA) following the manufacturer’s protocol. First-strand cDNA synthesis was performed by reverse transcription in a total volume of 20 µl reaction mixture containing 1 µg of RNA, 1x reaction buffer, 1 mM dNTP, 5 µM random primers, 20 units RNase inhibitor, and 20 units AMV reverse transcriptase (Promega, Madison, WI, USA). The reaction mixture was incubated at 42°C for 1 h, terminated by heating at 95°C for 5 min. TLR-2, -4, -5, -9, NOD-1 and -2 primers are shown in Table 1.
Table 3. Primers utilized for real-time RT-PCR.

Abbreviations - RT-PCR: real time-polymerase chain reaction; TLR: Toll-like receptor; NOD: nucleotide-binding oligomerization domain.

Real-time polymerase chain reaction (PCR) was performed using a Chromo4 Detector real-time system (Bio-Rad, Hercules, CA, USA) with the SsoFast EvaGreen supermix (Bio-Rad). PCR was performed with 2 µl of cDNA in a 20-µl reaction mixture of 10 µl SsoFast EvaGreen supermix, 2 µl primers and 6 µl PCR grade water. The reaction parameters were as follows: denaturation at 95°C for 30 sec followed by 45 cycles at 95°C for 5 sec, 55°C to 64°C for 12 sec.
The crossing point of TLR-2, -4, -5, -9, NOD-1, or -2 with β-actin was applied to the formula, $2^{(\text{target gene- } \beta \text{ actin})}$ and the relative amounts were quantitated. Difference of PRRs expression level by presence of bacteria, ventilation tube insertion rate, and effusion fluid character was assessed.

**Statistical Analysis**

Between group differences in PRRs expression, bacterial culture results, and frequency of ventilating tube insertion were compared using the Mann-Whitney U test. The relationship between the expression level of PRRs and characteristics of effusion fluid was analyzed by the Kruskal Wallis test. All statistical calculations were performed using SPSS 11.5 for Windows, and P-values lower than 0.05 were considered statistically significant.
RESULTS

The study sample consisted of 46 children with OME, 35 boys and 11 girls with a mean age 4.7 (± 2.1) years. Effusion fluids from all 46 of these patients were collected during the insertion of tympanostomy tubes. TLR-2, -4, -5, -9, NOD-1, and -2 were expressed in all middle ear exudates; TLR-9 showed the highest expression level, followed by TLR-2, TLR-4, TLR-5, NOD-2, and NOD-1. The characteristics of effusion fluid presented 17 mucoid cases, 16 serous cases, 10 mucopurulent cases and 3 purulent cases. There were no significant differences among mRNA levels of TLR-2, -4, -5, -9, NOD-1, and -2 regardless of their effusion characteristics (P>0.05, Table 4).

|       | mucoid     | serous     | mucopurulent | purulent   | p-value |
|-------|------------|------------|--------------|------------|---------|
| TLR2  | 0.061±0.051| 0.168±0.218| 0.151±0.160  | 0.197±0.151| 0.248   |
| TLR4  | 0.034±0.024| 0.083±0.072| 0.091±0.061  | 0.049±0.018| 0.064   |
| TLR5  | 0.034±0.038| 0.055±0.051| 0.060±0.052  | 0.051±0.013| 0.551   |
| TLR9  | 0.154±0.149| 0.298±0.268| 0.099±0.105  | 0.183±0.107| 0.140   |
| NOD1  | 0.003±0.002| 0.008±0.016| 0.005±0.006  | 0.004±0.003| 0.646   |
| NOD2  | 0.005±0.003| 0.039±0.070| 0.017±0.013  | 0.013±0.001| 0.297   |

Table 4. Expression of PRRs according to characteristics of exudates.

PRRs: Pattern recognition receptors; TLR: Toll-like receptor; NOD: nucleotide-binding oligomerization domain.
Of the 46 samples examined, 31 (67.4%) were negative for growth of bacteria, whereas the remaining 15 (32.6%) showed bacterial growth from the effusion fluid. Bacteria were cultured and identified; the bacterial species identified were coagulase-negative *Staphylococcus* (CNS), methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae*, *Micrococcus spp.*, *Pseudomonas aeruginosa*, methicillin-sensitive *Staphylococcus aureus*, *Streptococcus viridians*, and *Acinotobacter iwoffi* (Table 5).

| Bacteriology                        | Number (%) |
|-------------------------------------|------------|
| No growth                           | 31 (67.4%) |
| CNS                                 | 3 (6.5%)   |
| MRSA                                | 2 (4.3%)   |
| *Streptococcus pneumoniae*          | 2 (4.3%)   |
| *Micrococcus spp.*                  | 2 (4.3%)   |
| *Pseudomonas aeruginosa*            | 2 (4.3%)   |
| *Staphylococcus aureus*             | 2 (4.3%)   |
| *Streptococcus viridians*           | 1 (2.1%)   |
| *Acinotobacter iwoffi*              | 1 (2.1%)   |
| **Total**                           | **46**     |

Table 5. **Culture results from middle ear effusion fluid.**

CNS: coagulase-negative staphylococci; MRSA: methicillin-resistant *Staphylococcus aureus*.

We observed expressions of TLR-2, -4, -5, -9, NOD-1, and -2 mRNAs in all effusion samples. We observed no differences between the presence of
bacteria in exudates and the expression levels of TLR-9, NOD-1, and NOD-2 mRNA (p>0.05), (Table 6). In addition, expression levels of PRRs mRNA were not associated with the frequency of ventilating tube insertion (p>0.05), (Table 7). Similarly, there were no differences between frequencies of ventilation tube insertion and expression levels of PRRs, regardless of the presence of bacteria in middle ear fluid (p>0.05), (Table 8).

|             | Non-bacterial detection | Bacterial detection | p-value |
|-------------|-------------------------|---------------------|---------|
| TLR2        | 0.123±0.166             | 0.113±0.106         | 0.822   |
| TLR4        | 0.069±0.063             | 0.058±0.066         | 0.565   |
| TLR5        | 0.043±0.048             | 0.059±0.067         | 0.876   |
| TLR9        | 0.182±0.210             | 0.241±0.267         | 0.691   |
| NOD1        | 0.006±0.011             | 0.004±0.003         | 0.139   |
| NOD2        | 0.022±0.047             | 0.007±0.005         | 0.089   |

Table 6. Expression of PRRs according to bacterial culture results in effusion fluid.

PRRs: Pattern recognition receptors; TLR: Toll-like receptor; NOD: nucleotide-binding oligomerization domain.
|      | Once     | Twice or more | p-value |
|------|----------|---------------|---------|
| TLR2 | 0.115±0.155 | 0.136±0.090 | 0.525   |
| TLR4 | 0.066±0.066 | 0.063±0.043 | 0.449   |
| TLR5 | 0.044±0.051 | 0.072±0.061 | 0.128   |
| TLR9 | 0.175±0.217 | 0.183±0.196 | 0.437   |
| NOD1 | 0.006±0.012 | 0.004±0.006 | 0.868   |
| NOD2 | 0.021±0.049 | 0.013±0.008 | 0.811   |

Table 7. Expression of PRRs according to the frequency of ventilating tube insertion.

PRRs: Pattern recognition receptors; TLR: Toll-like receptor; NOD: nucleotide-binding oligomerization domain.
Table 8. Expression of PRRs according to culture results and the frequency of ventilating tube insertion.

PRRs: Pattern recognition receptors; TLR: Toll-like receptor; NOD: nucleotide-binding oligomerization domain.
DISCUSSION

Otitis media with effusion (OME) is a disease in which secreted fluid accumulates in the middle ear cavity and is a major cause of hearing loss in children (30,31). Among the possible causes of otitis media are: Eustachian tube dysfunction, bacterial or viral infection, family history, and allergies, with inflammatory reactions induced by pathogen stimulation thought to be important (32). Persistent infection can lead to the accumulation of inflammatory mediators, as well as their secretion into effusion fluid. Therefore, analysis of effusion fluid could be important in understanding the pathologic mechanism of OME. Although the immunological etiology of recurrent OME has been investigated thoroughly, it is not yet clear when the innate immunity system first reacts with pathogens invading the middle ear cavity.

Invading pathogens are recognized by PRRs, a class of proteins, which are distributed in extracellular, membrane, and cytoplasmic compartments. The major extracellular PRRs, including the complement components may be the first to encounter invading pathogens, opsonizing them for clearance by phagocytosis, a process mediated by membrane-associated phagocytic receptors. The major membrane-associated PRRs, the TLRs, are closely related type 1 transmembrane proteins that transduce signals in response to microbial intruders such as bacteria, fungi, protozoa and viruses. Although TLRs are crucial for innate immunity, they are also required for the induction of adaptive
immune responses that combat many infections. Pathogens that escape these two detection systems may be recognized by cytoplasmic PRRs, NLRs, which mediate antibacterial defenses (33).

These receptors in different cellular compartments such as on the cell surface, and in the endosomes, lysosomes or cytoplasm have been shown to differ in intracellular localization, recognition of different pathogenic motifs, and cell signaling (34). For example, TLR-2, -4, and -5 are located on the cell surface, TLR-9 is present on endosomal and lysosomal membranes, and NOD-1 and -2 receptors are located in the cytoplasm. TLR-2 and -4 have been reported to recognize pathogenic bacteria, whereas TLR-2 is the receptor for the cell wall components of Gram-positive bacteria including peptidoglycans, lipoteichoic acid and lipoproteins, and TLR-4 binds to the toxic pneumolysin ligand produced by Gram-positive bacteria, as well as binding to lipopolysaccharide (LPS), the major component of Gram-negative bacterial cell walls (35-37). Both NOD-1 and -2 have cytoplasmic caspase-recruiting effector domains and recognize peptidoglycan fragments. NOD-1 recognizes Gram-negative bacteria, whereas NOD-2 recognizes both Gram-positive and Gram-negative bacteria (38).

Inflammatory diseases caused by the abnormal immune control of nosocomical infections may be due to some problem with expression levels of PRRs. Despite the fact that PRR may play a crucial role in the innate immune response, little is known about how and when the innate immune system reacts with pathogens invading the middle ear cavity in pediatric patients with OME.
Thus, we not only assayed the expressions and relationships among PRRs, but we also aimed to discern whether there were differential expression levels of PRRs according to bacterial infection.

TLRs serve a sentinel role in early detection for invading pathogens so that the host can activate local inflammation through the expression of innate immune mediators such as the complement cascade. When there is a mutation in TLRs, not only otitis media, but periodontitis and osteomyelitis can also occur due to the reduced ability for bacterial cell wall recognition and an increased susceptibility to bacterial infection (39-43). TLR-2 and -4 were reported to be involved in defense mechanism of the middle ear cavity and in the bacterial alleviation or clearance of OME. Genetic mutation of TLR-2 or -4 was also reported to increase the susceptibility for bacterial infection in OME (44,45). In our study, we confirmed that TLR-2 and -4 were expressed in exudates of OME. TLR-5 and -9 that are related to the response to bacterial infection were also expressed in the middle ear effusion. These results imply that the difference of PRRs expression levels may be closely associated with the development of OME.

The role of cytoplasmic receptors becomes critical when pathogens that have the ability to escape from TLR recognition enter the body (33). The expression levels of many cytoplasmic receptors stay low under normal conditions, but when pathogens invade and pro-inflammatory cytokines are manifested, their expressions are highly induced. Although cytoplasmic receptor-
associated diseases have been described, detailed causes and treatment methods have not yet been determined. Failure of PAMP recognition or transformation of the function of cytoplasmic receptors by mutations in domains that send a signal lead abrogate immune reactions, even after infection by pathogens. In contrast these aberrant responses may provoke unnecessary inflammatory responses and inflammatory diseases by sending excess signals. For example mutations in NOD have been associated with chronic inflammatory diseases such as Crohn's disease and Blau syndrome (46,47). Our results showed simultaneous expression of the cytoplasmic receptors NOD-1 or NOD-2 along with the TLRs in all cases regardless of bacterial presence or species. This suggests that PRRs function cooperatively rather than individually in OME.

Previous studies on the immunological status of the host in otitis media have asserted that the independent local immunity of the middle ear cavity is one of the main causes of OME (48,49). The close relationship between bacterial infection and the innate immune response of OME can be deduced because bacteria were detected in exudates of the middle ear cavity, and children with a higher prevalence for upper airway infection were more likely to develop OME. These findings suggest that bacterial infection probably plays an important role in the development of OME (50).

Some have presented an association between the level of antibody in the middle ear effusions and bacterial infection, and suggested that the higher concentrations of immunoglobulin in middle ear effusions from patients with
acute otitis media are associated with lower detection rates of bacteria. Other research has showed that the serum levels of immunoglobulin from children with OME may be closely related to bacterial infection (49). These studies led to the examination of the relationship between bacterial infection and the acquired immune response in otitis media (9, 49). However, there was no correlation between effusion Ig concentration and the presence of bacteria in OME. In order to determine the relationship between bacterial infection and innate immunity, the expression levels of PRRs were compared between subjects who were detected of bacteria and those who were not detected of bacteria in exudates, and we found no relationship between bacterial infection and PRRs. However, it is still important to note the possible involvement of PRRs in immune response to otitis media, since PRRs were expressed in all exudate samples from otitis media regardless of bacterial detection.

**Study Limitations**

There are some limitations in this study. First, although our study group included patients with OME, all had normal immunity and were in a chronic, not an acute, state; and the study did not include children with early stage OME. Second, antibiotics had been used for 2 weeks to treat early stage of symptoms in all patients. Third, 67.4% showed negative growth despite bacterial infection possibly due to the use of various antibiotics prior to myringotomy and insertion
of ventilation tubes, creating a bacteriostatic condition and delaying the proliferation and growth of pathogens. Fourth, only the middle ear effusion was tested for bacteria and not the mucous membrane of the middle ear. The exudate contains only a few partially exfoliated mucosal epithelial cells and inflammatory cells; this would not fully reflect the immune cells in the middle ear mucosa during infection, but we obviously could not collect the middle ear mucosa of patients due to ethical issues. Finally, as the exudates used in this study were collected during surgery 2-3 months after the occurrence of the initial onset of otitis media, our study may not fully reflect the direct immune response in the middle ear cavity. PRRs were expressed in all exudates samples of otitis media regardless of bacterial detection. Our study showed similar PRRs expression values between the group that received reoperation from the recurrence of OME and the group with only one operation. This suggests that innate immunity may be involved in the middle ear cavity irrespective of the recurrence of OME.
This study confirmed that all exudates of OME patients showed some level of PRR expression related to innate immune response regardless of characteristics of effusion fluid, presence of bacteria in exudates, or frequency of ventilation tube insertion. Thus, variable expression levels of PRRs may be an important indicator for innate immune responses in patients with OME.
| Abbreviation                  | Full Name                                                   |
|------------------------------|-------------------------------------------------------------|
| Am J Otol.                    | American Journal of Otolaryngology                          |
| Annu Rev Biochem.            | Annual Review of Biochemistry                               |
| Arch Otolaryngol Head Neck Surg. | Archives of Otolaryngology-Head & Neck Surgery             |
| BMC Immunol.                 | BMC Immunology                                              |
| Clin Exp Immunol.            | Clinical & Experimental Immunology                          |
| Clin Exp Otorhinolaryngol.   | Clinical & Experimental Otorhinolaryngology                 |
| Clin Immunol Immunopathol.   | Clinical Immunology and Immunopathology                     |
| Cold Spring Harb Symp Quant Biol. | Cold Spring Harbor Symposia on Quantitative Biology       |
| Curr Allergy Asthma Rep.     | Current Allergy and Asthma Reports                         |
| Infect Immun.                | Infection and Immunity                                      |
| Int Immunol.                 | International Immunology                                    |
| Int J Pediatr Otorhinolaryngol. | International Journal of Pediatric Otorhinolaryngology.      |
| J Biol Chem.                 | Journal of Biological Chemistry                             |
| J Immunol.                   | Journal of Immunology                                       |
| J Infect Dis.                | Journal of Infectious Disease                               |
| J Leukoc Biol.               | Journal of Leukocyte Biology                               |
| J Mol Med.                   | Journal of Molecular Medicine                              |
| Med Hypotheses.              | Medical Hypotheses                                          |
| Mol Cells.                   | Molecules and Cells                                         |
| Journal Name                        | Magazine Title                                      |
|------------------------------------|-----------------------------------------------------|
| Nat Immunol.                       | Nature Immunology                                   |
| Nat Rev Immunol.                   | Nature Reviews Immunology                            |
| Otolaryngol Head Neck Surg.        | Otolaryngology- Head and Neck Surgery               |
| Pediatr Infec Dis J.               | The Pediatric Infectious Disease Journal            |
| Proc Natl Acad Sci USA.            | Proceedings of the National Academy of Sciences     |
| Trends Immunol.                    | Trends in Immunology                                |
REFERENCES

1. Resenfeld RM, Culpepper L, Doyle KJ, Grundfast KM, Hoberman A, Kenna MA, et al. Supplement to otolaryngology-head and neck surgery. Otolaryngol Head Neck Surg. 2004 May; 130(5):S95-117.

2. Ryan AF, Catanzaro A, Wasserman SI, et al. Complement depletion reduces immunologically mediated middle ear effusion and inflammation. Clin Immunol Immunopathol. 1986, 40:410-21.

3. Alper CM, Winther B, Mandel EM, et al. Temporal relationships for cold-like illness and otitis media in sibling pairs. Pediatr Infect Dis J. 2007, 26:778-781.

4. Shekelle P, Takata G, Chan LS, et al. Diagnosis natural history and late effects of otitis media with effusion. Evidence report/technology assessment no. 55. Rockville, MD: Agency for healthcare Research and Quality; 2003.

5. Tos M. Epidemiology and natural history of secretory otitis. Am J Otol 1984; 5:459-62.

6. Paradise JL, Rockette HE, Colborn DK, et al. Otitis media in 2253 Pittsburgh area infants: prevalence and risk factors during the first two years of life. Pediatrics 1997;99:318-33.

7. Casselbrant ML, Mandel EM. Epidemiology. In: Rosenfeld RM, Bluestone CD, editors. Evidence-based otitis media, 2nd ed. Hamilton, Ontario: BC Decker Inc; 2003:147-62.
8. Stool SE, Berg AO, Berman S, et al. Otitis media with effusion in young children. Clinical Practice Guideline, no. 12. Rockville, MD: Agency for Health Care Policy and Research, Public Health Services, US department of heath and human services; 1994

9. Yeo SG, Park DC, Lee SK, Cha CI. Relationship between effusion bacteria and concentrations of immunoglobulin in serum and effusion fluid in otitis media with effusion patients. Int J Pediatr Otorhinolaryngol. 2008 Mar;72(3):337-42.

10. Leichtle A, Lai Y, Wollenberg B, Wasserman SI, Ryan AF. Innate signaling in otitis media: pathogenesis and recovery. Curr Allergy Asthma Rep. 2011; 11:78-84.

11. Parkin J and Cohen B. An overview of the immune system. Lancet 2001; 357: 1777-89.

12. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006 Feb;124(4):783-801.

13. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature. 2007 Oct;449(7164):819-26.

14. Janeway CA, Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol. 1989;54(1):1-13.

15. Muzio M, Polentarutti N, Bosisio D, et al. Toll-like receptors: a growing family of immune receptors that are differentially expressed and regulated by different leukocytes. J Leukoc Biol. 2000 Apr; 67: 450-6.
16. Bowie A and O’Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: signal generators for pro-inflammatory interleukins and microbial products. J Leukoc Biol. 2000; 67: 508-14.

17. Oshiumi H, Matsumoto M, Funami K, et al. TICAM-1 an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. Nat Immunol. 2003;4: 161-7.

18. Yamamoto M, Sato S, Mori K, Hoshino K, et al. Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling. J Immunol. 2002;169:6668-72.

19. Fritz JH, Ferrero RL, Philpott DJ, and Girardin SE. NOD-like proteins in immunity, inflammation and disease. Nat Immunol. 2006 Dec; 7(12): 1250-7.

20. Girardin SE, et al. NOD2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem. 2003; 278: 8869-72.

21. Inohara N, et al. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn’s disease. J Biol Chem 2003; 278: 5509-12.

22. Chamaillard M et al. An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat Immunol. 2003; 4: 702-7.

23. Girardin SE et al. NOD1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. Science. 2003; 300: 1584-7.
24. Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, et al. NOD2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 2005; 307: 731-4.

25. Shuto T, Xu H, Wang B, et al. Activation of NF-kappa B by nontypeable Haemophilus influenza is mediated by toll-like receptor 2-TAK1-dependent NIK-IKK alpha/beta-1 kappa B alpha and MKK3/6-p38 MAP kinase signaling pathways in epithelial cells. Proc Natl Acad Sci USA. 2001; 98: 8774-9.

26. Trinchieri G and Sher A. Cooperation of Toll-like receptor signals in innate immune defense. Nat Rev Immunol. 2007; 7: 179-90.

27. Lee MS and Kim YJ. Signaling pathways downstream of pattern-recognition receptors and their cross talk. Annu Rev Biochem. 2007; 76: 447-80.

28. Kim MG, Park DC, Shim JS, Jung H, Park MS, Kim YI, Lee JW, Yeo SG: TLR-9, NOD-1, NOD-2, RIG-I and immunoglobulins in recurrent otitis media with effusion. Int J Pediatr Otorhinolaryngol 2010;12:1425-1429

29. Lee YC, Kim C, Shim JS, Byun JY, Park MS, Cha CI, et al. Toll-like receptors 2 and 4 and their mutations in patients with otitis media and middle ear effusion. Clin Exp Otorhinolaryngol 2008;4:189-195

30. Klein JO, Tos M, Hussl B, Naunton RF et al. Recent advances in otitis media. Definition and classification. Ann Otol Rhinol Laryngol. 1989; 139: 10.

31. Tos M and Bak PK. The pathogenesis of chronic secretory otitis media. Arch Otolaryngol Head Neck Surg. 1972; 95: 511-21.

32. Streatemans M, Van Heerbeek N, Tonnaer E, et al. A comprehensive model
for the aetiology of otitis media with effusion. Med Hypotheses. 2001; 57: 784-91.

33. Lee MS, Kim YJ. Pattern-recognition receptor signaling initiated from extracellular, membrane, and cytoplasmic space. Mol Cells. 2007 Feb; 23(1): 1-10.

34. Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol. 2009 Apr;21(4):317-37.

35. Vandermeer J, Sha Q, Lane AP, Schleimer RP. Innate immunity of the sinonasal cavity. Arch Otolaryngol Head Neck Surg. 2004 Dec;130(12):1374-80.

36. Aliprantis AO, Yang RB, Mark MR, Suggett S, Devaux B, Radolf JD, et al. Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. Science. 1999 Jul; 285(5428):736-9.

37. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity. 1999 Oct;11(4):443–51.

38. Kim MJ, Cho YK. Pattern recognition receptors in immune modulation. Biowave. 2006 Jun;8(12):1-22

39. Malley R, Henneke P, Morse SC, Cieslewicz MJ, Lipsitch M, Thompson CM, et al. Recognition of pneumolysin by Toll-like receptor 4 confers resistance to pneumococcal infection. Proc Natl Acad Sci USA. 2003 Feb;100(4):1966–71.
40. Shuto T, Imasato A, Jono H, Sakai A, Xu H, Watanabe T, et al. Glucocorticoids synergistically enhance nontypeable Haemophilus influenzae–induced Toll-like receptor 2 expression via a negative cross-talk with p38 MAP kinase. J Biol Chem. 2002 Mar;277(19):17263-70.

41. Agnese DM, Calvano JE, Hahm SJ, Coyle SM, Corbett SA, Calvano SE, et al. Human toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of gram-negative infections. J Infect Dis. 2002 Nov;186(10):1522-5.

42. Montes AH, Asensi V, Alvarez V, Meana A, Carton JA, Paz J, et al. The toll-like receptor 4 (Asp299Gly) polymorphism is a risk factor for gram-negative and haematogenous osteomyelitis. Clin Exp Immunol. 2006 Mar;143(3):404-13.

43. Schroder NWJ, Hermann C, Hermann J, Gobel UB, Hartung T, Schumann RR. High frequency of polymorphism Arg753Gln of the Toll-like receptor-2 gene detected by a novel allele-specific PCR. J Mol Med. 2003 Jun;81(6):368-72.

44. Lee YC, Kim C, Shim JS, Byun JY, Park MS, Cha CI, et al. Toll-like Receptors 2 and 4 and Their Mutations in Patients with Otitis Media and Middle Ear Effusion. Clin Exp Otorhinolaryngol. 2008 Dec;1(4):189-95.

45. Leichtle A, Hernandez M, Pak K, Webster NJ, Wasserman SI, Ryan AF. The toll-Like receptor adaptor TRIF contributes to otitis media pathogenesis and recovery. BMC Immunol. 2009 Aug;5:10-45.
46. Strober W, Murray PJ, Kitani A, Watanabe T. Signaling pathways and molecular interactions of NOD1 and NOD2. Nat Rev Immunol. 2006; 6: 9-20.
47. Martinon F and Tschopp J. NLRs joining TLRs as innate sensors of pathogens. Trends Immunol. 2005; 26: 447-54.
48. Jeep S. Correlation of immunoglobulins, the complement system and inflammatory mediators with reference to the pathogenesis of serous otitis media. Laryngorhinootologie. 1990 Apr;69(4):201-7.
49. Howie VM, Ploussard JH, Sloyer JL, Johnston RB. Immunoglobulin of the middle ear fluid in acute otitis media: relationship to serum immunoglobulin concentrations and bacterial cultures. Infect Immun. 1973 Apr;7(4):589-93.
50. Bluestone CD, Klein JO. Epidemiology: Otitis Media in Infants and children. 4th ed. Italy: BC Decker Inc. 2007: 93 p.
51. Merriam-Webster Visual Online Dictionary. 2013. http://visual.merriam-webster.com/human-being/sense-organs/hearing/structure-ear.php
52. A.D.A.M. Inc., 2013. American Accreditation HealthCare Commission. http://www.urac.org,
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PUBLICATIONS

Lee, S.Y., Ryu, E.W., Kim, J.B., & Yeo, S.G. (2011). “Clinical Approaches for Understanding the Expression Levels of Pattern Recognition Receptors in Otitis Media with Effusion: Clinical and Experimental Otorhinolaryngology
Shim, H.J., Lee, L.H., Huh, Y.B, Lee, S.Y., Yeo, S.G. (2011). Age-related changes in the expression of NMDA, Serotonin, and GAD in the Central Auditory System in the Rat: Acta-laryngologica
Ryu, E.W., Lee, S.Y., Park, M.S., Yeo, S.G. (in press). Clinical Manifestation and Prognosis of Patients of Ramsay-hunt Syndrome: American Journal of Otorhinolaryngology
Jung, S.Y., Lee, S.Y., Lee, S.K., Lee, H.Y., Yeo, S.G. (in revision). Comparison Result According to Steroid Administration Methods in Idiopathic Sudden Deafness: American Journal of Otorhinolaryngology
Hong, S.M., Lee, S.Y., & Yeo, S.G. (e-book publication on 2012). The Auditory Cortex: Anatomy, Function, and Disorders: NOVA Publisher
Chung, D.H., Park, D.C., Byun, J.Y., Park, M.S., Lee, S.Y., & Yeo, S.G. (2011), Prognosis of Recurrent Facial Palsy: European Archives of Otorhinolaryngology