

Acknowledgments

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References

- Stephens FD, Joske RA, Simmons RT. Megaureter with vesico-ureteric reflux in twins. *Aust N Z J Surg* 1955; **24**: 192–94.
- Bredin HC, Winchester P, McGovern JH, Degnan M. Family study of vesicoureteral reflux. *J Urol* 1975; **113**: 623–25.
- Aggarwal VK, Verrier Jones K. Vesicoureteric reflux: screening of first degree relatives. *Arch Dis Child* 1989; **64**: 1538–41.
- Kenda RB, Fetich JJ. Vesicoureteric reflux and renal scars in asymptomatic siblings of children with reflux. *Arch Dis Child* 1992; **67**: 506–08.
- Ericsson NO. Urologic viewpoints on the treatment of urinary tract infection in children. *Acta Paediatr* 1960; **49**: 196–202.
- Hellstrom A, Hanson E, Hansson S, Hjalmas K, Jodal U. Association between urinary symptoms at 7 years old and previous urinary tract infections. *Arch Dis Child* 1991; **66**: 232–34.
- Smellie JM. Childhood urinary infections and their significance. In: Asscher AW, ed. The management of urinary tract infections. Oxford: Medicine Publishing, 1980: 29–38.
- Bailey RR, Lynn KL. End-stage reflux nephropathy. In: Hodson CJ, Heptinstall RH, Winberg J, eds. Reflux nephropathy update 1983. Basle: Karger, 1984: 102–10.
- El-Khatib M, Packham DK, Becker GJ, Kincaid-Smith P. Pregnancy-related complications in women with reflux nephropathy. *Clin Nephrol* 1994; **41**: 50–55.
- Ransley PG, Risdon RA. Renal papillary morphology and intrarenal reflux in the young pig. *Urol Res* 1975; **3**: 105–09.
- Ring E, Nobel G. Urinary infections and malformations of the urinary tract in infancy. *Arch Dis Child* 1988; **63**: 818–20.
- Schimmmenti LA, Pierpont ME, Carpenter BL, Kashtan CE, Johnson MR, Dobyns WB. Autosomal dominant optic nerve colobomas, vesicoureteral reflux and renal anomalies. *Am J Med Genet* 1995; **59**: 204–08.
- Vernon S, Redfearn A, Pedler SJ, Lambert HJ, Coulthard MG. Urine collection on sanitary towels. *Lancet* 1994; **344**: 612.
- Scott JES, Lee REJ, Hunter EW, Coulthard MG, Matthews JNS. Ultrasound screening of newborn urinary tract. *Lancet* 1991; **338**: 1571–73.
- Uehling DT, Vlach RE, Pauli RM, Friedman AL. Vesicoureteric reflux in siblings. *Br J Urol* 1992; **69**: 534–37.

Early BCG vaccination and development of atopy

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Summary

Background The increase in atopic diseases may be partly explicable by a decline of certain infectious diseases, or changes in childhood vaccination programmes, or both. We investigated whether BCG vaccination against tuberculosis influences the development of atopy.

Methods We did a retrospective cohort study of 216 children with atopic heredity, born in Stockholm between 1989 and 1992, who received BCG vaccination when they were younger than 6 months, and 358 age-matched controls who had not been vaccinated. Both groups attended Sachs' Children's Hospital, Stockholm, Sweden, during 1995–96 for assessment of atopic history and clinical signs of atopic disease. All children also underwent skin-prick testing (SPT) and serum was analysed for allergen-specific IgE antibodies. Serum from parents was also analysed for IgE antibodies.

Findings 77 (36%) children in the BCG group and 145 (41%) in the control group had a positive history or clinical signs of atopic disease. In the vaccinated group, 26 (12%) children had one or more positive SPT, and 61 (31%) had circulating allergen-specific IgE antibodies, whereas in the control group, the numbers were 35 (10%) and 84 (27%) respectively. Atopy was confirmed by serology in parents of

almost two-thirds of the children in each group. Other risk factors for atopic disease were evenly distributed between the two groups.

Interpretation early BCG vaccination in children with atopic heredity does not seem to affect the development of atopic disease before school age.

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Introduction

The occurrence of atopic disease mediated by IgE antibodies is increasing in developed countries, and the proportion of children with asthma, atopic dermatitis, rhinoconjunctivitis, or food allergy, is now more than 30%.^{1–3} The reasons for this increase are largely unknown, but environmental factors are thought to have an important role. For example, associations with residence in industrial or urban areas and a western lifestyle have been found in comparisons of eastern and western Europe.^{3,4}

T-helper (Th) lymphocytes have a central regulatory influence on allergic inflammation and can be subdivided according to their production of cytokines.⁵ Th1 cells, when stimulated with virus or intracellular bacteria, secrete interferon- γ , which can inhibit IgE production by B cells.^{5,6} By contrast, Th2 cells, when stimulated with allergens or parasites, release interleukin-4, which promotes IgE production by B cells.^{5,6} Th2 activity dominates in atopic individuals.⁶

BCG vaccine is a potent adjuvant for induction of cell-mediated immunity⁷ and induces interferon- γ as one

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of the major cytokines.⁸ This action may cause a Th1-activity type immune response.⁵ BCG vaccine has been used in attempts to modulate the immune response and, thereby, interfere with the pathogenesis of diseases. Studies in diabetes-prone mice have shown that a single injection of BCG vaccine given at an early age can prevent the development of type 1 diabetes.^{9,10} In a trial of children with newly diagnosed type 1 diabetes, a single intracutaneous dose of BCG vaccine led to long-term clinical remission.¹⁰

The increase in atopic disease may be partly explained by a decline in certain infectious diseases, as well as changes in immunisation programmes. BCG vaccine was given to 95% of all newborn babies in Sweden until 1975, when vaccination was stopped because of side-effects and lower risk of tuberculosis exposure.¹¹ Vaccination is now offered only to high-risk groups, such as children who have a close relative with tuberculosis or immigrants from some areas, or upon parental request. Therefore, the vaccination rate is now less than 4% in Swedish-born children.¹¹ We assessed the influence of BCG vaccination in early life on the development of atopy in children with atopic heredity.

Methods

780 children born between 1989 and 1992 who received BCG vaccination before the age of 6 months were traced from Child Welfare Centre records in the catchment area of Sachs' Children's Hospital in south Stockholm (table 1). In this area, 99.7% of all families with infants visited the Child Welfare Centres.¹² Nurses at these centres were asked to exclude children of non-Nordic parents to keep potential confounding to a minimum. A questionnaire about atopic disease among the parents was sent to all these families. 650 (83%) of 780 responded, and 283 of these children had no atopic heredity. Among the remaining children, 87 who had been vaccinated after the age of 6 months were excluded, and 37 refused further participation, which left 243 children for clinical assessment.

A control group of children, age-matched by calendar year, was randomly selected from population registers among children living in the same area as the BCG group. After exclusion of non-Nordic infants, 1266 children remained. The control group received the same questionnaire as the BCG group. 980 (77%) replies were received (table 1). The parents of 475 of these infants reported no atopic symptoms. Among the remaining children, 40 were excluded because of previous BCG vaccination, and 73 refused further participation. Therefore, the control group consisted of 392 infants.

The parents of both groups of infants were mailed a second questionnaire about atopic symptoms as well as social and environmental details, and were asked to visit the outpatient ward at Sachs' Children's Hospital, Stockholm, Sweden, between October, 1995, and October, 1996, for a clinical assessment. At this point, in the BCG group consisting of 243 children (87% of those invited), two children had no vaccination scar, 11 had had late (>6 months) BCG vaccination, and 21 had non-Nordic parents. These 34 infants were excluded from further study, which left 209 infants. Seven controls had been vaccinated before the age of 6 months, and these were transferred to the BCG group, and, therefore, the BCG group consisted of 216 children after clinical assessment.

The mean age at BCG vaccination was 17 days (range 0–180) for girls (n=104), and 21 days (range 1–173) for boys (n=112). In the control group, 392 (84%) children were clinically assessed. After further exclusion of eight infants who were vaccinated at older than 6 months and 19 who had non-Nordic parents, 358 children remained in this group (172 girls and 186 boys). The mean age at examination was 5.5 years (range 3.1–7.2) and 5.4 years (range 2.9–7.2) in the BCG and control groups, respectively.

	BCG group	Control group
Screening stage		
Selected for screening	780	1266
Responded to screening questionnaire	650 (83%)	980 (77%)
Atopic heredity by history	367	505
Excluded because of late vaccination (>6 months)	87*	40*
Clinical assessment		
Invited	280	465
Attended	243 (87%)	392 (84%)
No visible BCG scar	2*	377
Late vaccination (>6 months)	11*	8*
Non-Nordic parent	21*	19*
BCG-vaccinated controls transferred to BCG group	7	..
Final study groups	216	358

*Excluded.

Table 1: Selection of children with early BCG vaccination and controls

All clinical assessments were done by the same doctor (JSA) who was not aware of the vaccination status or test results. The clinical diagnosis of atopic disease was based on information from the questionnaire and from the clinical assessment. The following criteria were used.

Bronchial asthma—three or more episodes of wheezing before the age of 2 years, or one episode at 2 years or older, or any episode of wheezing, independent of age, if combined with familial atopy or other atopic symptoms in the child.

Allergic rhinoconjunctivitis—rhinitis, or conjunctivitis, or both, at least twice after exposure to a particular allergen, and unrelated to infection.

Food allergy or allergic urticaria—acute onset of symptoms such as skin reactions, wheezing, vomiting, or diarrhoea on more than one occasion after ingestion or contact with a particular type of food or allergen.

Atopic dermatitis—as described by Hanifin and Rajka.¹³

The BCG vaccine used was attenuated Kb 1331 strain of *Mycobacterium bovis*, without preservative (Statens Seruminstitut, Copenhagen, Denmark).¹¹ In children younger than 1 year, this vaccine is given as 0.05 mL intracutaneously on the proximal

	BCG group (n=216)	Control group (n=358)
Clinical symptoms or history of atopic disease		
Bronchial asthma	22 (10%)	43 (12%)
Atopic dermatitis	58 (27%)	107 (30%)
Allergic rhinoconjunctivitis	31 (14%)	34 (9.0%)
Food allergy	6 (3.0%)	7 (2.0%)
Urticaria	10 (5.0%)	9 (3.0%)
Total with at least one symptom	77 (36%)	145 (41%)
Skin-prick test		
≥1 positive test	26 (12.0%)	35 (10%)
Cat	10 (4.8%)	13 (3.8%)
Dog	2 (1.0%)	2 (0.6%)
Horse	4 (1.9%)	7 (2.0%)
Birch	7 (3.3%)	12 (3.5%)
Timothy grass	6 (2.9%)	5 (1.5%)
Mugwort	0	0
Cladosporium	0	0
<i>D. pteronyssinus</i>	1 (0.5%)	0
Peanut	7 (3.3%)	10 (2.9%)
Egg white	2 (1.0%)	1 (0.3%)
Codfish	0	2 (0.6%)
Cow's milk	1 (0.5%)	0
Soy bean	1 (0.5%)	0
Number tested	210 (97%)	344 (96%)
Blood sample from children		
Any serology positive	61 (31%)	84 (27%)
Phadiatop positive only	20 (10%)	31 (10%)
fx5 positive only	17 (9%)	31 (10%)
Phadiatop and fx5 positive	24 (12%)	22 (7.0%)
Total with blood samples available	194 (90%)	317 (89%)

Table 2: Signs of atopy according to clinical examination, SPT, and serological tests in children with early BCG vaccination and controls

lateral part of the left upper arm without a previous tuberculin test.¹⁴ None of the children received a subsequent tuberculin test or revaccination. All children in the final BCG group had a scar at the site of vaccination, which was confirmed in the clinical assessment.

Skin-prick tests (SPT) (Soluprick, ALK, Copenhagen, Denmark) were done on the volar side of the child's lower arm. These included tests for birch, cat, cladosporium, *Dermatophagoides pteronyssinus*, dog, horse mugwort, and timothy grass (10 HEP), hen's-egg white (1/100 weight/volume), codfish (1/20), cow's milk (3% fat, standard foodstuff), peanut (1/20) and soy bean (Soja Semp, Semper AB, Stockholm, Sweden). Histamine chloride 10 mg/mL was used as the positive control and the allergen diluent as the negative control. An SPT was deemed positive if the weal was at least 3 mm in diameter and larger than or equal to the histamine-induced weal after 15 min. The same batches of each extracts were used and the SPTs were done throughout the study by the same nurse who was unaware of vaccination status.

A capillary blood sample was taken from each child and from the parent with the most probable atopic history. The blood samples were centrifuged and serum was separated and stored at -20°C until analysis, which included detection of circulating IgE antibodies (Pharmacia Diagnostics AB, Uppsala, Sweden) against 11 common inhalant allergens (Phadiatop) and six food allergens (fx5). Infants with at least one positive SPT to any of the selected food or inhalant allergens, or a positive Phadiatop, or a positive fx5, or any combination, were classed as atopic.¹⁵

Data were analysed by χ^2 tests or Fisher's exact test (mid p values) to compare proportions; for continuous data, the Mann-Whitney non-parametric *U* test was used. Significance was set at $p < 0.05$. Power calculations were done according to Casagrande and colleagues' calculations.¹⁶

The study was approved by the local research ethics committee, and informed consent was given by all parents.

Results

Positive history or clinical signs consistent with atopy were seen in 36% of the BCG group and in 41% of controls, of which the youngest were 19 months (range 0–51) and 24 months (1–72), respectively (table 2). The distribution of different atopic symptoms in the two groups did not differ significantly.

12% of infants in the BCG group had positive SPT reactions compared with 10% of controls, with an equal distribution for different allergens between the two groups (table 2). A positive reaction to cat antigens was most common, followed by birch, peanut, timothy grass, and horse.

Blood samples were available for nearly 90% of children (table 2). In the BCG group, 10% had positive Phadiatop tests and 9% had positive fx5 tests. The corresponding proportions in the control group were 10% for both tests. A greater proportion of the BCG group than of the control group had positive results in both in-vitro tests (12 *vs* 7%), but overall, the percentage of positive results was similar in the two groups. When results from STP and serological tests were combined, the rate of atopy according to Pepys' definition¹⁵ was 29% and 24% in the BCG group and the control group, respectively.

Demographic data and risk factors for atopic disease are presented in table 3. There were no clear differences between the BCG group and controls in birthweight (mean 3544 g and 3473 g), gestational age at birth (39.9 and 39.7 weeks), number of children in the family (2.2 in both groups), or other risk factors for atopic disease, such as heredity, sex, short duration of breastfeeding, parental smoking, and furred pets in the home. Parental atopy was

	BCG group (n=216)	Control group (n=358)
Characteristics of children		
M/F	108 (52%)/104 (48%)	186 (52%)/172 (48%)
Low birthweight (<2500 g)	4 (3.2%)	11 (3.1%)
Breastfed exclusively ≥ 4 months	124 (57.0%)	226 (63.0%)
Characteristics of home		
Maternal tobacco smoker (≥ 1 cigarette/day)	53 (25%)	93 (26%)
Live in flat	108 (50%)	166 (46%)
Furred pets in household	101 (47%)	147 (41%)
Moisture inside window panes at home	11 (5.0%)	15 (4.2%)
Family history of atopy		
Mother	76 (35%)	120 (33%)
Father	56 (26%)	99 (28%)
Both parents	84 (39%)	139 (39%)
Blood samples from parents	200 (93%)	337 (94%)
Positive atopy serology in parents	125 (63%)	221 (66%)
Origin of parents*		
Denmark	1	3
Finland	28	17
Norway	0	4
Total	29 (13.0%)	24 (6.7%)

*Other than Sweden

Table 3: Demographic data and risk factors for atopic disease in the BCG group and controls

verified by serology in 63% and 66% of parents in the BCG and control groups, respectively (table 3). Restriction of the analysis to the children of these parents did not result in significant differences between the groups for any outcome (data not shown).

Based on the proportions in the control group, the statistical power of the study to detect a 50% reduction in frequency of atopy was 99% for clinical symptoms or history of atopic disease, 48% for at least one positive SPT, and 89% for any positive serology.

Discussion

This study shows that early BCG vaccination has no primary preventive effect on the development of atopic disease before school age in children with atopic heredity. To increase the efficiency of our study, only children whose parents had a history of atopy were included. The statistical power was adequate to detect a 50% reduction in the frequency of clinical or serological signs of atopy among the children in the BCG group. The occurrence of clinical symptoms of atopy, and the frequency and distribution of positive SPTs and positive serological tests in the two groups are similar to those in other Swedish studies of children of similar age and with atopic heredity.^{17,18} The clinical part of the study was masked to reduce the risk of exposure-related misclassification of the outcomes. Non-response rates were similar for children who were and were not vaccinated and the occurrence of atopy is unlikely to differ greatly between the non-responders in the two groups. The distribution of risk factors for development of atopic disease was similar among the BCG group and controls, which reflects a lack of confounding by these factors. The distribution of risk factors was also similar to that found in other Swedish studies.^{19,20} A higher proportion of the BCG-group parents were born in Nordic countries other than Sweden, but most of these parents came from Finland, where the atopy rates are similar to those in Sweden.^{17,21}

The protective efficacy of BCG vaccine against tuberculosis is debated.²² Mantoux skin testing is the established method of checking the protection, although

the correlation is doubtful because of individual differences in the reactivity to tuberculin^{14,23} and cross-reactions to atypical mycobacteria.¹⁴ The presence of a scar is thought to be the best sign of a successful vaccination¹⁴ and we used scars to confirm vaccination in our study.

Shirakawa and colleagues²⁴ reported that positive tuberculin responses among Japanese schoolchildren aged 12–13 years correlated with a lower incidence of atopic disorders. Positive tuberculin responses (≥ 10 mm skin induration) were recorded in 3% of the children at age 3 months, 33% at age 6 years, and 58% at age 12 years. At each of these ages, negative responders were BCG vaccinated. The rate of atopic symptoms was significantly lower in children with positive tuberculin reactions. Much of the inverse association between tuberculin and atopy was due to children with strong tuberculin responses (>40 mm skin induration). An important difference between the children in the Japanese study and ours, apart from different vaccination programmes, is the environmental exposure to mycobacteria. The incidence of tuberculosis is about ten times higher in Japan than in Sweden.^{24,25} Natural exposure to mycobacteria but also a predisposition in the individual to a Th1 response may have implications for the interpretation of the Japanese results. The difference in outcome after vaccination and natural infection is illustrated in a retrospective cohort study from Guinea-Bissau, where measles infection, but not childhood measles vaccination, was associated with a reduction in atopy.²⁶ How the immunological responses to a measles infection and vaccination differ is not clear,^{27,28} but a protective effect against atopy was achieved only by real infection.

Early BCG vaccination in infants with atopic heredity seems to have no significant effect on the development of atopic disease before school age. If these findings are the same for older children and children without atopic heredity, vaccination may not constitute an effective primary preventive strategy for atopic diseases.

Contributors

Johan S Alm, Gunnar Lilja, Göran Pershagen, and Annika Scheynius were all involved in the planning of the study, data analysis, and manuscript preparation. The clinical part of the study was done by Johan S Alm. Paediatric allergology was dealt with by Gunnar Lilja. Göran Pershagen was responsible for matters related to epidemiology. Matters related to immunology were dealt with by Annika Scheynius.

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References

- 1 Taylor B, Wadsworth J, Wadsworth M, Peckham C. Changes in the reported prevalence of childhood eczema since the 1939–45 war. *Lancet* 1984; **ii**: 1255–57.
- 2 Burney PGJ, Chinn S, Rona RJ. Has the prevalence of asthma increased in children? Evidence from the national study of health and

growth 1973–86. *BMJ* 1990; **300**: 1306–10.

- 3 Bråbäck L, Breborowicz A, Dreborg S, Knutsson A, Pieklik H, Björkstén B. Atopic sensitization and respiratory symptoms among Polish and Swedish school children. *Clin Exp Allergy* 1994; **24**: 826–35.
- 4 Wichmann HE. Possible explanation for the different trends of asthma and allergy in east and west Germany. *Clin Exp Allergy* 1996; **26**: 621–23.
- 5 Romagani S. Induction of Th1 and Th2 responses: a key role for the “natural” immune response? *Immunol Today* 1992; **13**: 379–81.
- 6 Del Prete G. Human Th1 and Th2 lymphocytes: their role in the pathophysiology of atopy. *Allergy* 1992; **47**: 450–55.
- 7 Mehra VL, Salgame P, Snapper SB, Lugosi L, Jacobs W, Bloom BR. Vaccines against leprosy. In: Woodrow CG, Levine MM, eds. New generation vaccines. New York: Marcel Dekker, 1990: 611–17.
- 8 Sander B, Skansén-Saphir U, Damm O, Håkansson L, Andersson U. Sequential production of Th1 and Th2 cytokines in response to live bacillus Calmette-Guérin. *Immunology* 1995; **86**: 512–18.
- 9 Harada M, Kishimoto Y, Makino S. Prevention of overt diabetes and insulinitis in NOD mice by single BCG vaccination. *Diabetes Res Clin Pract* 1990; **8**: 85–89.
- 10 Shehadeh N, Calcinaro F, Bradley BJ, Bruchlim I, Vardi P, Laffert KJ. Effect of adjuvant therapy on development of diabetes in mouse and man. *Lancet* 1994; **343**: 706–07.
- 11 Romanus V, Svensson Å, Hallander HO. The impact of changing BCG coverage on tuberculosis incidence in Swedish-born children between 1969 and 1989. *Tuber Lung Dis* 1992; **73**: 150–61.
- 12 Aurelius G. The Child Health Care in Stockholm County Council: annual report 1995. Serafen, Stockholm: The Child Health Care in Stockholm County Council, 1995: 20.
- 13 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venereol* 1980; **92** (suppl): 44–47.
- 14 Larsson LO. Sensitivity to sensitins and tuberculin in Swedish children. Gothenburg, Sweden: Dissertation, Department of Pulmonary Medicine, University of Gothenburg, 1991: 6–13.
- 15 Pepys J. Clinical aspects of immunology, 3rd edn. Oxford: Blackwell Scientific Publications, 1975: 877–902.
- 16 Casagrande JT, Pike MC, Smith PG. An improved approximate formula for calculating sample size for comparing two binomial distributions. *Biometrics* 1978; **34**: 483–86.
- 17 Sigurs N, Hattveig G, Kjellman B. Maternal avoidance of eggs, cow's milk and fish during lactation: effect on allergic manifestations, skin-prick tests, and specific IgE antibodies in children at age 4 years. *Paediatrics* 1992; **89**: 735–39.
- 18 Lilja G, Öhman H, Johansson SGO. Development of atopic disease during childhood and its prediction by Phadiatop Paediatric. *Clin Exp Allergy* 1996; **26**: 1073–79.
- 19 Kjellman N-IM. Atopic disease in seven-year-old children. *Acta Paediatr Scand* 1977; **66**: 465–71.
- 20 Rylander E, Pershagen G, Eriksson M, Nordvall L. Parental smoking and other risk factors for wheezing bronchitis in children. *Eur J Epidemiol* 1993; **9**: 517–26.
- 21 Kajosaari M. Atopy prevention in childhood: the role of diet. *Pediatr Allergy Immunol* 1994; **5** (suppl 1): 26–28.
- 22 Colditz GA, Berkey CS, Mosteller F, et al. The efficacy of bacillus Calmette-Guérin vaccination of new-borns and infants in the prevention of tuberculosis: meta-analyses of the published literature. *Paediatrics* 1995; **96**: 29–35.
- 23 Al-Kassmi FA, Al-Hajjaj MS, Al-Orainey IO, Bamgboye EA. Does the protective effect of neonatal BCG correlate with vaccine-induced tuberculin reaction? *Am J Respir Crit Care Med* 1995; **152**: 1575–78.
- 24 Shirakawa T, Enomoto T, Shimazu S, Hopkin J. The inverse association between tuberculin responses and atopic disorder. *Science* 1997; **275**: 77–79.
- 25 Romanus V. The Swedish tuberculosis index 1991–94. Stockholm: The Swedish Institute for Infectious Disease Control and the Swedish Heart-Lung Foundation, 1996.
- 26 Shaheen SO, Aaby P, Hall AJ, Barker DJP, Heyes CB, Shiell AW. Measles and atopy in Guinea-Bissau. *Lancet* 1996; **347**: 1792–96.
- 27 Ward BJ, Johnson RT, Vaisberg A, Jauregui E, Griffin DE. Cytokine production in vitro and the lymphoproliferative defect of natural measles virus infection. *Clin Immunol Immunopathol* 1991; **61**: 236–48.
- 28 Ward BJ, Griffin DE. Changes in cytokine production after measles virus vaccination: predominant production of IL-4 suggests induction of a Th2 response. *Clin Immunol Immunopathol* 1993; **67**: 171–77.