

Increased IgM-Type Betalactoglobulin Antibodies in Children with Recurrent Otitis Media

ULLA M. SAARINEN, ERKKI SAVILAHTI and PIIRKKO ARJOMAA

Children's Hospital, University of Helsinki, Finland

Total concentrations of IgA, IgG, IgM and IgE, precipitating antibodies to cow milk, milk-specific IgE, as well as anti-milk antibodies and betalactoglobulin antibodies of the IgA, IgG and IgM classes determined by the ELISA method were studied and compared in children with recurrent otitis media, and children who had never had otitis. The children with recurrent otitis had a higher total IgE at 1 year of age and a higher total IgM at 3 years. The only difference in milk-specific antibodies was an increased titre of betalactoglobulin antibodies of IgM type in children with recurrent otitis. This new finding suggests that immunological mechanisms might play a part in recurrent otitis, which has been shown to be more common in bottle-fed than in breast-fed infants. So far, the role of betalactoglobulin antibodies in the pathogenesis of otitis media remains uncertain.

Key words: betalactoglobulin antibodies; breast milk; cow milk; immunoglobulin A; immunoglobulin E; immunoglobulin G; immunoglobulin M; milk antibodies.

Accepted for publication 3 May 1983

Several risk factors are known to predispose infants and small children to recurrent otitis media, e.g., enlarged adenoids, disturbed function of the eustachian tube (3), day nursery care (22), and atopic disease (7, 13). Milk-related influences have also been disclosed; recurrent otitis media has been associated with early bottle-feeding, in contrast to prolonged breast-feeding (4, 19, 21) which has provided long-term protection up to 3 years of age (19).

Chronic secretory otitis in particular has been attributed to allergy (7, 16, 23) or to a complication of allergy (17). Secretory otitis may sometimes be due to food allergy (23) with, e.g., cow milk as the aetiological factor (12, 23). Cow milk allergy in general is a disease with involvement of multiple systems, and most patients show a strong immune reaction to cow milk (1, 20). This reaction is mediated by either reaginic antibodies, immune complexes, or sensitized lymphocytes (1, 12, 20). Cow milk has also been suggested to cause symptoms solely in the respiratory tract, e.g., recurrent rhin-

orrhoea and bronchitis (9), chronic respiratory disease with circulating precipitins (10, 12), bronchial asthma (14), or secretory otitis media (12, 23).

The interesting finding that breast-feeding protects against otitis media prompted us to investigate the immunological background in order to find out whether cow milk hypersensitivity could be responsible for recurrent otitis in infants and small children.

SUBJECTS AND METHODS

The subjects of this study were initially selected from a series of 256 infants, who were prospectively followed up from birth to 3 years of age. The dietary regimen of the infants was exactly similar regarding solid foods during the first year, but differed in milk feeding because the breast-feeding periods varied. Details of the follow-up, dietary regimens, and recording of infections have been described elsewhere (19).

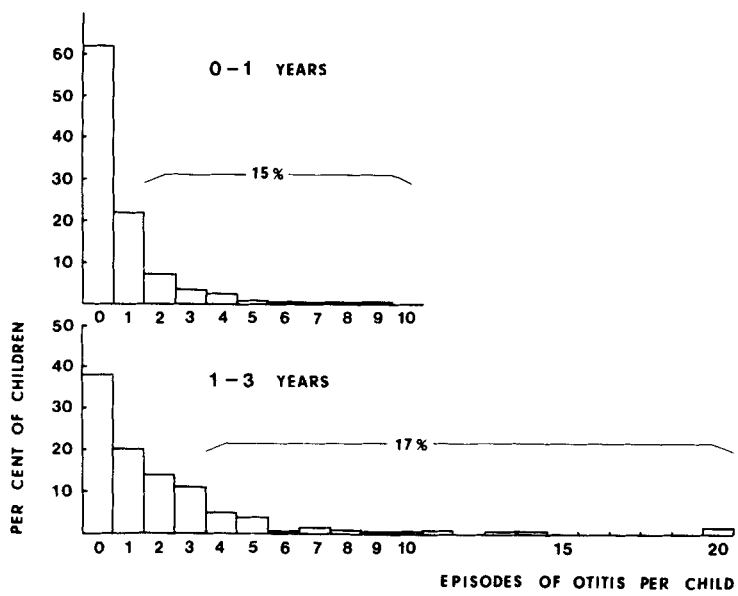


Fig. 1. Distribution of episodes of otitis media per child in the total series of children, evaluated during age intervals of 0-1 year and 1-3 years.

Definition of recurrent otitis media

The diagnosis of otitis media was based on otoscopic examination (19). The distribution of episodes of otitis per child is seen in Fig. 1. Recurrent otitis was defined as two or more episodes from birth to 1 year, and four or more episodes from 1 to 3 years of age.

Study groups

Only children with sufficient serum samples available could be included in the analysis.

Group I: Children with recurrent otitis. Group Ia comprised 36 children who had had otitis twice or more before 1 year of age. Group Ib comprised 31 children in whom otitis was diagnosed four times or more between 1 and 3 years of age. Group Ic comprised those 18 children who belonged to both Ia and Ib.

Group II: Otitis-free children. The control group consisted of the 46 children who were totally free from otitis media throughout the follow-up period from birth to 3 years of age.

Milk feeding in the study groups

In addition to the incidence of otitis, groups I and II differed in the duration of breast-feeding, as is shown in Table 1, and as was evident from the previous study (19).

Table 1

Duration of breast-feeding in the study groups

Study group	n	≥ 6 months (%)	2-6 months (%)	≤ 2 months (%)
Children with recurrent otitis:				
Group Ia	36	3 (8) ³	8 (22)	25 (69) ²
Group Ib	31	3 (10) ⁴	7 (23)	21 (68) ³
Group Ic	18	1 (5) ⁴	2 (11)	15 (83) ¹
Otitis-free children:				
Group II	46	12 (26)	14 (30)	20 (43)

¹ $P < 0.0005$
² $P < 0.025$
³ $P < 0.05$
⁴ $P < 0.10$ } compared with group II

Table 2

Comparison between mean serum immunoglobulin concentrations in children with recurrent otitis and in those with no episodes

Study group	n	IgA (g/l)		IgG (g/l)		IgM (g/l)		IgE ¹ (U/ml)	
		9 months	3 years	9 months	3 years	9 months	3 years	9 months	3 years
Children with recurrent otitis:									
Group Ia	36	0.29		7.2		0.53		12.5	
Group Ib	31		0.54		9.7		0.74 ²		16.9
Group Ic	18	0.27	0.54	6.8	9.6	0.49	0.69	14.7 ²	22.4
Otitis-free children:									
Group II	46	0.20	0.54	6.7	10.2	0.53	0.60	8.4	15.9

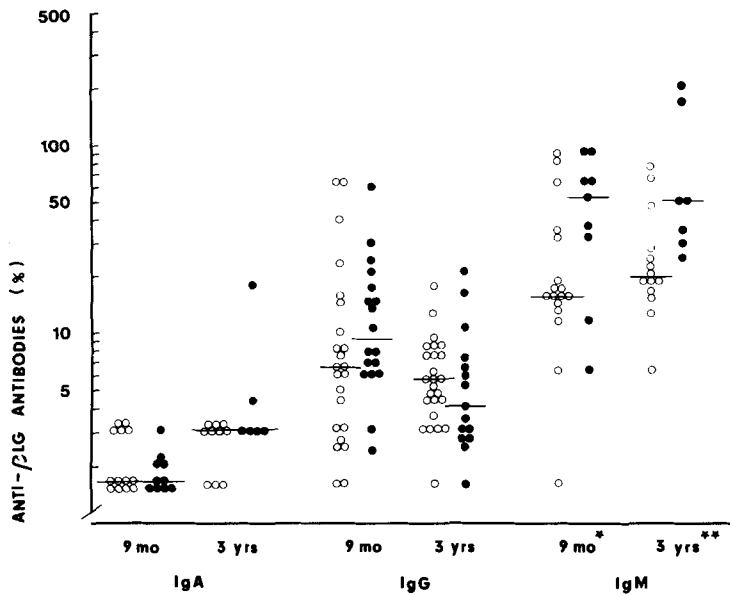
¹ The geometric means are stated.² $P < 0.05$ compared with corresponding value in group II.

Fig. 2. Betalactoglobulin antibodies of the IgA, IgG and IgM classes in children with recurrent otitis media compared with those free from otitis, determined by the ELISA method and expressed as a percentage of the reference serum concentration. Solid circles indicate children with recurrent otitis (at 9 months group Ia, at 3 years group Ib), and open circles indicate children free from otitis (group II). The medians are shown by the horizontal lines. Asterisks indicate significant differences between the study groups:

*Median test: $P < 0.05$; Mann-Whitney U-test: n.s.

** Median test: $P < 0.005$; Mann-Whitney U-test: $P < 0.01$.

Laboratory methods

Venous blood was drawn at scheduled age intervals in most of the children, and the serum was frozen and stored at -20° until analysed.

Serum total IgA, IgG and IgM concentrations were measured at 9 months and 3 years by a single radial immunodiffusion method (11). Serum total IgE was determined at 1 and 3

years with the Phadebas IgE PRIST radio-immunoassay (Pharmacia Diagnostics, Uppsala, Sweden). Precipitating antibodies to cow milk were determined semiquantitatively by a double diffusion micromethod (6).

The cow milk-specific IgE antibodies were determined at 6 and 12 months with the Phadebas RAST kit, using the 0 to 4 radio-allergosorbent test score system.

The specific antibody concentrations to cow milk proteins in the immunoglobulin classes IgA, IgG and IgM were studied at 9 months and 3 years by using the enzyme-linked immunosorbent assay (ELISA) with some modifications. Microtitre plates (Lindbro®; Flow Laboratories Inc., Connecticut) were coated with defatted dairy milk diluted with phosphate buffered saline (PBS) pH 7.4, 1:10 for IgA and IgG and 1:2 for IgM titration, or with betalactoglobulin (Sigma Pharmaceuticals) 1 mg/l in PBS. Coating was done at +4°C overnight. The serum samples were diluted with PBS, 1:40 for IgA and IgG and 1:10 for IgM antibody measurement, and were added to microtitre wells for 4 h at 37°C. After washing, an optimal concentration of phosphatase conjugated monospecific antihuman IgA, IgG and IgM serum (Orion Diagnostica, Espoo, Finland) was incubated overnight in the wells at room temperature. Attached phosphatase reacted with p-nitrophenylphosphate, and the colour was measured with Titertek Multiscan® (Flow Laboratories Inc.) at wavelength 405 nm. In the titrations, a pooled serum with a high titre of anti-cow milk antibodies was run in various dilutions in parallel with the serum to be tested. The titre of the unknown serum was expressed as a percentage of the antibody concentration of the pooled reference serum.

Statistical methods

Chi-square test and Student's *t* test were used in the statistical analyses, as well as the median test and the U-test of Mann and Whitney as nonparametric tests.

RESULTS

The children with recurrent otitis media had a higher serum total IgE at 1 year of age, and a higher total IgM at 3 years than the children free from otitis (Table 2). The concentrations of total IgA and IgG did not differ (Table 2).

Precipitating antibodies to cow milk occurred almost as frequently in children with recurrent otitis as in those without otitis at 3 years: 17% in groups Ia, Ib and Ic, and 24% in group II. At 9 months the figures were 6%, 13% and 13% in groups Ia, Ib and Ic, respectively, compared with 2% in group II; the difference was not significant.

The only milk-specific antibodies in which a difference was found were the betalactoglobulin (β LG) antibodies of the IgM class. These antibodies occurred in higher titres in children with recurrent otitis than in those without otitis at 9 months and 3 years of age (Fig. 2). The IgM-anti- β LG titres did not correlate with the duration of breast-feeding, when evaluated in otitis-free children (group II). There was no difference between study groups I and II regarding specific anti- β LG of IgA and IgG classes.

Titres against whole cow milk were generally lower than those against β LG; the study groups did not show any difference.

Cow milk-specific IgE antibodies were determined in 32 infants at 6 and 12 months of age. Only one child had detectable cow milk-specific IgE with a RAST score of only 0.5. This child belonged to the otitis group and also had atopic eczema.

DISCUSSION

Is cow milk hypersensitivity responsible for recurrent otitis media in children? In the present study we have found some evidence suggesting that immunological mechanisms might play a part in recurrent otitis of bottle-fed infants. Our specific finding was that children with recurrent otitis media had increased titres of β LG-antibodies of the IgM type. This was the only type of milk-specific antibodies in which we found a difference between children with and without otitis. The finding is unexpected, as there have

been no observations indicating the clinical importance of particularly the IgM class of cow milk antibodies (1, 20). The possible role of these β LG-antibodies in the pathogenesis of recurrent otitis media is difficult to settle, however, and requires further studies.

Bottle-feeding with cow milk-based formulas has been postulated to cause positional otitis media (2, 5, 8); if an infant is bottle-fed in the lying position, milk can regurgitate to the middle ear through the pharyngotympanic tube due to its inadequate function in infants, especially during respiratory illness. Local irritation in the tympanic cavity caused by cow milk may give rise to impaired function of the middle ear mucosa and thus increase the susceptibility to otitis media. Direct contact of the respiratory epithelium with cow milk might also stimulate the synthesis of milk-specific antibodies, as has been postulated regarding precipitating antibodies of the IgG class (10). One can speculate that also the IgM type β LG-antibodies might originate in this way. Whether the β LG-antibodies are in fact involved in the pathogenesis of recurrent otitis media or whether they represent a secondary phenomenon as a result of a wrong feeding position, remains uncertain.

Also the total IgM concentrations in serum were increased in children with recurrent otitis at 3 years of age (Table 2). β LG-antibodies contribute to the increase in serum total IgM, but their proportionate role is probably not very important in the total IgM. We believe that increased antiviral and antibacterial IgM antibodies account for this change in otitis-prone children.

Atopic disease may predispose to otitis (13), which could explain why the children with recurrent otitis had a higher serum total IgE at 1 year of age. However, no evidence could be found that particularly milk-specific IgE antibodies would play a part in recurrent otitis. We should like to explain that bottle-feeding with cow milk-based formula is the common background of both recurrent otitis (4, 19, 21) and atopic disease (15, 18), and the two conditions need not necessarily be dependent on each other.

To sum up, in a search for milk-specific antibodies in otitis-prone children we found increased β LG-antibodies of IgM type to be associated with recurrent otitis media. This is a new observation, and the role of these specific antibodies remains to be solved.

REFERENCES

1. Bahna, S. L. & Heiner, D. C.: Cow's milk allergy: Pathogenesis, manifestations, diagnosis and management. In Barness, L. A. (ed.): *Advances in pediatrics*, Vol. 25, pp. 1–36. Year Book Medical Publishers, Inc., Chicago, 1978.
2. Beauregard, W. G.: Positional otitis media. *J. Pediatr.* 79, 294–296, 1971.
3. Bluestone, C. D.: Eustachian tube function and allergy in otitis media. *Pediatrics* 61, 753–760, 1978.
4. Chandra, R. K.: Prospective studies of the effect of breast feeding on incidence of infection and allergy. *Acta Paediatr. Scand.* 68, 691–694, 1979.
5. Duncan, R. B.: "Positional otitis media". *Arch. Otolaryngol.* 72, 454–463, 1960.
6. Fällström, S. P., Ahlstedt, S. & Hansson, L. Å.: Specific antibodies in infants with gastrointestinal intolerance to cow's milk protein. *Int. Arch. Allergy Appl. Immunol.* 56, 97–105, 1978.
7. Fernandez, A. A. & McGovern, J. P.: Secretory otitis media in allergic infants and children. *South. Med. J.* 58, 581–585, 1965.
8. Forestier, E.: Liggvanor och otitfrekvens hos spädbarn. *Läkartidningen (Sweden)* 74, 2022–2023, 1977.
9. Gerrard, J. W.: Familial recurrent rhinorrhea and bronchitis due to cow's milk. *J. Am. Med. Assoc.* 198, 605–607, 1966.
10. Heiner, D. C., Sears, J. W. & Kniker, W. T.: Multiple precipitins to cow's milk in chronic respiratory disease. *Am. J. Dis. Child.* 103, 634–650, 1962.
11. Immonen, P.: Levels of the serum immunoglobulins γ A, γ G and γ M in the malabsorption syndrome in children. *Ann. Paediatr. Fenn.* 113, 115–153, 1967.
12. Jordan, R. E.: Allergy of middle ear and inner ear. *Arch. Otolaryngol.* 71, 212–215, 1960.
13. Kjellman, N.-I. M., Synnerstad, B. & Hansson, L. O.: Atopic allergy and immunoglobulins in children with adenoids and recurrent otitis media. *Acta Paediatr. Scand.* 65, 593–600, 1976.
14. Lee, S. K., Kniker, W. T., Cook, C. D. & Heiner, D. C.: Cow's milk-induced pulmonary disease in children. In Barness, L. A. (ed.): *Advances in pediatrics*, Vol. 25, pp. 39–57. Year Book Medical Publishers, Inc., Chicago, 1978.
15. Matthew, D. J., Taylor, B., Norman, A. P., Turner, M. W. & Soothill, J. F.: Prevention of eczema. *Lancet* i, 321–324, 1977.
16. Rapp, J. D. & Fahey, D. J.: Allergy and chronic secretory otitis media. *Pediatr. Clin. North. Am.* 22, 259–264, 1975.

17. Reisman, R. E. & Bernstein, J.: Allergy and secretory otitis media. Clinical and immunological studies. *Pediatr. Clin. North. Am.* 22, 251-257, 1975.
18. Saarinen, U. M., Kajosaari, M., Backman, A. & Siimes, M. A.: Prolonged breast-feeding as prophylaxis for atopic disease. *Lancet ii*, 163-166, 1979.
19. Saarinen, U. M.: Prolonged breast feeding as prophylaxis for recurrent otitis media. *Acta Paediatr. Scand.* 71, 567-571, 1982.
20. Savilahti, E.: Cow's milk allergy. *Allergy* 36, 73-88, 1981.
21. Schaefer, O.: Otitis media and bottle-feeding. An epidemiological study on infant feeding habits and incidence of recurrent and chronic middle ear disease in Canadian Eskimos. *Can. J. Publ. Health* 62, 478-489, 1971.
22. Strangert, K.: Respiratory illness in preschool children with different forms of day care. *Pediatrics* 57, 191-196, 1976.
23. Viscomi, G. J.: Allergic secretory otitis media: An approach to management. *Laryngoscope* 85, 751-758, 1975.

Address:

Ulla M. Saarinen, M.D.

Division of Pediatric Hematology-Oncology
Rainbow Babies and Children's Hospital
2101 Adelbert Road
Cleveland, Ohio 44106
U.S.A.