

Original article

The inverse association of salmonellosis in infancy with allergic rhinoconjunctivitis and asthma at school-age: a longitudinal study

Background: Respiratory allergies are inversely related to early acquisition of food-borne and fecal-oral infections, consumption of unpasteurized milk, early exposure to stables and high endotoxin concentrations in a farming environment. We tested therefore if infection by *Salmonella* in early life can protect from development of respiratory allergies later in life.

Methods: During 2003, we studied two groups of Sardinian children (age 6–18 years) who had been hospitalized before 4 years of age (during 1989–2001) with non-typhoid salmonellosis ($n = 148$) or acute enteritis of nonbacterial etiology (NB-enteritis) ($n = 167$). Allergic rhinoconjunctivitis (AR) and asthma were evaluated by telephonic interview with a ISAAC questionnaire; participants reporting AR and/or asthma were further examined through a complete diagnostic work-up to objectively confirm or exclude current disease. Kaplan–Meier curves and Cox proportional hazard models were used to analyze the role of different types of enteritis on the risk of developing allergic rhinoconjunctivitis or asthma over time.

Results: Children who had been hospitalized with salmonellosis had a lower prevalence of allergic rhinoconjunctivitis (eight of 148, 5.4% vs 23 of 167, 13.8%; $P = 0.019$) or asthma (five of 148, 3.4% vs 21 of 167, 12.6%; $P = 0.006$) than those who had been hospitalized with NB-enteritis. The proportional hazard of salmonellosis for asthma was 0.23 (95% CI: 0.08–0.67; $P < 0.01$) and for allergic rhinoconjunctivitis was 0.40 (95% CI: 0.17–0.95; $P = 0.04$), after adjusting for confounders.

Discussion: The strength of the observed associations suggests that *Salmonella* may contribute to shape the natural history of respiratory allergies. However, further studies are needed to test in other settings the association observed in Sardinian children. We speculate that clinical or subclinical infection by *Salmonella* may contribute to the atopy protective influence of a traditional farming environment or of areas endemic for food-borne and fecal-oral infections. Food hygiene and prevention of salmonellosis must remain however a public health priority.

U. Pelosi¹, G. Porcedda¹, F. Tiddia², S. Tripodi³, A. E. Tozzi⁴, V. Panetta³, C. Pintor¹, P. M. Matricardi⁵

¹Unità di Pneumologia e Malattie Allergiche, Clinica Pediatrica, Università di Cagliari, Cagliari;

²Laboratorio Microbiologia, Cagliari; ³Unità di Allergologia Pediatrica, Ospedale Sandro Pertini;

⁴Unità di Epidemiologia and ⁵Unità di Ricerca per le Allergie e l'Asma, UOC Allergologia, Ospedale Pediatrico Bambino Gesù, IRCCS, Roma, Italy

Key words: allergic rhinoconjunctivitis; asthma; endotoxin; hygiene hypothesis; *Salmonella*.

Paolo Maria Matricardi MD
Unità di Allergologia
Ospedale Pediatrico Bambino Gesù
IRCCS
Piazza Sant'Onofrio, 4
00165 Roma
Italy

Accepted for publication 12 August 2004

The reasons why allergic disorders have been increasing in westernized countries are still unknown and are probably multifactorial (1). A popular theory proposes that children can develop allergy more frequently if they are less exposed to microbes early in life (2–4). This 'hygiene hypothesis' has not been formally demonstrated yet (5, 6). Although many infections may contribute to prevent atopic sensitization and allergic inflammation through a range of different mechanisms (2, 4, 7–9), no specific infection has been demonstrated to prevent atopy (10). Nonetheless, atopy is less frequent among subjects who acquire food-borne and fecal-oral infections (11), in those exposed to stables and to high endotoxin concentrations, or consuming unpasteurized milk in a farming environment (12–14).

Therefore, an example of atopy-preventing infection may be found among gram-negative bacteria transmitted by contaminated food and the fecal-oral route, and by animals typical of farming environment. A number of infectious agents share these properties; among them, *Salmonella* can be better studied since they cause diseases that can be easily diagnosed even in early childhood.

We tested the hypothesis that acquisition of an infection by *Salmonella* in early childhood may counteract the development of respiratory allergies later in life. To this end, we compared the incidence of hay fever and asthma in a group of Sardinian school-aged children who had been hospitalized at preschool-age with salmonellosis to that observed in children who had been hospitalized with acute enteritis of nonbacterial etiology.

Materials and methods

Study population

Our target population included all children hospitalized before 4 years of age either with non-typhoid salmonellosis or with acute enteritis of nonbacterial etiology in the Pediatric Clinic of the University of Cagliari, Sardinia, between January 1989 and December 2001. Children resident outside Sardinia (e.g. tourists), those with a history of hospitalization with enteritis because of other enteropathogenic bacteria or with parasitic diseases or with major underlying diseases (e.g. primary or acquired immunodeficiencies), or whose clinical record was not available were excluded from the study.

In the period January 1989–December 2001, 201 children with salmonellosis and 510 with enteritis of nonbacterial etiology were admitted into the Pediatric Clinic of the University of Cagliari. A random sample of 201 children with enteritis of nonbacterial etiology was extracted to conduct the study.

The study design was approved by the Ethics Committee of ASL-8 in Cagliari, Sardinia.

Microbiologic data

Salmonella spp. had been routinely detected in stool specimens collected on the first day of hospitalization and plated on *Salmonella-Shigella* (SS) agar after enrichment in selenite F broth. Colonies suspicious for *Salmonella* spp. were further identified through chemical tests. Isolates were serologically typed using polyvalent and monovalent O or H antisera according to the Kauffman–White scheme. No major changes in this procedure occurred from 1989 to 2001. We arbitrarily labeled an enteritis as of nonbacterial etiology (hereafter 'NB-enteritis') if two or more consecutive stool specimens collected during hospitalization were all negative for enteropathogenic bacteria, always including *Salmonella*, *Campylobacter*, and *Shigella*. Socio-demographic data and information on fever peak and its duration, erythrocyte-sedimentation rate (ESR), blood eosinophils count, peak of loose stools per day, and antibiotic prescriptions were obtained from the hospitalization records.

Diagnosis of current allergic rhinoconjunctivitis and asthma

A validated Italian translation of the ISAAC core questionnaire (15) was administered to parents by computer-assisted telephonic interview by one of us (G.P.). Of the 201 families of children with salmonellosis, 148 (74%) were contacted by telephone and agreed to participate, whereas 167 of the 201 families of children with NB-enteritis (83%) were traced and participated in the study. Ninety-five percentage of nonparticipating families were not traced while 5% declined participation in the study.

All the participants with a family history of allergic diseases who responded negatively to all ISAAC questions on current symptoms of allergy were interviewed a second time after 2–3 months by an independent interviewer (U.P.). No new cases of allergic rhinoconjunctivitis or asthma were identified by this procedure. To objectively identify participants affected by current allergic rhinoconjunctivitis and asthma, all the subjects reporting current symptoms or a diagnosis of respiratory allergies at ISAAC interview were further examined; reported allergic rhinoconjunctivitis and/or asthma was clinically confirmed or excluded on the basis of standard criteria and of a complete diagnostic work-up for allergy and asthma including skin prick tests (SPT) for airborne allergens (see below). Criteria for diagnosis have been reported elsewhere (16). Briefly, allergic rhinoconjunctivitis was diagnosed in subjects with a history of rhinitis apart from colds, characterized by rhinorrhea and

sneezing and/or watery, itchy eyes, lasting for 3 weeks or more during allergy season, or occurring nonseasonally, but associated with exposure to specific triggers and related to their own sensitization to airborne allergens (13). Allergic asthma was diagnosed in subjects who presented a history of repeated episodes of coughing, dyspnea, wheezing and chest tightness or whistling, not caused by any organic condition, occurring during pollen season or recurrently, if continuously exposed to specific triggers and related to their sensitization to airborne allergens (16). For the purposes of the present study we labeled as 'current' a disease which symptoms occurred in the last 12 months.

Skin prick tests

A panel of airborne allergens, including mixed grass pollens, *Parietaria officinalis*, *Ambrosia*, *Artemisia vulgaris*, *Olea europaea*, *Cypripedium*, *Candida albicans*, *Alternaria alternata*, *Cladosporium*, *Aspergillus*, *Penicillium*, Cat, Dog, and *Dermatophagoides pteronyssinus* (Lofarma Allergeni, Milano, Italy) was used for immediate SPT. Histamine 0.1 mg/dl and glycerol solution were used as positive and negative controls, respectively. Morrow-Brown needles were used and wheals were read after 20 min. A wheal reaction greater than or equal to half the histamine diameter, after subtraction of the negative control, was regarded as positive.

Statistical analysis

Comparisons between the two groups of participants were examined by chi-square tests for dichotomous variables (or Fisher exact test when appropriate), and Student's *t*-test for continuous variables. Since the length of the follow up period differed among the patients, the analysis of the study factors associated with allergic rhinoconjunctivitis and asthma was first conducted at the univariate level drawing Kaplan–Meier curves and comparing them with the log-rank test; in addition, those variable significant at the $P < 0.10$ level were included in a Cox proportional hazard model to estimate the hazard ratio (HR) and their 95% confidence intervals (CIs). Statistical analysis was performed using STATA 7.

Results

Population

We found no significant differences in age (11.1 ± 3.1 vs 11.1 ± 3.2 years; $P = 0.97$) and in the time interval between hospitalization and interview (9.4 ± 3.2 vs 9.0 ± 3.0 years; $P = 0.35$) between patients with salmonellosis and those with NB-enteritis. Similarly, no major differences were observed in gender, residence, and a family history of atopy; by contrast, a lower socioeconomic status and a higher number of older siblings were observed in children with salmonellosis (Table 1).

Characteristics at hospitalization

Characteristics of patients at hospitalization are reported in Table 2. Children with salmonellosis had been hospitalized on average 4 months later in life than those with NB-enteritis. During hospitalization they had, as expected, a lower eosinophil count, a higher ESR, a higher

Table 1. Characteristics of the population sample

	NB-enteritis (n = 167)		Salmonellosis (n = 148)		P-value
	n	%	n	%	
Male gender	101	60.5	86	58.1	ns
Living in cities with >10 000 inhabitants	121	72.5	96	64.9	ns
First born	100	59.9	64	43.2	0.003
Nonmanual father's occupation	94	56.3	63	42.6	0.015
Atopy in the family	56	33.5	44	29.7	ns

Table 2. Age, eosinophils, erythrocyte-sedimentation rate (ESR) and fever in pre-school children with enteritis, by etiology

	NB-enteritis (n = 167)		Salmonellosis (n = 148)		P-value
	Mean	SD	Mean	SD	
Age at hospitalization	20.4	12.0	24.6	12.6	0.002
Blood eosinophils (n/mm ³)	133	214	63	118	<0.0001
Blood eosinophils (%)	1.39	3.3	0.65	1.2	<0.0001
ESR (mm/h)	16.0	11.2	31.6	16.8	<0.0001
Fever peak (°C)	38.0	1.3	39.2	1.3	<0.0001
Days with fever (n)	1.93	2.3	4.1	2.3	<0.0001

peak and a longer duration of fever (Table 2), a higher frequency of discharges per day (> 5 discharges/day: 103 of 148, 69.6% vs 60 of 167, 35.9%, $P < 0.0001$) and antibiotic treatment (62 of 148, 41.9% vs 25 of 167, 15.0%, $P < 0.0001$).

Allergic rhinoconjunctivitis and asthma

A total of 52 patients reported current symptoms or a diagnosis of respiratory allergies at the ISAAC questionnaire and underwent the diagnostic work-up to confirm the diagnosis. Allergic rhinoconjunctivitis and asthma were diagnosed in 26 and 31 of these participants, respectively. At interview, participants with salmonellosis reported significantly less current wheeze, asthma, current runny nose with itchy eyes, and hay fever (Table 3).

To exclude the possibility of selective underreporting of allergic symptoms, all the 74 participants with a reported history of allergic diseases in the family but with negative responses to ISAAC questions on current respiratory allergies were contacted again by phone 3 months after the first interview. No new cases of allergic rhinoconjunctivitis or asthma were identified by this procedure in this subgroup. All the 52 subjects with a positive response to at least one of the critical ISAAC questions on current allergic symptoms were further evaluated through a complete diagnostic work-up for respiratory allergies. This clinical procedure confirmed that participants with salmonellosis had a lower prevalence of allergic rhinoconjunctivitis (eight of 148, 5.4% vs 23 of 167, 13.8%;

Table 3. Respiratory allergies in schoolchildren hospitalized at preschool-age with enteritis, by etiology

	NB-enteritis (n = 167)		Salmonellosis (n = 148)		P-value
	n	%	n	%	
Wheeze in last 12 months	23	13.8	7	4.7	<0.01
Doctor's diagnosis of asthma*	21	12.6	3	2.0	<0.001
Runny nose and itchy eyes in last 12 months	25	15.0	8	5.4	<0.01
Hay fever ever	25	15.0	8	5.4	<0.01
Any†	36	21.6	16	10.8	0.01

*Lifetime prevalence.

†One or more affirmative answers to the questions in the Table.

$P = 0.019$) or asthma (five of 148, 3.4% vs 21 of 167, 12.6%; $P = 0.006$).

When studying risk factors over time, atopy in the family, salmonellosis, and eosinophil count resulted associated with allergic asthma at the univariate level. The inverse association of salmonellosis with allergic asthma was confirmed after adjusting for confounders (HR = 0.23; 95% CI: 0.08–0.67; $P < 0.01$). The Kaplan–Meier curves illustrating the cumulative proportion of asthma-free children over time in groups resulting from the combination of salmonellosis or NB-enteritis and presence or absence of atopy in the family is illustrated in Fig. 1. Children with NB-enteritis and with a family history of atopy developed asthma more frequently and more rapidly than all the other groups.

On the contrary, atopy in the family, salmonellosis, place of residence, and eosinophilic count were associated with allergic rhinitis at the univariate level. When adjusting for confounding, salmonellosis remained the only protective factor associated with allergic rhinitis (HR = 0.40; 95% CI: 0.17–0.95; $P = 0.04$). However,

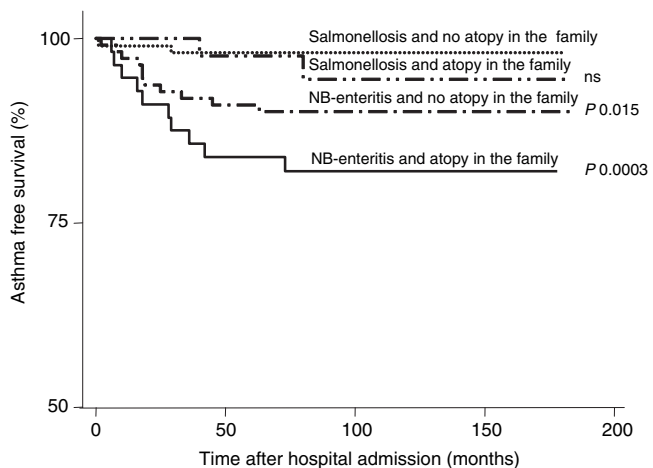


Figure 1. Asthma-free survival in children after hospitalization for enteritis, by etiology of enteritis and family history of atopy.

when asthma was introduced in the Cox model, asthma remained the only strong predictor of allergic rhinitis (HR = 16.33; 95% CI: 7.19–37.10; $P < 0.01$).

Discussion

We found that Sardinian children hospitalized for salmonellosis in preschool-age developed allergic asthma less frequently than children hospitalized for acute enteritis of nonbacterial etiology. Given the rationale of our study and the strength of the observed associations, it is tempting to speculate that acquisition of infection early in life by *Salmonella* may inhibit the development of atopic diseases and in particular of allergic asthma. *Salmonella* may contribute to prevent the development of respiratory allergies through a range of mechanisms acting on the innate immune system, in a critical period for the maturation of immune response against ubiquitous allergens (4).

The comprehension of the mechanisms of such a putative protective influence falls beyond the objectives of the present study. However, *Salmonella*, as mycobacteria, grow into the endosomes of macrophages and induce a strong activation of CD4+ T helper-1 lymphocytes, which is required to enhance intracellular killing and clearance (17, 18). This process is highly dependent on production of γ -interferon and interleukin-12, so that patients with selective deficiencies of these cytokines or their receptors develop fatal infections by *Salmonella* as well as mycobacteria (19). In addition, *Salmonella* play a transient regulatory role on adaptive immunity (20). Finally, the immune response to *Salmonella* is controlled by the natural resistance-associated macrophage protein 1 (Nramp-1) gene (21), which plays also a major role in regulation of atopic responses and airways allergic inflammation in rodents (22). These properties or other unknown mechanisms may provide biologic plausibility to the hypothesis that salmonellosis contributed to protect from allergic asthma and rhinoconjunctivitis the children we examined.

Alternative explanations of the observed associations must be considered. First, we cannot totally exclude that early acquisition of *Salmonella* may be related in our study area to acquisition of many other food-borne and fecal-oral infections (11, 16), to a different gut microflora composition (23) or to nonmicrobial dietary factors that may have partially or totally determined the

observed associations (24). On the contrary, our results may be an additional example of gene-by-environment interaction in the hygiene hypothesis (25, 26): it could be argued that a genetic predisposition to develop atopic responses protects from developing an enteritis severe enough to require hospitalization after infection by *Salmonella* (reverse causation). However, a family history of allergy was equally distributed between participants hospitalized with salmonellosis and those hospitalized with NB-enteritis. Finally, the prevalence of respiratory allergies among subjects with NB-enteritis may have been sustained by infections facilitating atopy; however, the prevalence of allergic asthma and rhinoconjunctivitis among controls felt in the range of values measured in the 1990s with the same questionnaire in 13 Italian cities (15).

The finding that the predictive role of asthma for allergic rhinitis is more important than that of salmonellosis is not surprising as in the majority of patients rhinitis was associated with asthma, in keeping with the concept 'one airway, one disease' (27).

Our observations highlight that *Salmonella* deserve attention in future investigations on the hygiene hypothesis. In particular, further longitudinal studies in different geographic areas and settings (traditional farming environment, developing countries) are necessary to discriminate if and how salmonellosis occurred before 4 years of age contribute to prevent respiratory allergies or if the associations we observed in Sardinia are made spurious by local factors. Anyway, food hygiene and prevention of salmonellosis must continue to be a public health priority.

It has been previously proposed that we may learn from microbes how to prevent or treat allergy without causing infectious diseases (28). Interestingly, molecules derived by *Salmonella* have been already examined as adjuvants in specific immunotherapy for respiratory allergies (29). Our results encourage further efforts to find new strategies to identify microbial products to prevent or treat allergic diseases (30).

Acknowledgments

Giulio Porcedda was supported by the Italian Ministry of Education. Authors are indebted with Dr Gabriele Porcu for technical assistance, Dr Francesco Rosmini for helpful discussion and Dr Andreina Santoro for her help in editing the text.

References

1. Matricardi PM, Bonini S. Why is the incidence of asthma increasing? In: Johnston SL, Holgate ST, editors. *Challenges in asthma*. London: Blackwell Science Ltd, 2002:3–17.
2. Holt PG. Environmental factors and primary T-cell sensitisation to inhalant allergens in infancy: reappraisal of the role of infections and air pollution. *Pediatr Allergy Immunol* 1995;6:1–10.
3. Strachan DP. Family size, birth order, and atopy. A decade of the hygiene hypothesis. *Thorax* 2000;55:S2–S10.

4. Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. *Lancet* 1999;**354**:12–15.
5. Strachan DP, Warner J, Pickup J, Schweiger M, Pennington H, Stanwell-Smith R et al. The hygiene hypothesis. *Pediatr Allergy Immunol* 2003;**14**:145–146.
6. Von Hertzen LC, Haahtela T. Asthma and atopy – the price of affluence? *Allergy* 2004;**59**:124–137.
7. Bach JF. The effect of infections on the susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002;**347**:911–917.
8. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites and the hygiene hypothesis. *Science* 2002;**296**:490–494.
9. McIntire JJ, Umetsu SE, Macaubas C, Hoyte EG, Cinnioğlu C, Cavalli-Sforza LL et al. Hepatitis A virus link to atopic disease. *Nature* 2003;**425**:576.
10. Matricardi PM, Bonini S. High microbial turnover rate preventing atopy: a solution to inconsistencies impinging on the hygiene hypothesis? *Clin Exp Allergy* 2000;**30**:1506–1510.
11. Matricardi PM, Rosmini F, Panetta V, Ferrigno L, Bonini S. Hay fever and asthma in relation to markers of infection in the United States. *J Allergy Clin Immunol* 2002;**110**:381–387.
12. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;**347**:869–877.
13. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001;**358**:1129–1132.
14. Von Mutius E. Environmental factors influencing the onset and progression of pediatric asthma. *J Allergy Clin Immunol* 2002;**109**:S525–S532.
15. International Study of Asthma and Allergies in Childhood (ISAAC). Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet* 1998;**351**:1225–1232.
16. Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ* 2000;**320**:412–417.
17. Mizuno Y, Takada H, Nomura A, Jin CH, Hattori H, Ihara K et al. Th1 and Th1-inducing cytokines in *Salmonella* infection. *Clin Exp Immunol* 2003;**131**:111–117.
18. Mastroeni P, Menager N. Development of acquired immunity to *Salmonella*. *J Med Microbiol* 2003;**52**:453–459.
19. de Jong R, Altare F, Haagen IA, Elferink DG, Boer T, van Breda Vriesman PJ et al. Severe mycobacterial and *Salmonella* infections in interleukin-12 receptor-deficient patients. *Science* 1998;**280**:1435–1438.
20. Rotta G, Edwards EW, Sangaletti S, Bennett C, Ronzoni S, Colombo MP et al. Lipopolysaccharide or whole bacteria block the conversion of inflammatory monocytes into dendritic cells *in vivo*. *J Exp Med* 2003;**198**:1253–1263.
21. Soo SS, Villareal-Ramos B, Anjam Khan CM, Hormaeche CE, Blackwell JM. Genetic control of immune response to recombinant antigens carried by attenuated *Salmonella typhimurium* vaccine strain: Nrp1 influences T-helper subset responses and protection against leishmanial challenge. *Infect Immun* 1998;**66**:1910–1917.
22. Smit JJ, van Loveren H, Hoekstra MO, Nijkamp FP, Bloksma N. Influence of the macrophage bacterial resistance gene, Nrp1 (Slc11a1), on the induction of allergic asthma in the mouse. *FASEB J* 2003;**17**:958–960.
23. Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? *Allergy* 1998;**53**:46:20–25.
24. Weiss ST. Diet as a risk factor for asthma. *Ciba Found Symp* 1997;**206**:244–257.
25. Martinez FD. Gene by environment interactions in the development of asthma. *Clin Exp Allergy* 1998;**28**:S5:21–25.
26. Baldini M, Vercelli D, Martinez FD. CD14: an example of gene by environment interaction in allergic disease. *Allergy* 2002;**57**:188–192.
27. Members of the workshops. ARIA in the pharmacy: management of allergic rhinitis symptoms in the pharmacy. Allergic rhinitis and its impact on asthma. *Allergy* 2004;**59**:373–387.
28. Matricardi PM. Infections preventing atopy: facts and new questions. *Allergy* 1997;**52**:879–882.
29. Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy* 2001;**56**:498–505.
30. Matricardi PM, Bjorksten B, Bonini S, Bousquet J, Djukanovic R, Dreborg S et al. Microbial products in allergy prevention and therapy. *Allergy* 2003;**58**:461–471.