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The future of research in immunobiology of suppurative otitis media

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Summary
Otitis media (OM) continued to be one of the most common reasons for children to see a physician and annually requires billions of dollars in healthcare expenditures for treatment in USA. Despite the high incidence, its complex multifactorial pathogenesis is not yet understood and controversies continued on the role of immunobiological factors particularly cytokines and immunoglobulins in the actiology or chronicity of otitis media. This review article discusses our research findings and reviews our knowledge up to date as found in published literature.

Keywords: *Immunoglobulins, cytokines, otitis media, future*

Résumé
L'otite media continue d'etre l'une des raisons les plus communes pour les enfants de rencontrer un médecin. A nos jours aux Etats Unis, des milliards de dollars sont dépensés en soins de santé pour le traitement. Malgré la grande incidence, sa pathogénèse multifactorielle complexe n'est pas encore comprise et les controverses/debats continuent sur le role des facteurs immunobiologiques particulièrement les cytokines et les immunoglobulines dans la sévérité de l'otite media.

Introduction

Otitis media has a complex multifactorial pathogenesis which can be provoked by different primary factors such as bacterial and viral infections, local allergic reactions and reflux. The typical inflammatory response being the accumulation of cellular and chemical mediators in middle ear [1]. However, there are controversies whether specific biochemical and immunochemical factors might be responsible for the aetiology or chronicity of otitis media [2]. OM continued to be one of the most common reasons for children to see a physician and annually requires billions of dollars in healthcare expenditures for treatment in USA. In addition, antibiotic resistance has made otitis media treatment more challenging. Despite these facts otitis media is relatively understudied [1].

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Epidemiology

Most studies have looked into the epidemiology, documenting the incidence up to 90% in children under 2 years of age [3,4] and 60% in preschool attendees [3]. In Nigeria, the prevalence is 21.2% in children and accounted for 25% of patient attendance at the otorhinolaryngologic clinic [5,6]. The Sociodemographic risk factors have been reported with differences in the significance and roles of the risk factors. Most of the works were point survey and review of community/ hospital data, but very few were prospective longitudinal study of children.

In our study, we followed up children for one year from birth and we found the prevalence of suppurative otitis media in the first year of life to be 37%. In addition we found that low levels of cord blood IFN gamma, retinol and Zinc were significant in development of ESOM [7]. Our hypothesis was that the mechanism of prenatal influence might be expressed through maternal undernutrition particularly in the trace elements and overwhelming viraemia passed down to the infant at birth and predisposing to OM. Serial measurement of *foetal immunobiologic* markers and the maternal serum level of these markers may also be important in future to establish relationship between maternal and foetal undernutrition and early otitis media. Indeed studies on prenatal predisposition to OM are few and these are areas for further studies in the immunobiology of the development of OM. In addition, we did not consider the effect of breastfeeding, although, all the children were on breastfeeding during the study. Chantry *et al* [8] concluded that infants who were fully breastfed for 4 to < 6 months had statistically significant increased risk for both pneumonia (odds ratio [OR]: 4.27; 95% confidence interval [CI]: 1.27-14.35) and > or = 3 episodes of OM (OR: 1.95; 95% CI: 1.06-3.59) in those who were fully breastfed for

4 to < 6 months compared with > or = 6 months. Hence this supports the current recommendations that infants should receive only breast milk for the first 6 months of life.

One other significant finding from our study was that the otorrhoea in the children were either pus or mucoid, we did not see the typical serous effusion as reported in cauccasians, despite the fact that we studied children in the first year of life. This further reinforced our earlier observation in the out – patient clinic. Earlier investigators have posited that otitis media with effusion was not commonly seen in this environment, the patients presented late and there was secondary bacterial infection [1,3,4,7]. However, our study was a prospective follow up design, thus obviating the problem of late presentation. This difference in the nature of otorrhoea needs further study to be clarified. This might also suggest the need to study the role of the eustachian tube factor in our patients. Probably, these findings may improve our knowledge of the pathogenesis of OM, and the clinical management.

Immunoglobulins and OM

The activity of immunoglobulins in chronic OME is evidence of chronic humoral inflammatory processes in the middle ear, which is obviously controlled by cytokines. The presence of the main types of immunoglobulins, IgM, IgG, IgA, secretory IgA and IgE, in effusions is indirect evidence that cytokines IL-2, IL-10, TGF-b, IL- 4 and IL-5, which are involved in regulation of immunoglobulin production and secretion also regulate humoral immune reactions during the course of middle ear inflammation [8-14]. Different types of immunoglobulins, namely IgM, IgG, IgA, secretory IgA and IgE, have been identified in effusions and middle ear fluid of chronic OME [15-18]. The immunologic investigation of effusions detected the immune complexes of IgG (IgG- ICs) and IgA (IgA- ICs) in both acute and chronic otitis media [19,20]. The highest level of IgG-ICs was found in sub-acute cases, whereas IgA-ICs were predominant in chronic OME. The immunoglobulin immune complexes have been speculated to prolong the inflammatory process in the middle ear. The presence of immunoglobulins in chronic OME was associated mainly with the bacterial infection [21-24]. IgG and IgA antibodies specific to *Hemophilus influenzae* and *S. pneumoniae*, IgG, IgM, IgA and secretory IgA antibodies specific to outer membrane antigens of *Moraxella catarrhalis*, and *Staphylococcus aureus*-harboured bacteria, intensely coated with secretory IgA and IgG antibodies were identified in chronic effusions [19-24]. However, only the secretory immunoglobulin, IgA, was identified in effusions infected with respiratory viruses [25]. High levels of MES Ig E and IFN gamma, and impaired middle ear immune response as shown by low levels of MES Ig A and serum Ig G were identified as factors of chronicity in OM [26,27]. The nature of otorrhoea has also been linked to the differences in the concentration of the immunoglobulins in the middle ear secretion. The mucoid type of effusions contained a high level of IgG, IgA and IgE [28]. However, our research findings showed higher Ig A in mucoid and lower Ig G and Ig E in mucoid compared to purulent otorrhoea [26,27]. Correlation between the levels and types of immunoglobulins and the immunoregulatory and allergy-associated cytokines in chronic OME has not been investigated. Comparison of the immunoglobulin levels measured in effusions and in sera showed that, in many cases, the effusion levels of secretory IgA [29] and IgE [30] were significantly higher than the corresponding serum levels, and this was hypothesized to be the evidence of local overproduction of immunoglobulins in the middle ear. It is still not known whether the immunoglobulin in the middle ear secretion is produced from the middle ear or from the nasopharynx and subsequently transported into the middle ear. Animal experiment may be required to confirm this. In addition, differences between the cellular constituent of the secretion and the mucosa may reveal the types of cells produced by the middle ear. Further investigative studies are required in this area.

Cytokines in OM

Cytokines are responsible for resolution of inflammation and can initiate local molecular processes leading to histopathological changes in the middle ear mucosa and submucosa, and the chronic condition of otitis media [1,2]. Different groups of cytokines have been identified in the middle ear effusions and mucosa: The pro-inflammatory TNF-a, IL-1b, IL-6 and IL-8; the immunoregulatory IL-2, IL-10 and TGF-b; and the allergy-associated IL-4, IL-5 and GM-CSF. The immunoregulatory cytokines and the allergy-associated cytokines have been considered the key regulators of the middle ear inflammation responsible for the molecular and cellular background of chronic OME [1]. The immunoregulatory cytokines IL-2, IL-10 and TGF-b initiated and supported molecular switching of the acute phase of inflammation in the chronic stage [1]. Whereas the allergy-associated cytokines IL-4, IL-

5 and GM-CSF probably provided the molecular and cellular background for chronic humoral, cell-mediated and allergic inflammatory processes in the middle ear, which lead to the chronicity of OM [1]. However, further studies are necessary in order to elucidate the molecular mechanisms of cytokine regulation of the middle ear inflammation; and the possibility of anti-cytokine therapy in clinical treatment of OM.

Animal models suggested that the inflammatory stimulus were bacterial endotoxins, which would stimulate TNFa production, and led to mucin production and mucous hyperplasia [31,32]. Yellon *et al* [33] detected interleukin-1 beta in 58% (44/75) middle ear effusions; interleukin-6, 83% (60/72); tumor necrosis factor alpha, 37% (28/75); and interferon gamma, 61% (45/74). High TNFa levels in middle ear effusions have been correlated with persistence of OME. One function of IL-8, in the middle ear as elsewhere, is to induce chemo-attraction of neutrophils and its production is directly controlled by TNFa and IL-1b. IL-8 has been found in higher levels in more viscous effusions and in effusions with bacteria on culture. TNFa and IL-1b are markers of the acute inflammatory response, whereas IL-8 may represent chronicity [34,35,36,37].

IL-2 is the up-regulating cytokine, which stimulates primarily the cell-mediated inflammatory response by promoting growth, proliferation and differentiation of T cells, B cells, natural killer (NK) cells, monocytes and macrophages [38,39]. It is secreted mainly by activated T cells. IL-2 induces cytokine production in T cells, including interferon (IFN)-gamma and IL-4 [38-42].

In contrast, IL-10 (known as the cytokine synthesis inhibitory and macrophage deactivating factor) down-regulates the immune reactions accompanying acute inflammation and limits the duration of inflammatory responses. IL-10 can also promote and regulate chronic inflammatory processes. IL-10 is produced by a variety of cell types, including CD4 - T cells, activated CD8- T cells and activated B cells. The main anti-inflammatory activities of IL-10 namely, inhibition of cytokine production in macrophages, neutrophils, T cells and NK cells [39-41], and inhibition of the macrophage - monocyte activation and the antigen presentation abilities of these cells lead to the resolution of inflammation [1,43]. However, if the acute inflammatory process has not been resolved, IL-10 can induce humoral inflammatory reactions such as the immunoglobulin isotype switching in B cells, differentiation of B cells into plasma cells and thus promote switching of inflammation in the chronic stage [9-12]. Identification of factors involved in chronicity appears to be an essential step in the treatment and ultimate prevention of chronic otitis media. Our finding revealed significant difference in the middle ear secretion levels of interferon gamma between resolved ASOM and CSOM, and between mucoid and purulent OM [43]. This suggests that the middle ear immune response may be a significant factor in the outcome of SOM. This is also needs to be studied in HIV/AIDS patients with OM to determine the difference in their middle ear immune response. Our further hypothesis is that persistence of SOM may be a result of deficient secretion of the pro – inflammatory cytokine compared to the immunoregulatory cytokines. The study of the ratio of these cytokines in SOM should constitute major areas of future research because of their clinical implications in the control of CSOM.

Allergy and OM

Allergy has been associated with OME in 35% to 45% of cases [44-46]. In addition, sensitivity to foods and inhalants has been reported in 92.3% and 100% respectively of patients with eustachian tube dysfunction [47,48]. Middle ear effusions contain mediators of the allergic response, such as IgE and eosinophil cationic protein [49-52]. However, from the relative concentrations of the mediators in the effusion and serum it is yet to be confirmed that they are produced locally [52,53]. In addition, the presence of IgE in chronic OME has not been associated with local allergic inflammation [53,54]. However, local overproduction of IgE was usually accompanied by local allergic reactions, such as degranulation of mast cells found in the middle ear biopsy specimens and expression of IgE on mast cells detected in nasal mucosa specimens from patients with OME [53,54]. In our study of 228 acute SOM subjects, we identified positive skin test to one or more of the allergens among dust, house dust mite, mould, cockroach and poultry feather in 105 compared to 15/71 controls. Allergy was found in 66/87 of CSOM and 39/141 of resolved ASOM. In an earlier study, we had reported our finding of significant difference in the mean middle ear secretion levels of Ig E between resolved ASOM and CSOM. All of these revealed that allergy was a significant factor in the persistence of SOM [55].

However, it was difficult to prove whether Ig E was produced in the middle ear or a result of migration into the middle ear space. In addition we did not study allergy associated cytokines in our study due to limited research funds.

Allergy associated cytokines (IL-4, IL-5, granulocyte macrophage colony-stimulating factor), as crucial molecular regulators, are responsible for chronic inflammation in the middle ear and the chronic condition of OME [1]. IL-4 was identified in the middle ear effusions of children with persistent OME [59] and in atopic children with OME undergoing myringotomy and ventilation tube placement [56]. The analysis of effusions showed a higher mean level of IL-4 in the allergy-positive group compared with the allergy negative group and a higher percentage of cells expressing IL-4 in atopic patients with OME compared with that seen in non-atopic patients [56,57]. A higher level of IL-4 in effusions correlated with predominance of T lymphocytes, which was the sign of chronic inflammation and was also related to the atopic background of patients with OME [56]. We speculate that the levels of the allergy associated cytokines may correlate with the middle ear Ig E; and persistence of SOM. These are areas for future research in addition to the role of IL 4 and IL 5 in the aetiology and potentiation of OM.

Mucin factor in OM

The chronic condition of otitis media is associated with proliferative changes in the middle ear tissues, especially in the surface middle ear mucosa, which is present in OM as a modified pseudostratified epithelium [58,59]. In addition, goblet cells are proliferating with enhanced secretory activity [60] and formation of mucus glands occurs [61,62]. Goblet cells produce and secrete mucin, which are important glycoprotein in the mucociliary transport system of the middle ear and are the main component of middle ear effusions, responsible for the viscous properties of effusions [63-68]. However, under disease conditions, the alterations that occur in the middle ear and eustachian tube in the mucin metabolism, the structure of mucin glycoproteins and in the glycoconjugate expression in cilia and goblet cells promote the dysfunction of the normal mucociliary transport system and the formation of effusion in the middle ear cleft [66-70]. The expression of several mucins has been detected in OME; However, overproduction of only two mucins has been observed in otitis media; namely, the membrane-bound MUC4 and the secreted MUC5B [66-68]. Another secreted mucin (MUC5AC) is always present in effusions, but in varying amounts [71,72] and its levels might be linked to the levels of the pro-inflammatory cytokines TNF-a, IL-6 and IL-8, which could promote different levels of MUC5AC secretion [71-73]. Our studies showed that hyporetinolaemia was significant in the development and potentiation of otitis media [74]. Similarly, earlier investigators have linked low retinoic acid with changes in the viscosity of the mucin in favour of development of OM [71,72]. Further role of retinol is in augmenting epithelial integrity, thus ensuring optimal epithelial recovery following inflammation. Thus we speculate that delayed epithelial recovery may be one of the ways in which hyporetinolaemia leads to persistence of SOM. However, further proof in support of this may require an animal experiment and an interventional study in SOM subjects. Interventional studies from India and other developing countries have reported improvement in the resolution of other RTI although this has not been done with otitis media, hence further studies are required in this area.

Biofilm theory of OM

The demonstration that otitis media, as a chronic infectious disease, is a human pathologic condition associated with biofilm development represent a major advance in the understanding of SOM. The concept that biofilms are the primary source of bacterial infection in chronic forms of otitis media is novel. The association of biofilms with other chronic forms of infection is certainly not 75-78].

It has long been recognized that most bacteria in nature exist in a biofilm state as opposed to the small percentage of bacteria that live in a planktonic or free-swimming state. In the planktonic state, bacteria are capable of producing host responses that are generally associated with acute infections. Our observation during the study revealed various subjects with recurrent otorrhoea, which were responsive to suction toileting and ear dressing with disinfectants. We speculate that these recurrences may be due to the bacterial biofilm; However, this needs scientific proof in future.

Evidence that chronic otitis media is the result of a biofilm infection has been demonstrated [79,80]. It has been postulated that each of these recurrent infections represent a new episode caused by a unique bacterial strain ascending through the nasopharynx and eustachian tube into the middle ear space [81]. However, these findings would suggest that viable bacteria are capable of residing as biofilms within the middle ear space in between acute exacerbations of bacterial infection and, may be responsible for the recrudescence of these acute infections through planktonic showering of bacteria from the biofilm.

Other studies have supported this hypothesis and have demonstrated that approximately 30% of

cases of recurrent otitis media result from relapses attributable to the original organism [82,83]. It has been concluded that the biofilm concept did not exclude other potential pathogenic factors associated with chronic otitis media. Important associations such as an antecedent viral upper respiratory infection, eustachian tube dysfunction, allergy, a genetically predisposed host, persistent inflammatory mediators and exacerbation by gastroesophageal reflux. Further studies into these might provide significant scientific advances toward understanding chronic otitis media and developing novel treatment regimens.

In our study, we employed the ELISA techniques and high performance liquid chromatography; however more sophisticated methods such as the Polymerase Chain reaction (PCR) and electron microscopy are commonly available in the developed world. The PCR technique has been used to identify microorganisms and their product while electron microscopy technique is used in visualizing the biofilm. It is suggested that these new technologies be employed in the investigation of middle ear secretions in the OM for microorganisms and their products

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