TAPIO SEISKARI

The Association Between Viral Infections, IgE Sensitization, and Type 1 Diabetes

■

ACADEMIC DISSERTATION
To be presented, with the permission of
the Board of the School of Medicine of the University of Tampere,
for public discussion in the Auditorium of Finn-Medi 5,
Biokatu 12, Tampere, on March 20th, 2015, at 12 o'clock.

UNIVERSITY OF TAMPERE

TAPIO SEISKARI

The Association Between Viral Infections, IgE Sensitization, and Type 1 Diabetes

Acta Universitatis Tamperensis 2021
Tampere University Press
Tampere 2015

UNIVERSITY
OF TAMPERE

ACADEMIC DISSERTATION
University of Tampere, School of Medicine
Fimlab Laboratories, Department of Clinical Microbiology
Finland

Supervised by
Professor Heikki Hyöty
University of Tampere
Finland

Reviewed by
Professor Erika Isolauri
University of Turku
Finland
Docent Anna Pelkonen
University of Helsinki
Finland

The originality of this thesis has been checked using the Turnitin OriginalityCheck service in accordance with the quality management system of the University of Tampere.

Cover design by
Mikko Reinikka

Distributor:
kirjamyynti@juvenes.fi
http://granum.uta.fi

Acta Universitatis Tamperensis 2021
ISBN 978-951-44-9708-7 (print)
ISSN-L 1455-1616
ISSN 1455-1616

Acta Electronica Universitatis Tamperensis 1510
ISBN 978-951-44-9709-4 (pdf)
ISSN 1456-954X
http://tampub.uta.fi

Suomen Yliopistopaino Oy – Juvenes Print
Tampere 2015

1. LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications referred to in the text by the Roman numerals I – IV:

I Seiskari T, Kondrashova A, Viskari H, Kaila M, Haapala AM, Aittoniemi J, Virta M, Hurme M, Uibo R, Knip M, Hyöty H and the EPIVIR study group (2007); Allergic sensitization and microbial load -- a comparison between Finland and Russian Karelia. Clin Exp Immunol 148:47-52.

II Seiskari T, Viskari H, Kaila M, Haapala AM, Koskela P and Hyöty H (2009); Time trends in allergic sensitisation and Helicobacter pylori prevalence in Finnish pregnant women. Int Arch Allergy Immunol 150:83-88.

III Seiskari T, Viskari H, Kondrashova A, Haapala AM, Ilonen J, Knip M and Hyöty H (2010); Co-occurrence of allergic sensitization and type 1 diabetes. Ann Med 42:352-359.

IV Seiskari T, Kondrashova A, Tauriainen S, Knip M, Viskari H, Haapala AM and Hyöty H (2012); Role of enteroviruses in IgE sensitization. J Med Virol 84:268-71.

2. ABBREVIATIONS

CAV	Coxsackie A virus
CBV	Coxsackie B virus
CI	Confidence interval
CMV	Cytomegalovirus
EBV	Epstein-Barr virus
EIA	Enzyme immunoassay
EV	Echovirus
FMC	Finnish Maternity Cohort
HAV	Hepatitis A virus
HHV6	Human herpes virus 6
HSV	Herpes simplex virus
IgE	Immunoglobulin E
MMR	Measles-mumps-rubella
OR	Odds ratio
PRR	Pattern recognition receptor
RSV	Respiratory syncytial virus
SPT	Skin prick test
Th1	T-helper type 1
Th2	T-helper type 2
TIM	T cell, immunoglobulin domain and mucin domain
TLR	Toll-like receptor
VZV	Varicella-zoster virus

3. ABSTRACT

During the past decades, the prevalence of allergic diseases has rapidly increased in developed countries. Simultaneously, the prevalence of autoimmune diseases such as type 1 diabetes has also increased.

Epidemiological data suggests that having older siblings or growing up in a farm environment is linked to a lower risk of allergic disorders. The reasons for these relationships remain unknown, but one possible explanation is 'the hygiene hypothesis'. Accordingly, the microbial stimulus to immune cells is hypothesised to guide the maturation of immune responses.
The hypothesis of this study was that diminished exposure to microbes in childhood can lead to impaired maturation of the regulatory elements of the immune system and impaired control of immune responses. This, in turn, leads to overwhelming inflammatory responses resulting in autoimmune and allergic diseases.

This study was carried out in two populations that lived close to each other – i.e. in Finland and Russian Karelia –and shared a similar genetic background, but were characterized by a conspicuous difference, for example, in their degree of affluence and their childhood microbial exposure. The prevalence of IgE mediated allergic sensitization and IgG antibodies against certain infective agents was studied in both populations. In addition, a possible association between type 1 diabetes and IgE sensitization was evaluated.

The prevalence of IgE mediated allergic sensitization as well as its association with infections were studied among 266 schoolchildren in the Oulu region of Finland and 266 schoolchildren in the Karelian Republic in Russia. In addition, 147 type 1 diabetic patients in Finland and 132 type 1 diabetic patients in Russian Karelia were included in the study. The association between type 1 diabetes and IgE mediated allergic sensitization was investigated among these diabetic children and healthy controls in both countries.

In addition, time trends in allergic sensitization and *H. pylori* infections were compared by analysing altogether 958 blood samples drawn from Finnish pregnant women in 1983, 1989, 1995 and 2001.

The prevalence of IgE sensitization was significantly higher in Finland compared to Russian Karelia, whereas microbial antibodies were more frequent in Russian Karelia. Furthermore, infections were associated with a lower risk of allergic sensitization in Russian Karelia, enterovirus showing the strongest protective effect. Differences between enterovirus types were observed when associations of allergic sensitization and enteroviruses were analysed in more detail.

A clear birth cohort effect was found in the prevalence of IgE mediated allergic sensitization in Finland, IgE sensitization being more frequent in recent birth cohorts than earlier ones. The prevalence of *H. pylori* antibodies, in turn, followed the opposite trend.

A positive association was observed between type 1 diabetes and IgE sensitization in Russian Karelia. Furthermore, the co-occurrence of type 1 diabetes and IgE sensitization in Russian Karelia was associated with a lack of antibodies against the hepatitis A virus.

Taken together, results imply that environmental factors strongly contribute to the development of allergic diseases. Yet, the mechanisms affecting the development of IgE sensitization may differ in populations characterized by different standards of hygiene. Exposure to microbes seems to be important environmental factor, and it is possible that some microbe-specific effects are involved.

4. FINNISH SUMMARY

Virusinfektioiden yhteys allergiseen herkistymiseen ja tyypin 1 diabetekseen.

Viime vuosikymmenten aikana allergiset taudit kuten allerginen nuha ja ihottuma ovat nopeasti yleistyneet kehittyneissä maissa. Samanaikaisesti myös useiden autoimmunitautien, kuten tyypin 1 diabeteksen, esiintyvyys on lisääntynyt. Epidemiologisissa tutkimuksissa sisarusten lukumäärän ja allergisten sairauksien esiintyvyyden välillä on todettu käänteinen yhteys. Myös maatilalla varttumisen on todettu suojaavan allergiselta herkistymiseltä. Syyt näiden yhteyksien taustalla ovat pitkälti tuntemattomia. Mahdollisen selityksen tarjoaa ”Hygienia hypoteesi”, jonka mukaan lapsuuden aikainen altistus mikrobeille suojaa allergiselta herkistymiseltä ohjaamalla immuunijärjestelmän kehittymistä.

Tämän tutkimuksen hypoteesina oli, että vähentynyt varhaislapsuuden aikainen mikrobialtistus ei riittävästi stimuloi kehittyvää immuunijärjestelmää, minkä seurauksena esiintyy lisääntyvässä määrin immuunivasteita hillitsevien CD4+CD25+ T-solujen toiminnan häiriöitä. Nämä puolestaan ovat altistavia tekijöitä sekä Th1-tyyppisten että Th2-tyyppisten vasteiden epätarkoituksenmukaiselle voimistumiselle, mikä voi johtaa allergiseen herkistymiseen tai autoimmuunitaudin kehittymiseen. Atopialla ja autoimmuunitaudeilla saattaa siis olla patogeneettinen yhteys, jonka selvittäminen tulee auttamaan näiden tautien ennaltaehkäisemisessä.
Tutkimuksen tavoitteina oli tutkia allergisen herkistymisen esiintyvyyttä Suomessa ja Karjalan tasavallassa Venäjällä, sekä allergisessa herkistymisessä viime vuosikymmenien aikana Suomessa tapahtuneita muutoksia. Tavoitteena oli myös selvittää allergisen herkistymisen ja mikrobialtistusten välisiä yhteyksiä. Lisäksi tavoitteena oli tutkia allergisen herkistymisen ja tyypin 1 diabeteksen välisiä yhteyksiä.

Suomen ja Venäjän Karjalan tasavallan välillä vallitsevat suuret erot esimerkiksi lapsuuden aikaisessa mikrobialtistuksen määrässä ja kansantaloudellisesti mitattavassa elintasossa. Toisaalta alueiden maantieteellinen läheisyys tarjoavat ainutlaatuisen lähtökohdan näiden ilmiöiden tutkimiseen.

Tutkimme allergisen herkistymisen esiintyvyyttä ja yhteyttä mikrobi-infektioihin 266:n Suomen Oulun alueen koululaisen ja 266:n Venäjän Karjalan tasavallan koululaisen aineistoilla. Suomalaisten tyypin 1 diabetespotilaiden (N=147) , Petroskoin sairaalassa hoidettujen diabetespotilaiden (N=132) ja verrokkiryhminä toimineiden koululaisten avulla vertailimme allergisen herkistymisen esiintyvyyttä tyypin 1 diabeetikoilla ja terveillä verrokeilla.

Lisäksi käytössämme oli yhteensä 958 verinäytettä suomalaisilta raskaina olleilta naisilta vuosilta 1983, 1989, 1995 ja 2001. Näiden näytteiden avulla tutkimme IgE-tasoissa (kokonais- ja allergeenispesifinen IgE) viime vuosikymmenten aikana tapahtuneita muutoksia, sekä yhteyksiä IgE-tasojen ja IgG-luokan Helicobakteeri pylori-vasta-aineiden esiintyvyyksien välillä.

Allerginen herkistyminen osoittautui Suomessa selvästi yleisemmäksi kuin Venäjän Karjalassa. Toisaalta vasta-aineet mikrobeja vastaan olivat Karjalan tasavallassa selvästi yleisempiä kuin Suomessa. Lisäksi Karjalan tasavallassa mikrobivasta-aineiden ja allergisen herkistymisen välillä oli todettavissa käänteinen yhteys, joka oli voimakkain enteroviruksen kohdalla.

Vuosien 1983 ja 2001 välillä allergisen herkistymisen todettiin lisääntyneen Suomessa *H. pylori* -negatiivisilla naisilla. Toisaalta *H.pylori* -vasta-aineiden esiintyvyydessä todettiin selvä lasku näiden vuosien välillä. Lisäksi oli todettavissa allergisen herkistymisen lisääntyminen syntymävuosikohorteissa: myöhemmin syntyneillä esiintyi selvästi useammin allergista herkistymistä kuin aiempina vuosina syntyneillä.

Tyypin 1 diabeteksen ja allergisen herkistymisen välillä todettiin yhteys Karjalan tasavallassa, muttei Suomessa. Lisäksi Karjalan tasavallassa todettiin käänteinen yhteys hepatiitti A

-virus-vasta-aineiden esiintyvyyden, sekä allergisen herkistymisen ja diabeteksen yhteisesiintyvyyden välillä.

Tutkiessamme tarkemmin enterovirusvasta-aineiden ja allergisen herkistymisen välisiä yhteyksiä Karjalan tasavallassa, totesimme eroavuuksia eri enterovirustyyppien välillä. Erityisesti Echovirus 11 liittyi alhaiseen allergisen herkistymisen esiintymiseen.

Kaiken kaikkiaan tulokset viittaavat siihen, että ympäristötekijöillä on suuri merkitys allergisen herkistymisen kehittymiseen. Kuitenkin allergista herkistymistä säätelevät mekanismit saattavat erilaisissa ympäristöissä poiketa toisistaan. Mikrobeille altistuminen näyttää olevan yksi tärkeä ympäristötekijä ja on mahdollista, että eri mikrobit eroavat toisistaan tässä suhteessa.

5. INTRODUCTION

During the past decades, we have seen a dramatic increase in the prevalence of allergic diseases in the industrialised Western countries, described as an allergy epidemic. Concurrently, the prevalence of autoimmune diseases such as type 1 diabetes has also increased.

The 'hygiene hypothesis' suggests diminished microbial exposure in childhood as a reason for these unfavourable prevalence trends (Strachan 1989, Bach 2002). Accordingly, microbial stimulus to immune cells is hypothesised to guide the maturation of immune responses. Consequently, a reduced exposure to microbes could lead to an overreaction of the immune responses involved in both autoimmune and allergic diseases (Bach 2005).

Epidemiological data provides evidence for this hypothesis, since having older siblings and growing up in a farm environment have repeatedly been shown to be inversely linked to the risk of allergic disorders (Strachan 2000, von Mutius and Vercelli 2010). Furthermore, exposure to certain microbes has been linked to a lower risk of allergic diseases, although also controversial data exists (von Hertzen and Haahtela 2004).

The hygiene hypothesis was first introduced from an epidemiological background, but since then, several plausible immunological mechanisms have been proposed to support this hypothesis (Bach 2005). Some of these mechanisms, such as the Th1/Th2 paradigm, imply that allergic and autoimmune diseases would probably affect different individuals, whereas other mechanisms, such as defects in regulatory pathways, would cause these diseases to co-occur more than expected. A number of studies have therefore investigated whether allergic conditions are inversely related to organ-specific autoimmunity. However, studies analysing the frequency of allergic manifestations in patients with type 1 diabetes have not shown a clear relationship (Cardwell et al. 2003).

The allergy epidemic is a serious health problem, and, in some European countries, up to 50% of children have IgE sensitization (Bousquet et al. 2011). A better understanding of potential risk factors and protective factors is needed to fight this epidemic. Although it seems clear that environmental factors – mainly environmental microbiota – play a key role, the exact mechanisms and potential microbe-specific factors remain unknown.

This study was set to investigate the hygiene hypothesis in two populations living close to each other – i.e. in Finland and Russian Karelia – and sharing a similar genetic background, but were characterized by a conspicuous difference, for example, in their degree of affluence and in their childhood infections. IgG antibodies against certain microbes were studied in both populations as markers for infectious pressure, and allergen-specific IgE antibodies were measured to evaluate the prevalence of allergic sensitization. In addition, the relationship between type 1 diabetes and IgE sensitization was evaluated.

6. REVIEW OF THE LITERATURE

6.1 Allergy epidemic

During the last few decades, the prevalence of allergic diseases has dramatically increased in Finland as well as in other industrialised Western countries. As an illustration, the prevalence of eczema more than doubled and prevalence of hay fever almost doubled in British adolescents between 1958 and 1970, outcome being based on parental reports (Butland et al. 1997). A similar trend for eczema was seen in Scotland between1964 and 1989, when the prevalence of hay fever almost tripled in the same area. Also the prevalence of asthma and wheeze increased in this Scottish survey, based again on parental reports (Ninan and Russel 1992). Furthermore, the prevalence of specific IgE increased significantly (26.5% vs. 33.9%) from 1990 to 1998 in Denmark (Linneberg et al. 2000). Although there are evidence from some areas suggesting that this epidemic might have reached a plateau (Zöllner et al. 2000, Braun-Fahrländer et al. 2004), in other areas, such as Finland, still-increasing prevalence trends have been recently reported. Furthermore, it seems that not only the prevalence of allergic sensitization in Finland has increased, but also the gap between Finland and adjacent Russian Karelia has widened, as the prevalence figures have remained low and constant in Russian Karelia (von Hertzen et al. 2006, Laatikainen et al. 2011). Similarly to Russian Karelia, low prevalence rates have been reported from other Eastern European countries as well (Kramer et al. 2009). Indeed, major variations exist globally in the prevalence and in the time trends of prevalence of allergic conditions between different regions of the world. These include, for example, a high prevalence of asthma, allergic rhinoconjunctivitis and eczema in New Zealand, Australia, USA, and some Latin American and European countries, whereas low prevalence rates have been reported from India, Indonesia and some Eastern European countries (Beasley R et al. 1998, Asher et al. 2006). Studies conducted on immigrants suggest that environmental rather than genetic factors are responsible for these variations. As an illustration, immigrants coming from

Ethiopia to Israel had no allergies upon arrival, but allergies developed after five to ten years from arrival (Geller-Bernstein and Kenett 2004).

6.2 Geographical variation

The International Study of Asthma and Allergies in Childhood (ISAAC) found huge worldwide variations in the prevalence of asthma, allergic rhinoconjunctivitis and atopic eczema. In this large international comparison, all three conditions showed low prevalence figures in Eastern European countries, India and China. On the other hand, the prevalence of these conditions was high in the UK and the USA. Furthermore, central and northern European countries showed much higher prevalence than Eastern and some southern European countries (Beasley R et al. 1998).

Since then, these variations have been related to dietary factors (Weiland et al. 1999, Ellwood et al. 2001), the tuberculosis notification rate (von Mutius at al. 2000), gross national product per capita (GNP) (Stewart AW et al. 2001), and climate (Weiland et al. 2004). These ecological relationships may not be direct, but rather reflect some underlying relationships that may well be consistent with the hygiene hypothesis. For example, diet has a considerable effect on the composition of gut microbiota, which in turn has effects on immunological and inflammatory responses (Maslowski and Mackay 2011). On the other hand, variations in tobacco smoking (Mitchell and Stewart 2001), immunization (Anderson et al. 2001), pollen exposure (Burr et al. 2003) and antibiotic sales (Foliaki S et al. 2004) do not seem to account for the worldwide variations in asthma and allergies to a significant degree.

It is worth noticing that the first ISAAC study was carried out in 155 collaborating centres in 56 countries, whereas some of the following studies included much smaller numbers of centres and countries. As an illustration, standardized data for tuberculosis notification rates was available from 23 countries, mainly leaving developing countries out (von Mutius at al. 2000).

Furthermore, there are some interesting exceptions to the above-mentioned patterns of allergy and asthma prevalence, such as a high prevalence of allergic rhinoconjunctivitis and atopic eczema in Nigeria, as well as in some Latin American countries in which asthma prevalence is high (Beasley R et al. 1998). These exceptions can be considered contradictory to the hygiene hypothesis. Yet, it may be that some symptoms regarded as asthmatic or allergic may actually be caused by infections with similar pattern of symptoms.

6.3 Hygiene hypothesis

The risk of hay fever has been reported to be inversely linked with family size and especially with the number of older siblings (Strachan 1989). The suggested explanation was that allergic diseases were prevented by infection in early childhood, and that these infections were transmitted by unhygienic contact with older siblings (Strachan 1989). Thereafter, this idea of a relationship between hygiene and allergic diseases has become known as 'the hygiene hypothesis'.

This hypothesis has also been proposed to explain the increase seen in the prevalence of autoimmune diseases such as type 1 diabetes (D'Angeli et al. 2010). Indeed, the risk of multiple sclerosis had been linked with a high level of sanitation more than 20 years before the hygiene hypothesis was proposed as an explanation for the increased prevalence of allergic diseases (Poskanzer et al. 1963, Leibowitz et al. 1966). The idea was that, in areas characterised by a high level of hygiene, the suspected infectious trigger of the disease would be acquired later on in life, when individuals are more susceptible to infection. Analogously with poliomyelitis, this would more often lead to a manifestation of an infection that would be represented as clinical multiple sclerosis.

The hygiene hypothesis has survived and been updated due to accumulating epidemiological data and the current understanding of complex immunological mechanisms. Since infectious exposures in childhood were initially considered as the main driving force guiding the

maturation of the immune system and its regulation, a possible relationship between infectious and allergic diseases has been studied intensively (Matricardi et al. 1997, Janson et al. 2007). However, in recent decades, this hypothesis has evolved to emphasise the role of the indigenous microbiome as a regulator of immune responses (Atarashi et al. 2013). Furthermore, the indigenous microbiome is related to the environmental microbiome, which, in turn, is connected with the environmental macrobiome, suggesting that the loss of natural environments and biodiversity may have a role in the allergy epidemic (Hanski et al. 2012).

Hence, different aspects of the hygiene hypothesis have been proposed: overt and invasive infections with viruses and bacteria or non-invasive microbial exposures – or both – may have a remarkable influence on human innate and adaptive immune responses and consequently on the development of allergies and autoimmune diseases as well.

6.3.1 Epidemiological considerations

The strongest epidemiological support for the hygiene hypothesis is derived from studies reporting lower prevalence of allergic diseases in farmers' families and studies reporting an inverse relationship between family size and risk of allergic diseases.

6.3.1.1 Family size

Family size has been inversely related to the risk of allergic diseases in numerous studies. In 1989, Strachan observed significant trends in the prevalence of hay fever in relation to position in the household: a high number of siblings protected from hay fever, and this protection was stronger with older siblings. Eczema in the first year of life was also related to the number of older, but not younger siblings (Strachan 1989). Similar inverse associations between family size and allergic conditions have been reported from several European countries (Strachan 2000). However, it seems

that changes in family size account for little of the reported increase in allergic diseases (Wickens et al. 1999, Upchurch et al. 2010).

6.3.1.2 Farm living

Although a prevalence of allergic diseases is more frequent in Westernized urban and affluent areas than in more rural areas, large differences in prevalence exist within rural areas as well. Namely, children who grow up on farms are at a lower risk of developing allergic diseases (Braun-Fahrländer et al. 1999, Kilpeläinen et al. 2000, Riedler et al. 2000). Although living conditions on farms may differ in many respects from living conditions in other families (Braun-Fahrländer et al. 1999), this reduced risk has been linked to certain protective exposures associated with a farming lifestyle. These exposures include contact with farm animals (Riedler et al. 2000, von Ehrenstein et al. 2000, Ege et al. 2007) and the consumption of unprocessed cow's milk (Riedler et al. 2001, Ege et al. 2007). The timing of these exposures is crucial, the strongest effects being associated with exposures that occur during the first years of life or even *in utero* (Riedler et al. 2001, Ege et al. 2006). Furthermore, maternal exposure to stables during pregnancy has been linked to upregulation of receptors of innate immunity in school-aged children (Ege et al. 2006).

One possible protective factor that might relate to the exposures in a farm environment is a greater exposure to microbes. Indeed, the level of environmental microbial exposure, such as endotoxin concentration measured from indoor mattresses, is higher in households where children have regular contact with farm animals (von Mutius et al. 2000). Furthermore, mattress dust can be regarded as a reflection of long-term microbial exposure (von Mutius and Vercelli 2010).

This suggestion of a farm environment protecting against allergic diseases through a greater microbial exposure is well in line with the hygiene hypothesis.

A striking change has occurred in farming during past century, mainly after World War II. As an illustration, farming in Finland has changed from being the most frequent source of livelihood in 1920 to being a rarity in 2004. Indeed, according to Finnish national registers, farming was the source of livelihood for 70% of workers in 1920, whereas in 2004 only 4% of workers were farmers (Statistics Finland 2007). It could be speculated that a change of this magnitude may have had a substantial effect on the increase seen in the prevalence of allergic diseases. To our knowledge, this effect has not been investigated in a similar manner to what has been done to look at the relationship between family size and allergic diseases (Wickens et al. 1999, Upchurch et al. 2010). Yet, one Swedish study reported that the increase in allergic diseases has occurred in both farming and non-farming environments and that the protective effect of farming appears to be a fairly recent phenomenon (Bråbäck et al. 2004). This implies that changes that have occurred in the popularity of farming as a livelihood account only partially for the reported increase in allergic diseases.

6.3.1.3 Day care attendance

One would easily think that attending day care at a young age would expose a child to infections and therefore – according to the hygiene hypothesis – would lead to a reduced risk of allergic diseases. However, studies investigating the association between day care attendance and allergic diseases have reported controversial results. Indeed, in a large Danish study, the incidence rate of atopic dermatitis was decreased if a child had attended day care before age of six months (Benn et al. 2004). On the other hand, Cramer et al. (2011) reported that day care attendance during the first two years of life was associated with an increased risk of eczema in Germany. Again, in a large Swedish study (Hagerhed-Engman et al. 2006), children in day care had more asthma and allergic symptoms than children in the home. The authors noted that most of the children stay at home until 18 months of age in Sweden. In a large European survey, day care attendance before the age of five

was associated with a lower prevalence of hay fever; no association was found with eczema (Svanes et al. 2002). In this study, as in other studies (Hagendorens et al. 2005, Hagerhed-Engman et al. 2006), wheezing was associated with day care attendance. Furthermore, in a Dutch survey, IgE sensitization occurred less frequently in children that had attended day care, but there was no relation to allergic symptoms (de Meer et al. 2005).

There may be many reasons for these inconsistent results, one being the age at which children start at a day care centre. As it seems that the timing of protective exposures is important (Riedler et al. 2001, Ege et al. 2006), it may well be that attending day care tends to start too late to have an effect. Indeed, it has been shown that the prevalence of IgE sensitization as measured by positive SPT or allergen-specific IgE was higher among children who started at a day care centre at an older age than it was in those who began attending at a younger age (Krämer et al. 1998). Another confounding factor may be possible differences between children who attend day care and those who remain at home. This phenomenon was investigated in Sweden, where children cared for at home were younger, had more siblings, more often lived in a rural area and in single-family houses, and more often had furred pets at home, compared with children attending day care (Hagerhed-Engman et al. 2006).

In addition, when considering epidemiological data in the light of the hygiene hypothesis, one should bear in mind that microbial exposure at a day care centre is probably very different from the microbial exposure encountered in farming activities, for example.

Furthermore, in the past few decades, a substantial increase has occurred concurrently in the prevalence of allergies and in the proportion of children who attend day care (Välimäki and Rauhala 2000). On a population level, this is in conflict with the idea that day care would provide protection against allergy, or at least it is not the answer to the problem.

6.3.1.4 Pet keeping

Farm living has a protective effect against allergic diseases, and this protection has been partly linked to contact with farm animals (Riedler et al. 2000, von Ehrenstein et al. 2000, Ege et al. 2007). This suggests that having pets might have a similar protective effect against IgE sensitization. However, controversial data exists, with some studies reporting a protective effect (Ownby et al. 2002, Benn et al. 2004) and other studies finding no effect at all (Remes et al. 2001, Campo et al. 2006).

The relationship between exposure to pets and allergic disease risk may vary with several factors, including the timing of the exposure and the number of pets. Indeed, the first year of life seems to be the critical period (Wegienka et al. 2011), and a higher number of pets seems to provide stronger protection (Ownby et al. 2002, Mandhane et al 2009).

Furthermore, while it seems that pet exposure may contribute to a lower risk of overall IgE sensitization at six to seven years of age (Ownby et al. 2002), this overall protective effect may not persist to age 18-20 (Wegienka et al. 2011). Yet, exposure to a cat or dog during the first year of life may be associated with a lower specific sensitization to these animals (Hesselmar et al. 1999, Wegienka et al. 2011).

There may also be confounding factors involved, such as higher endotoxin levels in homes with more pets (Campo et al. 2006). Another possible confounding factor is an avoidance of pets in families with known allergic conditions. Although allergic rhinitis has been associated with pet avoidance (Bertelsen et al. 2010), there are also controversial results that report no evidence of pet avoidance in allergic families (Mandhane et al 2009).

6.3.2 Infections and allergic sensitization

Certain infections have been linked to a lower prevalence of allergic diseases. The next few chapters provide an overview of some candidate microbes that have been suggested to protect against allergic diseases.

6.3.2.1 Viral infections and allergic sensitization

6.3.2.1.1 Measles

In 1996, young African adults who had been infected with the measles virus during an epidemic in 1979 were reported to have a markedly reduced prevalence (about half) of atopy, as defined by positive SPTs (Shaheen et al. 1996). Nevertheless, a substantial loss in follow-up, mainly due to a high mortality rate, invalidates the interpretation of the results of this study (Soothill JF 1996). Furthermore, a subsequent study of a cohort of younger children from the same community that had been struck by a measles epidemic in 1991 did not find a similar effect (Aaby et al. 2000). In the UK, a protective effect was seen for asthma, but not for hay fever or eczema (Bodner et al. 1998). A later report from the same group showed that childhood infections, including measles, did not protect from atopy in adulthood (Bodner et al. 2000). However, again in the UK, atopy – as defined by positive SPTs – was less common in a small number of adults who had a record of measles by the age of three (Cullinan et al. 2003). Yet another report from the UK found a reduced prevalence of hay fever among those who had previously had a measles infection, but there were confounding factors present, with birth order as the most significant confounder. In this report, the protective effect of a measles infection was enhanced by a growing number of older siblings (Lewis and Britton 1998). In Switzerland, an inverse association was found between the prevalence of asthma and a positive history of either a natural measles infection or vaccination against measles. In this

report, the protection was stronger with natural infection than with vaccination, but neither infection nor vaccination had any effect on the risk of atopy (Roost 2004).

In Sweden, pupils of anthroposophic (Steiner) schools were shown to have less IgE sensitization than their peers in control schools, but a measles infection did not significantly associate with IgE sensitization as defined by positive SPTs (Alm et al. 1999). However, in a larger multicentre study among Steiner schoolchildren, measles infection was associated with a lower risk of atopic eczema symptoms combined with IgE sensitization, but not with other allergic outcomes (Föistrup H et al. 2006).

No association between measles infection and allergic diseases was reported in an epidemiological study among Italian male cadets (Matricardi et al. 2000), whereas measles history was found to be a protective factor for mite sensitization in Turkish school children (Kuyucu S et al 2004).

However, a contradictory result was reported from a large Finnish study that found a substantially increased prevalence of allergic diseases among those who had had measles (Paunio et al 2000). It has been proposed that a differential misclassification of exposure could explain the reported positive association in this study, namely that an underdiagnosis of measles in nonallergic children and an overdiagnosis of measles in allergic children cannot be ruled out (Remes S et al 2000). Also, a Danish study found a positive association between measles infection in the first year of life and IgE sensitization as defined by allergen-specific IgE (Bager et al. 2002). Furthermore, a positive association was found between measles and 'any atopic disorder' (asthma, hay fever, eczema, or food allergy) in the Netherlands, but this was observed only among the MMR-vaccinated group. The authors discussed that 'this association is not necessarily causal, as it is not unlikely that children who fail to seroconvert have a higher risk of atopic disease' (Bernsen and van der Wouden 2008). Taken together, there are some reports suggesting a protective effect of measles infection against allergic diseases, but also controversial results exist. The studies that have

investigated this issue are largely cross-sectional or retrospective, which means that observed relationships may not be causal.

6.3.2.1.2 Rubella and mumps

Many studies that have investigated the relationships between measles infection and allergic diseases have also focused on the associations between rubella or mumps infections and allergies. In the UK, a slight increase in the risk of eczema was associated with having had rubella, but no association was observed with other allergic conditions. No association was found between mumps and allergies. Yet, a significant trend towards a greater risk of eczema and hay fever with increasing exposure to rubella, mumps, and varicella was observed (Bodner et al. 1998). Studies since then have not, however, found any relationship between rubella or mumps infection and the risk of allergy (Matricardi et al. 2000, Bodner et al. 2000, Bager et al. 2002). In Switzerland, mumps infection was associated with a decreased IgE sensitization, but not with hay fever or asthma, while rubella infection was not associated with any of these conditions (Roost et al. 2004). On the other hand, rubella infection was associated with lower prevalence of eczema and food allergy in Netherlands, where no association could be found between mumps infection and allergic diseases (Bernsen and van der Wouden 2008). Furthermore, a multicentre study from Austria, France, Germany, and Switzerland investigating relationships between cord blood IgE and maternal health conditions found an inverse association of cord blood IgE to allergens with maternal antibodies for the rubella virus. However, the presence of rubella antibodies in this report might not only have reflected natural immunity, but also previous vaccination (Ege et al. 2008).

6.3.2.1.3 Hepatitis A virus

Strong evidence to support the hygiene hypothesis came from a report showing that IgE sensitization and allergic rhinitis were less common among military students who had antibodies

against the hepatitis A virus (HAV) (Matricardi et al. 1997). Two years later, again by the same group, an inverse association between atopy and HAV seropositivity was observed also in San Marino (Matricardi et al. 1999). Again, a large study among US residents showed that HAV seropositivity was inversely related to asthma, hay fever, and IgE sensitization (Matricardi et al. 2002). Also, a Danish study found a lower prevalence of IgE sensitization and allergic rhinitis among those who were seropositive for HAV (Linneberg et al. 2003). In rural Spain, the presence of HAV antibodies was associated with a lower prevalence of IgE sensitization, but this association lost its statistical significance when adjusted for the confounding effect of age (Gonzalez-Quintela et al. 2005). However, contradictory results also exist: evidence coming largely from the UK, where no association between hepatitis A virus seropositivity and IgE sensitization could be found (Bodner et al. 2000). Three years later, another study in the UK showed no relation between HAV and atopy either (Cullinan et al. 2003), and again, a year later, a third UK study reported that no association existed between HAV antibodies and wheeze, hay fever, or IgE sensitization (Jarvis et al. 2004). In Ethiopia, the risk of wheeze was reduced by hookworm infection, but was not affected by HAV antibodies (Scrivener et al. 2001). In addition, exposure to HAV was not associated with protection against IgE sensitization in either Finland or Russian Karelia (von Hertzen et al 2006, von Hertzen et al. 2007). Furthermore, in a multicentre study including subjects from Iceland, Estonia and Sweden, HAV serology was not associated with IgE sensitization, although orofaecal-foodborne infections had a protective effect as a group (Janson et al. 2007). Recent reports from Brazil (Veiga et al. 2011) and Greece (Michos et al. 2011) showed no relationship between IgE sensitization and HAV serology.

Taken together, although many studies have reported an inverse relation between the hepatitis A virus and allergies, there are also contradictory results. Interestingly, genetic studies have shown that the HAV receptor TIM-1 is encoded by an important atopy susceptibility gene

family (*Tim*), which may explain these observed inverse relationships between HAV infection and the development of atopy (McIntire et al 2001).

6.3.2.1.4 Human herpes viruses

The Epstein-Barr virus (EBV) was first suspected to cause immunological changes resulting in the development of atopy, since increased antibody levels against EBV were observed in adults with a history of atopic dermatitis (Rystedt et al. 1984). In this study, no association was observed between atopic dermatitis and antibodies to the varicella-zoster virus (VZV) or herpes simplex virus (HSV) (Rystedt et al. 1984). However, Calvani et al. reported higher total IgE levels among EBV seronegative young children, whereas a similar association could not be found among older children (1997). This result suggested that EBV infection in early childhood could protect against the development of allergic diseases. On the other hand, the history of having had varicella (VZV) was associated with an increased risk of asthma, while no significant association was evident between VZV and hay fever or eczema in the UK (Bodner et al. 1998). No association was observed between atopy and seropositivity to the cytomegalovirus (CMV), HSV or VZV in Italy (Matricardi et al. 2000). Another study from Bodner et al. showed that VZV infection in childhood did not protect from atopy in adulthood (Bodner et al. 2000). On the other hand, hay fever was inversely associated with positive serology for HSV-1, but not for HSV-2 in the USA (Matricardi et al. 2002). A report from Denmark showed no association between VZV and the risk of atopy (Bager et al. 2002).

Another study investigating the association between EBV and allergy found no relationship between EBV and asthma or suspected allergic rhinitis: in this Swedish study, blood samples for serologic assays were drawn from 2561 children who were approximately four years (Sidorchuk et al. 2003). These children were further investigated by the same group, who tested for IgG antibodies to CMV and IgE antibodies to common airborne and food allergens. There were no

significant associations between CMV seropositivity and allergic outcomes, but CMV seropositivity in the absence of EBV antibodies was related to IgE antibodies to airborne and food allergens. An antagonism between these viruses to IgE sensitization was therefore suggested (Sidorchuk et al. 2004). Nevertheless, another Swedish study found an inverse relation between IgE sensitization and EBV seropositivity at two years of age, but, in this study, combined EBV and CMV seropositivity had even stronger protective effect. Other viruses included in this study had no effect on the risk of IgE sensitization. These other viruses included adenovirus, influenza virus, parainfluenza virus, CMV, HSV, human herpes virus 6 (HHV6), and VZV (Nilsson et al. 2005). In a multicentre study involving subjects from Iceland, Estonia, and Sweden, HSV-1 and CMV were more prevalent in nonatopic than in atopic subjects, whereas EBV had no effect on atopy (Janson et al. 2007). Similarly, HSV was inversely related to atopy also in Finland, but in Russia no similar association could be found (von Hertzen et al. 2007a).

In the USA, VZV infection during childhood was associated with protection against atopic dermatitis (Silverberg et al. 2010). The following study by the same group showed that VZV infection occurring before the age of eight also decreased the odds of asthma and allergic rhinoconjunctivitis, but not of food allergies. In addition, VZV infection was associated with decreased total IgE levels and IgE sensitization defined by allergen-specific IgE (Silverberg et al. 2012).

Another Swedish study showed that EBV infection before age of two had a protective effect against IgE sensitization, whereas contracting EBV after two years of age had the opposite effect. This result strongly supports the value of early-life microbial exposure for protection against IgE sensitization (Saghafian-Hendengren et al. 2010). Yet another Swedish study showed that seropositivity to HHV6 at 18 months of age was associated with reduced IgE sensitization, but no association with allergic disease was evident. The other herpes viruses included in this study (HSV, VZV, EBV and CMV) protected from IgE sensitization as a group, but low prevalence in

combination with the limited size of subjects did not allow statistical analysis of the relationship between these infections and IgE sensitization. Indeed, the seroprevalence of HHV6 was higher than that of the other herpes viruses in this study (Nordström et al. 2010). A Greek study found no relationship between IgE sensitization and the seroprevalence of HSV, CMV, EBV or VZV. However, a higher number of serological infectious markers (the aforementioned herpes viruses and HAV, *T. gondii*, *H. pylori*, RSV, *Mycplasma pneumopniae*, and *Bartonella henselae*) was associated with a higher odds ratio for IgE sensitization also in this study (Michos et al. 2011). In addition, seropositivity to HSV and EBV was inversely related to positive SPT results, whereas no relationship was observed between seropositivity to VZV and SPT results in Brazil (Alcantara-Neves et al. 2012).

Taken together, it seems that EBV infection occurring early in childhood may have a protective effect against IgE sensitization. Similarly, VZV infection in childhood may offer some protection. In addition, some reports indicate that HSV (mainly HSV-1) and CMV could also be protective. Although less investigated, HHV6 infection in early childhood has also been associated with a decreased risk of IgE sensitization.

6.3.2.2 Other infections and allergic sensitization

In addition to the viruses transmitted through the faecal-oral route (e.g. HAV), certain other microbes such as *Helicobacter pylori* and *Toxoplasma gondii* can be considered markers of hygiene level and infectious pressure. Matricardi and colleagues (2000) compared seroprevalence of foodborne and orofaecal microbes in atopic cases and non-atopic controls in Italy and found a lower prevalence of *T. gondii* and *H. pylori* antibodies in atopic participants. Different results were reported from the UK, where these microbes were not associated with atopy (Bodner et al. 2000). Thereafter, a number of studies have investigated whether these microbes have a protective effect against allergic diseases. A report from US showed an inverse association between *T. gondii* and

hay fever whereas *T. gondii* was not related to asthma or IgE sensitization (Matricardi et al. 2002). In Finland, an increase of IgE sensitization from 1973 to 1994 had occurred in the subgroup with no antibodies to *H. pylori*, but not among those who were seropositive for *H. pylori* indicating that *H. pylori* prevalence may indeed relate to the allergy prevalence (Kosunen et al. 2002). Nevertheless, in a Danish study showing an inverse relation between HAV and IgE sensitization, no such relation could be found between IgE sensitization and *H. pylori* or *T. gondii*. Yet, seropositivity to 2 or 3 of these microbes was associated with a lower prevalence of IgE sensitization in this study, prevalence declining from 45% to 21% (Linneberg et al. 2003). Somewhat similar results were observed in UK, where the sibling effect for atopy was unexplained by *H. pylori* infection, but record of gastrointestinal infections as a group was associated with lower prevalence of atopy (Cullinan et al. 2003). In a rural environment in Germany, neither *H. pylori* nor *T. gondii* were associated with atopy (Radon et al. 2004); another report from the UK showed no evidence that infection with HAV or *H. pylori* was related to IgE sensitization, except for an isolated finding of a negative association of *H. pylori* infection with sensitization to grass (Jarvis et al. 2004). Then again, a Finnish study found that the SPT positivity rate was lower in subjects with *H. pylori* antibodies, both among asthmatic and controls (Pessi et al. 2005). In a multicentre study including subjects from Sweden, Estonia and Iceland, *Toxoplasma* antibodies were not associated with IgE sensitization or lung function (Birgisdóttir et al. 2006), whereas a subsequent study using the same material found an inverse relation between H. pylori and atopy (Janson et al. 2007). Another study that investigated infections and allergy prevalence in Finland and in Russian Karelia found an inverse relation between *H. pylori* antibodies and SPT positivity in Russia but not in Finland, while *T. gondii* did not have an independent effect (von Hertzen et al. 2006). The following study comprising younger subjects from the same areas did not find relationships between these microbes and atopy, although the sample size in Russian Karelia was about the same (von Hertzen et al. 2007a). Nevertheless, another German study found an inverse association between eczema and *H. pylori* (Herbath et al.

2007), whereas *T. gondii* was found to be less frequent among atopic Norwegian military recruits compared to their nonatopic peers (Ellertsen et al. 2008).

Another large study from the USA found that *H. pylori* was associated with reduced risks of allergy and asthma, association being stronger with the *cagA+* strains of *H. pylori* (Chen and Blaser 2007), whereas another report from the same group showed that seropositivity to *cagA+ H. pylori* was inversely associated with asthma, but not with allergen-specific IgE (Reibman et al. 2008). These results suggest that infections with different strains of *H. pylori* might lead to different types of pathogen-host interaction, presence or absence of the *CagA* gene representing an important biologic and clinical dichotomy (Blaser 2005). Yet another report from the same group showed an inverse association between *H. pylori* and onset of childhood asthma before five years of age. In addition, an inverse association between *H. pylori* and allergic rhinitis was observed, whereas *T. gondii* was not associated with asthma or atopic conditions (Chen and Blaser 2008).

Furthermore, a multicentre study from Austria, France, Germany, and Switzerland investigating relationships between cord blood IgE and maternal health conditions found an inverse association of cord blood IgE to allergens with maternal antibodies to *T. gondii*. However, as maternal exposure to cats already in infancy was the strongest predictor for a positive *T. gondii* record, in most *T. gondii* positive mothers, the infection might have occurred early in their lives (Ege et al. 2008).

Again in the UK, Fullerton et al. (2009) found no association between *H. pylori* serological status and SPT positivity, asthma, or hay fever, whereas in Brazil seropositivity to *T. gondii* was inversely related to positive SPTs and specific IgE against aeroallergens. No association was reported between *H. pylori* and IgE sensitization in this study (Alcantara-Neves et al. 2011).

Thus, although many studies have shown an inverse relationship between allergic conditions and *T. gondii* or *H. pylori*, there have also been many studies that found no such

relationship. Yet, despite the vast number of studies, no report of a controversial positive association has been reported.

The association of *Bordetella pertussis* with allergic diseases has been investigated in several studies. A slight increase in IgE sensitization was associated with pertussis infection in a German study (Wjst et al. 1994). Another report showed that having had pertussis infection later than the age of three was associated with an increased risk of atopic eczema. However, no association was observed with other atopic diseases or if the infection was acquired before age of three (Bodner et al. 1998). In addition, a positive association with pertussis infection and asthma has been reported (Strachan et al. 1996). In the Netherlands, reported allergic diseases (asthma, hay fever and food allergy) were associated with an increased prevalence of reported pertussis infection in the pertussis-vaccinated group, but not among 622 unvaccinated subjects. The authors suggested that this positive association in pertussis-vaccinated children could be either interpreted as a synergistic effect of vaccination and infection or as due to reverse causation or diagnostic bias (Bernsen et al. 2007). However, it is unlikely that a higher infection prevalence due to a weaker response to pertussis vaccine in atopic children would explain this positive association, since pertussis antibody levels after vaccination are similar in atopic and non-atopic subjects (Blanco-Quiros et al. 2005). So it seems that there may be a positive relationship between pertussis infection and atopic conditions; this kind of positive association would not support the hygiene hypothesis. Nonetheless, the association between pertussis infection and atopy warrants further investigation.

Similarly, *Mycoplasma pneumoniae* appears to be associated with exacerbation of asthma, but the role of *M. pneumoniae* in the initial onset of asthma or IgE sensitization remains unclear (Hong 2012). However, a Greek study investigating the association of IgE sensitization with several infectious diseases found a positive association between IgE sensitization and seropositivity to *M. pneumoniae*, while no association was seen with other microbes (Michos et al 2011).

In a multicentre study including subjects from Sweden, Estonia and Iceland, another microbe causing respiratory tract infections, *Chlamydia pneumoniae*, was inversely associated with atopy (Janson et al. 2007).

A large study from East Germany reported an inverse association between parental reports of a prior worm infestation and atopic eczema. Also IgE sensitization to aeroallergens was inversely related to the history of worm infestation in this survey (Schäfer et al. 2005). Furthermore, a meta-analysis by Feary and colleagues (2010) found a consistent protective effect for current *Ascaris lumbricoides* infection against a positive skin prick test result. Similarly, *Tricuris trichuria* and Schistosomiasis were associated with a decreased prevalence of positive SPTs, while the protective effect of hookworm did not reach statistical significance. In addition, a reduction in the risk of atopy in individuals with any current intestinal parasite infection was seen, while no individual effect was seen with *Enterobius vermicularis*, *Giardia intestinalis*, or *Blastocystis hominis* (Feary et al. 2010).

Since this meta-analysis, similar results have been reported from Brazil, where the presence of *Ascaris lumbricoides* in faecal samples was inversely related to positive SPTs; no association was reported between IgE sensitization and *Trichuris trichiura*l (Alcantara-Neves et al. 2012).

Although it seems that helminth infections are associated with a decreased IgE sensitization, no increase in the prevalence of atopy was seen due to a deworming programme using albendazole treatment in rural Ecuador (Cooper et al. 2006). On the other hand, skin-test reactivity and serum levels of specific IgE antibody against environmental allergens markedly increased in children who underwent anthelmintic treatment in Venezuela (Lynch et al. 1993). Similarly, anthelminthic treatment of chronically infected children resulted in increased atopic reactivity also in Gabon (van der Biggelaar et al. 2004).

Reported gastrointestinal infections before the age of five years were associated with reduced IgE sensitization (defined by SPT) in adulthood in a UK study (Cullinan et al. 2003). In Denmark, on the other hand, seropositivity to two or three intestinal bacterial pathogens (*Clostridium difficile*, *Campylobacter jejuni* and *Yersinia enterocolitica*) was associated with a higher prevalence of IgE sensitization defined by specific IgE (Linneberg et al. 2003). In addition, it seems that gut microbiota plays a role in IgE sensitization. Nevertheless, at the moment it remains unclear whether there are specific microbes involved that are particularly protective or harmful (Penders et al. 2007).

6.3.3 Allergy and autoimmune diseases

Over past decades, not only allergic diseases but also autoimmune diseases have become more frequent in developed countries, and this phenomenon has been linked to a decreased prevalence of infectious diseases. The hygiene hypothesis suggests that infections play an important role in the maturation of the immune responses and regulate the risk of allergic and autoimmune diseases (Bach 2002, Bach 2005). Indeed, epidemiological studies have indicated that increased infectious pressure is related to a decreased risk of not only allergies (Strachan 1989), but also type 1 diabetes (Patterson et al. 1996) and multiple sclerosis (Leibowitz et al. 1966).

Since these organ-specific autoimmune diseases have been related to Th1-type immune response and allergic diseases to Th2-type immune response, and since these two types of immune responses are reciprocal to each other, a number of studies have tried to find an inverse relationship between allergic conditions and autoimmune diseases. Especially the relationship between allergies and type 1 diabetes has been under investigation. A meta-analysis in 2003 suggested that there is a small reduction in the prevalence of asthma among children with type 1 diabetes, whereas no clear relationship was seen between other atopic diseases and type 1 diabetes (Cardwell et al 2003).

6.3.4 Immunological mechanisms behind the hygiene hypothesis

6.3.4.1 Th1 – Th2 paradigm

T helper (Th) cells are key players in different immune responses. These cells can be divided into different subtypes according their different cytokine products. The characteristic cytokine products of Th1 (interferon-γ, interleukin[IL]-2) and Th2 (IL-4, IL-5) cells are inhibitory towards the differentation and effector functions of the reciprocal cell type. In other words, when an immune reaction involving Th cells (e.g. Th1) is triggered (e.g. by an infection), this immune response should down-regulate the action of other Th cells (e.g. Th2). For more than one and a half decades after their discovery, these two cell types dominated the immunological field. This has led to an immunological dichotomy in which organ-specific autoimmune diseases have been related to Th1-type immune responses and allergic diseases to Th2-type immune responses. This dichotomy is often referred to as the Th1 – Th2 paradigm (Zhu et al. 2010).

This paradigm fits in well with the hygiene hypothesis, and has in fact been its immunological core for many years. The idea itself is simple: when an infection causes an immune response of one Th-type, the reciprocal Th-type is inhibited. Hence, a stimulation of Th1 cells would lead to decreased allergic diseases and stimulation of Th2 cells to decreased organ-specific autoimmunity. Although autoimmune diseases and allergies may well be prevented by infections, some epidemiological findings do not favour the Th1 – Th2 paradigm. Namely studies that have tried to find an inverse association between autoimmune diseases and allergy have not been able to define a relationship between these conditions (Cardwell et al. 2003).

Since 2003, the discovery of IL-17 producing Th cells, i.e. Th 17 cells, and increasing knowledge of their function has shown the Th1 – Th2 paradigm to be a rough simplification of a much more complex immune system (Zhu et al. 2010). There seems also to be plasticity in different

response types, and Th1, Th2 and Th17 cells have complex interactions that modulate their activity (Zhu and Paul 2010).

6.3.4.2 Competition

Immune responses to single antigens are usually stronger than the response to antigens that are administered simultaneously with other antigens. Therefore, it is possible that strong immune responses to infectious agents compete with immune responses against weaker antigens such as allergens for homeostatic signals that are mandatory for lymfocyte proliferation and survival. These homeostatic signals include cytokines such as IL-7 and self-peptide MHC recognition (Theofilopoulos et al. 2001).

6.3.4.3 Regulation

It is currently accepted that the suppressive immunoregulatory effect induced by a defined antigen may extend to immune responses specific to other antigens (bystander suppression). Therefore, regulatory cells stimulated by infectious agents may also dampen autoimmune or allergic responses (Bach 2005). Indeed, T regs are capable of actively blocking immune responses, inflammation, and tissue destruction by suppressing the functions of multiple cell types, including CD4+ Th cells, B-cell antibody production and affinity maturation, CD8+ cytotoxic T-lymphocyte granule release, and also antigen presenting cell function and maturation state (Brusko et al. 2008).

6.3.4.4 Innate immunity

Innate immune cells express surface and intracellular pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) that recognise specific patterns of microbial components. In turn, these receptors regulate the activation of both innate and adaptive immunity (Takeda et al 2003). As an illustration, a TLR4-dependent suppression of allergic inflammation has been described in mice

(Nembrini et al 2011). Thus, microbial stimulation of the innate immune system may result in the suppression of adaptive immune responses.

Prenatal farm exposure has been related to enhanced gene expression of innate immunity receptors such as TLR2, TLR4 and CD14 and decreased IgE sensitization in school-age children (Ege et al. 2006). Furthermore, maternal contact with farm animals and an elevated expression of TLRs at birth has been linked with decreased atopic dermatitis (Roduit et al. 2011).

6.3.4.5 Epigenetics

Epigenetic mechanisms are known to regulate gene expression or cellular phenotype without altering the nucleotide sequence. These mechanisms include posttranslational modifications of histones, DNA methylation and the expression of noncoding RNAs (Kabesh 2014). Environmental factors such as tobacco smoking may have such epigenetic effects (Lutz et al 2011). Moreover, these epigenetic changes may even be heritable (Rehan et al 2012). Although the allergy epidemic is often considered to be too dramatic to be explained by genetic variation (altered nucleotide sequences), these epigenetic mechanisms provide means for rapid environmental effects on gene expression in a relatively short time period. As an illustration, should this altered gene expression involve inflammatory genes by shutting them down, the result would be decreased inflammatory responses. In addition, the expression of regulatory genes may be affected. Therefore, it has been speculated that prenatal exposure to a farm environment could alter methylation processes and gene expression *in utero* and exert its effect on the offspring (Ege et al. 2006).

7. AIMS

The aims of the study were to investigate the prevalence of IgE-mediated allergic sensitization and infections in Finland and in Russian Karelia. In addition, the study aimed to investigate the association between type 1 diabetes and IgE sensitization.

The study was carried out in two populations – i.e. Finland and Russian Karelia – that live close to each other and share similar genetic backgrounds, but are characterised by a conspicuous difference, for example, in their level of affluence and in their childhood microbial exposure.

The main aims of the study were:

1. To investigate the possible difference in the prevalence of IgE sensitization in Russian Karelia and Finland, and in addition, to investigate the association between infectious diseases and IgE sensitization (Substudy I).

2. To evaluate the possible time changes in the prevalence of IgE sensitization and evaluate whether these changes are related to changes in microbial exposure (Substudy II).

3. To investigate whether allergic and autoimmune diseases, namely type 1 diabetes, co-occur less or more than expected, and whether this co-occurrence is affected by microbial exposure (Substudy III).

4. To study the relationship between enterovirus infections and IgE sensitization in more detail (Substudy IV).

8. MATERIALS AND METHODS

8.1 Subjects

8.1.1 Substudy I

In Substudy I, the prevalence of IgE-mediated allergic sensitization and its association with infections was studied among schoolchildren in the Oulu region of Finland and the Karelian Republic in Russia. The cohorts were recruited as a part of the type 1 diabetes-related EPIVIR project (the EU INCO-Copernicus programme; contract number IC15-CT98-0316, Coordinator Prof. Hyöty). The initial Finnish cohort comprised a total of 3654 subjects; the Karelian cohort 2070 subjects. All 266 Karelian children whose parents were both of either Finnish or Karelian ethnicity were included in the analyses. For these Karelian children, a cohort of 266 Finnish children was matched pairwise by age, gender, and the date of the sample (no more than one month apart; different year). The study cohorts included a total of 114 boys and 152 girls from each country: the mean age at sampling was 11.4 years (range: 7-15 years) in both cohorts. Blood samples were taken during the months March, April and May in 1997-1999 in Karelia and in 1994 in Finland.

8.1.2 Substudy II

For Substudy II, coded (anonymous) serum samples were obtained from the Finnish Maternity Cohort (FMC) serum bank of the National Institute for Health and Welfare (THL) in Finland. Since 1983 in Finland, practically all (>98%) pregnant women have been screened for congenital infections (such as HIV, syphilis and hepatitis B) in a process organised by THL. The purpose of this screening is to find and prevent possible infectious diseases that would cause a threat to the health and life of the unborn child. Serum samples for screening are drawn from women at the maternity clinics during the first trimester of pregnancy (10-12 weeks of gestation). These samples

are stored (at -25°C) at the FMC serum bank. By the end of 2001, the FMC serum bank contained about 1,350,000 samples from 683,000 women.

A total of 958 randomly selected blood samples from the years 1983 (N=232), 1989 (N=240), 1995 (N=243) and 2001 (N=243) from Finnish pregnant women aged 17-42 (median 26), 16-42 (median 28), 16-43 (median 29) and 16-49 (median 29) respectively were included in the testing performed for Substudy III. The age distributions between these series were not equal, so the series were further divided into separate groups representing ages of less than 25, 25-30 and over 30, in which the medians for ages were 22, 27 and 33 respectively. In addition, testing was done in birth cohorts including women born in 1941-1949 (N=34), 1950-1954 (N=81), 1955-1959 (N=163), 1960-1964 (N=254), 1965-1969 (N=198), 1970-1974 (N=122), 1975-1979 (N=71) and 1980-1985 (N=35).

The study protocol for Substudy II was approved by THL ethical committee.

8.1.3 Substudy III

In Substudy III, the association between type 1 diabetes and IgE-mediated allergic sensitization was investigated among diabetic children and healthy controls in Finland and in Russian Karelia.

The 266 Finnish schoolchildren in Substudy I were included as a non-diabetic control group. For these controls, we were able to identify 147 Finnish diabetic patients matched for age, gender and season of sampling (no more than two months apart; year of blood sampling may differ). These diabetic patients were identified from the outpatient diabetes clinic of the University of Oulu's Department of Paediatrics: all of them were on daily insulin treatment, and all of them were included in the testing. Furthermore, 132 patients diagnosed with type 1 diabetes were identified from the records of the Republic Hospital in Petrozavodsk, Russian Karelia. The present study included all 132 patients diagnosed with type 1 diabetes in this hospital from 1990 to 1999. For these Russian Karelian diabetic patients, we were able to find 112 control children matched for

age, gender, and season of blood sampling, from the initial cohort of 2070 Russian Karelian schoolchildren. Unlike the Russian Karelian children, no data were available on allergic symptoms in the Finnish children.

8.1.4 Substudy IV

The study population for Substudy IV was also conducted from the Russian Karelian EPIVIR cohort and included subjects from Substudies I and III. Overall, this study cohort comprised 250 subjects, including 60 children, who had tested positive for allergen-specific IgE in Substudies I and III and 190 randomly selected IgE-negative schoolchildren representing the same age and gender distribution (mean age 12 years and age range 8-15 years; 53% males). Twelve of the IgE-positive children had type 1 diabetes and all IgE negative children were non-diabetic, yet diabetes was not a criterion for inclusion or exclusion.

All of the children had written parental consent to participate in the study. The study plan for Substudies I, II, and IV was approved by the ethical committee of the Faculty of Medicine at the University of Oulu in Finland and by the Ministry of Health in the Karelian Republic of the Russian Federation. The reported investigations were carried out in accordance with the principles of the Declaration of Helsinki.

8.2 Methods

8.2.1 Measurement of microbial antibodies

For Substudies I and III, IgG-class HAV antibodies were measured using Enzygnost® Anti-HAV commercial EIA kits, IgG-class *H. pylori* antibodies using Enzygnost® Anti-*H. pylori*/IgG assays, and Toxoplasma IgG using Enzygnost® Toxoplasmosis IgG assays according to manufacturer instructions (Dade Behring, Marburg, Germany). Behring Elisa Processor III was used for further processing of the tests and for calculating the antibody levels. For Substudy I, IgG class enterovirus

antibodies were measured using EIA against a highly purified coxsackievirus B4 antigen (CBV4) as previously described (Salminen et al. 2003).

For Substudy II, IgG antibodies against *H. pylori* were measured by an enzyme immunoassay (Pyloriset EIA-G III, Orion Diagnostica, Espoo, Finland) according to the manufacturer's instructions.

For Substudy IV, the presence of neutralizing antibodies against 12 different enterovirus serotypes including coxsackievirus A 4 (CAV4), CAV9, coxsackievirus B1 (CBV1), CBV2, CBV3, CBV4, CBV5, CBV6, echovirus 9 (EV9), EV11, EV26, and EV30 was analysed using the classical plague-neutralization assay as described previously (Roivainen et al. 1998, Viskari et al. 2005). A serum dilution of 1:4 was used to detect low levels of neutralizing antibodies, and the serum was considered antibody-positive if it blocked 80% or more of the virus infectivity. All virus strains were ATCC reference strains except CAV4, CBV3 and EV26, which were wild-type isolates from Finland.

8.2.2 Measurement of IgE antibodies

The levels of total IgE and allergen-specific IgE were measured using the ImmunoCAP® fluoroenzyme immunoassay (Phadia Diagnostics, Uppsala, Sweden). Specific IgE for two common inhalant allergens (birch and cat) and for egg albumin was analysed according to the manufacturer's instructions. For allergen-specific IgE, values of 0.35 kU/l or more were considered positive. Total IgE values of 100 kU/l or more were considered high, as values exceeding 100 kU/l have been considered markers of atopic predisposition in previous studies (Vartiainen et al. 2002).

8.2.3 HLA typing

HLA class II risk alleles (DQA1*05, DQB1*02, DQB1*0301, DQB1*0302, DQB1*0602 and DQB1*0603) were typed by polymerase chain reaction and microtitre well plate-based

hybridization with lanthanide-labelled oligonucleotide probes as previously described (Nejentsev et al. 1999). DQA1*05/DQB1*02 stands for the DR3-DQ2 haplotype, while DQB1*0302 stands for the DR4-DQ8 haplotype. For the analyses, the presence of diabetes-related HLA class II genotypes were categorized as follows: DR3-DQ2/DR4-DQ8, DR3-DQ2/x (x≠DR4-DQ8), DR4-DQ8/y (y≠DR3-DQ2) and z/z (z≠DR3-DQ2 and DR4-DQ8).

8.2.4 Assays for diabetes-associated antibodies

All diabetes-associated antibodies were analysed in the Department of Paediatrics research laboratory at the University of Oulu. Antibodies to glutamic acid decarboxylase (GADA) and to islet antigen 2 (IA-2A) were analysed using specific radiobinding assays (Savola et al. 1998, Savola et al. 1998). The cut-off values for GADA and IA-2A positivity were 5.36 relative units (RU) and 0.43 RU respectively, based on the 99th percentile in more than 370 non-diabetic children and adolescents. According to the 2005 Diabetes Autoantibody Standardization Program (DASP) workshop, the disease sensitivity was 82% and the disease specificity 96% for the GADA assay, while the corresponding figures for the IA-2A assay were 72% and 100%. Insulin antibodies were also measured using a specific radiobinding microassay with a cut-off value of 3.48 RU representing the 99th percentile in 370 non-diabetic children and adolescents (Ronkainen et al. 2001). The disease sensitivity of this assay was 58% and the disease specificity 98% in the 2005 DASP workshop.

8.2.5 Statistical methods

In Substudy I, statistical testing was performed using version 12.0 of the SPSS program (SPSS Inc., Chicago, IL, USA) and CIA (Altman D et al. 2000); SPSS program versions 14.0 and 20.0 were used for statistical analysis in other substudies (II, III, and IV). The 2010 version of Microsoft Excel

(Microsoft inc., Redmond, WA, USA) was used to calculate confidence intervals for proportions in Substudies II, III and IV; the Wilson method was applied in these calculations.

In Substudy I, the prevalence of specific IgE, high values (>100IU/l) of total IgE and microbial antibodies between two paired cohorts was compared using McNemar's test. Comparisons of total IgE levels (a skewly distributed continuous variable) between paired cohorts were performed using the Wilcoxon Signed Ranks test. Cross-tabulation and the Chi-square test or Fisher's exact test were used to examine associations between microbial antibodies, high values of total IgE and specific IgE. The Mann-Whitney U test was used when associations between total IgE levels and specific IgE (classified as positive or negative) were analysed and also in looking for associations between CBV4 IgG levels and specific IgE.

In Substudy II, cross-tabulation and Chi-square tests were used to find associations between classified variables. Kruskal-Wallis and Mann-Whitney tests were used in comparing skewly distributed continuous variables with classified variables. Logistic regression analysis adjusting for the effect of age and year of sampling was used in determining birth year effects, whereas cross-tabulation and the Mantel-Haenzel linear-by-linear association Chi-square test was used to analyse time trends.

In Substudy III, the prevalence of allergen-specific IgE was compared between the groups using cross-tabulation and the Chi-square test or the Fisher exact test, whereas cross-tabulation and the Mantel-Haenzel linear-by-linear association Chi-square test was applied in analysing associations between HAV antibodies and having neither diabetes nor IgE, having either, or having both of these conditions. The Mann-Whitney U test was used to compare antibody levels between various groups, whereas base-10 logarithmic values of these levels were compared using the t test. A linear regression model was applied in the further analysis of insulin antibody levels in a comparison between the two countries.

In Substudy IV, logistic regression was used to identify the independent effects of each virus on IgE sensitization. This model included type 1 diabetes as an interaction term. Testing was also conducted with and without including the diabetic patients.

In all substudies, logistic regression was used to identify the independent effect of each parameter when appropriate. The model selection was based on a forward stepwise procedure in which the limit to enter and to remove the term was equal to 0.10. The results were supported by the assessment of odds ratio (OR) and 95% confidence intervals (CI). If there were missing or indifferent values, cases were not included in the analyses involving those particular parameters. All analyses were two-sided, and statistically significant *P*-values (<0.05) were given.

8.2.6 Ethical aspects

The study plan for Substudies I, III, and IV was approved by the ethical committee of the faculty of Medicine at the University of Oulu in Finland and by the Ministry of Health in the Karelian Republic of the Russian Federation. All the children included in these studies had written parental consent to participate in the study. The study protocol for Substudy II was approved by the National Institute for Health and Welfare (THL) ethical committee. All the reported investigations were carried out in accordance with the principles of the Declaration of Helsinki.

9. RESULTS

Below, the major findings of the present study are given by substudy.

9.1. IgE sensitization and infections in Finland and Russian Karelia (Substudy I)

In Substudy I, the prevalence of IgE sensitization and its relation to microbial antibodies was investigated in Russian Karelian and Finnish schoolchildren. The prevalence of allergen-specific IgE was significantly lower in Russian Karelian children than in Finnish children. On the other hand, the prevalence of all microbial antibodies was significantly higher in the children in Russian Karelia than in the children in Finland (Table 1).

In addition, the presence of *H. pylori* and enterovirus (CBV4) antibodies was inversely related to IgE sensitization in Russian Karelia (Table 3 in original publication I), but not in Finland.

In Russian Karelia, only 1.2% (1/85) of the children who were seropositive for more than two microbes of the four tested had at least one positive specific IgE compared to 9.1% (16/176) of those who were seropositive for fewer microbes (OR: 0.12; 95% CI: 0.016-0.91; *P*=0.015). When logistic regression analysis was used to identify the independent effect of each microbial seropositivity, that of enterovirus (CBV4) had the strongest effect on IgE sensitization. A similar protective trend was also observed for *H. pylori* but not for *T. gondii* and HAV. For example, 22% of the children who were enterovirus seronegative had at least one positive specific IgE result, compared to 5% of seropositive children. The median enterovirus antibody level was 74 EIU (range: 0-224) in children who had no specific IgE, compared to 49 EIU (range: 0-154) in those who had at least one allergen-specific IgE (*P*=0.048).

In Finland, the number of *H. pylori*, *T. gondii* and HAV seropositive children was very low, which made it difficult to analyse their association with allergen-specific IgE. However,

enterovirus antibodies were frequent also in Finnish children. Nevertheless, unlike in Russian Karelia, they showed no association with allergen-specific IgE (18% of the enterovirus seronegative children had at least one positive specific IgE result compared to 23% of the seropositive children, N.S.).

Table 1. Total IgE levels and the prevalence (% and 95% CI) of allergen-specific IgE and microbial antibodies in schoolchildren in Finland and Russian Karelia.

	Finland (n=266)	Russian Karelia (n=266)	*P* value
Cat IgE	11% (8-16%)	2% (1-5%)	<0.001
Birch IgE	11% (8-16%)	2% (1-5%)	<0.001
Egg albumen IgE	6% (4-10%)	3% (2-6%)	0.065
At least one positive IgE	22% (17-27%)	6% (4-10%)	<0.001
Coxsackievirus B4	77% (72-82%)	93% (90-96%)	<0.001
Helicobacter pylori	5% (3-8%)	73% (64-78%)	<0.001
Toxoplasma gondii	5% (3-9%)	24% (19-29%)	<0.001
Hepatitis A virus	2% (1-5%)*	24% (19-29%)	<0.001

*Only 166 Finnish children were screened for HAV antibodies.

9.2. Time trends in IgE sensitization and Helicobacter pylori infections in Finland (Substudy II)

Substudy II was an investigation into the prevalence of *H. pylori* antibodies and of IgE sensitization in pregnant Finnish women. The study material comprised 958 pregnant women in 1983, 1989, 1995 and 2001, which enabled an analysis of time changes in *H. pylori* prevalence and IgE sensitization.

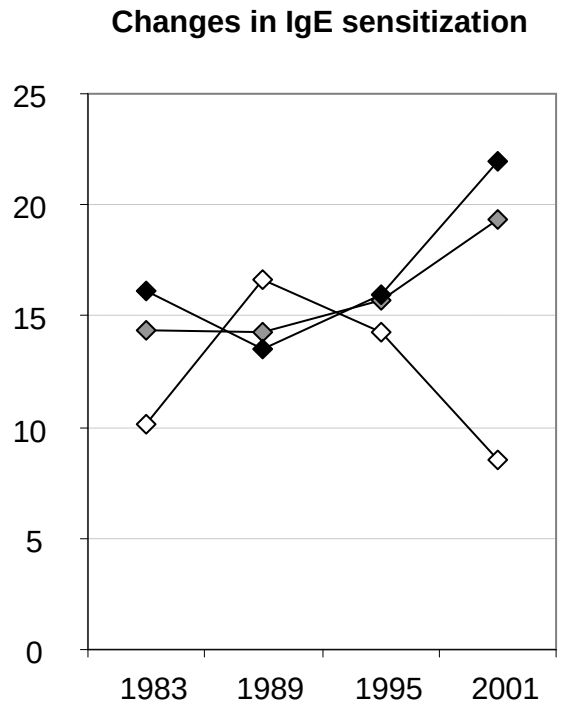
The proportion of the IgE-sensitized women increased during the study period (*P*=0.05). Nevertheless, this increase was quite modest and happened only among women who lacked antibodies against *H. pylori* (Figure 1). Altogether 12.3% of the *H. pylori* positive women had allergen-specific IgE, compared to 17.2% of the *H. pylori* negative women (not significant). However, in the last time series (2001), the *H. pylori* negative women had a significantly higher prevalence of specific IgE than the *H. pylori* positive women (8.5 vs. 21.9%, *P*=0.036). Women in the earlier birth cohorts had a clearly lower prevalence of IgE sensitization compared with women who were born more recently. Furthermore, birth year had an effect on IgE sensitization (*P*=0.001, OR for the effect of one year to the increase = 1.037; 95% CI 1.014-1.060). The logistic regression model was applied in this analysis, adjusting for the effects of age, *H. pylori*, and year of sampling. The effect of birth year on prevalence of specific IgE was evident among the *H. pylori* negative women, but not among the *H. pylori* positive women (Figure 2).

Older age groups had a lower prevalence of specific IgE (>0.35 kU/l): 11.4% of women aged over 30 years had specific IgE, compared to 15.8% of women aged 25-30 and 21.7% of women younger than 25 (*P*=0.001 for trend). This age-dependent effect was strongest in the first time series, which was collected in 1983: 7.4% of women aged over 30 had specific IgE, compared to 10.4% of those aged 25-30 and 23.1% of those younger than 25 (*P*=0.008 for trend). Other time series showed also similar kinds of age trends not being statistically significant. The highest

prevalence (26.2%) of allergen-specific IgE was observed among the youngest age group within the year 2001 cohort.

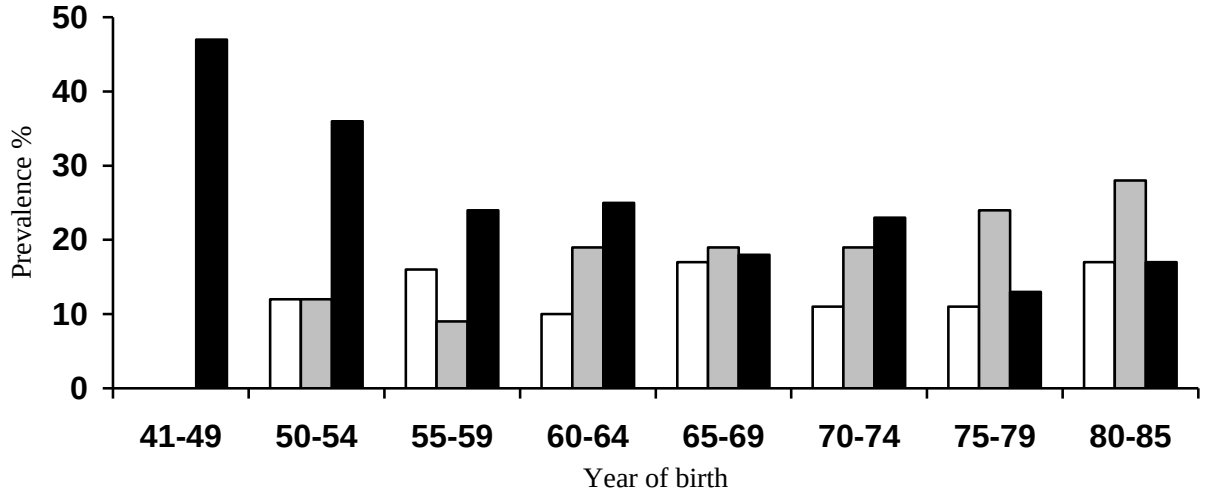
A clear decrease occurred in the prevalence of IgG antibodies against *H. pylori* between 1983 and 2001, the prevalence being 31% in 1983, 21% in 1989, 24% in 1995, and 19% in 2001 (P=0.003 for trend, OR for the increase by time series = 0.96; 95% CI 0.94-0.99). Analogously, women who were born more recently had fewer *H. pylori* antibodies (Figure 2). The *H. pylori* prevalence among women born between 1941 and 1955 was 39.1% (45/115), whereas among women born between 1975 and 1985 it was 14.6% (15/103; $P<0.001$; Figure 2).

Figure 1. Time trends in the prevalence of IgE sensitization in pregnant Finnish women in relation to the presence of *H. pylori* antibodies. Grey = all (*P*=0.05); white = *H. pylori* positive women (*P*=0.83); black = *H. pylori* negative women (*P*=0.04). Below the figure is the corresponding prevalence for each time point (and 95% CI for given prevalence) presented in table form.



Year	**1983**	**1989**	**1995**	**2001**
All women (grey symbols)				
IgE prevalence	14%	14%	16%	19%
95% CI	10%-20%	10%-20%	12%-21%	15%-25%
H. pylori positive women (white symbols)				
IgE prevalence	10%	17%	14%	9%
95% CI	5%-20%	8%-31%	7%-26%	3%-20%
H. pylori negative women (black symbols)				
IgE prevalence	16%	14%	16%	22%
95% CI	11%-23%	9%-20%	12%-23%	17%-28%

Figure 2. Prevalence (%) of allergen-specific IgE antibodies in relation to *H. pylori* antibodies among pregnant Finnish women according to the year of birth. White bars = *H. pylori* positive women (not significant); grey bars = *H. pylori* negative women (P=0.001, OR for the effect of one year to the increase = 1.041; 95% CI 1.016-1.068). Black bars represent *H. pylori* prevalence (P<0.001, OR for the effect of one year to the increase = 0.957; 95% CI 0.939-0.976; adjusted for the effects of age and year of sampling). Below the bar chart is the prevalence for each bar (and 95% CI for given prevalence) presented in table form.



Year of birth	**41-49**	**50-54**	**55-59**	**60-64**	**65-69**	**70-74**	**75-79**	**80-85**
H. pylori positive women (white bars)								
IgE prevalence	0%	12%	16%	10%	17%	11%	11%	17%
	0%	4%	8%	5%	7%	4%	2%	3%
95% CI	-24%	-30%	-31%	-21%	-34%	-28%	-44%	-56%
H. pylori negative women (grey bars)								
IgE prevalence	0%	12%	9%	19%	19%	19%	24%	28%
	0%	6%	5%	14%	13%	13%	15%	15%
95% CI	-20%	-24%	-16%	-26%	-26%	-29%	-36%	-46%
H. pylori prevalence (black bars)	47%	36%	24%	25%	18%	23%	13%	17%
	30%	16%	18%	20%	13%	16%	7%	8%
95% CI	-64%	-47%	-31%	-31%	-24%	-31%	-23%	-33%

9.3 Co-occurrence of IgE sensitization and type 1 diabetes (Substudy III)

Allergic sensitization was more common in Finnish children than in Karelian children, both among patients with type 1 diabetes and control subjects (25% of all Finnish children but only 10% of all Russian Karelian children had allergen-specific IgE against at least one of the three allergens; $P<0.001$). Among the Finnish children, allergic sensitization was equally frequent in diabetic and control children, the prevalence being 25% and 22% respectively. In contrast to Finnish children, the prevalence of allergen-specific IgE was conspicuously higher in Karelian patients with type 1 diabetes than in Karelian control subjects (Tables 2 and 3). Furthermore, Karelian children with type 1 diabetes also reported more allergic symptoms than control children (Table 2). Most frequently reported allergic symptoms were allergic eczema and asthma. The mean total IgE levels or the prevalence of high IgE levels (≥100 kU/l) did not differ between patients and control subjects in either of the countries.

Only 2% (1/59) of HAV-seropositive subjects had both diabetes and allergen-specific IgE, while 44% (26/59) had either diabetes or allergen-specific IgE and 54% (32/59) had neither of these conditions. Among HAV-seronegative subjects, the corresponding figures were 11% (19/181), 49% (89/181) and 40% (73/181) respectively ($P=0.016$ for trend).

There was also an inverse association between allergen-specific IgE and HAV antibodies, as 4% (1/25) of the IgE-positive children compared with 27% (58/215) of the IgE-negative children were HAV seropositive ($P=0.012$; OR=0.11, 95% CI:0.02-0.85). On the other hand, HAV antibodies did not differ between patients with type 1 diabetes and controls (Table 3). Overall, 25% (59/240) of all Russian Karelian children were HAV positive.

There was a strong association between allergic symptoms and allergen-specific IgE. When all Russian Karelian children were included in the analysis, 45% (21/47) of those reporting allergic symptoms had allergen-specific IgE, compared with 8% (19/230) of those without allergic symptoms ($P<0.001$; OR=8.97, 95% CI:4.27-18.84). Similarly, among patients with type 1

diabetes, a respective 39% (5/13) and 13% (15/119) of the children with or without allergic symptoms were sensitized to at least one allergen (P=0.014; OR=4.33, 95% CI:1.25-15.00). Again, among non-diabetic control children, the only child who reported symptoms was also sensitized, while only 4% (4/111) of the children without allergic symptoms were sensitised (P<0.001).

Diabetes-associated HLA haplotypes were conspicuously more frequent among type 1 diabetic patients than among controls in both countries. The DR3-DQ2/DR4-DQ8 risk genotype was more frequent in Russian Karelian patients with type 1 diabetes than in their Finnish peers (33% *vs*. 17%; P<0.001). The frequency of HLA genotypes did not differ between the non-diabetic control groups in the two countries. Yet, diabetes-associated genotypes had no effect on IgE sensitization. In the entire study population, the diabetes-associated genotypes were present in 57% of the study subjects. About 19% of these subjects were sensitized against at least one of the tested allergens, while 20% of the subjects without risk genotypes were sensitized.

Table 2. Prevalence of allergen-specific IgE, reported frequency of allergic symptoms, prevalence of high levels (≥100 kU/l) of total IgE, and frequency of diabetes-related HLA genotypes in patients with type 1 diabetes and in control subjects in Russian Karelia.

	Patients with type 1 diabetes (N=132)	Control subjects (N=112)	*P*	OR (95% CI)
Cat IgE present	8.3%	1.8%	0.039	5.00 (1.08-23.06)
Birch IgE present	5.3%	0.9%	0.09	6.25 (0.75-51.31)
Egg albumen IgE present	5.3%	1.8%	0.25	3.08 (0.63-15.14)
At least one allergen-specific IgE present	15.2%	4.5%	0.012	3.47 (1.34-10.44)
Allergic diseases[a]	9.8%	0.9%	0.019	11.70 (1.56-94.23)
Total IgE > 100 kU/l	36.8%	42.3%	0.65	0.86 (0.51-1.43)
HAV antibodies present	21%	29%	0.17	0.64 (0.35-1.15)
Frequency of diabetes-related HLA genotypes[b]	92%	37%	<0.001	0.48 (0.02-0.10)
DR3-DQ2/DR4-DQ8	33%	2%	<0.001	26.63 (6.28-113.0)
DR3-DQ2/x	18%	14%	0.31	1.26 (0.63-2.53)
DR4-DQ8/y	42%	21%	<0.001	2.77 (1.56-4.92)

[a]Reported allergic symptoms included asthma, allergic eczema and allergic rhinitis.

[b]DR3-DQ2/DR4-DQ8, DR3-DQ2/x(x≠DR4-DQ8), or DR4-DQ8/y (y≠DR3-DQ2)

Table 3. Prevalence of allergen-specific IgE, high levels (≥100 kU/l) of total IgE, and frequency of diabetes-related HLA genotypes in patients with type 1 diabetes and in control subjects in Finland.

	Patients with type 1 diabetes (N=147)	Control subjects (N=266)	*P*	OR (95% CI)
Cat IgE present	12%	11%	0.77	1.1 (1.08-23.06)
Birch IgE present	10%	11%	0.74	0.89 (0.46-1.72)
Egg albumen present	9.5%	6.4%	0.25	1.54 (0.74-3.23)
At least one allergen specific IgE present	25%	22%	0.53	1.16 (0.72-1.87)
Total IgE > 100 kU/l	32%	26%	0.22	1.32 (0.85-2.05)
Frequency of diabetes-related HLA genotypes[a]	85%	38%	<0.001	0.11 (0.06-0.18)
DR3-DQ2/DR4-DQ8	17%	2%	<0.001	13.1 (4.35-39.5)
DR3-DQ2/x	18%	14%	0.97	0.99 (0.51-1.93)
DR4-DQ8/y	42%	21%	<0.001	3.58 (2.25-5.69)

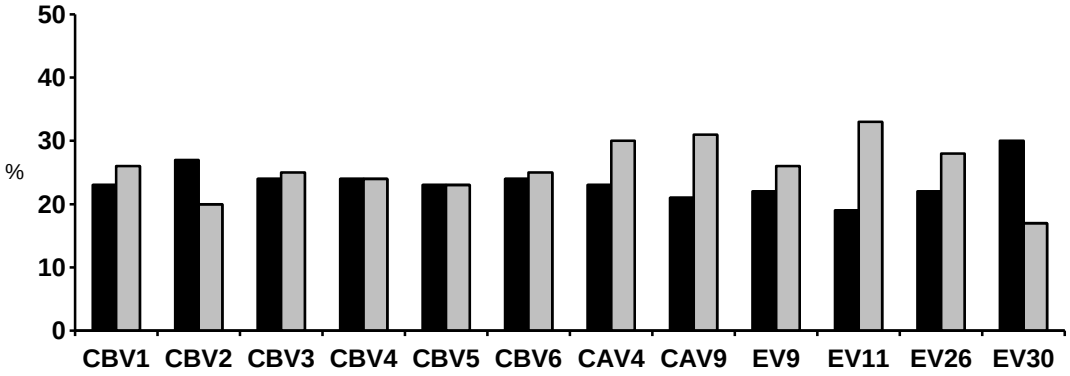
[a]DR3-DQ2/DR4-DQ8, DR3-DQ2/x(x≠DR4-DQ8), or DR4-DQ8/y (y≠DR3-DQ2)

9.4 Role of enteroviruses in IgE sensitization (Study IV)

Among the 60 IgE-positive children included in the substudy IV, 67% had IgE against cat allergen, 37% against birch allergen and 27% against egg albumen. The prevalence of antibodies against different enterovirus serotypes showed conspicuous variation ranging from 21% for CBV6 to 92% for CAV4 among non-sensitized children. The EV11 serotype was significantly more frequent in non-sensitized children, prevalence being 52% among the IgE-sensitized and 70% among the non-sensitized children. On the other hand, 19% of the EV11-positive children had allergen-specific IgE, while IgE prevalence among the EV11 negative children was 33% (*P*=0.001; OR 3.7, 95% CI 1.8-7.9), making it the strongest single protective serotype. None of the CBV serotypes was associated with protection against IgE sensitization. Only one virus (EV30) was associated with increased IgE sensitization, the prevalence of allergen-specific IgE being 30% among EV30 positive children and 17% among EV30 negative children (*P*=0.018; OR 0.16, 95% CI 0.034-0.73). Logistic regression model was applied in these analyses adjusting for the possible effect of diabetes and age.

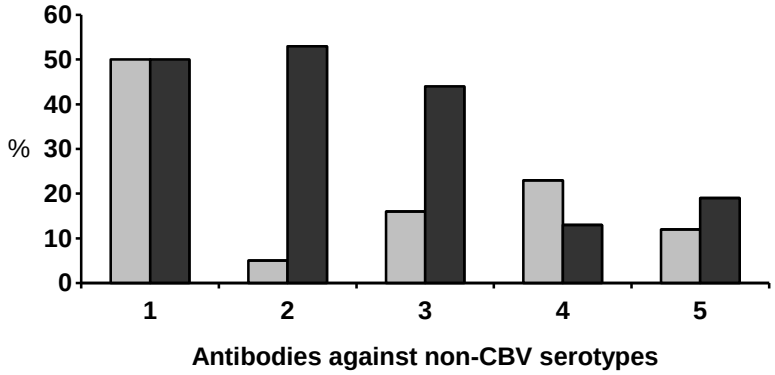
Non-CBV serotypes seemed to have a protective effect as a group, and having antibodies against several non-CBV serotypes other than EV30 was indeed associated with lower risk of IgE sensitization (*P*=0.017; OR=0.73, 95% CI: 0.56-0.94; Figure 1 in original publication IV). This trend was even stronger among EV30-positive children (*P*=0.003; OR=0.57, 95% CI: 0.39-0.82, Figure 4). Furthermore, among EV30-positive children, EV11 and CAV9 showed an independent protective effect (*P*=0.02; OR=0.35, 95% CI: 0.14-0.86 and *P*=0.003; OR=0.23, 95% CI: 0.086-0.61 respectively; Figure 2 in original publication IV).

Figure 3. Prevalence of allergen-specific IgE in relation to enterovirus antibodies. Black bars = enterovirus-positive children; grey bars = enterovirus-negative children. CBV, coxsackievirus B; CAV, coxsackievirus A; EV, echovirus. Below, the IgE prevalence (%) for each bar (and 95% CI for each given prevalence) is presented in table form.



Enterovirus serotype	CBV1	CBV2	CBV3	CBV4	CBV5	CBV6	CAV4	CAV9	EV9	EV11	EV26	EV30
Enterovirus positive (black bars)												
IgE prevalence	23%	27%	24%	23%	23%	24%	23%	21%	22%	19%	22%	30%
	17%	20%	17%	18%	17%	14%	18%	16%	16%	19%	17%	12%
95% CI	-30%	-35%	-31%	-31%	-31%	-37%	-29%	-28%	-29%	-25%	-29%	-39%
Enterovirus negative (grey bars)												
IgE prevalence	26%	20%	24%	24%	23%	24%	30%	31%	26%	33%	28%	17%
	17%	13%	17%	16%	17%	19%	15%	22%	19%	24%	19%	11%
95% CI	-37%	-29%	-34%	-34%	-32%	-31%	-52%	-42%	-36%	-43%	-39%	-25%

Figure 4. Prevalence of allergen-specific IgE antibodies in relation to the number of antibodies against enterovirus serotypes other than CBV (non-CBV). Grey bars = echovirus 30 negative children (not significant); black bars = echovirus 30 positive children (*P* for trend = 0.003).



10. DISCUSSION

10.1 Infections and IgE sensitization

The present study shows clearly that IgE sensitization is more frequent in Finland than in Russian Karelia. Furthermore, an inverse association between infectious diseases and IgE sensitization was seen in Russian Karelia. Although a similar association was not evident in Finland, the present study found a link between *H. pylori* infection and increasing allergy prevalence in Finland. Namely, IgE sensitization has increased among women without *H. pylori* antibodies. Among the microbes analysed in the present study, HAV, *H. pylori* and *T. gondii* have previously been linked to a reduced risk of atopy (Matricardi et al 2000, Kosunen et al 2002). However, this was the first study to show an association between enterovirus infection and a reduced risk of IgE sensitization. Indeed, the prevalence of allergen-specific IgE was four times higher in enterovirus-negative than in enterovirus-positive subjects in Russian Karelia. In this analysis, an EIA assay detecting antibodies against coxsackievirus B4 antigen was used. This assay may not be specific for CBV4, but more likely reflects the presence of enterovirus antibodies against a wider group of enteroviruses in general, thus indicating the overall exposure to different enteroviruses. Therefore, a highly specific neutralization assay to measure antibodies against individual enterovirus serotypes was used in the following study. It showed that the reduced risk of IgE sensitization was related only to certain enterovirus serotypes and that especially certain echovirus types were associated with protection against allergy. Hence, these viruses can be considered potential indicators of a protective environment.

Another finding emphasizing the different patterns of IgE sensitization in Finland and Russian Karelia is the co-occurrence of IgE sensitization and type 1 diabetes. Namely, in Russian Karelian these conditions co-occurred more than expected, whereas in Finland no similar pattern could be found. Furthermore, the co-occurrence of allergic sensitization and type 1 diabetes in Russian Karelia was associated with lack of HAV antibodies, suggesting that hygiene-related

factors are involved. Although it seems likely that changes in environmental exposures have led to the allergy epidemic seen in the Westernized world, the association between these environmental factors and allergic conditions seem to be more striking in less urbanized surroundings. The present findings raise two major questions that are discussed below.

10.1.1 IgE sensitization in Finland and in Russian Karelia – why the different patterns?

The Karelian Republic lies in northwestern Russia, adjacent to the Finnish border. Even though these two areas share the same geographic and climatic environment, they differ markedly in culture, economy and standards of living. As an illustration, the GNI per capita was about USD 47,720 in Finland compared with USD 9,900 in the Russian Federation in 2010. Furthermore, when 100% of the Finnish urban population had access to improved sanitation facilities in year 2000, only 85% of the urban population in the Russian Federation were in a similar situation (http://data.worldbank.org/country/finland and http://data.worldbank.org/country/russian-federation, accessed 2 January 2012). There may also be other hygiene-related differences. Indeed, a high microbial content in drinking water has been reported to be inversely associated with atopy, based on a survey in Russian Karelia (von Hertzen et al 2007b).

The present study shows markedly reduced IgE sensitization in Russian Karelia compared with Finland. A similar finding has been reported from other studies as well (von Hertzen et al 2006, Laatikainen et al 2011). The fact that the samples from Russian Karelia and Finland were taken in different years might have affected this result. However, since the samples from the Finnish children were collected before the samples from the Russian Karelia children, and the prevalence of allergy has been increasing in Finland, it might be expected that the temporal interval of about four years between the sample collections would rather have diminished than increased the observed differences between the two regions (Laatikainen et al 2011). Similarly, autoimmune diseases such as type 1 diabetes, celiac disease and thyroid autoimmunity are much more frequent

in Finland than in Russian Karelia (Kondrahova et al 2005, Kondrashova et al 2008a, Kondrashova et al 2008b). On the other hand, infectious pressure is much higher in Russian Karelia than in Finland. As an illustration, the present study found the prevalence of enterovirus, *H. pylori*, *T. gondii* and HAV to be conspicuously higher in Russian Karelia. These results are consistent with the hygiene hypothesis.

Interestingly, an inverse association between infections and IgE sensitization was found in Russian Karelia, but not in Finland. This could be explained by the assumption that Finnish children are infected at an older age, as the circulation of enteroviruses and other microbes is conspicuously lower in Finland. According to the hygiene hypothesis, infections occurring during the first months of life may be the most important ones, as at this age they can have a marked effect on the maturation of the immune responses (Nordtröm et al 2010, Saghafian-Hedengren et al 2010). Hence, it is possible that Finnish children are infected with the studied microbes mainly after IgE sensitization has already developed, and therefore inverse association is not seen.

It also seems possible that the clean and urban environment in prosperous countries (such as Finland) predisposes to the development of allergic and autoimmune diseases, leading to clinical disease in a large proportion of those who are genetically susceptible. This would be consistent with the results of the present study: diabetes-associated risk genotypes were less frequent among Finnish patients with type 1 diabetes than in Russian Karelian diabetic patients, suggesting that even a smaller genetic risk leads to the development of clinical disease in Finland. Similar results have been reported previously, and, indeed, type 1 diabetes may now develop in subjects with weaker HLA-conferred susceptibility than a few decades ago (Hermann et al 2003). The same may well apply for allergic diseases, and there may well be epigenetic mechanisms involved. If this was the case, it could be expected that genetic characteristics in the population would play a major role in the prevalence of IgE sensitization in Finland, whereas protective environmental factors (preventing the development of disease in a high percentage of those who are

genetically susceptible) might have a more obvious role in Russian Karelia. Yet, it seems that especially the environmental factors – or the lack of them – are responsible for the increase that has occurred in the IgE sensitization in Western countries. The present finding of increasing allergy prevalence among *H. pylori* negative Finnish women fits in well with this theory. This subgroup of women likely represents women who have grown up in more hygienic circumstances than *H. pylori* positive ones.

The assumption that genetic susceptibility leads to a clinical disease at different rates in Finland and Russian Karelia is also supported by other present finding: the observation that IgE sensitization occurs more often than expected in Russian Karelian diabetic patients, but not in their Finnish peers. This issue is discussed in more detail below, in Chapter 10.2.

10.1.2 Why would enterovirus serotypes differ in their relation to IgE sensitization?

The present results suggest that different enterovirus types differ in their relation to IgE sensitization. Twelve common enterovirus serotypes representing different genetic subgroups were included in neutralization assays, and interesting differences were observed between the tested viruses. There may be microbe-specific effects involved, such as different immunomodulatory effects of different virus types, or the differences could be explained by the different epidemiologies of these viruses. Viruses in the CBV group did not seem to be associated with IgE sensitization, whereas EV11 was associated with a reduced risk of IgE sensitization. Non-CBV EVs appeared to be associated with reduced risk when analysed as a group.

Unlike any other enterovirus, EV30 was associated with an increased risk of IgE sensitization. This may indicate that this virus is different from all other viruses either biologically or epidemiologically. In fact, most EV30 infections have been shown to occur among young adults and older children (Khetsuriani et al 2006), i.e. too late to protect against the development of IgE

sensitization. In contrast to EV30, EV11 and CAV9 predominantly affect young children aged less than one year (Khetsuriani et al 2006).

The biological basis of the possible protective effect conferred by certain enterovirus types may be related to the immunomodulatory effect of these viruses. Indeed, enteroviruses induce strong regulative cytokine responses such as IL-10 (Hofmann et al., 2001), which may lead to bystander suppression of allergic responses. This kind of effect can be particularly strong for enteroviruses, which replicate in the cells of the gut immune system, where they can activate tolerogenic dendritic cells. Interestingly, previous studies suggest that CBV cannot infect dendritic cells, whereas at least some echoviruses and possibly other non-CBV viruses can cause productive infection in these cells (Kramer et al 2007; Lin et al 2009). In addition, certain CBV strains can induce strong inflammatory responses by interacting with plasmocytoid dendritic cells (Hämäläinen et al. 2014). Accordingly, it is possible that the association between a reduced risk of IgE sensitization and certain EV infections is mediated by viral interactions with the gut immune system. In addition, replication lasts quite long, usually several weeks, which can lead to long-term immunological changes.

10.2 Type 1 diabetes and IgE sensitization

A strong positive association between type 1 diabetes and IgE sensitization was evident in Russian Karelia, but not in Finland. This kind of positive association suggests that the mechanisms leading to the development of these diseases could be partly overlapping. Such a mechanism could well be related to the induction of immune regulation by a variety of infections in early childhood.

Nonetheless, the finding of markedly increased IgE sensitization among diabetic patients in Russian Karelia runs counter to some previous findings. A meta-analysis in 2003 suggested that there is a small reduction in the prevalence of asthma among children with type 1 diabetes, while the association between other atopic diseases and type 1 diabetes was less

conclusive (Cardwell et al 2003). However, when previous studies are reviewed in more detail, a pattern that is consistent with our observation can be seen. Namely, an inverse correlation or no association between type 1 diabetes and allergic symptoms has been reported, mainly from affluent countries such as Italy (Caffarelli et al 2004), the Netherlands (Meerwaldt et al 2002), Finland (Mattila et al 2002), Germany (Rosenbauer et al 2003), Norway (Stene et al 2004), Denmark (Olesen et al 2001, Thomsen et al 2011), Sweden (Stromberg et al 1995), Austria and the UK (The EURODIAB Substudy 2 Study Group 2000). In addition, an extensive Israeli study reported an inverse relationship between type 1 diabetes and asthma (Tirosh et al 2006). A large Canadian study reported a positive relationship between asthma and type 1 diabetes, but this was seen only in those aged over 40 and not in younger age groups (Dales et al 2005). The EURODIAB Substudy 2 Study Group reported an inverse association between atopic diseases and the development of type 1 diabetes when eight European centres were combined, but individually only Austria and the UK showed a significant inverse relationship. In contrast, Bulgaria was the only country where there was a significant positive association (The EURODIAB Substudy 2 Study Group 2000). In addition, an association between a positive family history for allergic diseases and risk of type 1 diabetes has been reported from former Yugoslavia (Sipeti et al 2002). Thus, it seems that where income is low (mainly countries of low prevalence of allergic and autoimmune diseases), a positive association between these diseases is more likely to be found. Hence, the present finding of a positive relationship between type 1 diabetes and allergen-specific IgE responses in Russian Karelia can be seen to be in line with the trend seen in these other studies.

It seems likely that a large proportion of those who are genetically susceptible develop clinical diabetes in prosperous surroundings (such as Finland), while a large proportion of genetically susceptible individuals avoid clinical disease in more rural areas. The present finding of diabetes-associated risk genotypes being less frequent among Finnish patients with type 1 diabetes than in their Russian Karelian peers fits in well with this scenario. It seems that even a smaller

genetic risk leads to the development of clinical disease in Finland. Similar results have been reported previously, and indeed it seems that, in affluent countries, type 1 diabetes now develops in subjects with weaker HLA-conferred susceptibility than was the case a few decades ago (Hermann et al 2003). There may be a similar pattern with allergic diseases as well. If there are no protective factors in the environment, or if these protective factors are encountered too late, it would be likely that the penetrance of a disease would reflect the genetic susceptibility rather well. On the other hand, in an environment where there are protective factors present, the disease prevalence would more likely reflect the effect of these protective factors.

The conclusion is that mechanisms responsible for the possible associations between allergic diseases and type 1 diabetes may differ in different surroundings. As suggested by Thomsen and colleagues (2011), genetic associations may be important in affluent countries. Nevertheless, other factors may be more important in countries of low income.

11. LIMITATIONS OF THE PRESENT STUDY

The major limitation in the present study is that it is based on a cross-sectional study design, which makes it difficult to evaluate possible causal relationships underlying the observed associations. As an illustration, with this kind of study protocol, we cannot say whether a certain enterovirus infection has occurred before or after the subject has developed an allergic immune response. Therefore, possible causal relationships and their mechanisms remain to be explored in further studies.

The low prevalence of HAV, *T. gondii* and *H.pylori* antibodies in Finland made it difficult to analyse their association with allergen-specific IgE. This does not rule out the possibility that there is an association we were unable to show in this study.

In addition, the two cohorts in Substudy III differed slightly in their age distributions and were recruited during different time periods, i.e. Finland and Russian Karelia. The median age of the Finnish children was 10.7 years, compared with 11.7 in the Russian Karelian children. The children were recruited and samples collected in 1991-1997 in Finland and in 1997-2001 in Russian Karelia. As a result, these study cohorts were analysed separately. However, it seems unlikely that these differences would explain the different findings regarding the co-occurrence of type 1 diabetes and IgE sensitization in these two populations.

12. FUTURE PROSPECTS

Prospective study protocols are needed to investigate the causal relationship between infectious agents and allergic diseases. Currently, such studies are in progress in both Finland and in the Karelian Republic in Russia. Furthermore, it would be important to investigate the mechanisms that could mediate the immunomodulatory effects of certain microbes. For example, the reasons why certain enterovirus types are associated with protection against allergic sensitization could be studied further. In theory, this could be related to their ability to infect certain cells of the immune system (such as dendritic cells) or their ability to induce regulatory cytokines during the infection. In any case, the present study shows the great value of studies carried out in contrasting environments in attempting to fully understand the complex mechanisms of microbe-host interactions that can modulate the risk of allergic diseases. Comparisons between Finnish and Russian Karelian children will offer a unique resource for future research in this field.

13. CONCLUSIONS

The present results support the hygiene hypothesis by showing an inverse relationship between IgE sensitization and microbial infections in Russian Karelia. Furthermore, results indicate that the increasing prevalence of IgE sensitization that has occurred in the Westernized world (e.g. in Finland) is associated with hygiene-related factors. As an illustration, the prevalence of IgE sensitization has increased among women who are seronegative for *H. pylori*, i.e. women who likely grew up in surroundings characterized by high standards of hygiene. This increase may not have reached its peak yet, since the youngest cohort in Substudy II had the highest prevalence of IgE sensitization. Results also show a strong association between allergy and type 1 diabetes in Russian Karelia and therefore imply that allergic diseases and diabetes may have common pathogenic mechanisms that could be linked to the role of microbes in immune regulation. Furthermore, there may be microbe-specific factors involved in the protection that microbes provide against allergic diseases, since different enterovirus types differ markedly in their relationship to IgE sensitization. Enteroviruses, especially certain echoviruses, present a new candidate for an indicator of an environment that confers protection against developing allergic diseases.

Further studies are needed to uncover the exact mechanisms, as this may open up possibilities for clinical interventions aimed at preventing the development of allergies and type 1 diabetes.

14. ACKNOWLEDGEMENTS

This study was carried out at the Department of Virology of the Medical School at the University of Tampere and at the Department of Clinical Microbiology of Fimlab Laboratories.

I would like to acknowledge the many people who made this dissertation possible. First, I wish to express my deepest gratitude to my supervisor, Professor Heikki Hyöty, who gave me the original idea for this thesis and the opportunity to join his research group. I am grateful for his encouragement and expertise, which have been a source of inspiration for me and vital to this thesis.

I would also like to express my gratitude to the official reviewers of my thesis, Professor Erika Isolauri and Docent Anna Pelkonen, for their expertise and advice in shaping this thesis into its final form.

I also wish to express my profound thanks to Professor Mikael Knip for his involvement in this thesis. I am particularly grateful for his ideas and valuable comments in helping to refine the publications into their final forms.

In addition, I would like to express my deep gratitude to Doctor Hanna Viskari, who helped me a great deal in this work, especially in the earlier years of the project.

I am also grateful to Doctor Anita Kondrashova, whose knowledge and work contributed a great deal to this project.

I would like to thank Docent Anna-Maija Haapala for her valuable contribution, especially to the IgE measurements in this thesis.

I would also like to thank Professor Minna Kaila for her advice and expertise in this project.

Additionally, I wish to thank my co-authors Janne Aittoniemi, Sisko Tauriainen, Mikko Hurme, Jorma Ilonen, Miia Virta, Pentti Koskela and Raivo Uibo for their valuable contribution to the original publications.

I express my sincere thanks to everyone I worked with at the Department of Virology. I am deeply grateful to researchers Maarit Oikarinen, Hanna Honkanen and Sami Oikarinen for their help and support. I would also like to express my special thanks to Eveliina Jalonen, Mervi Kekäläinen, Sari Valovirta, Anne Karjalainen, Eeva Tolvanen and Tanja Kuusela for their skilful technical assistance in the laboratory work and for their fun and supportive attitude. It has been a privilege to work on this team.

I would also like to thank many friends and colleagues for their support. I am especially thankful to Janne Aittoniemi for his continual pressure on me to complete this task.

I wish to express my thanks to my parents Ritva and Jorma for their support and encouragement. I am also grateful to my sisters Terhi and Meri and also to Petri Koskela for the interest they have shown in this thesis.

Finally, I want to express my gratitude to my beloved Susan, who has supported me in writing this thesis.

This study received financial support from the EU as a part of the EPIVIR project (ICO-Copernicus Program, contract number IC15-CT98-0316) and the DiabImmune project (FP7-202063), from the Päivikki and Sakari Sohlberg Foundation, from the Tuberculosis Foundation in Tampere, from the Academy of Finland, from the University of Tampere, and from the Medical Research Fund of Tampere University Hospital.

15. REFERENCES

Aaby S, Shaheen SO, Heyes CB, Goudiaby A, Hall AJ, Shiell AW, Jensen H and Marchant A (2000); Early BCG vaccination and reduction in atopy in Guinea-Bissau. Clin Exp Allergy 30:644-50.

Alcantara-Neves NM, Veiga RF, Dattoli VCC, Fiaccone RL, Esquivel R, Cruz ÁA, Cooper PJ, Rodriques LC and Barreto ML (2012); The Effect of single and multiple infections on atopy and wheezing in children. J Allergy Clin Immunol 129:359-67.

Alm JS, Swartz J, Lilja G, Scheynius A and Pershagen G (1999); Atopy in children of families with an anthroposophic lifestyle. Lancet 353:1485-8.

Altman D, Altman DG, Bryant T, Gardner M, Gardner MJ and Machin D (2000); Statistics with Confidence. BMJ Books, London.

Anderson HR, Poloniecki JD, Phil D, Strachan DP, Beasley R, Björkstén B, and Asher I for the ISAAC phase 1 study group (2001); Immunization and symptoms of atopic disease in children: results from the international study of asthma and allergies in childhood. Am J Public Health 91:1126-9.

Asher MI, Montefort S, Björkstén B, Lai CKW, Strachan DP, Weiland SK, Williams H and the ISAAC Phase Three Study Group (2006); Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivits, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. Lancet 368:733–43.

Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle H, Sakaguchi S, Taniguchi T, Morita H, Hattori M and Honda K (2013); Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 500:232-6.

Bach JF (2002); The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 347:911-20.

Bach JF (2005); Infections and autoimmune diseases. J autoimmun 25:74-80.

Bager P, Westergaard T, Rostgaard K, Hjalgrim H and Melbye M (2002); Age at childhood infections and risk of atopy. Thorax 57:379-82.

Beasley R, Keil U, von Mutius E, Pearce N and the International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee (1998); Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 351:1225–32.

Benn CS, Melbye M, Wohlfahrt J, Björkstén B and Aaby P (2004); Cohort study of sibling effect, infectious diseases, and risk of atopic dermatitis during first 18 months of life. BMJ 328:1223

Bernsen RMD, Nagelkerke NJD, Thijs C and van der Wouden JC (2007); Reported pertussis infection and risk of atopy in 8- to 12-yr-old vaccinated and non-vaccinated children. Pediatr Allergy Immunol 19:46-52.

Bernsen RMD and van der Wouden JC (2008); Measles, mumps and rubella infections and atopic disorders in MMR-unvaccinated and MMR-vaccinated children. Pediatr Allergy Immunol 19:544-51.

Bertelsen RJ, Carlsen KCL, Granum B, Carlsen K-H, Håland G, Devulapalli CS, Munthe-Kaas MC, Mowinckel P and Løvik M (2011); Do allergic families avoid keeping furry pets? Indoor Air 20:187-95.

Birgisdóttir A, Asbjörnsdottir H, Cook E, Gislason D, Jansson C, Olafsson I, Gislason T, Jogi R and Thjodleifsson B (2006); Seroprevalence of Toxoplasma gondii in Sweden, Estonia and Iceland. Scand J Infect Dis 38:625-31.

Blanco-Quiros A, Garcia-Marcos L, Garrote JA, Martinez-Torres AE and Leon A (2005); Antibody levels to Bordetella pertussis in 10-yr-old children with atopy and atopic asthma. Pediatr Allergy Immunol 16:637-40.

Blaser MJ (2005); The biology of cag in the Helicobacter pylori-human interaction. Gastroenterology 128:1512-5.

Bodner C, Godden D, Seaton A on behalf of the Aberdeen WHEASE Group (1998); Family size, childhood infections and atopic diseases. Thorax 53:28-32.

Bousquet J, Anto J, Auffray C, Akdis M, Cambon-Thomsen A, Keil T, Haahtela T, Lambrecht BN, Postma DS, Sunyer J, Valenta R, Akdis CA, Annesi-Maesano I, Arno A, Bachert C, Ballester F, Basagana X, Baumgartner U, Bindslev-Jensen C, Brunekreef B, Carlsen KH, Chatzi L, Crameri R, Eveno E, Forastiere F, Garcia-Aymerich J, Guerra S, Hammad H, Heinrich J, Hirsch D, Jacquemin B, Kauffmann F, Kerkhof M, Kogevinas M, Koppelman GH, Kowalski ML, Lau S, Lodrup-Carlsen KC, Lopez-Botet M, Lotvall J, Lupinek C, Maier D, Makela MJ, Martinez FD, Mestres J, Momas I, Nawijn MC, Neubauer A, Oddie S, Palkonen S, Pin I, Pison C, Rancé F, Reitamo S, Rial-Sebbag E, Salapatas M, Siroux V, Smagghe D, Torrent M, Toskala E, van Cauwenberge P, van Oosterhout AJM, Varraso R, von Hertzen L, Wickman M, Wijmenga C, Worm M, Wright J and Zuberbier T. (2011); MeDALL (Mechanisms of the Development of ALLergy): an integrated approach from phenotypes to systems medicine. Allergy 66: 596-604.

Braun-Fahrländer CH, Gassner M, Grize L, Neu U, Sennhauser FH, Varonier HS, Vuille JC, Wüthrich B and the SCARPOL team (1999); Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. Clin Exp Allergy 29:28-34.

Braun-Fahrländer C, Gassner M, Grize L, Takken-Sahli K, Neu U, Stricker T, Varonier HS, Wuthrich B, Sennhauser FH and the Swiss Study on Childhood Allergy and respiratory symptoms, Air Pollution (SCARPOL) team (2004); No further increase in asthma, hay fever and atopic sensitisation in adolescents living in Switzerland. Eur Respir J 23:407-413.

Brusko TM, Putnam AL and Bluestone JA (2008); Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. Immunol Rev 223:371-90.

Bråbäck L, Hjern A and Rasmussen F (2004); Trends in asthma, allergic rhinitis and eczema among Swedish conscripts from farming and non-farming environments. A nationwide study over three decades. Clin Exp Allergy 34:38-43.

Burr ML, Emberlin JC, Treu R, Cheng S, Pearce NE and the ISAAC phase one study group (2003); Pollen counts in relation to the prevalence of allergic rhinoconjunctivitis, asthma and atopic eczema in the international study of asthma and allergies in childhood (ISAAC). Clin Exp Allergy 33:1675-80.

Butland BK, Strachan DP, Lewis S, Bynner J, Butler N and Britton J (1997); Investigation into the increase in hay fever and eczema at age 16 observed between the 1958 and 1970 British birth cohorts. BMJ 315:717-721.

Caffarelli C, Cavagni G, Pierdomenico R, Chiari G, Spattini A and Vanelli M (2004); Coexistence of IgE-mediated allergy and type 1 diabetes in childhood. Int Arch Allergy Immunol 134:288-94.

Calvani M, Alessandri C, paolone G, Rosengart L, Di Caro A and De Franco D (1997); Correlation between Epstein Barr virus antibodies, serum IgE and atopic disease. Pediatr Allergy Immunol 8:91-6.

Campo P, Kalra HK, Levin L, Reponen T, Olds R, Lummus ZL, Cho S-H, Khurana Hershey GK, Lockley J, Villareal M, Stanforth S, LeMasters G and Bernstein DI (2006); Influence

of dog ownership and high endotoxin on wheezing and atopy during infancy. J Allergy Clin Immunol 118:1271-8.

Cardwell CR, Shields MD, Carson DJ and Patterson CC (2003); A meta-analysis of the association between childhood type 1 diabetes and atopic disease. Diabetes Care 26:2568-74.

Chen Y and Blaser MJ (2007); Inverse association of Helicobacter pylori with asthma and allergy. Arch Intern Med 167:821-7.

Chen Y and Blaser MJ (2008); Helicobacter pylori colonization in inversely associated with childhood asthma. J Inf Dis 198:553-60.

Cooper PJ, Chico ME, Vaga MG, Moncayo AL, Bland M, Mafla E, Sanzhes F, Rodrigues LC, Strachan DP and Griffin GE (2006); Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. Lancet 367:1598-603.

Cramer C, Link E, Bauer CP, Hoffman U, von Berg A, Lehmann I, Herbarth O, Borte M, Schaaf B, Sausenthaler S, Wichmann HE, Heinrich J and Krämer U, for the LISAplus study group (2011); Association between attendance of day care centres and increased risk of eczema in the German birth cohort study LISAplus. Allergy 66:68-75.

Cullinan P, Harris JM, Newman Taylor AJ, Jones M, Taylor P, Dave JR, Mills P, Moffat SA, White CW, Figg JK, Moon AM and Barnes MC (2003); Can early infection explain the sibling effect in adult atopy? Eur Respir J 22:956-61.

Dales R, Chen Y, Lin M and Karsh J (2005); The association between allergy and diabetes in the Canadian population: Implications for the Th1-Th2 hypothesis. Eur J Epidemiol 20:713-7.

D'Angeli MA, Merzon E, Valbuena LF, Tirschwell D, Paris CA and Mueller BA (2010); Environmental factors associated with Childhood-onset type 1 diabetes. Arch Pediatr Adolesc Med 164:732-8.

de Meer G, Janssen NAH, Brunekreef B (2005); Early childhood environment related to microbial exposure and the occurrence of atopic disease at school age. Allergy 60:619-25.

Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Üblagger E, Schram-Bijkerk D, Brunekreef B, van Hage M, Scheynius A, Pershagen G, Benz MR, Lauener R, von Mutius E, Braun-Fahrländer C and the PARSIFAL Study team (2006); Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J Allergy Clin Immunol 117:817-23.

Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR, Weiss G, Nyberg F, van Hage M, Pershagen G, Brunekreef B, Riedler J, Lauener R, Braun-Fahrländer C, von Mutius E and the PARSIFAL Study team (2007); Not all farming environments protect against the development of asthma and wheeze in children. J Allergy Clin Immunol 119:1140-7.

Ege MJ, Herzum I, Büchele G, Krauss-Etschmann S, Lauener RP, Bitter S, Roponen M, Remes S, Vuitton DA, Riedler J, Brunekreef B, Dalphin J-C, Braun-Fahrländer C, Pekkanen J, Renz H, von Mutius E and the PASTURE study group (2008); Specific IgE to allergens in cord blood is associated with maternal immunity to Toxoplasma gondii and rubella virus. Allergy 63:1505-11.

Ellertsen LK, Hetland G and Løvik M (2008); Specific IgE to Respiratory allergens and IgG Antibodies to Toxoplasma gondii and Streptococcus pneumoniae in Norwegian military recruits. Scand J Immunol 67:496-500.

Ellwood P, Asher MI, Björkstén B, Burr M, Pearce N, Robertson CF, and the ISAAC phase one study group (2001); Diet and asthma, allergic rhinoconjunctivitis and allergic eczema symptom prevalence: an ecological analysis of the international study of asthma and allergies in childhood (ISAAC) data. Eur Respir J 17:436-43.

Feary J, Britton J and Leonardi-Bee J (2010); Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 66:569-78.

Föistrup H, Swartz J, Bergström A, Alm JS, Scheynius A, van Hage M, Waser M, Braun-Fahrländer C, Schram-Bijkerk D, Huber M, Zutavern A, von Mutius E, Üblagger E, Riedler J, Michaels KB, Pershagen G and the PARSIFAL study group (2006); Allergic disease and sensitization in Steiner school children. J Allergy Clin Immunol 117:59-66.

Foliaki S, Kildegaard Nielsen S, Björkstén B, von Mutius E, Cheng S, Pearce N, and the ISAAC phase I study group (2004); Antibiotic sales and the prevalence of symptoms of asthma, rhinitis, and eczema: The international study of asthma and allergies in childhood (ISAAC). Int J Epidemiol 33:558-63.

Fullerton D, Britton JR, Lewis SA, Pavord ID, McKeever TM and Fogarty A (2009); Helicobacter pylori and lung function, asthma, atopy and allergic disease—a population-based cross-sectional study in adults. Int J Epidemiol 38:419-26.

Geller-Bernstein C and Kenett R (2004); Allergies in immigrants. Eur Ann Allergy Clin Immunol 36:313-6.

Gonzalez-Quintela A, Gude F, Boquete O, Aguilera A, Rey J, Meijide LM, Fernandez-Merino MC and Vidal C (2005); Association of hepatitis A virus infection with allergic sensitization in a population with high prevalence of hepatitis A virus exposure. Allergy 60:98-103.

Hagendorens MM, Bridts CH, Lauwers K, van Nuijs S, Ebo DG, Vellinga A, De Clerck LS, Van Bever HP, Weyler JJ and Stevens WJ (2005); Perinatal risk factors for sensitisation, atopic dermatitis and wheezing during the first year of life (PIPO study). Clin Exp Allergy 35:733-40.

Hagerhed-Engman L, Bornehag CG, Sundell J and Åberg N (2006); Day-care attendance and increased risk for respiratory and allergic symptoms in preschool age. Allergy 61:447-53.

Hanski I, von Hertzen L, Fyhrquist N, Koskinen K, Torppa K, Laatikainen T, Karisola P, Auvinen P, Paulin L, Mäkelä MJ, Vartiainen E, Kosunen TU, Alenius H and Haahtela T (2013); Environmental biodiversity, human microbiota, and allergy are interrelated. PNAS 109:8334-9.

Herbath O, Bauer M, Fritz GJ, Herbath P, Rolle-Kampczyk U, Krumbiegel P, Richter M and Richter T (2007); Helicobacter pylori colonisation and eczema. J Epidemiol Community Health 61:638-40.

Hermann R, Knip M, Veijola R, Simell O, Laine AP, Åkerblom HK, Groop PH, Forsblom C, Pettersson-Fernholm K, Ilonen J and FinnDiane study group (2003); Temporal changes in the frequencies of HLA genotypes in patients with type 1 diabetes – indication of an increased environmental pressure? Diabetologia 46:420-5.

Hesselmar B, Åberg N, Åberg B, Eriksson B and Björkstén B (1999); Does early exposure to cat or dog protect against later allergy development? Clin Exp Allergy 29:611-7.

Hofmann P, Schmidtke M, Stelzner A and Gemsa D (2001); Suppression of proinflammatory cytokines and induction of IL-10 in human monocytes after coxsackievirus B3 infection. J Med Virol 64:487-98.

Hong SJ (2012); The role of Mycoplasma pneumoniae in asthma. Allergy Asthma Immunol Res 4:59-61.

Hämäläinen S, Nurminen N, Ahlfors H, Oikarinen S, Sioofy-Khojine A-B, Frisk G, Oberste MS, Lahesmaa R, Pesu M and Hyöty H (2014); Coxsackievirus B1 reveals strain specific differences in plasmacytoid dentritic cell mediated immunogenicity. J Med Virol 86:1412-20.

Janson C, Asbjornsdottir H, Birgisdottir A, Sigurjonsdottir RB, Gunnbjörnsdottir M, Gislason D, Olafsson I, Cook E, Jögi R, Gislason T and Thjodleifsson B (2007); The effect of infectious burden on the prevalence of atopy and respiratory allergies in Iceland, Estonia, and Sweden. J Allergy Clin Immunol 120:673-9.

Kabesh M (2014); Epigenetics in asthma and allergy. Curr Opin Allergy Clin Immunol 14:62-8.

Khetsuriani N, LaMonte-Fowlkes A, Oberste S, Pallansch M (2006); Enterovirus surveillance—United States, 1970-2005. MMWR Surveill Summ 55:1-20.

Kilpeläinen M, Terho EO, Helenius H and Koskenvuo M (2000); Farm environment in childhood prevents the development of allergies. Clin Exp Allergy 30:201-8.

Kondrashova A, Reunanen A, Romanov A, Karvonen A, Viskari H, Vesikari T, Ilonen J, Knip M and Hyöty H (2005); A six-fold gradient in the incidence of type 1 diabetes at the eastern border of Finland. Ann Med 37:67-72.

Kondrashova A, Mustalahti K, Kaukinen K, Viskari H, Volodicheva V, Haapala A-M, Ilonen J, Knip M, Mäki M, Hyöty H and the EPIVIR study group (2008a); Lower economic status and inferior hygienic environment may protect against celiac disease. Ann Med 40:223-231.

Kondrashova A, Viskari H, Haapala A-M, Seiskari T, Kulmala P, Ilonen J, Knip M and Hyöty H (2008b); Serological evidence of thyroid autoimmunity among schoolchildren in two different socioeconomic environments. J Clin Endocrinol Metab 93:729-34.

Kosunen TU, Höök-Nikanne J, Salomaa A, Sarna S, Aromaa A and Haahtela T (2002); Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish

population and a possible relationship to Helicobacter pylori infections. Clin Exp Allergy 32:373-8.

Kramer M, Schulte BM, Toonen LW, de Bruijni MA, Galama JM, Adema GJ, van Kuppeveld FJ (2007); Echovirus infection causes rapid loss-of-function and cell death in human dendritic cells. Cell Microbiol 9:1507-18.

Kramer MS, Matush L, Bogdanovich N, Dahhou M, Platt RW and Mazer B (2009); The low prevalence of allergic disease in Eastern Europe: are risk factors consistent with the hygiene hypothesis? Clin Exp Allergy 39:708-16.

Krämer U, Heinrich J, Wjst M and Wichmann HE (1998); Age of entry to day nursery and allergy in later childhood. Lancet 352:450-4.

Kuyucu K, Saraclar Y, Tuncer A, Sackesen C, Adalıoglu G, Sümbüloglu V and Şekerel BE (2004); Determinants of atopic sensitization in Turkish school children: effects of pre- and post-natal events and maternal atopy. Pediatr Allergy immunol 15:62-71.

Laatikainen T, von Hertzen L, Koskinen J-P, Mäkelä MJ, Jousilahti P, Kosunen TU, Vlasoff T, Ahlström M, Vartiainen E and Haahtela T (2011); Allergy gap between Finnish and Russian Karelia on increase. Allergy 66:886-92.

Leibowitz U, Antonovsky A, Medalie JM, Smith HA, Halpern L and Alter M (1966); Epidemiological study of multiple sclerosis in Israel. II. Multiple sclerosis and level of sanitation. J Neurol Neurosurg Psychiatry 29:60-8.

Lin YW, Wang SW, Tung YY, Chen SH (2009); Enterovirus 71 infection of human dendritic cells. Exp Biol Med 234:1166-73.

Linneberg A, Nielsen NH, Madsen F, Frølund L, Dirksen A and Jørgensen T (2000); Increasing prevalence of specific IgE to aeroallergens in an adult population: Two cross-sectional surveys 8 years apart. J Allergy Clin Immunol 106:247-52.

Linneberg A, Østergaard C, Tvede M, Andersen LP, Nielsen NH, Madsen F, Frølund L, Dirksen A and Jørgensen T (2003); IgG antibodies against microorganisms and atopic disease in Danish adults: The Copenhagen Allergy Study. J Allergy Clin Immunol 111:847-53.

Breitling LP, Yang R, Korn B, Burwinkel B and Brenner H (2011); Tobacco-smoking-related Differential DNA Methylation: 27K Discovery and Replication. Am J Hum Genet 88:450-7.

Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R and Alvarez N (1993); Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. J Allergy Clin immunol 92:404-11.

Maslowski KM and Mackay CR (2011); Diet, gut microbiota and immune responses. Nat immunol 12:5-9.

Matricardi P, Rosmini F, Ferrigno L, Nisini R, Rapicetta M, Chionne P, Stroffolini T, Pasquini P and D'Amelio R (1997); Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. BMJ 314:999-1003.

Matricardi P, Rosmini F, Rapicetta M, Gasbarrini G and Stroffolini T on behalf of the San Marino study group (1999); Atopy, hygiene, and antroposophic lifestyle. Lancet 354:430.

Matricardi P, Rosmini F, Riondino S, Fortini M, Ferrigino L, Rapicetta M, Bonini S (2000); Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. BMJ320:412-7.

Matricardi P, Rosmini F, Panetta V, Ferrigno L and Bonini S (2002); Hay fever and asthma in relation to markers of infection in the United States. J Allergy Clin Immunol 110;381-7.

Mattila PS, Tarkkanen J, Saxen H, Pitkaniemi J, Karvonen M and Tuomilehto J (2002); Predisposition to atopic symptoms to inhaled antigens may protect from childhood type 1 diabetes. Diabetes Care 25:865-8.

McIntire JJ, Umetsu SE, Akbari O, Potter M, Kuchroo VK, Barsh GS, Freeman GJ, Umetsu DT and DeKruyff H (2001); Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. Nature Immunol 2:1109-16.

Meerwaldt R, Odink RJ, Landaeta R, Aarts F, Brunekreef B, Gerritsen J, Van Aalderen WM and Hoekstra MO (2002); A lower prevalence of atopy symptoms in children with type 1 diabetes mellitus. Clin Exp Allergy 32:254-5.

Michos A, Terzidis A, Kanariou M, Kalampoki V, Koilia C, Giannaki M, Liatsis M, Pangalis A and Petridou E (2011); Association of allergic sensitization with infectious diseases in Roma and non-Roma children. Pediatr Allergy Immunol 22:243-8.

Mitchell EA and Stewart AW on the behalf of the ISAAC phase one study group (2001); The ecological relationship of tobacco smoking to the prevalence of symptoms of asthma and other atopic diseases in children: The international study of asthma and allergies in childhood (ISAAC). Eur J Epidemiol 17:667-73.

Nebrini C, Sichelstiel A, kisielow J, Kurrer M, Kopf M and Marsland BJ (2011); Bacterial induced protection against allergic inflammation through a multicomponent immunoregulatory mechanism. Thorax 66:755-63.

Nejentsev S, Sjöroos M, Soukka T, Knip M, Simell O, Lövgren T and Ilonen J (1999); Population based genetic screening for the estimation of type 1 diabetes mellitus risk in Finland: selective genotyping of markers in the HLA-DQB1, HLA-DQA1 and HLA DRB1 loci. Diabet Med 16:985-92.

Nilsson C, Linde A, Montgomery SM, Gustafsson L, Näsman P, Blomberg MT and Lilja G (2005); Does early EBV infection protect against IgE sensitization? J Allergy Clin immunol 116:438-44.

Ninan TK and Russell G (1992); Respiratory symptoms and atopy in Aberdeen schoolchildren: Evidence from two surveys 25 years apart. BMJ 304:873-5.

Nordström I, Rudin A, Adlerberth I, Wold A, Saalman R, Hesselmar B, Åberg N, Liljeqvist J-Å and Eriksson K (2010); Infection of infants with human herpesvirus type 6 may be associated with reduced allergic sensitization and T-helper type 2 development. Clin Exp Allergy 40:882-90.

Olesen AB, Juul S, Birkebaek N and Thestrup-Pedersen K (2001); Association between atopic dermatitis and insulin-dependent diabetes mellitus: A case-control study. Lancet 357:1749-52.

Ownby DR, Johnson CC and Peterson EL (2002); Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. JAMA 288:963-72.

Patterson CC, Carson DJ and Hadden DR (1996); Epidemiolgy of childhood IDDM in Northern Ireland 1989-1994: low incidence in areas with highest population density and most household crowding. Northern Ireland Diabets study Group. Diabetologia 39:1063-9.

Paunio M, Heinonen OP, Virtanen M, Leinikki P, Patja A and Peltola H (2000); Measles history and atopic diseases. JAMA 283:343-6.

Penders J, Stobberingh EE, van den Brandt PA and Thijs C (2007); The role of the intestinal microbiota in the development of atopic disorders. Allergy 62:1223-36.

Pessi T, Virta M, Karjalainen J, Rautelin H, Kosunen TU, and Hurme M (2005); Genetic and environmental factors in the immunopathogenesis of atopy: interaction of Helicobacter pylori infection and IL4 genetics. Int Arch Allergy Immunol 137:282-8.

Poskanzer DC, Schapira K and Miller H (1963); Multiple sclerosis and poliomyelitis. Lancet 2:917-21.

Radon K, Windstetter D, Eckart J, Dressel H, Leitritz L, Reichert J, Schmid M, Praml G, Schosser M, von Mutius E and Nowak D (2004); Farming exposure in childhood, exposure to markers on infections and the development of atopy in rural subjects. Clin Exp Allergy 34:1178-83.

Rehan VR, Liu J, Naeem E, Tian J, Sakurai R, Kwong K, Akbari O and Torday JS (2012); Perinatal nicotine exposure induces asthma in second generation offspring. BMC Med 10:129.

Reibman J, Marmor M, Filner J, Fernandez-Beros M-E, Rogers L, Perez-Perez GI and Blaser MJ (2008). Asthma is inversely associated with helicobacter pylori status in an urban population. Plos One 3:e4060.

Remes S, Mäkelä M and Marshall J (2000); Measles and atopy in Finland. Allergy 55:973-4.

Remes ST, Castro-Rodriquez JA, Holberg CJ, Martinez FD and Wright AL (2001); Dog exposure in infancy decreases the subsequent risk of frequent wheeze but not of atopy. J Allergy Clin Immunol 108:509-15.

Riedler J, Eder W, Oberfeld G and Schreuer M (2000); Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin Exp Allergy 30:194-200.

Riedler J, Braun-Fahrländer C, Eder W, Schreuer M, Waser M, Maisch S, Carr D, Schierl E, Nowak D, von Mutius E and the ALEX Study Team (2001); Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet 358:1129-33.

Roduit C, Wohlgensinger J, Frei R, Bitter S, Bieli C, Loeliger S, Bùchele G, Riedler J, Dalphin JC, Remes S, Roponen M, Pekkanen J, Kabesch M, Schaub B, von Mutius E, Braun-Fahrländer C, Lauener R and the PASTURE Study Group (2011); Prenatal animal

contact and gene expression of innate immunity receptors at birth are associated with atopic dermatitis. J Allergy Clin Immunol 127:179-85.

Roivainen M, Knip M, Kulmala P, Hiltunen M, Vähäsalo P, Hovi T, Åkerblom H and The Childhood Diabetes in Finland (DiMe) Study Group (1998); Several different enterovirus serotypes can be associated with prediabetic autoimmune episodes and onset of overt IDDM. J Med Virol 56:74-8.

Ronkainen M, Hämäläinen A-M, Koskela P, Åkerblom HK, Knip M and the Finnish TRIGR Study Group (2001); Pregnancy induces non-immunoglobulin insulin-binding activity in both maternal and cord blood serum. Clin Exp Immunology 124:190-6.

Roost HP, Gassner M, Grize L, Wüthrich B, Sennhauser FH, Varonier HS, Zimmerman H, Braun-Fahrländer Ch and the SCARPOL team (2004); Influence of MMR-vaccinations and diseases on atopic sensitization and allergic symptoms in Swiss schoolchildren. Pediatr Allergy Immunol 15:401-7.

Rosenbauer J, Herzig P and Giani G (2003); Atopic eczema in early childhood could be protective against type 1 diabetes. Diabetologia 46:784-8.

Rystedt I, Strannegård IL and Strannegård O (1984); Increased serum levels of antibodies to Epstein-Barr virus in adults with history of atopic dermatitis. Int Arch Allergy Appl Immunol 75:179-83

Saghafian-Hedengren S, Sverremark-Ekström E, Linde A, Lilja G and Nilsson C (2010); Early-life EBV infection protects against persistent IgE sensitization. J Allergy Clin Immunol 125:433-8.

Salminen K, Sadeharju K, Lönnrot M, Vähäsalo P, Kupila A, Korhonen S, Ilonen J, Simell O, Knip M and Hyöty H (2003); Enterovirus infections are associated with the induction of beta-cell autoimmunity in a prospective birth cohort study. J Med Virol 69:91-8.

Savola K, Bonifacio E, Sabbah E, Kulmala P, Vähäsalo P, Karjalainen J, Tuomilehto-Wolf E, Meriläinen J, Akerblom HK and Knip M (1998); IA-2 antibodies--a sensitive marker of IDDM with clinical onset in childhood and adolescence. Childhood diabetes in Finland study group. Diabetologia 41:424-9.

Savola K, Sabbah E, Kulmala P, Vahasalo P, Ilonen J and Knip M (1998); Autoantibodies associated with type I diabetes mellitus persist after diagnosis in children. Diabetologia 41:1293-7.

Schäfer T, Meyer T, Ring J, Wichmann HE and Heinrich J (2005); Worm infestation and the negative association with eczema (atopic/nonatopic) and allergic sensitization. Allergy 60:1014-20.

Scrivener S, Yemaneberhan H, Zebenigus M, Tilahun D, Girma S, Ali S, McElroy P, Custovic A, Woodcock A, Pritchard D, Venn A and Britton J (2001); Independent risk of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. Lancet 358:1493-99.

Shaheen SO, Aaby P, Hall AJ, Barker DJ, Heyes CB, Shiell AW and Goudiaby A (1996); Measles and atopy in Guinea-Bissau. Lancet 347:1792-6.

Sidorchuk A, Lagarde F, Pershagen G, Wickman M and Linde A (2003); Epstain-Barr virus infection is not associated with development of allergy in children. Pediatr Infect Dis J 22:642-7.

Sidorchuk A, Wickman M, Pershagen G, Lagarde F and Linde A (2004); Cytomegalovirus infection and development of allergic diseases in early childhood: interaction with EBV infection? J Allergy Clin Immunol 114:1434-40.

Silverberg JI, Norowitz KB, Kleiman E, Silverberg NB, Durkin HG, Joks R and Smith-Norowitz TA (2010); Association between varicella zoster virus infection and atopic dermatitis in early and late childhood. J Allergy Clin immunol 126:300-5.

Silverberg JI, Kleiman E, Silverberg NB, Durkin HG, Joks R and Smith-Norowitz TA (2012); Chickenpox in childhood is associated with decreased atopic disorders, IgE, allergic sensitization, and leukocyte subsets. Pediatr Allergy Immunol 23:50-58.

Sipeti S, Vlajinac H, Kocev N, Marinkovi J, Radmanovi S and Deni L (2002); Family history and risk of type 1 diabetes mellitus. Acta Diabetol 39:111-5.

Soothill JF (1996); Measles and atopy in African children. Lancet 348:825.

Statistics Finland (2007); Kaskipelloilta palveluyhteiskuntaan – 90 vuotta elinkeinorakenteen muutosta. Tilastokeskus. Available from: http://www.stat.fi/tup/suomi90/helmikuu.html (accessed 18 May 2011).

Stene LC, Joner G and Norwegian Childhood Diabetes Study Group (2004); Atopic disorders and risk of childhood-onset type 1 diabetes in individuals. Clin Exp Allergy 34:201-6.

Stewart AW, Mitchell EA, Pearce N, Strachan DP and Stephan WK on behalf of the ISAAC Steering committee (2001); The relationship of per capita gross national product to the prevalence of symptoms of asthma and other atopic diseases in children (ISAAC). Int J Epidemiol 303:173-9.

Strachan DP (1989); Hay fever, hygiene, and household size. BMJ 299:1259-60.

Strachan DP, Butland BK and Anderson HR (1996); Incidence and prognosis of asthma and wheezing illness from early childhood to age 33 in a national British cohort. Br Med J 312:1195-9.

Strachan DP (2000); Family size, infection and atopy: the first decade of the 'hygiene hypothesis'. Thorax 55 (Suppl. 1):S2-10.

Stromberg LG, Ludvigsson GJ and Bjorksten B (1995); Atopic allergy and delayed hypersensitivity in children with diabetes. J Allergy Clin Immunol 96:188-92.

Svanes C, Jarvis D, Chinn S, Omenaas E, Gulsvik A and Burney P (2002); Early exposure to children in family and day care as related to adult asthma and hay fever: results from the European Community Respiratory Health Survey. Thorax 57:945-50.

Takeda K, Kaisho T and Akira S (2003); Toll-like recptors. Annu Rev Immunol 21:335-76.

The EURODIAB substudy 2 study group (2000); Decreased prevalence of atopic diseases in children with diabetes. J Pediatr 137:470-4.

Theofilopoulos AN, Dummer W and Kono DH (2001); T cell homeostasis and systemic autoimmunity. J Clin Invest 108:335-40.

Tirosh A, Mandel D, Mimouni FB, Zimlichman E, Shochat T and Kochba I (2006); Autoimmune diseases in asthma. Ann Intern Med 144:877-83.

Thomsen SF, Duffy DL, Kyvik KO, Skytthe A and Backer V (2001); Relationship between type 1 diabetes and atopic diseases in a twin population. Allergy 66:645-7.

Upchurch S, Harris JM and Cullinan P (2010); Temporal changes in UK birth order and the prevalence of atopy. Allergy 65:1039-41.

Van den Biggelaar AH, Rodriques LC, van Ree R, van der Zee JS, Hoeksma-Kruize YC, Souverijn JH, Missinou MA, Borrmann S, Kremsner PG and Yazdanbakhsh M (2004); Long-term treatment of intestinal helmiths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 189:892-900.

Vartiainen E, Petäys T, Haahtela T, Jousilahti P and Pekkanen J (2002); Allergic diseases, skin prick test responses, and IgE levels in North Karelia, Finland, and the republic of Karelia, Russia. J Allergy Clin Immunol 109:643-8.

Wegienka G, Johnson CC, Havstad S, Ownby DR, Nicholas C and Zoratti EM (2011); Lifetime dog and cat exposure and dog- and cat-specific sensitization at age 18 years. Clin Exp Allergy 41:979-86.

Veiga RV, Cunha SS, Dattoli VCC, Cruz ÁC, Cooper PJ, Rodriques LC, Barreto ML and Alcantara-Neves NM (2011); Chronic virus infections supress atopy but not asthma in a set of children from a large latin american city: a cross sectional study. BMC Pulm Med 11:24.

Weiland SK, von Mutius E, Hüsing A, Asher MI, on behalf of the ISAAC steering committee (1999): Intake of trans fatty acids and prevalence of asthma and allergies in Europe. Lancet 353:2040-1.

Weiland SK, Hüsing A, Strachan DP, Rzenak P, Pearce N, and the ISAAC phase one study group (2004); Climate and the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema in children. Occup Environ Med 61:609-15.

Wickens K, Crane J, Pearce N and Beasley R (1999); The magnitude of the effect of smaller family sizes on the increase in the prevalence of asthma and hay fever in the United Kingdom and New Zealand. J Allergy Clin Immunol 104:554-8.

Viskari H, Ludvigsson J, Uibo R, Salur L, Marciulionyte D, Hermann R, Soltesz G, Füchtenbusch M, Ziegler AG, Kondrashova A, Romanov A, Kaplan B, Laron Z, Koskela P, Vesikari T, Huhtala H, Knip M and Hyöty H (2005); Relationship between the incidence of type 1 diabetes and maternal enterovirus antibodies: time trends and geographical variation. Diabetologia 48:1280-7.

Wjst M, Dold S, Reitmeir P, Fritzsch C, von Mutius E and Thiemann HH (1994); Pertussis infection and allergic sensitization. Ann Allergy 73:450-4.

von Ehrenstein OS, von Mutius E, Illi S, Baumann L, Böhm O and von Kries M (2000); Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy 30:187-93.

von Hertzen L and Haahtela T (2004); Asthma and atopy – the prize of affluence? Allergy 59:124-37.

von Hertzen LC, Laatikainen T, Mäkelä MJ, Jousilahti P, Kosunen TU, Petäys T, Pussinen PJ, Haahtela T and Vartiainen E (2006); Infectious burden as a determinant of atopy – a comparison between adults in Finnish and Russian Karelia. Int Arch Allergy Immunol 140:89-95.

von Hertzen LC, Pekkarinen P, Laatikainen T, Mäkelä MJ and Haahtela T for the Karelian allergy study group (2007a); Herpes simplex virus and atopy in Finnish and Russian Karelian children. Eur Respir J 30:809-10.

von Hertzen L, Laatikainen T, Pitkänen T, Vlasoff T, Mäkelä MJ, Vartiainen E and Haahtela T (2007b); Microbial content of drinking water in Finnish and Russian Karelia – implications for atopy prevalence. Allergy 62:288-92.

von Hertzen L, Mäkelä MJ, Petäys T, Jousilahti P, Kosunen TU, Laatikainen T, Vartiainen E and Haahtela T (2006); Growing disparities in atopy between the Finns and the Russians: A comparison of 2 generations. J Allergy Clin immunol 117:151-7.

von Mutius E and Vercelli D (2010); Farm living: effects on childhood asthma and allergy. Nat Rev Immunol 10:861-8.

von Mutius E, Braun-Fahrländer C, Schierl R, Riedler J, Ehlermann S, Maisch S, Waser M and Nowak D (2000); Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin Exp Allergy 30:1230-4.

Von Mutius E, Pearce N, Beasley R, Cheng S, von Ehrenstein O, Björkstén B, and Weiland S on behalf of the ISAAC steering committee (2000); International pattern of tuberculosis and the prevalence of symptoms of asthma, rhinitis, and eczema. Thorax 55:449-53.

Välimäki AL and Rauhala PL (2000); Lasten päivähoidon taipuminen yhteiskunnallisiin murroksiin Suomessa. Yhteikuntapolitiikka 65:387-405.

Zhu J, Yamane H and Paul WE (2010); Differentation of effector CD4 T cell populations. Annu Rev Immunol 28:445-89.

Zhu J and Paul WE (2010); Heterogeneity and plasticity of T helper cells. Cell Res 20:4-12.

Zöllner IK, Weiland SK, Piechotowski I, Gabrio T, von Mutius E, Link B, pfaff G, Kouros B and Wuthe J (2005); No increase in the prevalence of asthma, allergies, and atopic sensitisation among children in Germany: 1992-2001. Thorax 60:545-548.

16. ORIGINAL PUBLICATIONS

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2007.03333.x

Allergic sensitization and microbial load – a comparison between Finland and Russian Karelia

T. Seiskari,* A. Kondrashova,*†‡ H. Viskari,*†§ M. Kaila,¶** A.-M. Haapala,§ J. Aittoniemi,§ M. Virta,†† M. Hurme,§†† R. Uibo,‡‡ M. Knip,†§§¶¶ H. Hyöty*†§ and The EPIVIR study group***

*Department of Virology, University of Tampere, Finland, †JDRF Centre for Prevention of Type 1 Diabetes in Finland, ‡Department of Paediatrics, University of Petrozavodsk, Russia, §Department of Clinical Microbiology, Centre for Laboratory Medicine, Pirkanmaa Hospital District, Tampere, Finland, ¶Centre for General Practice, Pirkanmaa Hospital District, Tampere, Finland, **Paediatric Research Centre University of Tampere, Finland, ††Department of Microbiology and Immunology, University of Tampere, Finland, ‡‡Department of Immunology, University of Tartu, Estonia, §§Hospital for Children and Adolescents, University of Helsinki, Finland, and ¶¶Department of Paediatrics, Tampere University Hospital, Finland

Accepted for publication 3 January 2007
Correspondence: Tapio Seiskari, Department of Virology, University of Tampere, Biokatu 10, 33520 Tampere, Finland.
E-mail: tapio.seiskari@uta.fi

***For the list of EPIVIR Study Group contributors, please see the list at the end of the paper.

Summary

Epidemiological data have indicated that some infections are associated with a low risk of allergic diseases, thus supporting the idea (hygiene hypothesis) that the microbial load is an important environmental factor conferring protection against the development of allergies. We set out to test the hygiene hypothesis in a unique epidemiological setting in two socio-economically and culturally markedly different, although genetically related, populations living in geographically adjacent areas. The study cohorts included 266 schoolchildren from the Karelian Republic in Russia and 266 schoolchildren from Finland. The levels of total IgE and allergen-specific IgE for birch, cat and egg albumen were measured. Microbial antibodies were analysed against enteroviruses (coxsackievirus B4), hepatitis A virus, *Helicobacter pylori* and *Toxoplasma gondii*. Although total IgE level was higher in Russian Karelian children compared to their Finnish peers, the prevalence of allergen-specific IgE was lower among Russian Karelian children. The prevalence of microbial antibodies was, in turn, significantly more frequent in the Karelian children, reflecting the conspicuous difference in socio-economic background factors. Microbial infections were associated with lower risk of allergic sensitization in Russian Karelian children, enterovirus showing the strongest protective effect in a multivariate model. The present findings support the idea that exposure to certain infections, particularly in childhood, may protect from the development of atopy. Enterovirus infections represent a new candidate to the list of markers of such a protective environment. However, possible causal relationship needs to be confirmed in further studies.

Keywords: allergy, atopy, bacteria, viruses, viral immunity, immunoglobulins

Introduction

In recent decades an increase in the prevalence of allergic diseases such as allergic rhinitis and eczema has been documented in developed countries [1–3]. There is also accumulating evidence that the prevalence of atopy, as measured by skin prick testing and specific IgE, is increasing [4–6]. Several studies have revealed an East–West gradient in the prevalence of symptoms of allergic diseases [7–11], as well as in the prevalence of atopy across European countries [9–11].

A series of environmental factors has been proposed to account for the increasing prevalence of allergic diseases and the variation between geographically adjacent areas. An inverse association between the number of siblings and allergic diseases has been documented in epidemiological surveys [12,13]. Growing up on a farm seems to be associated with a lower prevalence of allergic rhinitis and sensitization [11,14,15]. Children who do not live on a farm but have regular contact with livestock had also a lower prevalence of allergic sensitization [14]. The underlying reasons behind these associations are largely unknown, but the 'hygiene hypothesis' provides a possible explanation suggesting that exposure to a variety of microbes in childhood protects against atopic diseases

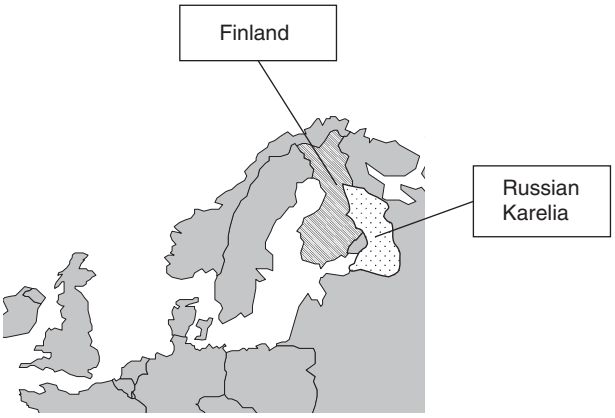


Fig. 1. Geographic location of the two study areas.

by promoting the maturation of the immune system [13,16].

Several studies have suggested a role for T helper 2 (Th2)-polarized $CD4^+$ T cells in the pathogenesis of asthma and allergy [17–19], but the exact immunological mechanisms regulating allergic sensitization are not known. Th1-biased immune responses may down-regulate the effects of Th2 cells [17] or regulatory T cells may control the Th1/Th2 balance [18,19]. There is also epidemiological evidence that some infections, such as hepatitis A virus (HAV) [20–23] *Toxoplasma gondii* [22,23] and *Helicobacter pylori* [4,22,23], and bacterial components [24–26] are associated with reduced risk of atopic diseases, thus supporting the hypothesis that the microbial load is an important environmental factor conferring protection against the development of allergies in childhood [27].

The objective of this study was to investigate variations and associations in the prevalence of microbial infections and allergic sensitization in children in Finland and Russia, in order to test the hygiene hypothesis in a unique epidemiological setting in two socio-economically and culturally markedly different, although geographically adjacent, areas (Fig. 1).

The Karelian Republic is situated in the north-western part of Russia adjacent to the Finnish border. Even though these two areas share the same geographical and climatic environment, they differ markedly in culture, economy and standards of living (e.g. the gross national product per capita is about US$32 790 in Finland compared to US$3410 in Russia). Consequently, many lifestyle-related factors, such as the general level of hygiene and related exposures to infections and commensal microbes, are perceived to differ between these two regions. To test the effect of these presumed differences in hygiene levels and microbial load on the risk of allergic sensitization, we analysed the prevalence of microbes that are considered commonly to reflect the level of hygiene, such as HAV, enterovirus, *H. pylori* and *T. gondii* infections as well as allergen-specific and total IgE in the general population (schoolchildren) in these two regions. This unique epidemiological setting makes it possible to study the role of environmental factors in allergic sensitization, particularly because the two study populations had similar ethnical and genetic background, the children from Russian Karelia being of Finnish–Karelian ancestry.

Methods

Subjects

Both the Russian Karelian study cohort and the Finnish study cohort comprised 266 schoolchildren. The children represented the mainstream populations and were not selected according to possible allergic or other diseases. All the children from the Karelian Republic had both parents of either Finnish or Karelian ethnicity, which confers an ethnic background close to that of the children in Finland [28]. Altogether, the study cohorts included 114 boys and 152 girls from each country. The mean age at sampling was 11·4 years (range 7–15 years) in both cohorts.

The Karelian cohort was recruited as a part of the type 1 diabetes-related EPIVIR-project (EU INCO-Copernicus programme, contract number IC15-CT98-0316, Coordinator Professor Hyöty). In this project blood samples were taken from a total of 1988 randomly selected schoolchildren in Karelia in the period 1997–99. The ethnic backgrounds of both the mother and father were recorded, and all children whose both parents were of either Finnish or Karelian ethnicity were included in the present study ($n = 266$). Samples were taken during the months March, April and May. For the Karelian children, a cohort of Finnish children was matched pairwise by age, gender and date of the sample (no more than 1 month apart), thus minimizing the effect of the season on exposure to microbes and allergens. The Finnish cohort was recruited in the same way as the Karelian cohort and initially included 3654 schoolchildren living in the Oulu region of Finland [29]. Blood samples were taken in 1994. All children had parental consent to participate in the study. The study was approved by the ethical committee of the Faculty of Medicine, University of Oulu, Finland, and by the Ministry of Health in the Karelian Republic of Russia.

IgE and microbial antibodies

The levels of total IgE and allergen-specific IgE were measured using the UniCAP® fluoroenzyme immunoassay (Pharmacia Diagnostics, Uppsala, Sweden). Specific IgE for two common inhalant allergens (birch and cat) and for egg albumin was analysed according to the manufacturer's instructions. These allergens were selected because the exposure to them can be expected to be quite similar in both populations (e.g. mite allergens were not included because the strength of exposure may differ). For allergen-specific IgE, values of 0·35 IU/l or more were considered positive. In previous studies total IgE values exceeding 100 IU/l have

Table 1. Total IgE levels and the prevalence (% and 95% CI) of allergen-specific IgE in schoolchildren in Finland and Russian Karelia.

	Finland ($n = 266$)	Russian Karelia ($n = 266$)	P-value
Total IgE			
Median IU/l (range)	39 (0–3328)	77 (2–3522)	< 0·001
> 100 IU/l (%)	26 (21–32)	40 (34–46)	0·001
Cat (%)	11 (8–16)	2 (1–5)	< 0·001
Birch (%)	11 (8–16)	2 (1–5)	< 0·001
Egg albumen (%)	6 (4–10)	3 (2–6)	0·093
At least one positive (%)	22 (17–27)	6 (4–10)	< 0·001

been considered as markers of atopic predisposition [9]. Microbial antibodies were analysed against coxsackievirus B4 (representing enteroviruses), HAV, *H. pylori* and *T. gondii*. IgG class enterovirus antibodies were measured using enzyme immunoassay (EIA) against a highly purified coxsackievirus B4 antigen (CBV4), as described previously [30]. IgG class HAV antibodies were measured using Enzygnost® anti-HAV commercial EIA kit, IgG class *H. pylori* antibodies Enzygnost® anti-*H. pylori*/IgG assay and *Toxoplasma* IgG by Enzygnost® toxoplasmosis IgG assay, according to the manufacturer's instructions (Dade Behring, Marburg, Germany). A Behring enzyme-linked immunosorbent assay (ELISA) Processor III was used for further processing of the tests and for the calculation of the antibody levels.

Statistical methods

Statistical analyses were performed with the SPSS program version 12·0 (SPSS Inc., Chicago, IL, USA) and confidence interval analyses (CIA) [31]. Prevalence of specific IgE, high values (> 100 IU/l) of total IgE and microbial antibodies between two paired cohorts was compared using McNemar's test. Comparisons of total IgE levels (skewly distributed continuous variable) between paired cohorts were performed using Wilcoxon's signed-ranks test. Cross-tabulation and χ^2 test or Fisher's exact test were applied for the analyses of associations between microbial antibodies, high values of total IgE and specific IgE in Russian Karelia and Finland. The Mann–Whitney *U*-test was used when associations between total IgE levels and specific IgE (classified as positive or negative) were analysed and also in the analyses of associations between CBV4 IgG levels and specific IgE. As a multivariate technique, logistic regression was applied to identify the independent effect of each parameter when appropriate. The model selection was based on a forward stepwise procedure, where the limit to enter and to remove the term was equal to 0·10. The results are supported by the assessment of odds ratio (OR) and 95% confidence intervals (CI). If there were missing or indifferent values, cases were not included in the analyses involving those particular parameters. The number of such cases was small; for example, for microbial serologies in Russian Karelian children, 0–2 missing cases per each microbe analysed. All analyses were two-sided. Statistically significant P-values (< 0·05) are given.

Results

Total IgE concentrations and the prevalence of allergen-specific IgE antibodies among Finnish and Russian Karelian subjects are shown in Table 1. Total IgE levels and the frequency of high total IgE were significantly higher in Russian Karelian than in Finnish children. In contrast, the prevalence of allergen-specific IgE was significantly lower in Russian Karelian children. In both geographical areas total IgE was significantly higher among children who were positive for at least one allergen than in children with no specific IgE responses, median IU/l-values (range) being 140 (12–3328) and 29 (0–2842) in Finland ($P < 0·001$) and 460 (31–3522) and 70 (2–2678) in Russian Karelia ($P < 0·001$), respectively.

The prevalence of all microbial antibodies was significantly higher in children in Russian Karelia than in children in Finland (Table 2). In addition, in Russian Karelia allergic sensitization was more rare in children who had a high number of microbial antibodies (Table 3). Only 1·2% (1/85) of the children who were seropositive for more than two microbes of the four tested had at least one positive specific IgE compared to 9·1% (16/176) of those who were

Table 2. Prevalence (% and 95% CI) of microbial antibodies in schoolchildren in Finland and Russian Karelia.

	Finland ($n = 266$)	Russian Karelia ($n = 266$)	P-value
Coxsackievirus B4	77 (72–82)	93 (90–96)	< 0·001
Helicobacter pylori (%)	5 (3–8)	73 (64–78)	< 0·001
Toxoplasma gondii (%)	5 (3–9)	24 (19–29)	< 0·001
Hepatitis A virus (%)	2 (1–5)*	24 (19–29)	< 0·001

*Only 166 Finnish children were screened for HAV antibodies.

Table 3. Proportion of children (% and 95% CI) positive for at least one allergen-specific IgE in relation to seropositivity for microbial antibodies in schoolchildren in Russian Karelia.

				Logistical model*	
	Microbe-seropositive	Microbe-seronegative	*P*-value	OR (95% CI)	*P*-value
Coxsackievirus B4	13/247 (5%; 3–9%)	4/18 (22%; 9–45%)	0·020	0·16 (0·04–0·6)	0·006
Helicobacter pylori	9/194 (5%; 3–9%)	8/72 (11%; 6–20%)	0·055	0·33 (0·1–0·9)	0·037
Toxoplasma gondii	1/64 (2%; 0–9%)	16/201 (8%; 5–13%)	0·082		n.s.
Hepatitis A virus	5/63 (8%; 3–17%)	12/201 (6%; 3–10%)	0·579		n.s.

n.s.: Not significant. *Forward stepwise model (*P* for entry and removal 0·10).

seropositive for fewer microbes (OR: 0·12; 95% CI: 0·016–0·91; $P = 0·015$). When the logistic regression analysis was applied to identify the independent effect of each microbial seropositivity, that of enterovirus (CBV4) had the strongest effect on allergic sensitization. A similar trend was also observed for *H. pylori* but not for *T. gondii* and HAV. For example, 22% of the children who were enterovirus seronegative had at least one positive specific IgE result compared to 5% of seropositive children. The median enterovirus antibody level was 74 enzyme immunoassay units (EIU) (range: 0–224) in children who had no specific IgE compared to 49 EIU (range: 0–154) in those who had at least one allergen-specific IgE ($P = 0·048$).

In Finland the number of *H. pylori*, *T. gondii* and HAV seropositive children was very low (Table 2), which made it difficult to analyse their association with allergen-specific IgE. However, enterovirus antibodies were frequent in the Finnish children but, in contrast to those in Russian Karelia, they showed no association with allergen-specific IgE responses (18% of the enterovirus seronegative children had at least one positive specific IgE result compared to 23% of the seropositive children).

In Russian Karelia the total IgE levels were higher in children who were seropositive for *T. gondii* compared to seronegative children: 51% (95% CI: 39–63%) *versus* 36% (95% CI: 30–43%) of the children had IgE > 100 IU/l, respectively ($P = 0·041$). Other microbial antibodies showed no association with total IgE. In Finland, none of the microbial antibodies correlated with total IgE, but the number of seropositive children was too small for proper comparisons (except for children positive for enterovirus antibodies).

Discussion

In the present study, the prevalences of microbial antibodies perceived as markers of microbial exposure and poor hygiene were significantly higher in Russian Karelian children than in Finnish children. Among the microbes analysed in this study, HAV, *H. pylori* and *T. gondii* have been linked previously to a reduced risk of atopy [21–23]. In line with this, we observed that atopic sensitization was indeed significantly less common in Russian Karelian children than in Finnish children, and the number of positive microbial serologies correlated inversely with the prevalence of allergic sensitization to the tested allergens in the children in Russian Karelia. In addition to the humoral immune response to the three above-mentioned microbes, we also measured antibodies against enteroviruses, which are transmitted mainly through the faecal–oral route and can be used as an indicator of hygiene and overcrowding. Interestingly, the strongest inverse association in this study was observed between the prevalence of enteroviruses (CBV4) and atopy in Karelia. On the other hand, HAV had no protective effect at all, which is in contrast with some previous surveys [21–23]. Accordingly, the results support the hygiene hypothesis suggesting that certain microbial infections are associated with lower risk of atopic sensitization. We found that enteroviruses may represent a new candidate for a marker of such a protective environment.

In previous studies, food-borne and faecal–oral microbes such as HAV, *T. gondii* and *H. pylori* rather than airborne viruses have been related to reduced risk of atopy [22,23,27]. On the other hand, seropositivity for intestinal bacterial pathogens (*Clostridium difficile*, *Campylobacter jejuni* and *Yersinia enterocolitica*) was associated with a higher prevalence of atopy among Danish adults [23]. Moreover, an independent inverse association was observed between the number of gastrointestinal infections before the age of 5 years and the risk of atopy in the United Kingdom [32]. Recently, Benn *et al.* reported that infectious diseases during the first 6 months of life (mainly upper respiratory infections) increase the risk of atopy [33], and Bager and collaborators observed a growing risk of atopy with an increasing number of infections caused by airborne viruses (measles, rubella, mumps and varicella) before the age of 1 year [34]. In the present study HAV infections, which are transmitted through the faecal–oral route, were not associated with protection against atopic sensitization, while other microbes with faecal–oral transmission showed a protective effect (e.g. enteroviruses). This suggests that there may be microbe-specific effects possibly linked to other microbe–host interactions than the transmission route *per se,* or that there are only certain specific food-borne or faecal–oral infections which are relevant and which still remain uncovered.

The fact that an association between infections and prevalence of atopy was observed only in Russian Karelian children but not in the Finnish children could be explained by the assumption that Finnish children are infected at an older

age, as the circulation of enteroviruses and other microbes is conspicuously lower in Finland. According to the hygiene hypothesis, infections occurring during the first months of life may be the most important ones, as at this age they can have a marked effect on the maturation of the gut-associated immune system and the developing Th1/Th2 balance. On the other hand it is also possible that some other factors, which are associated with these infections in the Russian but not in the Finnish population, are responsible for the effect. Thus, the effect of enteroviruses is not necessarily specific, but may be linked to some unknown factors which are not present in the Finnish population. In addition, enterovirus infections may have a different pattern of transmission in Russia compared to Finland, due to differences in the hygiene levels (for example, in Russia the faecal–oral transmission route may predominate, whereas respiratory transmission may be more common in Finland). At this point it remains open whether associations between infections and risk of atopy observed in Russian Karelian children are causal or merely reflect other environmental factors that play a role in the development of atopy.

The two study cohorts comprised children of similar ancestry, which minimizes the possible confounding effect of genetic factors on the risk of atopy and makes it possible to analyse the role of environmental factors in atopic sensitization. In a previous study we have shown that the distributions of human leucocyte antigen (HLA-DQ) genotypes does not differ between the two populations [28] supporting their genetic relationship. However, we cannot exclude small differences in the frequency of non-HLA genes, which may have an influence on microbe–host interactions, even though it is unlikely that such small variations in allele frequencies could explain the observed marked difference in allergic sensitization between the two populations.

The fact that samples from Russian Karelia and Finland were taken during different years might affect the results. However, the samples from the Finnish children were collected before the samples from the Russian Karelia children. Assuming that the prevalence of allergy is increasing [4] and that the prevalence of microbial infections is decreasing in Finland [4,35], it might be expected that the temporal interval of about 4 years between the sample collections would diminish rather than increase the observed differences between the two regions. Hypothetically, it is also possible that atopic children have a defective immune response against microorganisms, which could lead to false seronegativity in microbe antibodies. This kind of defective immune response has been described in vaccine studies [36].

Our results are in line with earlier observations suggesting lower prevalence of allergy, as measured by skin prick test or IgE concentrations to common aeroallergens, in Russian Karelian than in Finnish subjects [9,11,37]. Thus, we were able to replicate this observation, and found that it correlates with the difference in microbial exposures. In contrast to allergen-specific IgE, the levels of total IgE showed an opposite difference between the regions compared, being significantly higher in Russian Karelia. The reason for the high levels of total IgE in a region characterized by a low prevalence of allergic sensitization is not clear. In the present study we observed that high total IgE levels were associated with *T. gondii* infections, suggesting that the difference in total IgE concentrations can be explained partly by the higher prevalence of parasite infections in Russian Karelia compared to Finland.

In conclusion, the present study shows that allergic sensitization is conspicuously more common in Finland than in Russian Karelia. These two populations are living in geographically adjacent areas and are related genetically, but they differ markedly for several other factors linked to the sharp gradient in prosperity across the border. This suggests that environmental factors related to these differences in standard of living between the two populations are important in allergic sensitization, and according to the present observations microbial infections may play an important role in this process. Thus, the results support the hygiene hypothesis and enterovirus infections represent a new candidate to be added to the list of markers of such a protective environment. This epidemiological setting provides unique new possibilities to study further the role of microbe–host interactions in atopic sensitization. Such studies are currently under way.

The EPIVIR study group

The EPIVIR study group includes the following researchers: H. Hyöty (coordinator), M. Knip and H. Viskari, University of Tampere, Finland; J. Ilonen, University of Turku, Finland, A. Reunanen, National Public Health Institute, Helsinki, Finland; R. Uibo (scientific coordinator), University of Tartu, Estonia; J. Ludvigsson, University of Linköping, Sweden, D. Marciulionyte, University of Kaunas, Lithuania; R. Hermann, G. Soltesz, University of Pécs, Hungary; M. Fuechtenbusch and A. Ziegler, Munich, Germany; A. Kondrashova and A. Romanov, University of Petrozavodsk, Russia.

Acknowledgements

This study received support from the European Union as a part of the EPIVIR project (INCO-Copernicus Program, contract number IC15-CT98-0316) and has been supported by grants from the Päivikki and Sakari Sohlberg Foundation, the Academy of Finland, the Tuberculosis Foundation in Tampere, the University of Tampere and the Medical Research Fund of Tampere University Hospital. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the data for the study and made the final decision to submit for publication.

References

1 Butland BK, Strachan DP, Lewis S, Bynner J, Butler N, Britton J. Investigation into the increase in hay fever and eczema at age 16 observed between the 1958 and 1970 British birth cohorts. BMJ 1997; **315**:717–21.

2 Ninan TK, Russell G. Respiratory symptoms and atopy in Aberdeen schoolchildren: evidence from two surveys 25 years apart. BMJ 1992; **304**:873–5.

3 Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T. Increasing prevalence of allergic rhinitis symptoms in an adult Danish population. Allergy 1999; **54**:1194–8.

4 Kosunen TU, Hook-Nikanne J, Salomaa A, Sarna S, Aromaa A, Haahtela T. Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections. Clin Exp Allergy 2002; **32**:373–8.

5 Linneberg A, Jorgensen T, Nielsen NH, Madsen F, Frolund L, Dirksen A. The prevalence of skin-test-positive allergic rhinitis in Danish adults: two cross-sectional surveys 8 years apart. The Copenhagen Allergy Study. Allergy 2000; **55**:767–72.

6 Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T. Increasing prevalence of specific IgE to aeroallergens in an adult population: two cross-sectional surveys 8 years apart: the Copenhagen Allergy Study. J Allergy Clin Immunol 2000; **106**:247–52.

7 Williams H, Robertson C, Stewart A *et al.* Worldwide variations in the prevalence of symptoms of atopic eczema in the international study of asthma and allergies in childhood. J Allergy Clin Immunol 1999; **103**:125–38.

8 Bjorksten B, Dumitrascu D, Foucard T *et al.* Prevalence of childhood asthma, rhinitis and eczema in Scandinavia and Eastern Europe. Eur Respir J 1998; **12**:432–7.

9 Vartiainen E, Petays T, Haahtela T, Jousilahti P, Pekkanen J. Allergic diseases, skin prick test responses, and IgE levels in North Karelia, Finland, and the Republic of Karelia, Russia. J Allergy Clin Immunol 2002; **109**:643–8.

10 Nicolai T, Bellach B, Mutius EV, Thefeld W, Hoffmeister H. Increased prevalence of sensitization against aeroallergens in adults in West compared with East Germany. Clin Exp Allergy 1997; **27**:886–92.

11 von Hertzen L, Mäkelä M, Petäys T *et al.* Growing disparities in atopy between the Finns and the Russians: a comparison of 2 generations. J Allergy Clin Immunol 2006; **117**:151–7.

12 Karmaus W, Botezan C. Does a higher number of siblings protect against the development of allergy and asthma? A review. J Epidemiol Commun Health 2002; **56**:209–17.

13 Strachan DP. Family size, infection and atopy: the first decade of the 'hygiene hypothesis'. Thorax 2000; **55** (Suppl. 1):S2–10.

14 Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin Exp Allergy 2000; **30**:194–200.

15 Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. Clin Exp Allergy 2000; **30**:201–8.

16 Strachan DP. Hay fever, hygiene, and household size. BMJ 1989; **299**:1259–60.

17 Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 1996; **17**:138–46.

18 Umetsu DT, Akbari O, Dekruyff RH. Regulatory T cells control the development of allergic disease and asthma. J Allergy Clin Immunol 2003; **112**:480–7.

19 Curotto de Lafaille MA, Lafaille JJ. CD4(+) regulatory T cells in autoimmunity and allergy. Curr Opin Immunol 2002; **14**:771–8.

20 McIntire JJ, Umetsu SE, Macaubas C *et al.* Immunology: hepatitis A virus link to atopic disease. Nature 2003; **425**:576.

21 Matricardi PM, Rosmini F, Ferrigno L *et al.* Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. BMJ 1997; **314**:999–1003.

22 Matricardi PM, Rosmini F, Riondino S *et al.* Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. BMJ 2000; **320**:412–7.

23 Linneberg A, Ostergaard C, Tvede M *et al.* IgG antibodies against microorganisms and atopic disease in Danish adults: the Copenhagen allergy study. J Allergy Clin Immunol 2003; **111**:847–53.

24 Gereda JE, Leung DY, Liu AH. Levels of environmental endotoxin and prevalence of atopic disease. JAMA 2000; **284**:1652–3.

25 Gereda JE, Leung DY, Thatayatikom A *et al.* Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. Lancet 2000; **355**:1680–3.

26 von Mutius E, Braun-Fahrlander C, Schierl R *et al.* Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin Exp Allergy 2000; **30**:1230–4.

27 Matricardi PM, Ronchetti R. Are infections protecting from atopy? Curr Opin Allergy Clin Immunol 2001; **1**:413–9.

28 Kondrashova A, Romanov A, Reunanen A *et al.* A six-fold gradient in the incidence of type 1 diabetes at the eastern border of Finland – evidence of a critical role of environment in the disease pathogenesis. Ann Med 2005; **37**:67–72.

29 Mäki M, Mustalahti K, Kokkonen J *et al.* Prevalence of celiac disease among children in Finland. N Engl J Med 2003; **348**:2517–24.

30 Salminen K, Sadeharju K, Lönnrot M *et al.* Enterovirus infections are associated with the induction of beta-cell autoimmunity in a prospective birth cohort study. J Med Virol 2003; **69**:91–8.

31 Altman D, Altman DG, Bryant T, Gardner M, Gardner MJ, Machin D. Statistics with confidence. London: BMJ Books, 2000.

32 Cullinan P, Harris JM, Newman Taylor AJ *et al.* Can early infection explain the sibling effect in adult atopy? Eur Respir J 2003; **22**:956–61.

33 Benn CS, Melbye M, Wohlfahrt J, Bjorksten B, Aaby P. Cohort study of sibling effect, infectious diseases, and risk of atopic dermatitis during first 18 months of life. BMJ 2004; **328**:1223.

34 Bager P, Westergaard T, Rostgaard K, Hjalgrim H, Melbye M. Age at childhood infections and risk of atopy. Thorax 2002; **57**:379–82.

35 Viskari H, Ludvigsson J, Uibo R *et al.* Relationship between the incidence of type 1 diabetes and maternal enterovirus antibodies: time trends and geographical variation. Diabetologia 2005; **48**:1280–7.

36 Arkwright PD, Patel L, Moran A, Haeney MR, Ewing CI, David TJ. Atopic eczema is associated with delayed maturation of the antibody response to pneumococcal vaccine. Clin Exp Immunol 2000; **122**:16–9.

37 Klemola T, St Masyuk V, von Hertzen L, Haahtela T. Occurrence of atopy among russian and finnish schoolchildren. Allergy 2004; **59**:465–6.

Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2009;150:83–88
DOI: 10.1159/000210384

Received: June 25, 2008
Accepted after revision: November 20, 2008
Published online: April 2, 2009

Time Trends in Allergic Sensitisation and *Helicobacter pylori* Prevalence in Finnish Pregnant Women

Tapio Seiskari[a, b] Hanna Viskari[a] Minna Kaila[c, d] Anna-Maija Haapala[b]
Pentti Koskela[e] Heikki Hyöty[a, b]

[a]Department of Virology, University of Tampere, [b]Department of Clinical Microbiology, Centre for Laboratory Medicine, Pirkanmaa Hospital District, [c]Finohta, STAKES, Satellite Office Tampere, and [d]Department of Paediatrics, Tampere University Hospital, Tampere, and [e]National Public Health Institute, Oulu, Finland

Key Words
Birth cohort · Environmental factors · *Helicobacter pylori* · Hygiene hypothesis · IgE antibodies · Pregnancy · Sensitisation

Abstract
Background: An increase in the prevalence of allergic conditions has been documented in Finland, correlating with the diminishing prevalence of *Helicobacter pylori* infections. We investigated whether the increase of allergic sensitisation still continues and correlates with the prevalence of *H. pylori* infections. ***Methods:*** The sera from 958 pregnant women in 1983, 1989, 1995 and 2001 were analysed for the presence of antibodies against *H. pylori*. In addition, allergen-specific IgE antibodies and total levels of IgE antibodies were measured. ***Results:*** A clear birth cohort effect was found in the prevalence of allergic sensitization: allergen-specific IgE was more frequent among recent birth cohorts than earlier ones ($p = 0.001$). The frequency of *H. pylori* antibodies followed the opposite trend ($p < 0.001$) and the increase in allergic sensitisation was only seen among *H. pylori*-negative women. A modest increase was also seen in allergic sensitisation between the 4 time series among the *H. pylori*-negative subjects ($p = 0.04$). Total IgE levels did not differ between birth cohorts or time series. ***Conclusion:*** The results suggest that hygiene-related environmental factors have played a role in the increase of allergic sensitisation during the last decades.

Introduction

An increase in the prevalence of immunoglobulin E-mediated atopy and allergic diseases has been documented in industrialised countries [1–4]. There is also evidence that in some countries this increase might have reached a plateau [5, 6]. Western lifestyle and urban environment have been related to atopy [4, 7–10], and environmental factors such as childhood exposure to microbes have been proposed to influence allergic sensitisation [7, 9, 11, 12]. Kosunen et al. [1] demonstrated an increase in the levels of allergen-specific IgE antibody from 1973 to 1994 in Finnish subjects, particularly in males. Moreover, they found an inverse relation between allergic sensitisation and antibodies against *Helicobacter pylori* [1].

We investigated this further in a different set of subjects. Our aim was to find out whether the increase in allergen-specific IgE levels has continued beyond the pe-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/09/1501–0083$26.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Tapio Seiskari
Department of Clinical Microbiology
Centre for Laboratory Medicine, Pirkanmaa Hospital District
Biokatu 4, FI–33521 Tampere (Finland)
Tel. +358 3 3117 3276, Fax +358 3 3117 5260, E-Mail tapio.seiskari@uta.fi

Table 1. The prevalence (%) of specific IgE antibodies against at least 1 of the tested allergens (cat, birch, egg albumin) in the years 1983, 1989, 1995 and 2001 among Finnish pregnant women with and without IgG antibodies against *H. pylori*

Subjects	Year				p value for trend	Adjusted odds ratio (95% CI)[1]
	1983	1989	1995	2001		
All	30/209 (14)	30/210 (14)	37/235 (16)	47/243 (19)	0.05	1.03 (1.0–1.06)
H. pylori +	6/59 (10)	7/42 (17)	8/56 (14)	4/47 (9)[2]	NS	
H. pylori –	24/149 (16)	22/163 (13)	28/172 (16)	43/196 (22)[2]	0.04	1.03 (1.0–1.07)

NS = Non-significant.

[1] Odds ratio for increase in 1 year. Analyses were carried out using a logistic regression model that excluded the effects of age and year of birth.

[2] The proportion of women having allergen-specific IgE differs between *H. pylori*-positive and -negative women in the year 2001 (8.5 vs. 21.9%, p = 0.036).

riod previously studied, and if this still correlates with the prevalence of *H. pylori* infections. In addition, we wanted to investigate whether this increase has also happened among Finnish women, resembling that previously reported in males.

Material and Methods

Subjects

For this study, coded (anonymous) serum samples were obtained from the FMC-serum bank of the Finnish National Public Health Institute (KTL). Since 1983, in Finland practically all (>98%) pregnant women have participated in screening for congenital infections (such as HIV, syphilis and hepatitis B) organised by KTL. The purpose of this is to find and prevent possible infectious diseases that would cause a threat to the health and life of the unborn child. Serum samples for screening are drawn from women at the maternity clinics during the first trimester of pregnancy (10–12 weeks of gestation). These sera are stored (–25°C) at the FMC-serum bank. By the end of 2001, the FMC-serum bank contained about 1,350,000 samples from 683,000 women.

A total of 958 randomly selected blood samples from pregnant Finnish women were included in the analysis. The breakdown of the numbers of samples and age of subjects by the year in which the sample was taken was as follows. 1983: n = 232, age 17–42 years (median 26); 1989: n = 240, age 16–42 years (median 28); 1995: n = 243, age 16–43 years (median 29), and 2001: n = 243, age 16–49 (median 29). Age distributions between these series were not equal, owing to an observed increase in median ages from 1983 to 2001. This is probably due to the increasing mean age of Finnish pregnant women [13]. Because of these observed differences, the series were further separated into the following age groups: less than 25 years, 25–30 and over 30. The median ages within these groups were 22, 27 and 33 years, respectively. In addition, we analysed subjects by their birth cohort, grouping the following years of birth: 1941–1949 (n = 34), 1950–1954 (n = 81), 1955–1959 (n = 163), 1960–1964 (n = 254), 1965–1969 (n = 198), 1970–1974 (n = 122), 1975–1979 (n = 71) and 1980–1985 (n = 35).

The study protocol was approved by the KTL ethical committee.

IgE and H. pylori *Antibodies*

Total IgE levels were measured by a microparticle enzyme immunoassay (IMx® Total IgE, Abbott Laboratories) according to the manufacturer's instructions.

The levels of allergen-specific IgE were measured using the ImmunoCap® fluoroenzyme immunoassay (Phadia Diagnostics, Uppsala, Sweden). Specific IgE for 2 common inhalant allergens (birch and cat) and for egg albumin was analysed according to the manufacturer's instructions. These allergens were selected because we wanted to include important representatives of animal and pollen allergens as well as food allergens in this study.

For each allergen-specific IgE, 0.35 kU/l was considered as the cut-off level for positive. In line with earlier studies, total IgE values exceeding 100 kU/l were considered high and possible markers of atopic predisposition [7, 14].

IgG antibodies against *H. pylori* were measured by an enzyme immunoassay (Pyloriset EIA-G III, Orion Diagnostica, Espoo, Finland) according to the manufacturer's instructions. Values 20 U/ml or more were considered to be positive.

Statistical Methods

Statistical analyses were performed with the SPSS program version 14.0 (SPSS Inc., Chicago, Ill., USA). Cross-tabulation and χ^2 tests were applied for the analyses of associations between classified variables. For these purposes, the presence of allergen-specific IgE in the sera was classified as having specific IgE against at least 1 of the tested allergens that exceeded the cut-off levels mentioned above. Kruskal-Wallis and Mann-Whitney tests were applied for the comparisons of continuous variables with skewed distributions against classified variables. As a multivariate technique, logistic regression was applied to identify the independent effect of each parameter when appropriate. In this, the model selection was based on a forward stepwise procedure, where the limit to enter and to remove the term was equal to 0.10.

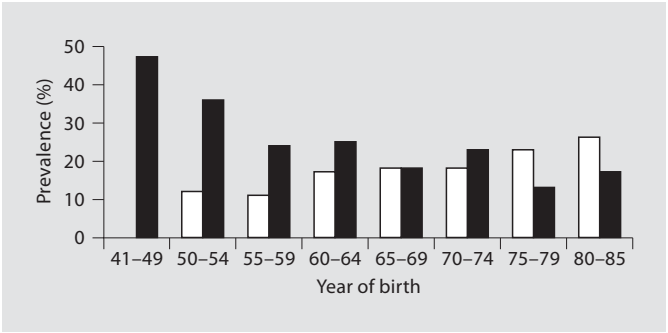


Fig. 1. Prevalence (%) of IgE antibodies against at least 1 of tested allergens (cat, birch and egg albumin) and IgG antibodies against *H. pylori* among pregnant Finnish women. Subjects were classified according to their year of birth. Blood samples were collected in years 1983, 1989, 1995 and 2001. White bars = allergen-specific IgE >0.35 kU/l (p = 0.001 for trend); black bars = *H. pylori* IgG >20 U/ml (p < 0.001 for trend).

Table 2. Prevalence (%) of IgG class *H. pylori* antibodies among different age groups of Finnish pregnant women

Age groups (years)	Year				All	p value for trend	Adjusted OR (95% CI)[1]
	1983	1989	1995	2001			
<25	20/86 (23)[2]	8/61 (13)	14/53 (26)	9/65 (14)	51/265 (19)	NS	
25–30	21/78 (27)	21/98 (21)	14/83 (17)	14/72 (19)	70/331 (21)	NS	
>30	29/65 (45)	21/75 (28)	28/100 (28)	24/106 (23)	102/346 (29)	0.003	0.95 (0.91–0.98)
All	70/229 (31)	50/234 (21)	56/236 (24)	47/243 (19)	223/942 (24)	0.002	0.96 (0.94–0.99)

NS = Non-significant.

[1] OR for increase in 1 year. Analyses were done by a logistic regression model that excluded the effect of age.

[2] Increase in *H. pylori* antibody prevalence with age: p = 0.002 for trend in all, p = 0.006 for trend in 1983 time series, p = 0.036 for trend in 1989 time series, non-significant for other time series.

If there were missing or indifferent values, cases were not included in the analyses involving those particular parameters. The number of subjects in different analyses may therefore differ. As an illustration, table 1 shows 210 subjects of the 1989 time series with valid allergen-specific IgE results, whereas 205 subjects of the same cohort have valid *H. pylori* test result. All testing was 2-sided. p < 0.05 was considered statistically significant.

Results

The proportion of the women positive for serum allergen-specific IgE increased during the study period (p = 0.05). Nevertheless, this increase was quite modest and happened only among women who lacked antibodies against *H. pylori* (table 1). Altogether, 12.3% of *H. pylori*-positive women had allergen specific IgE, compared to 17.2% of *H. pylori*-negative women (non-significant). However, in the last time series (2001) *H. pylori*-negative women had significantly higher prevalence of specific IgE than *H. pylori*-positive women (table 1).

Women in the earlier birth cohorts had a clearly lower prevalence of specific IgE compared to women who were born more recently (fig. 1). Furthermore, birth year had an effect on specific IgE prevalence. This effect was confirmed in a logistic regression model excluding the effects of age, *H. pylori* antibodies and year at the time of blood sampling (p = 0.001, OR for the effect of 1 year to the increase = 1.037, 95% CI 1.014–1.060). The effect of birth year on prevalence of specific IgE was evident among *H. pylori*-negative women (p = 0.001, OR for the effect of 1 year to the increase = 1.041, 95% CI 1.016–1.068). In contrast, it was not seen among *H. pylori*-positive women.

Older age groups had lower prevalence of specific IgE (>0.35 kU/l): 11.4% of women aged over 30 years had specific IgE, compared to 15.8% of women aged 25–30 years and 21.7% of women aged less than 25 years (p = 0.001 for trend). This age-dependent effect was strongest in the first time series collected in the year 1983: 7.4% of women aged over 30 had specific IgE, compared to 10.4% of those aged 25–30 years and 23.1% of those younger than 25

years ($p = 0.008$ for trend). Other time series also showed similar kinds of age trends (not statistically significant). The highest prevalence (26.2%) of allergen-specific IgE was observed among the youngest age group within the year 2001 cohort.

A clear decrease occurred in the prevalence of IgG antibodies against *H. pylori* between the years 1983 and 2001 (table 2). Analogously women who were born more recently had fewer *H. pylori* antibodies (fig. 1). The effect of birth year on prevalence of *H. pylori* antibodies was confirmed in a logistic regression model excluding the effects of the age and year at the time of blood sampling ($p < 0.001$, OR for the effect of 1 year to the increase = 0.957, 95% CI 0.939–0.976). *H. pylori* prevalence among women born between 1941 and 1955 was 39.1% (45/115), whereas among women born between 1975 and 1985 it was 14.6% (15/103, $p < 0.001$; fig. 1).

Total IgE levels did not correlate with *H. pylori* IgG. Altogether, 44.8% of women who had a total IgE value exceeding 100 kU/l had specific IgE against at least 1 of the tested allergens, compared to 8.7% among those with lower levels of total IgE ($p < 0.001$). High (at least 100 kU/l) total IgE levels were most frequent in the youngest age groups: 26.2% of women aged less than 25 years had a high value of total IgE, compared to 20.0% of those aged 25–30 years and 16.3% of those aged over 30 years ($p = 0.003$ for trend). When different time series were analysed separately, only the second time series showed a significant trend between the age groups: 32.8% of women aged less than 25 years had high total IgE, compared to 18.4% of those aged 25–30 years and 16.0% of those aged over 30 ($p = 0.021$ for trend). Other time series showed similar kinds of trends (not statistically significant). Total IgE levels showed no differences between different time series or birth cohorts.

Discussion

In the present study a clear birth cohort-effect was seen in the prevalence of allergen-specific IgE, indicating that allergic sensitization has kept increasing during the last decades. A similar increasing trend was also observed between different time series. Altogether, these findings do not herald a release from the growing burden of allergic diseases.

The prevalence of antibodies against *H. pylori* decreased between 1983 and 2001. The prevalence rate did not markedly decline by year of birth among women who were born after the 1960s. In contrast, this kind of decrease was seen in women born before the 1960s (fig. 1). In addition, we observed an inverse relation between the presence of *H. pylori* antibodies and allergic sensitisation in the most recent time series (year 2001).

H. pylori prevalence presumably reflects the standards of general hygiene [15]. Thus, the women without *H. pylori* antibodies are likely to represent a group that has lived in environment with high standards of hygiene. There may be some (yet unidentified) factors associated with poor hygiene that protect against allergic sensitisation. If so, the group of *H. pylori*-positive women might have been exposed to these protective factors. *H. pylori*-positive women would therefore be protected against allergy even in the current era when allergies are increasing due to the increasing proportion of population living in highly hygienic conditions. This may also be the explanation for our finding that allergic sensitisation has kept increasing among *H. pylori*-negative women but not among *H. pylori*-positive women. This finding is consistent with previous results by Kosunen et al. [1]. The small number of *H. pylori*-positive women in this study makes it difficult to find an increase in allergic sensitisation. However, as nothing suggested an increase in allergic sensitisation among *H. pylori*-positive women, it is unlikely that a larger sample size would have shown such trend.

The present study shows an increase in allergen-specific IgE among Finnish women in an era beyond the period previously studied. Kosunen et al. have reported an increase of allergen-specific IgE antibodies between 1973 and 1994 in Finland [1]. They measured IgE against 4 inhalant allergens (birch, timothy, cat and dog) in Finnish subjects aged 15–54 years, further distributed to age groups representing ages 15–24, 25–34, 35–44 and 45–54. Of these groups, only the youngest showed a statistically significant increase in the prevalence of allergen-specific IgE. This increase was most remarkable in the subgroup without antibodies against *H. pylori*. The increase was apparent among male subjects, whereas it was not statistically significant among females. Our results indicate that marked increase in allergic sensitisation has also occurred in females, resembling that previously reported in males.

Several studies have focused on the possible role of infections as factors providing protection against allergic sensitisation [7, 9, 11, 12, 16]. *H. pylori*, representing a chronic infection in gastric mucosa, might have microbe-specific potency in diminishing immune responses that lead to allergic sensitisation. *H. pylori* can induce production of IL-10. This suppresses T cell proliferation and cy-

tokine production and can therefore play a role in *H. pylori* persistence by damping the host's immune response [17]. IL-10 is also secreted by allergen-specific T regulatory cells and can suppress IgE production [18]. Moreover, *H. pylori* can suppress the production of IL-6 [17] and thereby promote differentiation of CD4 T cells into Foxp3+ regulatory T cells [19, 20]. On the other hand, in the whole study population allergic sensitisation among *H. pylori*-positive women was not significantly lower than among *H. pylori*-negative women. This suggests that possibly not *H. pylori* itself but some other associated factors may be protecting *H. pylori*-positive women from increasing allergic sensitisation. It is also possible that atopic children have a defective immune response against *H. pylori*, which could lead to false seronegativity in microbe antibodies. This kind of defective immune response has previously been described [21].

We observed higher total IgE levels among youngest age groups. This is in line with previous observations showing an inverse connection with allergic sensitisation and age [1, 3]. However, Jarvis et al. [22] demonstrated that there are only low net changes in sensitisation as the subjects grow older. Hence, the observed age-related differences in this study are probably due to subjects having been born in a different era. In fact, we observed that birth cohort had a stronger effect on the prevalence of allergen-specific IgE than did age.

The present study included only women. Nonetheless, no major differences have been reported between genders in allergic sensitisation, even though some studies have suggested higher prevalence of allergen-specific IgE among male subjects [1, 10]. As previously reported, the increase in the prevalence of allergic sensitisation in Finland during years 1973 to 1994 [1] was largely explained by an increase among male subjects. Atopic eczema is more common among females, while hay fever seems to affect more boys, with a change to a female predominance in adolescence [2, 23]. Furthermore, sex differences have been documented in total IgE levels, males having higher levels irrespectively of age or atopic status [24]. These sex differences can at least partly be explained by differences involving the secretion and function of sex hormones [23].

Pregnancy might have influenced IgE responses detected in the current series. In fact, the prevalence of specific IgE for cat (7.7%) and birch (11.1%) in the 1995 cohort is much higher than observed previously, in 1994 (3.6% for cat and 5.4% for birch) [1]. This might reflect the fact that during pregnancy immune responses may shift from Th-1 responses towards Th-2 responses [25]. Amoudrez et al. [26] showed that during pregnancy, there is a significantly higher spontaneous in vitro production of IL-1β, IL-6 and IL-10 by peripheral blood mononuclear cells and that total IgE levels are elevated. Hence, pregnancy must be considered as a potential confounding factor, which may have increased the number of IgE positives in the present study.

It is unlikely that all sensitised study subjects were identified using the present panel of 3 allergens in IgE measurements. Nevertheless, cat and birch allergens are among the clinically most relevant allergens in Finland. Furthermore, they are among the allergens most frequently causing sensitisation in Finland [14, 27, 28]. This suggests that the current panel can be used to detect changes in allergic sensitization in this kind of large epidemiological survey.

Theoretically, the long storage time of the samples may have caused the observed change in IgE levels. However, this is unlikely because earlier studies have shown that allergen-specific IgE sustains its stability during long-term storage at –20°C [3, 29].

In conclusion, our results indicate an increase in prevalence of allergic sensitisation among Finnish women. Moreover, certain environmental factors may provide protection against the development of allergy, and *H. pylori* infection possibly reflects this kind of environment. Our results provide no evidence of allergy epidemic reaching a plateau. In fact, the highest prevalence of allergen-specific IgE was observed among the most recent birth cohorts.

Acknowledgments

This study has received support from the Päivikki and Sakari Sohlberg Foundation, the Academy of Finland, the Tuberculosis Foundation in Tampere, the University of Tampere and the Medical research fund of Tampere University Hospital.

References

1 Kosunen TU, Hook-Nikanne J, Salomaa A, Sarna S, Aromaa A, Haahtela T: Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections. Clin Exp Allergy 2002;32:373–378.

2 Butland BK, Strachan DP, Lewis S, Bynner J, Butler N, Britton J: Investigation into the increase in hay fever and eczema at age 16 observed between the 1958 and 1970 British birth cohorts. BMJ 1997;315:717–721.

3 Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T: Increasing prevalence of specific IgE to aeroallergens in an adult population: Two cross-sectional surveys 8 years apart: the Copenhagen allergy study. J Allergy Clin Immunol 2000; 106:247–252.

4 von Hertzen L, Mäkelä M, Petäys T, Jousilahti P, Kosunen T, Laatikainen T, Vartiainen E, Haahtela T: Growing disparities in atopy between the Finns and the Russians: a comparison of 2 generations. J Allergy Clin Immunol 2006;117:151–157.

5 Zöllner IK, Weiland SK, Piechotowski I, Gabrio T, von Mutius E, Link B, Pfaff G, Kouros B, Wuthe J: No increase in the prevalence of asthma, allergies, and atopic sensitisation among children in Germany: 1992–2001. Thorax 2005;60:545–548.

6 Braun-Fahrländer C, Gassner M, Grize L, Takken-Sahli K, Neu U, Stricker T, Varonier HS, Wuthrich B, Sennhauser FH; Swiss Study on Childhood Allergy and respiratory symptoms, Air Pollution (SCARPOL) team: No further increase in asthma, hay fever and atopic sensitisation in adolescents living in Switzerland. Eur Respir J 2004;23:407–413.

7 Seiskari T, Kondrashova A, Viskari H, Kaila M, Haapala AM, Aittoniemi J, Virta M, Hurme M, Uibo R, Knip M, Hyöty H; the EPIVIR study group: Allergic sensitization and microbial load – a comparison between Finland and Russian Karelia. Clin Exp Immunol 2007;148:47–52.

8 Bjorksten B, Dumitrascu D, Foucard T, Khetsuriani N, Khaitov R, Leja M, Lis G, Pekkanen J, Priftanji A, Riikjarv MA: Prevalence of childhood asthma, rhinitis and eczema in Scandinavia and Eastern Europe. Eur Respir J 1998;12:432–437.

9 von Hertzen L, Haahtela T: Asthma and atopy – the price of affluence? Allergy 2004;59: 124–137.

10 Nicolai T, Bellach B, Mutius EV, Thefeld W, Hoffmeister H: Increased prevalence of sensitization against aeroallergens in adults in West compared with East Germany. Clin Exp Allergy 1997;27:886–892.

11 Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, Bonini S: Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. BMJ 2000;320:412–417.

12 Linneberg A, Ostergaard C, Tvede M, Andersen LP, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T: IgG antibodies against microorganisms and atopic disease in Danish adults: the Copenhagen allergy study. J Allergy Clin Immunol 2003;111: 847–853.

13 Kinnunen TI, Luoto R, Gissler M, Hemminki E: Pregnancy weight gain from 1960s to 2000 in Finland. Int J Obes 2003;27:1572–1577.

14 Vartiainen E, Petays T, Haahtela T, Jousilahti P, Pekkanen J: Allergic diseases, skin prick test responses, and IgE levels in North Karelia, Finland, and the Republic of Karelia, Russia. J Allergy Clin Immunol 2002;109: 643–648.

15 Malaty HM: Epidemiology of *Helicobacter pylori* infection. Best Pract Res Clin Gastroenterol 2007;21:205–214.

16 Bach JF: The effect of infections on susceptibility to autoimmune and allergic disease. N Engl J Med 2002;347:911–920.

17 Bergman MP, Engering A, Smits HH, van Vliet SJ, van Bodegraven AA, Wirth HP, Kapsenberg ML, Vandenbroucke-Grauls CM, van Kooyk Y, Appelmelk BJ: *Helicobacter pylori* modulates the T helper cell 1/T helper cell 2 balance through phase-variable interaction between lipopolysaccharide and DC-SIGN. J Exp Med 2004;200:979–990.

18 Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA: Mechanisms of immune suppression by interleukin-10 and transforming growth factor-β: the role of T regulatory cells. Immunology 2005;117:433–442.

19 Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy M: Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity 2006;24:677–688.

20 Cua DJ, Kastelein RA: TGF-β, a 'double agent' in the immune pathology war. Nat Immunol 2006;7:557–559.

21 Arkwright PD, Patel L, Moran A, Haeney MR, Ewing CI, David TJ: Atopic eczema is associated with delayed maturation of the antibody response to pneumococcal vaccine. Clin Exp Immunol 2000;122:16–19.

22 Jarvis D, Luczynska C, Chinn S, Potts J, Sunyer J, Janson C, Janson C, Svanes C, Kunzli N, Leynaert B, Heinrich J, Kerkhof M, Ackermann-Liebrich U, Anto JM, Cerveri I, de Marco R, Gislason T, Neukirch F, Vermeire P, Wjst M, Burney P: Change in prevalence of IgE sensitization and mean total IgE with age and cohort. J Allergy Clin Immunol 2005;116:675–682.

23 Osman M: Therapeutic implications of sex differences in asthma and atopy. Arch Dis Child 2003;88:587–590.

24 Barbee RA, Halonen M, Lebowitz M, Burrows B: Distribution of IgE in a community population sample: correlations with age, sex, and allergen skin test reactivity. J Allergy Clin Immunol 1981;68:106–111.

25 Williams DJ: Immunological changes during pregnancy; in Warrell DA, Cox TM, Firth JD, Benz EJ (eds): Oxford Textbook of Medicine, ed 4. Oxford, Oxford University Press, 2003, vol 2, p 384.

26 Amoudrez P, Minan TJ, Sundström Y, Nilsson C, Lilja G, Troye-Blomberg M, Sverremark-Ekstrom E: Pregnancy, but not allergic status, influences spontaneous and induced Interleukin-1β (IL-1β), IL-6, IL-10 and IL-12 responses. Immunology 2006;119:18–26.

27 Pekkarinen PT, von Hertzen L, Laatikainen T, Mäkelä MJ, Jousilahti P, Kosunen TU, Pantelejev V, Vartiainen E, Haahtela T: A disparity in the association of asthma, rhinitis, and eczema with allergen-specific IgE between Finnish and Russian Karelia. Allergy 2007;62:281–287.

28 von Hertzen LC, Laatikainen T, Pennanen S, Mäkelä MJ, Haahtela T; the Karelian Allergy Study Group: Is house dust mite monosensitization associated with clinical disease? Allergy 2008;63:379–381.

29 Paganelli R, Ansotegui IJ, Sastre J, Lange CE, Roovers MH, de Groot H, Lindholm NB, Ewan PW: Specific IgE antibodies in the diagnosis of atopic disease. Clinical evaluation of a new in vitro test system, UniCAP, in six European allergy clinics. Allergy 1998;53: 763–768.

Journal of Medical Virology 84:268–271 (2012)

Role of Enterovirus Infections in IgE Sensitization

Tapio Seiskari,[1,2]* Anita Kondrashova,[1,3] Sisko Tauriainen,[1] Mikael Knip,[4,5,6] Hanna Viskari,[1,7] Anna-Maija Haapala,[2] and Heikki Hyöty[1,2]

[1]*Department of Virology, University of Tampere, Tampere, Finland*
[2]*Department of Clinical Microbiology, Centre for Laboratory Medicine, Pirkanmaa Hospital District, Tampere, Finland*
[3]*Department of Pediatrics, University of Petrozavodsk, Petrozavodsk, Russia*
[4]*Children's Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland*
[5]*Folkhälsan Research Centre, Helsinki, Finland*
[6]*Department of Pediatrics, Tampere University Hospital, Tampere, Finland*
[7]*Department of Internal Medicine, Tampere University Hospital, Tampere, Finland*

Among other infectious agents, enteroviruses have been associated with protection against allergic diseases. The aim of the present study was to confirm these findings using a highly sensitive and specific neutralization antibody assay and to investigate whether the protective effect is related to certain enterovirus serotypes. Antibodies against 12 enterovirus serotypes were measured in 60 children who were positive for allergen-specific IgE and in 190 control children. Echoviruses seemed to be more protective than coxsackie-B-viruses and echovirus 11 had the strongest independent protective effect ($P = 0.001$; OR = 0.35, 95% CI: 0.18–0.67). The results support previous observations suggesting that infections by certain enterovirus types are associated with protection against IgE sensitization. ***J. Med. Virol. 84: 268–271, 2012.*** © 2011 Wiley Periodicals, Inc.

KEY WORDS: allergy; hygiene; infection; microbes; neutralizing antibodies

INTRODUCTION

Improved socioeconomic standards and diminishing exposure to microbes have been linked to the increasing prevalence of allergic diseases [von Hertzen and Haahtela, 2004; Seiskari et al., 2007]. This phenomenon has been studied previously in two neighboring populations living in contrasting socioeconomic environments in Russian Karelia and in Finland. An inverse relation between seropositivity to infectious agents and IgE sensitization was observed, enteroviruses showing the strongest protective effect among the microbes studied. Indeed, in Russian Karelia the prevalence of IgE sensitization was four times higher among enterovirus seronegative than among seropositive subjects [Seiskari et al., 2007]. The possible protective effect of enteroviruses is feasible biologically, since they replicate in the cells of the intestinal immune system, which is known to be crucial in the development of immunoregulatory responses, and further, they can also infect white blood cells including dendritic cells [Kramer et al., 2007; Lin et al., 2009].

In the present study, the association between enteroviruses and allergic sensitization was analyzed further. When previous studies were performed using EIA methods which detect antibodies against enteroviruses as a group, the present study applied a highly sensitive and specific plaque-neutralization assay to measure antibodies against individual enterovirus serotypes in children who were positive for allergen-specific IgE and in control children.

MATERIALS AND METHODS

Subjects

The study cohort was recruited in the Karelian Republic of Russia as a part of the type 1 diabetes—related EPIVIR-project as described in detail previously [Kondrashova et al., 2005; Kondrashova et al., 2007; Seiskari et al., 2010]. The present study cohort comprised 250 subjects including 60 schoolchildren who had tested positive for allergen-specific IgE in

Grant sponsor: Finnish Academy; Grant sponsor: Tampere Tuberculosis Foundation; Grant sponsor: The Päivikki and Sakari Sohlberg Foundation; Grant sponsor: EU (DIABIMMUNE project); Grant number: HEALTH-F2-202063.

*Correspondence to: Tapio Seiskari, MD, Department of Clinical Microbiology, Centre for Laboratory Medicine, Pirkanmaa Hospital District, P.O. Box 2000, FI-33521 Tampere, Finland.
E-mail: tapio.seiskari@uta.fi

Accepted 27 October 2011
DOI 10.1002/jmv.23186
Published online in Wiley Online Library (wileyonlinelibrary.com).

earlier studies [Seiskari et al., 2007, 2010] and 190 randomly selected IgE-negative schoolchildren representing the same age and gender distribution (mean age 12 years, range 8–15 years; 53% males). Twelve of the IgE-positive children had type 1 diabetes while all IgE-negative children were non-diabetic. The whole EPIVIR project included 2,070 non-diabetic children and adolescents who were randomly recruited in schools in Karelian Republic of Russia as well as a separate cohort of 132 children with type 1 diabetes who were recruited in the Children's Hospital, City of Petrozavodsk (capital of the Karelian Republic). All of the children had written parental consent to participate in the study. The study plan was approved by the ethical committee of the faculty of Medicine, University of Oulu, Finland, and by the Ministry of Health in the Karelian Republic of the Russian Federation. The reported investigations were carried out in accordance with the principles of the Declaration of Helsinki.

Neutralizing Antibodies Against Enteroviruses

The presence of neutralizing antibodies against 12 different enterovirus serotypes including coxsackievirus A 4 (CAV4), CAV9, coxsackievirus B1 (CBV1), CBV2, CBV3, CBV4, CBV5, CBV6, echovirus 9 (EV9), EV11, EV26, and EV30 was analyzed using the classical plague-neutralization assay as described previously [Roivainen et al., 1998; Viskari et al., 2005]. Serum dilution 1/4 was used to detect low levels of neutralizing antibodies, and the serum was considered antibody-positive if it blocked 80% or more of the virus infectivity. All virus strains were ATCC reference strains except CAV4, CBV3, and EV26, which were wild-type isolates from Finland.

Allergen-Specific IgE

The ImmunoCAP® fluoroenzyme immunoassay (Phadia Diagnostics, Uppsala, Sweden) was used for the measurement of allergen-specific IgE for two common inhalant allergens (birch and cat) and for egg albumin according to the manufacturer's instructions. Values of 0.35 kU/L or more were considered positive.

Statistical Analyses

Statistical analyses were performed with the SPSS program version 14.0 (SPSS Inc., Chicago, IL). As a multivariate technique, logistic regression was applied to identify the independent effect of each enterovirus type on IgE sensitization. The model selection was based on a forward stepwise procedure, and age and gender were included in these models. Since type 1 diabetes could represent a possible confounding factor, analyses were performed also without including the diabetic patients. The results are supported by the assessment of odds ratio (OR) and 95% confidence intervals (CI). Statistically significant P-values (<0.05) are given (all tests were two-sided).

RESULTS

Among the 60 IgE-positive children, 67% had IgE against cat allergen, 37% against birch allergen, and 27% against egg albumen. The prevalence of antibodies against different enterovirus serotypes showed conspicuous variation ranging from 21% for CBV6 to 92% for CAV4 (Table I). The EV11 serotype was significantly more frequent in non-sensitized children ($P = 0.001$) making it the strongest single protective serotype. None of the CBV serotypes was associated with protection against IgE sensitization. Only one virus (EV30) was more frequent in sensitized children (Table I). Having antibodies against several non-CBV serotypes other than EV30 was associated with lower risk of IgE sensitization ($P = 0.017$; OR = 0.73, 95% CI: 0.56–0.94, Fig. 1). This trend was even stronger among EV30-positive children ($P = 0.003$; OR = 0.57, 95% CI: 0.39–0.82). Furthermore, among EV30 positive children EV11 and CAV9 showed an independent protective effect ($P = 0.02$; OR = 0.35, 95% CI: 0.14–0.86, and $P = 0.003$; OR = 0.23, 95% CI: 0.086–0.61, respectively). The association between seropositivity for these viruses and IgE sensitization among EV30 positive children is shown in Figure 2.

Since diabetes could have a confounding effect, analyses were also done by excluding diabetic children from the cohort. EV11 seroprevalence was higher (70% vs. 46%) in non-sensitized children also when diabetic patients were not included ($P = 0.002$; OR = 0.002, 95% CI: 0.20–0.71). Similarly, excluding the diabetic patients did not change the positive association between EV30 antibodies and IgE sensitization: 45% of non-sensitized compared to 62% of sensitized children had neutralizing antibodies against EV30 ($P = 0.04$, OR = 2.0, 95% CI: 1.04–3.90).

TABLE I. Prevalence of Enterovirus Antibodies in Relation to Allergen-Specific IgE

Virus	IgE+ (N = 60) (%)	IgE− (N = 190) (%)	Adj. P-value	OR (95% CI)
CBV1	66	69	NS	
CBV2	68	58	NS	
CBV3	58	59	NS	
CBV4	65	65	NS	
CBV5	54	53	NS	
CBV6	21	21	NS	
CAV4	89	92	NS	
CAV9	61	72	NS	
Echo9	57	62	NS	
Echo11	52	70	0.001	0.35 (0.18–0.67)
Echo26	63	69	NS	
Echo30	63	45	0.015	2.2 (1.2–4.3)

Logistic regression model was applied in the analyses. NS = non-significant.

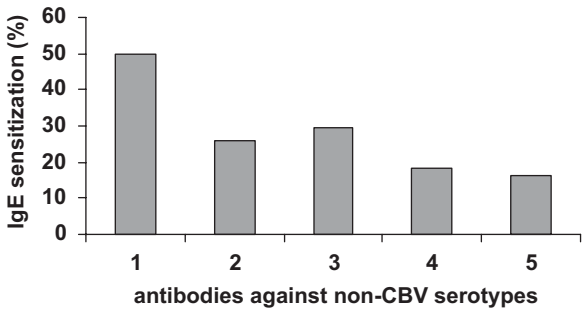


Fig. 1. Proportion (%) of subjects positive for allergen-specific IgE in relation to the number (1–5) of positive plaque-neutralization findings against non-CBV serotypes (Echovirus 30 not included, $P = 0.017$; OR = 0.73, 95%CI: 0.56–0.94).

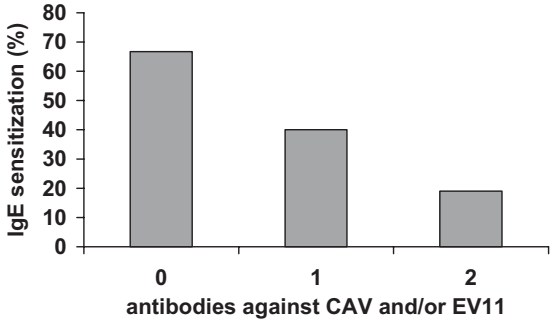


Fig. 2. Proportion (%) of IgE sensitized subjects among echovirus 30 seropositive subjects in relation to the presence of antibodies against both coxsackievirus A9 and echovirus 11 (2), against either one of these viruses (1), or against neither one of these viruses (0) ($P = 0.001$; OR = 0.35, 95% CI: 0.18–0.67).

DISCUSSION

The present study confirms previous findings suggesting that enteroviruses are associated with reduced risk of IgE sensitization. This study was also able to identify possible differences between different enterovirus types. Twelve common enterovirus serotypes representing different genetic subgroups were included and interesting differences were observed between the tested viruses. Viruses in the CBV group did not seem to associate with IgE sensitization, while EV11 had a protective effect. The whole non-CBV group appeared to have a protective effect also as a group.

Unlike any other enterovirus, EV30 was associated with increased risk of IgE sensitization. This may indicate that this virus can be different from all other viruses either biologically or epidemiologically. In fact, most EV30 infections have been shown to occur among young adults and older children [Khetsuriani et al., 2006], i.e., too late to protect against the development of IgE sensitization. Indeed, the timing of protective environmental exposures is crucial, the strongest effects being linked to exposures that occur in utero and during the first years of life [Riedler et al., 2001; Ege et al., 2006]. Consistent with this, infection of infants with human herpesvirus 6 has been reported to reduce IgE sensitization in young children [Nordström et al., 2010]. Furthermore, in a recent study Epstein–Barr virus infection before 2 years of age was protective against IgE sensitization, whereas infection after 2 years of age increased the risk [Saghafian-Hedengren et al., 2010]. This situation is also closely reflected by the results in animal models where LPS exposure prior to allergen challenge has a well-documented preventive effect. In contrast, LPS exposure at a later stage, when allergen sensitization has already been established, may exacerbate and promote the inflammatory response [Renz and Herz, 2002]. In contrast to EV30, EV11, and CAV9 predominantly affect young children aged less than 1 year [Khetsuriani et al., 2006]. Furthermore, EV30 is antigenically related with EV6, which may lead to crossreactivity in neutralization assay [Zeichhardt, 1986].

The biological basis of the possible protective effect conferred by certain enterovirus types is not known. One possibility is that it is related to immunomodulatory effect of these viruses. Indeed, enteroviruses induce strong regulative cytokine responses such as IL-10 [Hofmann et al., 2001], which may lead to bystander suppression of allergic responses. This kind of effect can be particularly powerful for enteroviruses, which replicate in the cells of the gut immune system where they may activate tolerogenic dendritic cells. Interestingly, previous studies suggest that CBV can not infect dendritic cells while at least some echoviruses and possibly other non-CBV viruses can cause productive infection in these cells [Kramer et al., 2007; Lin et al., 2009]. Accordingly, it is possible that the strong protective effect of certain non-CBV serotypes is associated with their specific interactions with the gut immune system. In addition, replication lasts quite long, usually several weeks, which can lead to long-term immunological changes.

However, in this type of epidemiological study the possibility cannot be excluded that the protective effect is due to some third factor which is linked to enterovirus infections. Poor hygiene could be one such factor, as enteroviruses are transmitted easily in non-hygienic conditions (fecal-oral transmission route). Nonetheless, if this was the case, one would expect to see that all enterovirus types should be similarly associated with protection against IgE sensitization.

The present findings support the hypothesis that poor hygiene may protect against allergy [Strachan, 1989, 2005]. In addition, the results indicate that there may be microbe-specific factors involved in this protection. If so, the investigation of such factors may open up new possibilities for clinical interventions to prevent the development of allergic diseases. Nevertheless, possible causal relationships and their mechanisms remain to be explored in further studies.

ACKNOWLEDGMENTS

The collection of the biobank has been funded by the EU as a part of the EPIVIR project (INCO-Copernicus Program, contract number IC15-CT98-0316). Skilful technical assistance from Anne Karjalainen was much appreciated.

REFERENCES

Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Üblagger E, Schram-Bijkerk D, Brunekreef B, van Hage M, Scheynius A, Pershagen G, Benz MR, Lauener R, von Mutius E, Braun-Fahrländer C, The PARSIFAL Study team. 2006. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J Allergy Clin Immunol 117:817–823.

Hofmann P, Schmidtke M, Stelzner A, Gemsa D. 2001. Suppression of proinflammatory cytokines and induction of IL-10 in human monocytes after coxsackievirus B3 infection. J Med Virol 64:487–498.

Kramer M, Schulte BM, Toonen LW, de Bruijni MA, Galama JM, Adema GJ, van Kuppeveld FJ. 2007. Echovirus infection causes rapid loss-of-function and cell death in human dendritic cells. Cell Microbiol 9:1507–1518.

Khetsuriani N, LaMonte-Fowlkes A, Oberste S, Pallansch M. 2006. Enterovirus surveillance—United States, 1970– 2005. MMWR Surveill Summ 55:1–20.

Kondrashova A, Reunanen A, Romanov A, Karvonen A, Viskari H, Vesikari T, Ilonen J, Knip M, Hyöty H. 2005. A six-fold gradient in the incidence of type 1 diabetes at the eastern border of Finland. Ann Med 37:67–72.

Kondrashova A, Viskari H, Kulmala P, Romanov A, Ilonen J, Hyöty H, Knip M. 2007. Signs of beta-cell autoimmunity in nondiabetic schoolchildren: A comparison between Russian Karelia with a low incidence of type 1 diabetes and Finland with a high incidence rate. Diabetes Care 30:95–100.

Lin YW, Wang SW, Tung YY, Chen SH. 2009. Enterovirus 71 infection of human dendritic cells. Exp Biol Med 234:1166–1173.

Nordström I, Rudin A, Adlerberth I, Wold A, Saalman R, Hesselmar B, Åberg N, Liljeqvist J-Å, Eriksson K. 2010. Infection of infants with human herpesvirus type 6 may be associated with reduced allergic sensitization and T-helper type 2 development. Clin Exp Allergy 40:882–890.

Renz H, Herz U. 2002. The bidirectional capacity of bacterial antigens to modulate allergy and asthma. Eur Resp J 19:158–171.

Riedler J, Braun-Fahrländer C, Eder W, Schreuer M, Waser M, Maisch S, Carr D, Schierl E, Nowak D, von Mutius E, The ALEX Study Team. 2001. Exposure to farming in early life and development of asthma and allergy: A cross- sectional survey. Lancet 358:1129–1133.

Roivainen M, Knip M, Kulmala P, Hiltunen M, Vähäsalo P, Hovi T, Åkerblom H, The Childhood Diabetes in Finland (DiMe) Study Group. 1998. Several different enterovirus serotypes can be associated with prediabetic autoimmune episodes and onset of overt IDDM. J Med Virol 56:74–78.

Saghafian-Hedengren S, Sverremark-Ekström E, Linde A, Lilja G, Nilsson C. 2010. Early-life EBV infection protects against persistent IgE sensitization. J Allergy Clin Immunol 125:433–438.

Seiskari T, Kondrashova A, Viskari H, Kaila M, Haapala AM, Aittoniemi J, Virta M, Hurme M, Uibo R, Knip M, Hyöty H, The EPIVIR study group. 2007. Allergic sensitization and microbial load - a comparison between Finland and Russian Karelia. Clin Exp Immunol 148:47–52.

Seiskari T, Viskari H, Kondrashova A, Haapala AM, Ilonen J, Knip M, Hyöty H. 2010. Co-occurrence of allergic sensitisation and type 1 diabetes. Ann Med 42:352–359.

Strachan DP. 1989. Hay fever, hygiene, and household size. BMJ 299:1259–1260.

Strachan DP. 2005. Family size, infection and atopy: The first decade of the 'hygiene hypothesis'. Thorax 55:S2–10.

Viskari H, Ludvigsson J, Uibo R, Salur L, Marciulionyte D, Hermann R, Soltesz G, Füchtenbusch M, Ziegler AG, Kondrashova A, Romanov A, Kaplan B, Laron Z, Koskela P, Vesikari T, Huhtala H, Knip M, Hyöty H. 2005. Relationship between the incidence of type 1 diabetes and maternal enterovirus antibodies: Time trends and geographical variation. Diabetologia 48:1280–1287.

von Hertzen L, Haahtela T. 2004. Asthma and atopy – the price of affluence? Allergy 59:124–137.

Zeichhardt H. 1986. In: Enteroviruses, Specter S, Lancz GJ, editors. Clinical virology manual. New York: Elsevier Science Publishing Company, Inc. pp 283–299.