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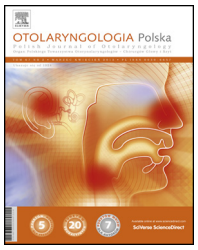

Original research article/Artykuł oryginalny

Expression of Toll-like receptors on peripheral blood white cells in acute otitis media

Krzysztof Trzpis [1,*], *Edwina Kasprzycka* [2], *Bożena Skotnicka* [1], *Elżbieta Hassmann-Poznańska* [1], *Jolanta Wysocka* [2]

[1] *Department of Pediatric Otolaryngology, Medical University, Bialystok, Poland*
[2] *Department of Pediatric Laboratory Diagnostics Medical University, Bialystok, Poland*

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ABSTRACT

Objective: From 10 to 15% of children suffer from recurrent acute otitis media (AOM). An association between polymorphism in TLRs and their co-receptor CD14 with otitis media proneness has been described in children. Moreover, the experiments on animal models have shown that TLRs and their signaling molecules are critical for timely resolution of bacterial otitis. **Aim:** The aim of this study was to determine the expression of TLR1, TLR2 and TLR4 on lymphocytes, monocytes and granulocytes in peripheral blood in children with recurrent or persistent AOM. **Methods:** The study was performed on a group of 25 children hospitalized for recurrent AOM, failures of previous treatments and/or acute mastoiditis. The results were compared to the control group of healthy children at the same age. The expression of TLRs on peripheral blood white cells was measured by flow cytometric analysis. The results were expressed as mean fluorescence intensity (MFI). The statistical analysis was performed using the Mann–Whitney *U* test. **Results:** The highest expression of TLR was found on monocytes, the lowest on lymphocytes in both groups of children (AOM and the control one). The expression of TLR1 was the lowest and expression of TLR4 was the highest on all examined cells. The expression of all examined TLRs on monocytes was significantly higher in the AOM group. **Conclusions:** Peripheral blood monocytes are characterized by increased expression of TLRs in the course of recurrent AOM.

Acute otitis media (AOM) is the most common bacterial infection in preschool children. Largest part of the episodes occur in the first years of life. The majority of AOM cases resolve spontaneously, but approximately 10–15% of children experience four or more episodes in the following year, which allow the diagnosis of recurrent otitis media. AOM may also occasionally result in serious complications, as mastoiditis.

There are several known risk factors for recurrent or persistent AOM and the disease is considered to be multifacto-

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* *Corresponding author at*: Klinika Otolaryngologii Dziecięcej, UM, ul. Waszyngtona 17, 15-274 Białystok, Poland. Tel.: +48 85 7 450 832; fax: +48 85 7 450 832.
E-mail address: krzysztof_trz@op.pl (K. Trzpis).

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rial. Environmental factors (day-care attendance, poor socioeconomic status), diseases associated with poor Eustachian tube function, genetic and immune factors all contribute to predisposition for recurrent AOM. Although it is apparent that AOM is commonly associated with viral infection of upper respiratory tract, bacterial infection is considered to be the most significant in the etiology of this disease. The most frequent bacterial pathogens of AOM are *Streptococcus pneumoniae* and *Haemophilus influenzae*.

The human immune system is comprised of two interrelated branches: innate and adaptive immunity. Those branches use different strategies to recognize pathogens. Whereas the innate, immediate, immune system detects molecular structures unique to microbes using a limited number of encoded pattern recognition receptors (PRR), the adaptive immune system uses clonally expressed, highly specific receptors [1, 2].

Humans cells are provided with various PRRs, including Toll-like receptors (TLRs) expressed on cells of the innate immune system as macrophages and dendritic cells but they are also present on many other cells including epithelial [3]. TLR2 responds to the cell wall components of Gram-positive bacteria as peptoglycans, lipoteichoic acid and lipoproteins. TLR4 binds to the toxic pneumolysin ligand produced by Gram-positive bacteria, as well as to lipopolisaccharide (LPS), the major component of Gram negative bacteria cell walls [4, 5]. TLR4 has also been shown to respond to the fusion protein of respiratory syncytial virus [6].

Activation of TLRs, leads to activation of the complement cascade and proinflammatory cytokine production [7–9]. TLRs induced signals also control adaptive immunity at various checkpoints regulating the selection of antigens presented by dendritic cells, type of response, its magnitude and duration and the production of long term memory [1, 10].

Increased expression of those receptors have been found in the mucosa and effusion from middle ears during the course of experimental forms of otitis media [11–14]. Therefore, it can be presumed that increased expression of those molecules would also be found on circulating immune cells from children with recurrent otitis media. Accordingly, the study was undertaken to define the expression of TLR 1, 2 and 4 on peripheral blood monocytes, lymphocytes and granulocytes in patients suffering from recurrent or persistent otitis media and compare them with other parameters of inflammatory reaction. The aim of this study was to explain the role of TLR's in the immunological response and their potential influence on the intensity of inflammatory reaction. This may provide new insight into the pathology of AOM and possibly modify required intervention.

Materials and methods

Twenty-five pediatric patients (mean age 3.5 years) hospitalized because of acute otitis media (group AOM) in the Department of Pediatric Otorhinolaryngology, Medical University, Bialystok, participated in the study. The reasons for hospitalizations were recurrent acute otitis media (3 and more episodes during 6 months or 4 or more during 1 year), persistent otitis media (failures of previous treatment) or complications of acute otitis media (mastoiditis). All patients fulfill the AAP criteria for diagnosis of AOM (acute onset of signs and symptoms, presence of middle-ear inflammation as indicated by earache and distinct erythema of the tympanic membrane and presence of effusion in the middle ear indicated by bulging of tympanic membrane or otorrhea).

The control group consisted of 25 healthy children (mean age 3.54 years), with no history of recurrent upper respiratory tract and ear infections, admitted to the Department of Pediatric Surgery for planned minor surgical procedures. The blood samples in this group was taken before the surgery, together with the probes for routine examinations.

The study was approved by the Ethics Committee of Medical University, Bialystok. The purpose of the study was explained to the parents and written informed consent was obtained from all of them. On the day of admission, before starting antibiotic and/or surgical treatment or up to 8 h after it, from all patients peripheral blood samples were obtained (1.8 ml) to test-tubes with K3EDTA anticoagulant for the purpose of assessing the expression of TLRs on granulocytes, monocytes and lymphocytes of peripheral blood. Also blood count in peripheral blood smear, erythrocyte sedimentation rate and C-reactive protein (CRP) level were assessed in all collected samples. C-reactive protein (CRP) level was performed using Cobas Integra 800 Roche, blood count in peripheral blood smear using Sysmex XT2000i hematologic analyzer and erythrocyte sedimentation rate using Roller 20 Alifax. Criteria for normal CRP values (ranged between 0 and 0.5 mg/dl) used in our Department of Pediatric Laboratory Diagnostics were defined for Cobas Integra 800 (turbidimetric immunoassay) by Roche.

Flow cytometry was performed on all the blood samples (using Cytomics FC500 by Beckman Coulter cytometer). Prior to staining the cells for cytometric analysis, cell viability was assessed by trypan blue exclusion. Only the samples with at least 98% of cells viability were selected for the study. 10 thousand cells were assessed in every collected sample. All the blood samples were incubated for 15 min in the room temperature, in the darkness, using monoclonal antibodies conjugated with proper fluorochromes (anti-human CD 281 (TLR1) PE, IgG1, clone CD2. F4; anti-human CD 282 (TLR2) PE, IgG2a, clone TL2.1; anti-human CD 284 (TLR4) PE, IgG2a, clone HT A 125 produced by eBioscience company and anti-human CD14 FITC, IgG2a clone TUK4 produced by DakoCytomation company). The negative control was applied in accordance with the class of used monoclonal antibodies. After the incubation all the samples underwent the process of lysis in automatic lysis station (Epics Immunology Work Station) using Multi-Q-Prep set. Lymphocytes and granulocytes were separated using the gate in FS/SS system, monocytes were signed with the usage of anti-CD14 antibodies. All the cytometric analyzes were performed in maximum 60 min after the incubation. The results were given as MFI (mean fluorescence intensity), that indirectly equals the level of receptors' density on the cell surface. All the analyses were performed at maximum 2 h after collecting the sample.

Statistical analysis of the results was performed using of non-parametrical Mann–Whitney *U* test in Statistica 5.0.