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ORIGINAL ARTICLE

Otitis Media with Effusion and BCG Vaccine: Cytokines and Immunoglobulins

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OBJECTIVE: In this study, we investigated the effects of bacille Calmette-Guérin vaccine, known to activate the immune response through T lymphocytes and macrophages, on the course of otitis media with effusion.

MATERIALS AND METHODS: We evaluated 2042 first-grade students of state-run basic-education schools at the centers of their provinces. These children had similar socioeconomic indicators and were considered clinically healthy. The children who were diagnosed with otitis media with effusion were invited to our clinic. Of the 55 students who accepted, 46 were recruited to this study. We drew 5 mL of venous blood from these children and vaccinated them with bacille Calmette-Guérin. Following vaccination, the patients were scheduled for control visits at 1-month intervals. Ear, nose, and throat examinations of the patients were repeated at each visit. Following otoscopic examination, tympanometric examinations were performed. On the third visit, an additional 5-mL venous blood sample was obtained (fourth month). The children were followed for 6 months after vaccination.

The control group consisted of healthy children (n = 30) of the same age group not vaccinated since infancy with bacille Calmette-Guérin, living in the same region, and sharing similar socioeconomic indicators.

Interferon gamma, interleukin 4, total immunoglobulin E, G, M, and A serum levels of the children with otitis media were measured before and 4 months after vaccination with bacille Calmette-Guérin and compared with the corresponding serum levels of the control group.

RESULTS: In the 46 children with otitis media with effusion who participated in the study, a significant difference existed among serum levels of interferon gamma, interleukin 4, and total immunoglobulin E, G, M, and A before and 4 months after vaccination with bacille Calmette-Guérin compared with the control group ($P < .05$). However, 4 months after vaccination, the difference between the IgA levels of the study and control groups was not significant ($P > .05$).

CONCLUSION: Bacille Calmette-Guérin vaccine is an effective stimulant for cell-mediated immunity. In this study, we identified a short-lasting positive effect of bacille Calmette-Guérin vaccine (which activates an immune response mechanism through macrophages and T lymphocytes) on the course of otitis media with effusion.

Otitis media with effusion (OME) is the most commonly encountered health problem in children of preschool and school age. OME is diagnosed in nearly 18% of children younger than 5 years who present to healthcare institutions.[1, 2]

The reasons underlying the accumulation of fluid behind the healthy ear drum have been discussed for many years. Functional disturbances of the eustachian tube, insufficient mastoid pneumatization, craniofacial anomalies, infections, immune system problems, and allergic etiologies all play a role in its pathophysiology.[1, 3]

Of the lymphocytes circulating in the blood, 35% to 60% are T helper (T_H) cells with their heterogeneous population. T_H cells play a critical role in controlling the immune response. Based on their cytokine-producing properties, T_H cells are categorized into 2 functional subgroups: T_H1 and T_H2.[4-7] T_H1 cells predominantly produce interleukin (IL) 2, interferon gamma (IFN-γ), and tumor necrosis factor β. T_H2 cells are responsible for the production of IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. T_H1 cells initiate cell-mediated immunity, and they play a critical role in pathologic processes like diabetes mellitus. In contrast, T_H2 cells have different response patterns to antibody production and allergy.[8] The late phase of the allergic response usually occurs along with increasing levels of T_H2-type cytokines like IL-4 and IL-5. While IL-4 isotype increases IgE synthesis, when IL-4 and IL-5 occur together the antibody response shifts toward IgA.[8, 9]. Furthermore, by secreting IFN-γ, T_H1 cells cause a down-regulation in the production of T_H1 cytokines.[8-10] The differentiation between T_H1 and T_H2 is related to the dose and structure of the antigens, resemblance to different co-stimulator molecules, types of cells presenting antigenic peptides, and the presence of cytokines unique to the microenvironment.[11, 12] With the increase in T_H2 cells, T_H1-T_H2 balance is hindered. This demonstrates the presence of IgE production and the beginning of allergic reactivity.

Bacille Calmette-Guérin (BCG) is a Mycobacterium bovis strain that possesses all the structural properties of the tuberculosis bacillus yet is deprived of its disease-generating capabilities. It is a live vaccine with low virulence. [13, 14]

In the medical treatment of OME, nasal and systemic decongestants are added to antibiotics, systemic steroids, topical nasal steroids, antihistamines, and mucolytics.[2] However, it is not likely that the use of one or more of these agents will result in cure. In OME, the purposes of medical treatment are to eliminate infection, diminish inflammation, and provide aeration of the middle ear. To explain the pathophysiologic process underlying OME, several hypotheses are being investigated, and controversy about ideal treatment continues.

Immunologic impairment and allergy are seriously considered in the etiology of OME. The aims of our study were to hamper the course of OME through administration of BCG vaccine and to investigate its efficacy.

MATERIALS AND METHODS

We evaluated 2042 first graders from state-run basic-education schools. These children lived in the same region, shared similar socioeconomic indicators, and were clinically healthy. The children shared similar parental economic status, lifestyle and traditions, numbers of siblings, housing type, and climate. A total 1072 boys (52.4%) and 970 (47.5%) girls were evaluated (mean age, 84 ± 2.7 months). The same team of ear, nose, and throat (ENT) clinicians performed the examinations of all students. Only the students with OME were invited to enter the study.

Diagnosis of OME was based on criteria defined at IVth International Symposium on the Recent Developments in Otitis Media.[15] Following otoscopic examination, children who were diagnosed with OME had tympanometric examinations using the Interacoustic AZ-26 Acoustic Impedance System (Interacoustic, Assens, Denmark), and the diagnosis was confirmed. Plateau, nonpeaking, low-compliance tympanogram appearance was defined as type B based on Jerger classification.[16] In instances where the

otoscopic and tympanogram results did not correlate, the otoscopic findings were used.

The children's teachers were educated about OME. With their support, all children with OME were invited (via a letter sent to their families) to the ENT department of Fırat University Hospital, in Elazığ, Turkey. We ascertained whether any of the children had been immunized with BCG. Presence of leukemia and lymphoma, use of steroids and immunosuppressants, history of radiotherapy or injection of BCG within the past couple of years, and history of allergy were criteria for exclusion. The families of the children invited into the study were also educated about OME. We provided them with information about BCG vaccine and the study. Informed written consent was obtained from the parents prior to inclusion in accordance with the ethical standards of the ethics committee of Firat University.

Of 55 children who accepted our invitation, 46 enrolled; parents of the other 9 children refused permission.

The control group consisted of 30 child volunteers (mean age, 82 ± 3.2 months) who had never been vaccinated with BCG vaccine since infancy. Their socioeconomic indicators matched those of the study group. Informed written consent was obtained from each volunteer's family.

Students with OME had ENT examinations. After we had obtained a 5-mL venous blood sample, we vaccinated children in the OME group with BCG. Following the vaccination, the patients were scheduled for visits at 1-month intervals. ENT examinations were repeated at every visit. At the third visit (fourth month), another 5-mL venous blood sample was collected. The children with OME were followed for 6 months after BCG vaccination.

All blood samples were transferred to the immunology laboratories within half an hour. Venous samples were centrifuged for 2 minutes at a rate of 1000/min. Serum samples were stored at –20°C and –70°C in deep freezers for immunoglobulin and cytokine level measurements and were thawed only once.

Total serum IgE levels were measured using the enzyme-linked immunosorbent assay (ELISA) technique according to manufacturer's instructions (ImmunoDiagnostics, Woburn, Mass, USA). Serum IFN-γ and IL-4 also were evaluated by the ELISA method (Central Laboratory for Blood Transfusion, Amsterdam, Netherlands). The results are expressed as pg/mL. IgG, IgM, and IgA levels were measured with a Beckman Array 360 Systems (Beckman Coulter, Inc., Fullerton, Calif, USA) using a nephelometric technique, using reagents from the same company, and are expressed as g/L.

The data from before and after BCG vaccination were evaluated statistically to obtain mean standard deviation. IFN-γ, IL-4, and total IgE, IgG, IgM, and IgA serum levels from the treatment group before and after BCG vaccination were compared with those of the controls. We used the Student t test to evaluate the serum levels of the children with OME from before and after the vaccination and of the control group. A paired Student t test was used to compare serum levels before and after BCG vaccination.

RESULTS

The study was carried out from November 2004 to May 2005. The difference between IFN-γ, IL-4, and total IgE, IgG, IgM, and IgA serum levels of 46 children with OME before and 4 months after BCG vaccination was found to be significant ($P < .05$; Table 1). When serum levels of IFN-γ, IL-4, and total IgE, IgG, and IgM before and 4 months after BCG vaccination were compared with those of controls, the difference was still of statistical significance ($P < .05$; Table 2). However, the difference between total IgA serum levels 4 months after BCG vaccination and the total IgA levels of the control group was not significant ($P > .05$; Table 2).

The tympanometry data of the 46 children with OME obtained during the monthly follow-up visits after the immunization with BCG are given in Table 3.

DISCUSSION

Biochemical and immunologic studies carried out on animal models have revealed several agents with inflammatory potential for middle ear effusion. The

Table 1. IFN-γ, IL-4, and total IgA, IgE, IgG, and IgM serum levels in children with OME before and 4 months after BCG vaccination

	Before BCG Vaccine (n = 46)	After BCG Vaccine (n = 46)	P value*
IFN-γ	0.37 ± 0.35	4.48 ± 2.15	< .001
IL-4	1.98 ± 0.96	0.23 ± 0.36	< .001
IgE	121.18 ± 98.41	100.48 ± 189.04	< .01
IgG	22.82 ± 8.38	4.85 ± 5.06	< .001
IgM	1.83 ± 1.34	4.33 ± 2.28	< .001
IgA	2.60 ± 1.37	1.56 ± 3.33	< .001

*P < .05, paired Student t test.

IFN = interferon; IL = interleukin; Ig = immunoglobulin; OME = otitis media with effusion; BCG = bacillus Calmette-Guérin.

Table 2: IFN-γ, IL-4, and total IgA, IgE, IgG, and IgM serum levels in children with OME, before and 4 months after BCG vaccination and in the control group

	Before BCG Vaccine (n = 46)	After BCG Vaccine (n = 46)	Control Group (n = 30)
IFN-γ	0.37 ± 0.35*	4.48 ± 2.15*	6.66 ± 2.71
IL-4	1.98 ± 0.96*	0.23 ± 0.36*	1.23 ± 0.64
IgE	121.18 ± 98.41*	100.48 ± 189.04*	42.06 ± 21.78
IgG	22.82 ± 8.38*	4.85 ± 5.06*	11.32 ± 1.74
IgM	1.83 ± 1.34*	4.33 ± 2.28*	0.98 ± 0.12
IgA	2.60 ± 1.37*	1.56 ± 3.33†	1.69 ± 0.42

*P < .05, Student t test.

†P > .05, Student t test.

IFN = interferon; IL = interleukin; Ig = immunoglobulin; OME otitis media with effusions; BCG = bacille Calmette-Guérin.

Table 3. Tympanometry data of children with OME before and after BCG vaccine

Tympanogram	OME Before BCG Vaccine (n)		OME After BCG Vaccine (n)									
			1.month		2.month		4.month		5.month		6.month	
	▲	■	▲	■	▲	■	▲	■	▲	■	▲	■
Type A	—	—	2	3	14	15	26	28	10	8	4	5
Type B	46	46	39	39	12	10	6	6	25	25	38	35
Type C	—	—	5	4	20	21	14	12	11	13	4	6

▲ = right ear; ■ = left ear.

OME = otitis media with effusion; BCG = bacille Calmette-Guérin.

identification of mediators such as the kallikrein-kinin system, cytokines, and immunoglobulins supports the hypothesis that inflammation is the result of interactions among these mediators.[17, 18] Whether inflammation in the middle ear is a component of systemic immune response or whether the middle ear is an organ capable of independent immune response are the questions to be answered.

Recently, there have been several studies highlighting the definitive role of the balance between T_H1 and T_H2 cell groups in cellular and humoral responses. This phenomenon, which is also called TH polarization, has helped us delineate the etiopathogenesis of several disease states; the potential to direct this polarization through treatment has resulted in emergence of new treatment protocols.[5-8]

T_H1 subset cells direct B cells to synthesize IgG1 and IgG3 antibodies that bind to high-affinity Fcγ receptors and complement components. The major functions of T_H1 cells are to strengthen the phagocytosis and microbial agent-killing capacities of macrophages through T_H1-type cytokines and to provide a defense for infections. Thus, by mobilizing cellular immunity, T_H1 cells are responsible for providing a defense against viral, bacterial, fungal, and protozoal infections.[6-8]

In contrast, T_H2-subset cells direct B cells to synthesize IgM, noncomplement-fixating IgG4, and IgE. Lymphocytes in the T_H2 subpopulation participate effectively in antibody production, including IgE, and induce eosinophilia.[5-8]

In our study, the level of IFN-γ, a TH1-type cytokine, was low in the serum of children with OME when compared with that of children in the control group. However, the level of IL-4, a T_H2-type cytokine, was higher in the serum of children with OME when compared with that of the control group. This difference was statistically significant ($P < .05$). It is known that the fluid that accumulates in the middle ear of children with OME is not sterile.[19] The low levels of T_H1-type cytokines measured in these patients may explain their insufficient defense mechanisms against viral, bacterial, fungal, and protozoal infections, In our study, the children with OME had high levels of serum total IgG when compared with that of the control group. Because the T_H1-cytokine levels of these patients are low, we can naturally anticipate that they have low levels of IgG as well. However, our results did not show low levels of serum IgG levels. Since we did not measure the levels of subtypes of IgG, there might be decrease in the levels of some of the subtypes of IgG. Furthermore, total IgM serum levels of children with OME were high compared with children in the control group. Because these patients had high levels of T_H2 cytokine, we would expect to see high levels of IgM as well.

Children diagnosed with OME had high serum levels of total IgE when compared with levels found in the control group ($P < .05$). This high level of total IgE in children with OME can be explained because they also have high levels of serum IL-4, which is a T_H2-type cytokine.

IL-4 plays a major role in diverting the immune response toward IgE, whereas when IL-4 is combined with IL-5, there is a predominance of IgA response.[4, 5] When total IgA levels of the patient group before BCG vaccination were compared with the control group, the difference was statistically significant ($P < .05$). Four months after the administration of BCG vaccine, the difference between the total IgA levels of the 2 groups was not statistically significant ($P > .05$). T_H1-T_H2 balance, which favored T_H2 before BCG vaccination, shifted toward T_H1 following vaccination. This finding correlates with the data in the literature.

As in infection caused by the virulent bacilli, together with BCG vaccination, an effective immune response to tuberculosis is developed by the activation of T lymphocytes and macrophages.[13] From this perspective, BCG is a powerful stimulus for cellular immunity. In atopic patients, it can be used to decrease allergic reactivity and to modify the immune response.[13, 20] Furthermore, the triggering of T_H1-cell predominance by BCG vaccine is a simple approach to reducing infection-related complications.[8] In our study, planned with this rationale in mind, there was a difference of statistical significance between first and second graders regarding the frequency of OME.[21] When we researched immunization history in these

children, we found that the first graders have not yet received immunization; however, the second graders had their BCG vaccinations nearly 4 months earlier. In the children with OME, the T_H1-T_H2 balance that favored T_H2 before BCG vaccination shifted toward T_H1 after vaccination. This change was statistically significant in some of the children with OME. Serum levels of the T_H1-type cytokine IFN-γ was different before and after the BCG vaccination when compared with the control group ($P < .05$). There was also a statistically significant difference between groups for IL-4, a T_H2-type cytokine ($P < .05$).

In our study, the control group consisted of the healthy, nonvaccinated children with no OME. We could have enrolled BCG-vaccinated children in same age group. Although this may seem contradictory, it has been stated in many studies that BCG vaccine activates the immune system via T lymphocytes and macrophages in immunocompromised patients. We think that the major problem in patients with OME is associated with the immune system.

In Turkey, the vaccination program for newborns starts with BCG vaccine, and BCG vaccine is routinely administered 2 months after birth. A repeat dose of BCG vaccine is given to first graders in primary schools, in spring. The reasons behind the continuation of the statistically significant difference (despite the decrease) between the control group and the study group before administering BCG are as follows: the age of nonvaccinated individuals, the time of vaccination, the period during which the effects of the vaccine are evaluated, and the differences between doses of repeat vaccine among individuals.

Our study was prospective. Therefore, it was not possible to determine the incidence of recurrent OME in children included in the study. Perhaps, if our study had been carried out in children with recurrent OME, the results might be more significant. Our research could guide other studies on this topic.

That the T_H1-T_H2 balance favored T_H2 in children with OME before BCG vaccination shifted toward T_H1 following vaccination and that this was reflected in the clinical course was pleasing indeed. The otoscopic and tympanometric evaluations were specifically positive at the second and fourth months after vaccination. However, this positive effect of BCG vaccination disappeared from the fourth month on. We observed an increase in the recurrence rates of OME during follow-up of these patients 6 months after BCG vaccination.

One would expect to find an increase in IgG levels and a decrease in IgM levels when T_H1-type cytokine profile shifts to T_H2 following BCG vaccination. This expectation may not be valid because the beneficial effects of BCG vaccination only last up to 4 months and serum levels of IgG subtypes were not determined in our study. Therefore, our findings may not necessarily support this assumption.

In investigating possible risk factors, we found that the children with OME had higher incidences of upper respiratory tract infections and allergy when compared with the general population, Currently, there are several research studies investigating the relationship between allergy and OME.[17, 22] T_H1-T_H2 balance disturbed by the increase in T_H2 can cause increases in IgE, resulting in allergic activity. Living in urban and industrialized regions, changing lifestyles, and decreasing incidences of microbial infections are listed as causes for increased allergies in the Turkish communities within the last 20 years. A significant T_H1 cytokine production is an indicator of a systemic response to mycobacterium. The tendency toward increased production of T_H1 cytokines in T_H1-T_H2 balance may be responsible for a conflicting relationship between tuberculosis and allergic disease.[23]

We have to acknowledge the limitation of our study. We evaluated 2 groups of children. One group consisted of children with OME and were vaccinated; the other group included healthy, nonvaccinated children of similar age and socioeconomic status. During the design of the study, we might have considered additional groups of patients with OME. Although, we might have added a third group-patients with OME not receiving BCG vaccine, we selected healthy subjects to constitute the control group. Initially, our aim was to assess the difference between the level of cytokines and immunoglobulins in children with and without OME. By populating the control group with healthy subjects,

we think that we avoided a possible bias by measuring the level of cytokines in healthy patients instead of measuring the cytokines in patients with OME. We assumed that cytokines in the control group would be regarded as a more standard measurement, reflecting the normal cytokine levels in healthy subjects. Nevertheless, adding 1 more group, patients with OME but not vaccinated with BCG, might have made the results of our study more reliable. We have expanded our research to include evaluation of the serum cytokine levels of this third group of children.. We plan to have more comparable results after collecting the data and will share our results in a forthcoming paper.

CONCLUSIONS

With the use of an appropriate antigenic stimulus, T_H1 and T_H2 can be selectively suppressed. BCG vaccine is an effective stimulus for cell-mediated immunity. In our study, in a disease state like OME where the roles of immunologic problems and allergy are seriously questioned, we investigated the effects of BCG vaccine through activation of immune-response mechanism via T lymphocytes and macrophages on the course of the disease. However, the effects of BCG vaccine on the immune systems of OME patients were of short duration. We believe that this study might provide a basis for future treatment protocols that may play an important role on the course of OME by suppressing the production of IL-4 and IL-13, being blocked by antibodies, or providing the predominance of T_H1 via IFN-γ application.

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