Immunodeficiencies in children with chronic post tympanic otorrhoea

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ABSTRACT

INTRODUCTION: A minority of children treated with ventilation tubes develop chronic otorrhoea. To test the hypothesis that this condition might be caused by an underlying primary immunodeficiency, the immunological status was examined in a group of children with longstanding otorrhoea.

MATERIAL AND METHODS: Eighteen children who had suffered from otorrhoea for a minimum of six months and who did not respond to relevant therapy were included. Thorough cleansing and suction was performed including removal of ventilation tubes. Swabs were obtained for microbiology and blood was collected for immunological analyses.

RESULTS: One child out of 18 had a normal immune status. Five demonstrated isolated humoral deficiencies, four had isolated cellular deficiencies, whereas combined defects were identified among eight children. The humoral deficiencies consisted of selective or partial immunoglobulin A deficiencies, immunoglobulin G subclass and mannan-binding lectin deficiencies. The cellular deficiencies most often involved the cytotoxic T cells and the natural killer cells.

CONCLUSION: Primary immunodeficiencies were very prevalent in a highly selected group of children suffering from longstanding post tympanic otorrhoea. The condition should therefore be considered in case of chronic, refractory otorrhoea. The serostatus should be followed carefully to obtain information of the prognosis.

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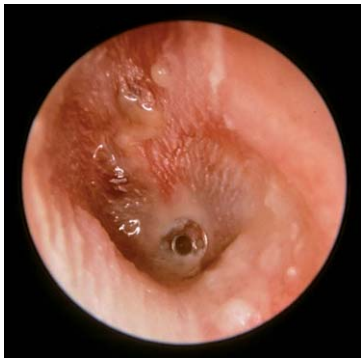
Next to acute rhinitis, otitis media is the most frequent disease in childhood [1, 2]. Both acute otitis media (AOM) and otitis media with effusion (OME) are very prone to spontaneous regression. Thus, in nine out of ten cases of AOM, the acute symptoms disappear in three days and only one out of eight recurrent AOM (rAOM) cases continue to have rAOM after six months of observation [3]. In addition, approximately half of OME incidents will resolve within three months [3]. Insertion of ventilation tubes (VT) due to rAOM and OME has become the most common surgical intervention among children in Denmark. Thus, some 60,000-70,000 tubes are inserted annually in Denmark. A well-functioning open tube produces symptomatic relief, but the child may still develop middle ear infection and inflammation that gives rise to otorrhoea (**Figure 1**). Early post tympanic otorrhoea develops in 10-20% and late post tympanic otorrhoea occurs in 20-40% of children [4-6]. In most cases, the discharge is temporary and unproblematic. However, chronic post tympanic otorrhoea has been reported in 1-10% of children with VTs that need regular cleansing and suction [4, 7]. Despite these efforts, a subpopulation develops a very resource-demanding course with more or less persistent discharge from the middle ear and acute exacerbations. In Denmark such cases were traditionally admitted to the ear-, nose-, and throat (ENT)-departments for elective mastoidectomy. Yet, the efficacy of this intervention was questioned in a previous study [8]. As a consequence, we requested an alternative strategy. First, it should be considered whether the child suffers from undiagnosed comorbidity, e.g., upper airway allergy, anatomical anomalies or biofilm on the VT. Second, it seems reasonable to pay attention to the possibility of an underlying primary immunodeficiency (PID) [9, 10].

PIDs may be humoral accounting for 65 % of all PIDs; or they may involve both the cellular and the antibody responses as is the case in 15 % of PID cases [9]. The most common PIDs in children are transient hypogammaglobulinaemia, immunoglobulin G (IgG) subclass deficiency and selective/partial antibody deficiency [10].

FIGURE 1

Otorrhoea through a ventilation tube in the left tympanic membrane.



ORIGINAL ARTICLE

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Furthermore, lack of mannan-binding lectin (MBL), a component of the innate immune system, has been suggested as a risk factor [11, 12]. It has been demonstrated that the mentioned deficiencies are associated with recurrent upper airway infections, otitis media, sinusitis, atopic and autoimmune diseases [9, 10]. In addition, chronic otorrhoea is related to an increased relative risk of specific antibody deficiency [13].

We therefore hypothesized that PIDs may be present in children with persistent middle ear post tympanic otorrhoea. Accordingly, the aims of the present study were to perform immunological examinations in a selected group of children with post tympanic otorrhoea and to suggest appropriate diagnostic and therapeutic strategies.

MATERIAL AND METHODS

Children younger than 15 years admitted to the ENT Department, Aarhus University Hospital, Denmark from 2003 to 2008 (six years) were included according to the following criteria: post tympanic otorrhoea for more than six months with only temporary/partial effect of relevant topical treatment and regular cleansing and suction by a ENT specialist practitioner.

Initially, swabs from the middle ear fluid were obtained in general anaesthesia during which thorough cleansing was performed by an ear surgeon and presence of other middle ear pathology was assessed. VTs were removed to exclude possible biofilm involvement. The specimens were cultivated under aerobe and anaerobe conditions for up to five days and growth of bacteria as well as fungi was identified. Topical treatment in accordance with the microbiological findings was initiated: ciprofloxacin in case of bacterial infection and miconazole if the ears were affected by fungi. The following analyses were conducted on peripheral blood samples: (a) concentrations of immunoglobulin A (IgA), immunoglobulin M (IgM) and immunoglobulin G (IgG), including the subclasses IgG1-4, (b) concentration of mannan-binding lectin (MBL), (c) capacity for activation of complement (classical, alternative or MBL-induced) (Cat.no: COMPL300, Euro-Diagnostica, AB), (d) lymphocyte transformation using phytohaemagglutinin, pokeweed mitogen, allogene cells or antigens from Candida albicans, Staphylococcus aureus, tuberculin and tetanus toxin, and (e) concentrations of lymphocyte subpopulations. The MBL assay has previously been described in detail [14]. In brief, microtitre wells were coated with mannan, and diluted samples were then incubated in the wells. The bound MBL was subsequently detected with anti-MBL antibodies. The analyses for the other parameters were performed using standard procedures. All results were listed according to age-specific reference intervals [15, 16]. The study did not fulfil the criteria for trial registration.

Trial registration: not relevant

RESULTS

During the 6-year study period, 18 patients were enrolled according to the inclusion criteria. The population

TABLE 1

Concentrations of the immunoglobulins and mannan-binding lectin and activation of complement for each of the 18 patients.

Pt. no	Age, months	IgA (g/l)	IgM (g/l)	IgG (g/l)	IgG1 (g/l)	IgG2	IgG3	IgG4 (g/l)	MBL (mg/l)	Complement
1	20	↓ (< 0.01)	N	↓ (5.3)	N	N	N	↓ (< 0.06)	N	N
2	18	↓ (< 0.01)	N	N	N	N	N	N	N	N
3	20	↓ (0.45)	N	N	N	N	N	N	↓ (< 0.01)	N
4	26	N	N	N	N	N	N	N	↓ (< 0.01)	↓ (clas + alt)
5	8	↓ (0.27)	N	↓ (5.1)	N	N	N	↓ (0.06)	N	N
6	18	N	N	N	N	N	N	↓ (< 0.08)	↓ (0.03)	(↑)
7	84	N	N	N	N	N	N	N	N	N
8	19	↓ (0.34)	N	N	N	N	N	N	N	N
9	20	↓ (0.51)	N	N	N	N	N	N	Missing	N
10	62	N	N	N	N	N	N	N	↓ (0.2)	↓ (MBL)
11	14	N	↓ (0.29)	↓ (5.6)	N	N	N	N	↓ (< 0.01)	↓ (clas)
12	65	N	N	N	N	N	N	↓ (0.08)	↓ (0.5)	↓ (clas + alt)
13	148	N	N	N	N	N	N	N	N	N
14	180	N	N	N	N	N	N	N	N	N
15	144	N	N	N	N	N	N	N	N	N
16	120	↓ (0.62)	N	↓ (5.0)	↓ (2.9)	N	N	N	↓ (0.5)	N
17	168	N	N	↓ (6.2)	↓ (4.9)	N	N	N	↓ (0.6)	N
18	172	↓ (0.67)	N	N	↓ (4.2)	N	N	N	N	N

Age = the age at the time of diagnosis; Alt = alanine aminotransferase; Clas = classical; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; MBL = mannan-binding lectin; N = normal range; ↓ = below the lowest reference value; ↑ = beyond than the highest reference value, the number in parenthesis gives the actual value.

TABLE 2

The results of measuring the cellular immune system in each of the 18 patients.

Pt. no	Lym Trans	Tot Leuco (10^9/l)	% Lym	Tot Lym (10^9/l)	Tot T cells (10^9/l)	Act T cells (10^9/l)	T helper (10^9/l)	Cytotox T cells (10^9/l)	Tot B cells (10^9/l)	NK cells (10^9/l)
1	N	N	↓ (33)	↓ (2.2)	N	↓ (0.06)	N	↓ (0.4)	↓ (0.4)	↓ (0.1)
2	N	N	↑ (67)	N	N	N	N	N	N	N
3	N	N	N	N	N	N	N	N	N	N
4	N	↑ (12)	↓ (29)	↑ (3.5)	N	N	N	↓ (0.6)	↑ (0.9)	N
5	N	N	↑ (70)	N	N	↓ (0.05)	N	N	N	N
6	N	N	↑ (74)	N	N	N	N	N	N	N
7	N	N	↑ (76)	↑ (46)	N	N	↑ (1.4)	↑ (1.1)	N	N
8	N	N	N	N	N	N	N	N	N	↓ (0.1)
9	N	N	↑ (49)	N	↓ (1.6)	N	N	↓ (0.6)	↓ (0.25)	N
10	N	N	N	N	↓ (1.5)	↓ (0.03)	N	↓ (0.5)	N	↓ (0.1)
11	N	↑ (13)	↓ (21)	N	N	↓ (0.07)	N	N	N	↓ (0.1)
12	N	N	↓ (37)	↓ (2.0)	N	N	↓ (0.8)	↓ (0.5)	↓ (0.3)	↓ (0.1)
13	N	N	↓ (33)	N	↓ (1.5)	↓ (0.06)	N	↓ (0.5)	N	N
14	N	↓ (4.2)	↓ (26)	↓ (1.1)	↓ (0.7)	↓ (0.02)	↓ (0.4)	↓ (0.3)	N	↓ (0.1)
15	N	N	N	N	↓ (1.4)	N	N	↓ (0.5)	N	↓ (0.1)
16	N	↓ (4.1)	N	↓ (1.8)	↓ (1.2)	↓ (0.03)	N	↓ (0.5)	N	↓ (0.1)
17	N	↓ (4.4)	N	N	↓ (1.3)	↓ (0.03)	N	↓ (0.5)	N	↓ (0.1)
18	N	N	N	N	N	↓ (0.03)	N	↓ (0.5)	N	↓ (0.0)

% Lym = percentage of lymphocytes; Act T cells = activated T cells; Cytotox T cells = cytotoxic T cells; Lym Trans = lymphocyte transformation test; NK = concentration of natural killer cells; Tot B cells = total concentration of B cells; T Helper = T helper cells; Tot Leuco = total leukocyte concentration; Tot T cells = total concentration of T cells; Tot Lym = lymphocytes.

consisted of 11 boys and seven girls with a median age of five years (range: eight months-15 years). In addition, these children had frequent upper airway infections, i.e. more than six per year. Eleven patients had previously been treated with ventilation tubes, six children more than four times. Two had had recurrent mastoiditis and one had had acute otogenic meningitis demanding acute mastoidectomy. None of the children had known allergic diseases or other co-morbidity.

On average patients had at least one visit per month at the out-patient clinic of the ENT Department during the observation period (1-7 years). At these follow-ups, the children underwent several microbiological examinations due to persistent or recurrent middle ear discharge. Usually, a mixture of bacteria from the upper airways (pneumococci, haemolytic streptococci, *Haemophilus influenzae*) and bacteria entering from the ear canal, i.e., *Staphylococcus aureus* and *Pseudomonas aeruginosa,* including *Candida* species were identified.

Table 1 lists the concentrations of IgA, M, G and IgG subclasses, MBL and activation of complement. In summary, normal humoral responses including MBL concentrations and activation of complement were seen in four of the 18 children (24%). Among the remaining children, subnormal concentrations of IgA were demonstrated in eight patients, two of whom had undetectable levels. In eight individuals, the MBL level was below the lowest reference value, and the level was undetectable in three. Subnormal IgG and IgG4 concentrations were found in five and four children, respectively. Combined deficiencies affecting the various immunoglobulins and/or MBL were present in nine of the 18 children.

Table 2 shows the results of the lymphocyte transformation test, the concentrations of the various lymphocyte subpopulations and natural killer (NK) cells. The transformation tests were normal in all cases, whereas the total number of leucocytes and/or lymphocytes was affected in most children. The most striking finding was the relatively frequent reduction of the cytotoxic T cells and/or NK cells (13 out of 18). Three children had isolated cellular deficiencies affecting the cytotoxic T cells, especially the cluster of differentiation 8 (CD8)-positive cells, and the remaining eight children had combined humoral/cellular deficiencies.

In summary, only one child (number seven) out of 18 had completely normal profiles.

Eleven children were younger than six years, none of whom had an isolated cellular deficiency (five humoral, five combined, one normalized). Among the children who were older than six years, none exhibited isolated humoral deficiencies (one normal, three cellular, three combined).

At present most of the children are followed by an otolaryngologist and all have been conferred with a paediatrician to establish individual plans for the patients. Repeated immunological tests are scheduled or already being conducted. Thus, child number eight who suffers from an initial combined deficiency developed

normal values after one year. In case of selective IgA defects, re-testing has included detection of antibodies against IgA, which has been found in patient number one.

DISCUSSION

In the present study, a PID was found in among 89% of children with chronic post tympanic otorrhoea. In most cases, a cellular deficiency was demonstrated alone or combined with a humoral deficiency. The humoral deficiencies consisted of selective or partial IgA, IgG subclass and/or MBL deficiencies. The cellular deficiencies affected especially the cytotoxic T cells dominated by CD8 cells and the NK cells.

Previous studies have demonstrated that the onset of recurrent infections among patients with combined deficiencies occurred within the first six months of life [17]. However, the age and onset of infections vary considerably among patients with cellular deficiencies, which may explain the median age of five years observed in the present study [17].

The most striking result is the prevalence of PIDs among our patients. In the international literature, the prevalence ranges from 1/500 to 1/500,000 in the general population depending on the criteria and resources used [9]. This may be a somewhat underestimated figure in the light of the prevalence of 1:330 of IgA deficiency found in blood donors [18]. Even in the light of the extreme selectivity of our study group, the findings were surprising. A more diffuse infectious burden involving various organs would have been anticipated in cases of PIDs. Despite the diversity of deficiencies, the main and frequently the only manifestation was middle ear inflammation. Usually, several infectious diseases including allergies and other comorbidities are listed as the general picture of patients suffering from PIDs [10, 17, 19].

In most of the children, the cellular immunity was affected either alone or in combination with a humoral deficiency. In contrast, patients attending immunological clinics present with another profile than otorrhoea and they usually suffer from humoral deficiencies [10, 17]. This discrepancy may be due to our selection criteria which included longstanding otorrhoea which is not representative of the entire PID population. The cell lines most often affected in our study were the cytotoxic CD8-positive cells and/or the NK cells. These cell lines are usually involved in intracellular infections due to virus and mycobacteria and probably also fungal infections [3]. However, pneumococci and *Haemophilus influenzae* were the typical findings in our children. Some of the patients also had infections of the external ear canal due to *Candida* species, which is interpreted as secondary super-infections. The expected increased susceptibility to viral infections based on the cellular deficiencies may actually have been present in the study group, but this was not specifically tested. However, the general concept in the understanding of otitis media with effusion involves a primary upper airway infection with virus and a secondary bacterial infection of the middle ear. In addition, the included children had more than six episodes of acute rhinitis per year.

Another explanation of the affected cellular immunity may be the existence of an ongoing infection at the time of blood sampling. The only sign of infection was chronic ear discharge which seems unlikely to result in such a systemic response. Furthermore, the number of T-cells and NK cells had only normalized in one out of six retested children a minimum of one year after the primary testing.

The distribution of the humoral findings is in accordance with previous results [10, 17]. Thus, isolated or partial IgA deficiencies were the most prevalent findings followed by IgG subclass deficiencies. Two children with humoral deficiencies demonstrated undetectable levels of IgA, one of whom (patient number one) produced antibodies against IgA. The patient had never received blood-derived products that could explain this phenomenon. Eight children had subnormal or undetectable MBL concentrations. As a result, the complement activation via the MBL pathway was affected in these patients [13]. The literature on MBL and disease association is increasing, and there is evidence that MBL has a composite function in many diseases, both in relation to disease susceptibility and modulation of disease severity [19, 20]. Although most people with MBL insufficiency appear healthy, probably owing to redundancy of the complement system, MBL insufficiency is associated with recurrent and life-threatening disease in some people. In the present population, none of the children older than six years exhibited signs of an isolated humoral deficiency. This may indicate that the humoral response normalizes with age.

In general, the interpretation of the present results raises the question if the PID is the direct cause of the severity of the ear disease in these children. In a previous study, half of children suffering from humoral immunodeficiencies had normalized levels of immunoglobulins at a mean age of 6 years which was accompanied by a decreased frequency of infections [17, 19]. However, there might be a risk that some of the patients with humoral deficiencies develop common variable immunodeficiency (CVID) which, besides the recurrent infections, is associated with increased risk of autoimmunity and neoplasia [9]. As some of the humoral deficiencies are transient, the cellular deficiencies may also be an expression of delayed maturation in more cell lines. Alternatively, it may be secondary to the recurrent

infections. Thus, our patient group will be followed with repeated serological tests which will clarify these questions in the future.

In conclusion, longstanding, almost retractive post tympanic otorrhoea may be caused by an underlying immunodeficiency. Thus, we recommend that the immunological status be clarified in such cases. If anomalies are found, the immunological parameters should be followed to identify those with a lifelong deficiency and the need for supplemental Ig. Furthermore, presence of comorbidity and need for vaccines should be determined, and acute bacterial infections ought to be treated promptly with relevant antibiotics.

Finally, this group of children should be treated by a team of paediatricians, immunologists and otorrhinolaryngologists.

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CONFLICTS OF INTEREST: none

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