

Association of allergic sensitization with infectious diseases burden in Roma and non-Roma children

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The hypothesis whether exposure to certain infections protects from atopy remains equivocal. To further investigate this, we compared serologic markers of infection and allergic sensitization prevalence in Roma children, who live under unfavorable hygienic conditions that facilitate the spread of infections, and non-Roma children who live in the same area. Analyses included 98 Roma and 118 non-Roma children. Serum IgG antibodies for 13 foodborne- airborne- and bloodborne infectious agents were determined, and a cumulative index of exposure was calculated by adding one point for each positive infection. Specific serum IgE to certain common food- and aero-allergens was also tested, and positivity to any of them was defined as indication of atopy. Roma children were found significantly more seropositive for *T. gondii*, *Hepatitis A*, *H. pylori*, *HSV-1*, *CMV*, and *Hepatitis B* ($p < 0.0001$). Non-Roma children were found more seropositive for *RSV* and *M. pneumoniae* ($p < 0.0001$). Regarding the overall prevalence of atopy or the specific IgE responses to the allergens tested, no statistically significant differences were found between Roma and non-Roma children. A positive association of the cumulative index of exposure to infections with atopy was found in the non-Roma children (OR: 1.38, 95% CI: 1.08–1.75, $p = 0.01$) and in the total population (OR: 1.42, 95% CI: 1.11–1.83, $p = 0.01$). Regarding the specific infectious agents tested, a statistically significant positive association of atopy with seropositivity was found for *M. pneumoniae* in the non-Roma children (OR: 3.93, 95% CI: 1.39) as well as in the total population studied (OR: 2.83, 95% CI: 1.32–6.07, $p = 0.01$). Despite the higher burden of exposure to the battery of the infectious agents tested among Roma children, no protective effect for allergic disease development was evident. On the contrary, a positive association of exposure to infections with evidence of atopy was found, especially evident in the non-Roma children.

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The prevalence of atopy has been rising steadily in developed countries during the last decades (1). There is accumulating evidence from epidemiologic studies that there is higher prevalence of atopy and allergic diseases in urban areas with 'westernized' style of life rather than rural areas and developing countries (2).

The 'hygiene hypothesis' postulates that the rise of allergic disease in recent decades is attributable to a decrease in childhood infections (3). This theory has been prompted by evidence that overcrowding, unhygienic conditions, and larger family size were associated with a lower prevalence of atopy, eczema, hayfever, and

asthma (4, 5). The most commonly quoted immune mechanism is that reduced microbial exposure results in not educating the T lymphocytes toward Th1 response. Th2 response remains with a consequent increase in allergic sensitization. An alternative explanation is that lower microbial burden is decreasing the activity of T-regulatory cells (6, 7).

The debate whether infection precipitates or prevents immune dysregulation remains a contentious one (8, 9). Although several studies support the protective effect of infections exposure, evidence is inconsistent. Most of these studies compared populations who live in different environments (urban versus rural) or in different countries; therefore, control for confounding is more cumbersome (10–13). Because the relationship between infections and atopy is complex, it is unlikely that studies of single microbes will shed light to unravel the mechanism of immune modulation (10). By contrast, a more pertinent epidemiologic model is to study accumulated effects of multiple infections.

The aim of this study was to investigate the association of microbial exposure with prevalence of atopy among Roma and non-Roma children who reside in the same deprived area, although experiencing profound differences in living conditions and lifestyles. Roma are a conglomerate of genetically isolated founder populations and comprise a translational minority of Indo-European origin with an estimated population worldwide of around 12 million; almost eight million reside in Europe, scattered mainly in the Balkans and the Carpathian region (14, 15). Most of them live under unfavorable life and hygienic conditions that could facilitate the spread of infections. The seroprevalence of 13 orofecal-, foodborne-, contact-, airborne- and bloodborne infectious agents, assessed in Roma and non-Roma children, was regarded as surrogate marker for microbial exposure. This unique epidemiological setting makes it possible to study the ‘hygiene hypothesis’ in two distinct childhood populations, who live in the same area.

Methods

Population

The study was conducted in 2002, in two elementary public schools of an industrial and rather deprived suburb of western major Athens, following approval from the Ministry of Education, the local Municipality, and the University of Athens Medical School Ethics Committee; informed consent was obtained prior to study

enrollment. Participation in the study was voluntary and reached a high ~90%. As described elsewhere (15), the study population comprised 216 school-age children, 5–15 year old; of them, 118 were Roma children attending a school in a proximal area to the one attended by the 98 non-Roma children. Roma or non-Roma origin was defined by the children’s parents, and schools were selected based on the ethnic group of pupils, with one school being attended mostly by Roma and the second in the vast majority by non-Roma population. An extensive questionnaire covering demographic and socioeconomic characteristics, atopic symptoms, medications, and health care delivery parameters was completed by the parents of the study participants in collaboration with the study group. The variables under investigation were age, gender, sibship size of the family, birth order of the index child, crowding index (number of rooms per person usually living in the residence), child health insurance, routine primary health care (at least one visit during the last 2 years), and availability of three house hygiene facilities variables, namely indoors toilet, electricity supply and central water supply, as well as duration of breastfeeding. A detailed history including atopic symptoms, family history for atopy, and medications was obtained. However, in most Roma children no medical documentation was available, and details of history were missing. Therefore, it was decided that the definition of atopy for the purposes of this study was exclusively based on laboratory findings.

Laboratory investigation

Serum samples were tested for total IgE and specific IgE to certain food- and aero-allergens in the Department of Immunology & Histocompatibility, ‘Aghia Sophia Children’s Hospital’ using UniCAP and ImmunoCAP systems (*Phadia*) for total IgE and specific IgE levels, respectively. Specifically, serum samples were tested for IgE specific to a mixture of 6 food allergens (fx5 mixture of: f1-eggwhite, f2-milk, f3-fish, f4-wheat, f13-peanuts, and f14-soybean) and to aero-allergens T9 (olea europea), W19 (parietaria), house dust (hx2, a mixture of: h2 -Hollister-Stier laboratories house dust, d1-Dermatophagoides pteronyssinus, d2- Dermatophagoides farinae, i6-Blatella germanica) and Phadiatop (a mixture of inhaled environmental allergens:t3-Betula verrucosa, t9- Olive tree pollen, g6-Phleum pratense,w6-Artemisia vulgaris, w19-Parietaria, e1- cat epithelium and dander, e3-horse dander, e5-dog dander,d1- Dermatophagoides pteronyss-

sinus). These allergens were selected because exposure to them is quite common in Greece and Europe. Positivity to any of the allergens tested was defined as evidence of atopy.

Serum IgG antibodies for 13 foodborne, airborne and bloodborne infectious agents, namely *T. gondii*, *Hepatitis A*, *Helicobacter pylori* (*H. pylori*), *Epstein-Barr virus (EBV)*, *Herpes simplex virus (HSV-1)*, *Varicella-Zoster virus (VZV)*, *Respiratory Syncytial virus (RSV)*, *Cytomegalovirus (CMV)*, *Mycoplasma pneumoniae* (*M. pneumoniae*), *Leishmania sp.*, *Hepatitis B*, *Hepatitis C*, and *Bartonella henselae* (*B. henselae*) were measured in the Microbiology Laboratory, 'Aghia Sophia Children's Hospital' using commercially available assays.

Children detected positive for HAV IgG could theoretically have had either past disease or vaccination. HAV vaccination was minimal among non-Roma children and practically non-existent among Romas, as documented in the vaccination booklets and confirmed by the local pediatricians in case the booklet was not available. Thus, for the purpose of this study, HAV seropositivity was virtually considered as past HAV infection. Regarding *Hepatitis B*, children who tested anti-HBc positive were regarded as seropositive.

Statistical analysis

Prevalence of positive serology for the tested food- and aero-allergens and the 13 infectious agents in Roma and non-Roma was calculated. The cutoff value for total IgE was set at 200 KU/l based on mean normal values + 2 s.d. for the Greek children population of this age (16). Standard statistical procedures (χ^2 and χ^2 for trend) were used to assess the differences in demographic, lactation, and infections score variables of 47 atopic and 169 non-atopic children. The overall seropositivity score (0–13) was calculated by adding one point for each positive infectious agent.

Subsequently, the data were modeled through multiple logistic regression analysis using atopy status as the dependent variable and ethnic group, gender, age, sibship size, birth order, crowding index, hygiene facilities, public health insurance, routine primary health care, and duration of lactation as predictor variables in a core model. Seropositivity to each infectious agent as well as the overall seropositivity score (1 degree more) was introduced in the models alternatively. The SAS statistical package (SAS Institute Inc, Cary, NC, USA) was used in all analyses.

Results

Distribution of study participants by demographic and socioeconomic variables is shown in Table 1, detailed also elsewhere (15). Roma children were statistically significantly more likely to belong to families with larger sibship size, reside in more crowded houses with worse hygiene facilities, and lack public health insurance and routine primary health care (for all parameters $p < 0.0001$). It is worth noting that they were also reported to be breast fed for longer periods ($p < 0.0001$).

Roma children were found to have higher levels of total IgE both assessed through comparison of the median values ($p = 0.02$) and also

Table 1. Distribution of 118 Roma and 98 Non-Roma school-age children by demographic and socioeconomic variables

Variable	Roma, N (%)	Non-Roma, N (%)	P-value for contrast/trend
Gender			
Boy	37 (31.4)	40 (40.8)	0.15*
Girl	81 (68.6)	58 (59.2)	
Age (years) (mean $\pm$ s.d.)	9.30 $\pm$ 2.13	8.82 $\pm$ 2.03	0.10
Sibship size (children)			
1–2	8 (6.8)	49 (50.0)	<0.0001**
3–4	51 (43.2)	42 (42.9)	
5–6	34 (28.8)	7 (7.1)	
7+	25 (21.2)	0 (0.0)	
Birth order			
1	25 (21.2)	27 (27.6)	<0.0001**
2	33 (28.0)	50 (51.0)	
3	25 (21.2)	11 (11.2)	
4+	35 (29.6)	10 (10.2)	
Crowding Index (room/person)			
≤ 0.25	55 (46.6)	0 (0.0)	<0.0001**
0.25–0.49	55 (46.6)	14 (14.3)	
0.50–0.74	8 (6.8)	60 (61.2)	
0.75+	0 (0.0)	24 (24.5)	
Score of home hygiene facilities: indoors toilet, electricity, water supply			
0	39 (33.1)	0 (0.0)	<0.0001**
1	17 (14.4)	0 (0.0)	
2	10 (8.5)	6 (6.1)	
3	52 (44.0)	92 (93.9)	
Child public health insurance			
Yes	96 (81.4)	97 (99.0)	<0.0001***
No	22 (18.6)	1 (1.0)	
Routine primary health care			
Yes	87 (73.7)	98 (100.0)	<0.0001***
No	31 (26.3)	0 (0.0)	
Duration of lactation (months)			
No lactation	15 (12.6)	21 (21.4)	<0.0001**
≤ 3	18 (15.3)	42 (42.9)	
4–6	20 (17.0)	21 (21.4)	
7–9	19 (16.1)	8 (8.2)	
10+	46 (39.0)	6 (6.1)	

Data are presented as N (%) or mean $\pm$ s.d.

*p-value derived from χ^2 test.

**p-value derived from test for trend.

***p-value derived from χ^2 Fisher's exact test.

values exceeding 200 Ku/L ($p = 0.05$) (Table 2). Differences in the prevalence of atopy (at least one specific IgE allergen positive) between Roma and non-Roma children did not reach statistical significance ($p = 0.12$). Regarding certain allergens, more non-Roma children tested positive to Phadiatop (mixture of inhaled environmental allergens), whereas more Roma tested positive to the aeroallergen W19 (parietaria). Nevertheless, none of the associations nominally reached statistical significance ($p = 0.13$ and 0.10 , respectively).

Prevalence of positive serology for foodborne, orofecal, airborne, and bloodborne infections is

Table 2. Prevalence of allergen-specific IgE and total IgE in 118 Roma and 98 non-Roma children

Variable	Seropositive Roma	Seropositive Non-Roma	p-value
fx5*, n (%)	17 (14.4)	16 (16.3)	0.70
Phadiatop*, n (%)	13 (11.0)	18 (18.4)	0.13
T9*, n (%)	8 (6.8)	7 (7.1)	0.92
W19*, n (%)	8 (6.8)	2 (2.0)	0.10
hx2*, n (%)	14 (11.9)	8 (8.2)	0.37
Total IgE, median (sd)	107.5 (544.8)	71.5 (244.2)	0.02
IgE>200 Ku/L, n (%)	35 (29.7)	18 (18.4)	0.05
Atopic (at least 1 positive), n (%)	21 (17.8)	26 (26.5)	0.12

*fx5 mixture of: f1-eggwhite, f2-milk, f3-fish, f4-wheat, f13-peanuts, f14-soybean) T9 (olea europea), W19 (parietaria), hx2, a mixture of: h2 -Hollister-Stier laboratories house dust, d1-Dermatophagoides pteronyssinus, d2- Dermatophagoides farinae, i6-Blatella germanica and Phadiatop (a mixture of inhaled environmental allergens): t3-Betula verrucosa, t9- Olive tree pollen, g6-Phleum pratense, w6-Artemisia vulgaris, w19-Parietaria, e1- cat epithelium and dander, e3-horse dander, e5-dog dander, d1- Dermatophagoides pteronyssinu.

presented in Table 3. Roma children were found significantly more seropositive for *T. gondii*, *Hepatitis A*, *H. pylori*, *HSV*, *CMV*, and *Hepatitis B* ($p < 0.0001$). Non-Roma children were found more seropositive for *RSV* and *M. pneumoniae* ($p < 0.0001$). No differences in seropositivity were found for *EBV*, *VZV*, and *B. henselae*, whereas no children were infected with *Leishmania* and *Hepatitis C*.

Within the Roma children population, multiple logistic regression analysis for comparison of seropositivity to specific infectious agents between atopic and non-atopic individuals showed no differences for any of the infections tested (data not shown). For the non-Roma children, however, the same analysis found that seropositivity for *M. pneumoniae* infection is associated with the presence of atopy (OR: 3.93, 95% CI: 1.39–11.08, $p = 0.01$). Increased risk for atopic sensitization was also associated with seropositivity for *M. pneumoniae* infection in the total population studied (OR: 2.83, 95% CI: 1.32–6.07, $P = 0.01$) (Table 4).

A positive association of higher cumulative index of exposure to infections with atopy was found in the non-Roma population (OR: 1.38, 95% CI: 1.08–1.75, $p = 0.01$) as well as in the total population studied (OR: 1.42, 95% CI: 1.11–1.83, $p = 0.01$) (Table 4).

Discussion

The debate over whether exposure to infections precipitates or prevents immune dysregulation remains a contentious one. This study, conducted

Table 3. Prevalence of positive serology for infections in 118 Roma and 98 non-Roma children

Variable	Roma (N = 118) Seropositivity N (%)	Non-Roma (N = 98) Seropositivity N (%)	p-value (χ^2 or χ^2 for trend)	Seropositivity All N (%)
Orofecal/foodborne infections				
<i>Toxoplasma gondii</i>	21 (17.8)	2 (2.0)	<0.0001	23 (10.6)
<i>Hepatitis A</i>	116 (98.3)	32 (32.7)	<0.0001	148 (68.5)
<i>Helicobacter pylori</i>	105 (89.0)	11 (11.2)	<0.0001	116 (53.7)
Contact/airborne infections				
<i>Epstein-Barr Virus</i>	58 (49.0)	46 (46.9)	0.75	104 (48.1)
<i>Herpes Simplex Virus-1</i>	110 (93.2)	35 (35.7)	<0.0001	145 (67.1)
<i>Varicella-Zoster Virus</i>	56 (47.5)	58 (59.2)	0.09	114 (52.8)
<i>Respiratory Syncytial Virus</i>	60 (50.9)	73 (74.5)	<0.0001	133 (61.6)
<i>Cytomegalovirus</i>	117 (99.1)	34 (34.7)	<0.0001	151 (69.9)
<i>Mycoplasma pneumoniae</i>	20 (17.0)	40 (40.8)	<0.0001	60 (27.8)
<i>Leishmania</i>	0 (0.0)	0 (0.0)	–	0 (0.0)
Other infections				
<i>Hepatitis B</i>	26 (22.0)	0 (0.0)	–	26 (12.0)
<i>Hepatitis C</i>	0 (0.0)	0 (0.0)	–	0 (0.0)
<i>Bartonella henselae</i>	18 (15.3)	16 (16.3)	0.83	34 (15.7)
Score of infections				
0–3	3 (2.5)	52 (53.1)	<0.0001	
4–5	38 (32.2)	32 (32.6)		
6+	77 (65.3)	14 (14.3)		

Table 4. Multiple logistic regression derived odds ratios (ORs) and 95% confidence interval (95% CIs) for atopy among study participants by demographic variables and additionally by individual infectious agents tested

Variable	Category or Increment	ORs	95% CIs	p-value
Ethnic group	Non-Roma	1.60	0.44–5.86	0.48
Gender	Girl	0.91	0.45–1.84	0.79
Age	2 years more	1.08	0.75–1.54	0.69
Sibship size	2 children more	1.06	0.57–1.96	0.87
Birth order	1 child	0.74	0.47–1.16	0.19
Crowding index	0.25 room/person	1.36	0.75–2.47	0.31
Score of home hygiene facilities	1 facility more	0.72	0.48–1.08	0.11
Child public health insurance	No	2.08	0.67–6.47	0.21
Routine primary health care	No	1.13	0.37–3.43	0.83
Duration of lactation	3 months more	0.88	0.67–1.14	0.33
Additional, alternatively introduced variables				
Score of infections	1 infection more	1.42	1.11–1.83	0.01*
Toxoplasma gondii	Yes	2.74	0.91–8.22	0.07
Hepatitis A	Yes	1.70	0.66–4.39	0.27
Helicobacter pylori	Yes	1.66	0.57–4.88	0.36
Epstein–Barr virus	Yes	1.39	0.71–2.75	0.34
Herpes Simplex Virus- 1	Yes	0.87	0.36–2.09	0.76
Varizella-Zoster Virus	Yes	1.40	0.70–2.77	0.34
Respiratory Syncytial Virus	Yes	1.76	0.81–3.82	0.15
Cytomegalovirus	Yes	2.19	0.83–5.74	0.11
Mycoplasma pneumoniae	Yes	2.83	1.32–6.07	0.01*
Bartonella henselae	Yes	0.69	0.25–1.88	0.47

*p-value <0.05.

in a unique epidemiological setting that presumably maximizes contrast of hygiene conditions, showed that previous increased infectious diseases exposure, documented through seropositivity to a battery of common infectious agents, did not confer a protective effect on the development of allergic sensitization. The latter was manifested through IgE positivity to specific aero- and food-allergens. In the infections tested, we included foodborne- airborne- and bloodborne-infectious agents as different ways of exposure could have dissimilar effects in the regulation of immune system.

The initial assumption, that Roma comparing with non-Roma children, would probably have much higher prevalence for markers of past infections, caused by adverse hygienic conditions, was corroborated, as they had statistically significant differences in 6 of 8 specific infectious agents in which differences were identified. Although seroprevalence of different types of infectious agents was tested, there was no difference as far as for protective effect for atopy prevalence regarding separately orofecal, foodborne-, airborne- or bloodborne microbes in both populations studied.

Much higher total IgE values were noted among Roma children, probably because of increased exposure to parasites. Interestingly enough, although Roma children had longer

period of breastfeeding, no significant protective effect was noted.

Regarding the specific infectious agents tested, a statistically significant positive association of atopy with seropositivity was evident only for *M. pneumoniae* in the non-Roma and in the total population, whereas the sizeable associations with *T. gondii*, *CMV*, and *RSV* did not reach nominally statistical significance. However, because of relatively limited study population, it is possible that other positive associations of infectious agents with atopy did not come up.

In contrast to the hypothesis that exposure to certain infectious agents could exert a protective effect for allergic sensitization, a positive association of the cumulative index of exposure to infections with atopy was found in the total population studied and especially in the non-Roma children.

Several risk factors for the development of allergic disease in children have been described. However, evidence of the influence of pathogen exposure on the development of atopy is equivocal. Previous studies addressed the issue of infectious diseases burden and atopy based on children or adult populations using different designs. Some studies used indirect surrogates of increased exposure to microbes like comparing children from foster versus physical families (17)

or rural versus urban children (13) to provide evidence that conditions that favor exposure to infections may protect from atopy.

Regarding cross-sectional studies referring to specific infectious agents, a recent study that included children from Finland-Russian region (11) found significant protective effect of *H. pylori* and enterovirus infection for atopy development, but no effect of *HAV* or *T. gondii*. In adult studies, foodborne and orofecal infections like *HAV*, *H. pylori*, and *T. gondii* rather than airborne viruses have been linked previously to a reduced risk of atopy (18–21). In a Danish study (20), seropositivity to 2 or 3 intestinal pathogens (*Clostridium difficile*, *Campylobacter jejuni*, and *Yersinia enterocolitica*) was associated with a higher prevalence of atopy.

As far as for airborne pathogens, there are studies indicating that early exposure to viruses, like RSV, during 1st year of life, could increase the risk of atopy, although evidence is inconsistent (22–25). The role of atypical airway pathogens, like *M. pneumoniae* or *Chlamydophila pneumoniae*, as potentially predisposing factors to atopy is still under investigation. Although they have been implicated for new-onset wheezing, exacerbation of prevalent asthma, and long-term lung sequelae, their role as risk factors is still poorly understood (26, 27). This study provided some evidence that *M. pneumoniae* may predispose to atopy that needs further investigation.

Conflicting evidence also exists regarding contribution of parasites to the development of atopy. There are studies in Africa and Latin America supporting a protective role of helminths for atopic disease (28, 29), albeit results depend on the kind of parasite and the geographic region (12, 30).

Because the relationship between infections and atopy is not a simple one, it is not surprising that therapeutic interventions in clinical trials, such as administration of probiotics for atopic dermatitis, have shown contradictory results (8, 31–34).

Limitation of this study is that is a cross-sectional study and cause-effect associations should be interpreted cautiously. Also, we studied two children populations that are probably genetically different, and even though we controlled for ethnic group in regression analysis, genetic predisposition should be taken into consideration. In addition, although both populations live in the same area, exposure to several environmental allergic factors might be different, and as we cannot rely on the medical history, there is no information about clinical manifestations of atopy.

In conclusion and in line with recent literature (9), data from this study indicate that increased exposure to infectious agents does not exert a protective effect on atopic sensitization; hence these results do not seem to support the 'hygiene hypothesis'. The link, if present, between increased exposure to infections and allergy development is likely to be more complex, depending critically on the timing, type of exposure, and genetic background of the population. Future studies will need to dissect the timing, magnitude, and type of such infectious stimulation to clarify those that are protective, neutral, or harmful. Further understanding of immune mechanisms is also essential for interpretation of epidemiologic data and is a prerequisite for the implementation of preventive measures.

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