

Inflammatory mediators of hypertrophic adenoids in children with otitis media with effusion

Mediatory zapalne przyrosłych migdałkach gardłowych u dzieci chorych na wysiękowe zapalenie ucha

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Summary

Adenoid is lymphoid tissues in the pharynx that play an important role in host defense against invading antigens. The structures of adenoid consist of well-defined microcompartments which all participate in the immune response: the lymphoepithelium, the follicular germinal center, the mantle zone, the interfollicular area. Otitis media with effusion (OME) is a common middle ear disease in children, but etiology of this disease is not clearly understood. Immunologic reaction within adenoid, pro-inflammatory cytokines (Th1 and Th2), decrease of lymphocytes T and B, metalloproteinase may lead to chronic infection.

Keywords: *lymphocytes T and B, adenoid, cytokine, VEGF, MMP, apoptosis*

Streszczenie

Migdałek gardłowy wchodząc w skład obwodowych narządów limfaticznych jest miejscem pierwszego kontaktu z antygenami, docierającymi drogą oddechową i pokarmową.

Migdałek gardłowy zawiera komórki aktywne immunologiczne niezbędne do odpowiedzi immunologicznej komórkowej oraz humoralnej. Komórki te zajmują trzy główne przedziały limfoidalne: limfonabłonek, grudkowe ośrodki rozmnażania wraz ze strefą osłonową tych ośrodków oraz przestrzeń międzygrudkowa.

Wysiękowe zapalenie ucha środkowego (OME) jest częstą chorobą ucha środkowego występującą u dzieci. Jednak etiologia tej choroby nie jest do końca poznana. Reakcje immunologiczne toczące się w obrębie migdałka gardłowego, udział cytokin prozapalnych typu Th1 i Th2, limfocytów T i B oraz metalloproteinaz może prowadzić do przewlekłego zakażenia w obrębie ucha środkowego u dzieci.

Słowa kluczowe: *limfocyty T i B, migdałek, cytokiny, VEGF, MMP, apoptoza*

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Adenoid

Adenoid play an important role in host defenses of the upper respiratory tract against invading antigens. Lymphoid tissue develops after birth. It reaches maximum size during early childhood and starts to regress at the age of 7-10 years [1]. These tissues have four histological compartments which are very important in the immune response: the follicular germinal center, the mantle zone, the interfollicular area and the crypt epithelium [2] (Figure.1). Germinal center reaction is characterized by large amounts of rapid clonal expansion and is generally polarized into two distinct zones. The dark zone is proximal lymphocytes T and contains proliferating B cells, called centroblasts, while the light zone contains noncycling B cells called centrocytes (Figure 1). Lymphocytes T provide help to B cells

via CD40-CD40L interaction and cytokine production. Lymphocytes B provide –MHC class II complexes (pMHCII) in the context to stimulatory production T cells and cytokine. Dendritic cells (DC) are found in the region between T and B cell zone [3]. The role of dendritic cells is to participate in polarization of immunological response to Th1 or Th2 which is necessary of rolling inflammatory process in middle ear [4]. Analysis of plasmoid DCs (CD 141⁺, CD 123⁺, CD 11c) and myeloid mDCs adenoid show that CD123⁺ pDCs as well as CD1c⁺, CD141⁺, and CD1c⁺CD141⁻ mDCs were detected in adenoid and lymph nodes. [5].

The etiology of hypertrophic adenoid in children with or without otitis media with effusion is not exactly known. It is estimated that more than 50% of children are diagnosed with OME by the age of 1 year, and up to 90% of

children by the time they have school age. [6]. The literature shows that there is an increased expression of several inflammatory response mediators of adenoid which may lead to hypertrophy and chronic infections and Eustachian tube dysfunction [2].

The role of Th1 and Th2 cytokines in hypertrophic adenoid

Effusion fluid in the middle ear contained acid phosphatase, mucin, alkaline phosphatase, cytokines, creatine phosphokinase and immunoglobulin producing cells [7]. Bacteria can trigger cytokine secretion of macrophages and monocytes as well as TLR expression. Gram-negative bacteria tend to induce expression of IL-6, IL-10, IL-8 but gram-positive bacteria tend to induce expression of TNF- α , IFN- γ and IL-12 [8]. Subgroup T helper cells-Th17 producing proinflammatory IL-17 and developmentally, different from Th1 and Th2 cells, which play an important role in antimicrobial immunity [9].

In respond to infection adenoid starts production in crypt epithelium proinflammatory Th1 cytokines, including TNF- α , IFN- γ , IL-2 and later secrete Th2 type cytokine (IL-4, IL-5, IL-6, IL-10, IL-13) [9]. Todorovic et al. demonstrated higher production of Th-1 cytokines (TNF- α , IFN- γ) in recurrent tonsillitis compared in tonsillar hypertrophy. In both forms of tonsilitis the production of Th-1 type cytokine was higher compoared to the production of Th-2- type cytokines. Valenzuela et al. showed that IL-1 β , TNF- α , TGF- β , IL-17 and IFN- γ levels were similar in the tonsils of patients and controls. IL-4 gene expression in the tonsils was lower ompared to the controls. However, IL-4 cytokine gene expression in the tonsils and peripheral blood eosinophils suggesting a potential pathogenesis pathway based on an inhibition of Th2 responses [10]. High concentrations of TNF- α , IL-8, IL-1 β and IL-6 were found in children with chronic otitis media with effusion but in the non-atopic group. In the non-atopic group with bacterial isolate,

TNF- α and IL-1 β levels positively correlated with the number of past acute otitis media. High concentrations of the proinflammatory cytokine are involved locally in the zone of inflammation [11]. Lymphocytes T CD4⁺ and T follicular helper (Tfh) are responsible for assisting B lymphocytes to generate antibodies. These cells interact with ligands on the surface of lymphocytes B and start secretion of IL-21 and IL-4, IL-6, IL-8 and IgE which may be involved in the onset of adenoidal hypertrophy combined with secretory otitis media in children [12].

Cell division cycle 42 (CDC42) is a small hydrolase of guanosine triphosphate (GTPase) with is fundamental regulator of various biological procedur end inflammation response. CDC42 promotes the differentiation of CD4⁺ T cell into T helper (Th) 2 cell. CDC42 would aggravate the imbalance of Th1/Th2 cell and involved in the OME pathogenesis and development. Wu et al. demonstrated that CDD42 and IL-4 are positively intercorrelated and their up-regulated levels reflect elevated effusion viscosity as well as heating loss degree in OME patients [13].

The role of VEGF in hypertrophic adenoid

Pro-inflammatory cytokines such as TNF- α , transforming growth factor-beta (TGF- β), IL-1 can induce production of VEGF (vascular endothelial growth factor) from epithelial cells, vascular endothelial cells or monocytes. Vitamin D deficiency causes an increase in VEGF expression in the tonsillar tissue which plays a role in the pathogenesis of chronic tonsillitis. VEGF expression in recurrent chronic tonsillitis may be a potential target for anti VEGF therapeutics [14].

VEGF can also impact endothelial cell expression of immunologically important molecules, decreasing expression of VCAM-1 (vascular cell adhesion molecule-1) increasing expression of FasL, leading to apoptosis [15]. VEGF is produced by the middle ear mucosa and its concentration increases in the exudative and middle ear mucosa in patients with otitis media. Sekiyama et al. showed the higher con-

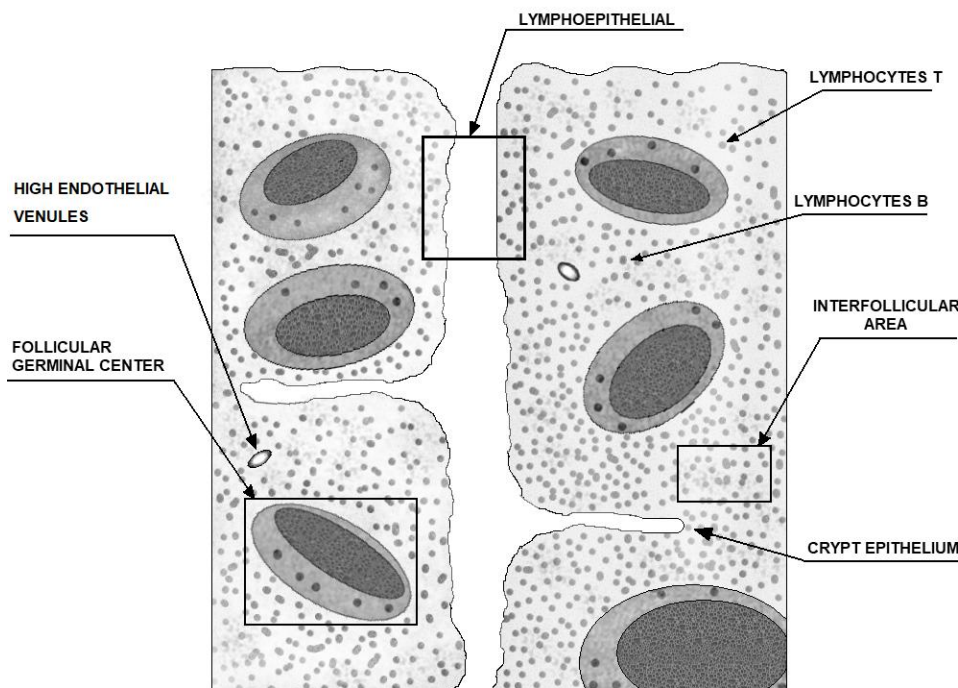


Figure. 1. The schema construction of adenoid with lymphoid interval

centration of endotoxin and IL-8 in most middle ear effusion samples. In their study the concentration of endotoxin were significantly correlated with VEGF levels. In contrast, here was no correlation between IL-8 and VEGF levels or between endotoxin and IL-8. Their results suggest that VEGF is produced in the middle ear in response to stimulation with endotoxin and hypoxic conditions [16]. Bacterial infections increase the levels of both VEGF and its receptors. Elevated cytokine concentrations lead to increased vascular permeability and elevated secretion of exudative fluid. Moreover, they affect the infection of inflammatory cells of the mucous membrane, thus leading to neovascularization within the middle ear [17]. The mean concentration levels of VEGF-A and TGF- β in hypertrophic adenoid in children with adenoid hypertrophy and otitis media with effusion were significantly higher compared to patients with adenoid hypertrophy. High levels of this cytokine may indicate a bacterial pathogen as one of the causes of exudative otitis media in children [18].

Apoptosis in hypertrophic adenoid

Germinal center of adenoid plays an important role in cell death and cell proliferation [19]. The increase in size and more number of germinal centers was found in patients with hypertrophy adenoid compared to those without hypertrophy. Onal et al. determined that the increased adenoid apoptosis was associated with age. The significantly increased of apoptosis in hypertrophy adenoid compared to chronic adenoid. The authors felt that increased apoptosis with increasing age might account for the noted involution of lymphoid tissue as patients get older [20]. The number of lymphocytes T and B in adenoid and their cooperation in the immunologic and morphological involution depends on their proliferation and migration status. Apoptosis as the process that regulates lymphocyte homeostasis is to a large extent responsible for the immune function of the hypertrophic adenoid [21]. Tumor necrosis factor receptor superfamily member (Fas receptor, CD95) is a death receptor localized on the surface of cells, which triggers a signal transduction pathway leading to apoptosis. Fas-mediated apoptosis is depending on cytokine-2 (IL-2). The interaction between Fas and ligand FasL (FasL/CD95L) regulates pathological and physiological processes. In the Fas-mediated apoptotic pathway, binding of FasL drives Fas clustering and binding of Fas to FADD. FADD recruits caspase-8 and caspase-10 form the death-inducing signaling complex (DISC) [22]. The antiapoptotic molecule Bcl-2 and its homolog Bcl-xL in lymphocytes accumulate greatly increased number of T and B cells [23]. These molecules control transport through the pores on the external mitochondrial membrane and prevent the exit of cytochrome c from mitochondria and apoptosis initiation. Germinal center cells do not display the expression of Bcl-2, but in the mantle zone cells express a high level of Bcl-2 [24].

BMP (bone morphogenetic protein) can affect B-cell development at different stages [23]. BMP-2, -4, -6 and -7 reduced CD40L- and IL-21-induced Ig production in human naïve and memory B cells. The mechanism for BMP-induced suppression differed between the BMPs, as BMP-6 potently inhibited plasmacell differentiation, BMP-7 mainly induced

apoptosis. Bollum et al. identified BMP-7 as a negative regulator of GC B-cell survival. Hence adding another component to the regulation of B cells undergoing GC reaction. They suggested that BMP-7-induced apoptosis could be influenced by the TGF- β receptor ALK5 in an indirect manner, further demonstrating the receptor complexity within the TGF- β superfamily [25].

The role of metalloproteinase in hypertrophic adenoid

Matrix metalloproteinases (MMP) are zinc-dependent endopeptidases which have a key role in the degradation of extracellular matrix. (MMP) and their inhibitors (TIMP) are produced during the inflammatory process by lymphocytes, neutrophils, macrophages, mast cells and eosinophils. The coordinate expressions of MMP and TIMP are involved, tissue remodeling cell transmigration during inflammatory disease, proliferation and apoptosis [26]. The literature shows that level of MMPs and TIMPs in relation to inflammatory and hypertrophic process in adenoids and the resulting otitis media with effusion. Guedez et al. demonstrated that TIMP-1 expressed by isolated germinal center B cells, but antiapoptotic mechanism is also not mediated by Bcl-2 but likely inducing Bcl-xL [27]. Another author showed diffuse dissemination of MMPs activity in adenoid tissue. The level of MMP-7 and MMP-9 was higher of hypertrophied tissue, but no differences were observed in the level of TIMP-1 and MMP-2. The concentrations of MMP-8 and MMP-9 and TIMP-1 in children with adenoid hypertrophy and secretory otitis media reached statistically significantly higher concentrations than those in children belonging to the reference group [28]. This result suggest that MMPs promotes of the extracellular matrix responded to inflammatory changes in adenoid tissue.

Conclusion

The different mediators of inflammation like cytokines, VEGF-A and TGF- β involved in the pathogenesis of otitis media with effusion in children. Increased levels of pro-inflammatory cytokines (Th1) suggest that abnormalities in the immune system triggering chronic, uncontrolled, inflammation may be a driver of disease. Elevated MMPs and TIMPs levels may induce an increased remodeling of the extracellular matrix. Indicate enhanced apoptosis of activated lymphocytes T and B, can lead to disturbances in local immune response involving the middle ear.

Accurate recognition of inflammatory markers activity may become, a viable preventative or treatment in children with otitis media with effusion.

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