Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2006.03235.x

Asthma onset prior to multiple sclerosis and the contribution of sibling exposure in early life

A.-L. Ponsonby,*† T. Dwyer,* I. van der Mei,† A. Kemp,‡ L. Blizzard,† B. Taylor,§ T. Kilpatrick¶ and R. Simmons**

*Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia, †Menzies Research Institute, University of Tasmania, Hobart, Australia, ‡University of Sydney Discipline of Paediatrics and Child Health, The Children's Hospital at Westmead, Sydney, Australia, §Christchurch School of Medicine and Health Sciences, University of Otago, New Zealand, ¶Centre for Neuroscience, University of Melbourne, Australia, and **The Canberra Hospital, Australia

Accepted for publication 22 September 2006
Correspondence: Anne-Louise Ponsonby, Murdoch Childrens Research Institute, 9th Floor, The Royal Children's Hospital, Parkville, Victoria 3052, Australia.
E-mail: anne-louise.ponsonby@mcri.edu.au

Summary

Higher sibling exposure is associated with a reduced risk of asthma and other T helper 2 (Th2)-type disorders, possibly through a beneficial effect of higher infection load. The effect on Th1 disorders such as multiple sclerosis (MS) is less clear. Here we examine the association between asthma and MS, taking into account early life sibling exposure. A population-based case–control study in Tasmania, Australia based on 136 cases of magnetic resonance imaging (MRI)-confirmed MS and 272 community controls, matched on sex and year of birth. Study measures include cumulative exposure to total, older or younger siblings by age 6 years, history of doctor-diagnosed asthma and serological IgG responses to herpes viruses. MS cases were more likely ($P = 0{\cdot}02$) than controls to have asthma which began before age of onset of MS symptoms compared to the corresponding age for controls. The absence of younger sibling exposure by age 6 years potentiated ($P = 0{\cdot}04$) the association between asthma and MS. Compared to those with younger sibling exposure and no asthma, the adjusted odds ratio for MS for those with asthma and no younger sibling exposure was 7·22 (95% CI: 2·52, 20·65). Early life sibling exposure was associated with altered IgG serological responses to Epstein–Barr virus (EBV) and herpes simplex virus 1 (HSV1) in adulthood. Reduced early life sibling exposure appeared to contribute to the excess of asthma among MS cases by the time of MS onset. MS development may reflect factors that relate to a general immuno-inflammatory up-regulation of immune activity as well as disease specific factors. The link between early life sibling exposure and the immune response to herpes group viral antigens is consistent with a protective role for early life infections.

Keywords: asthma, herpes virus, hygeine hypothesis, multiple sclerosis, sibling

Introduction

Paralleling the increasing incidence of immune disorders over the past 50 years, there has been a decrease in some common infectious diseases of childhood [1]. In 1989, the 'hygiene hypothesis' was proposed to explain the observation that higher birth order or smaller sibship size was associated with an increase in atopic disease [2,3]. The hypothesis proposed that this observation was due to a reduction in early life infection. One model (missing immune deviation) suggested that reduced exposure to infections resulted in a failure to shift from the T helper 2 (Th2) immune profile observed at birth to a Th1 immune profile [4]. An alternative model (reduced immune suppression) has also been proposed to account for the marked temporal increase in both Th1 and Th2 disorders that appears inconsistent with the deviation model [5]. Thus it is possible that there is first an immune abnormality leading to general immuno-inflammatory up-regulation, encompassing both Th1 and Th2 pathways, followed by other factors that predispose towards a specific immune disease.

The evidence that higher sibling exposure in early life is associated with reduced Th2-type atopic disease is compelling. More than 50 studies have provided evidence that markers of higher exposure to other children in early life (e.g. higher birth order indicating the influence of older siblings, other aspects of sibship structure and day care attendance) are associated with a reduced risk of atopic disorders [6]. However, for multiple sclerosis (MS), although higher younger sibling exposure has been associated with

reduced MS risk [7,8] including in this study sample [8], the pattern for older siblings or birth order is less clear [9]. Here we examine the association between asthma, MS and sibling exposure in a population-based case–control study. Further, we examine the association between sibling exposure in early life and adult viral antibody immune responses.

Methods

Participants

The source population consisted of residents under the age of 60 years in Tasmania, Australia, with at least one grandparent who was born in Tasmania [10]. Eligible cases had cerebral magnetic resonance imaging (MRI) abnormalities consistent with MS [11] and clinically definite MS based on neurological review [12]. Cases also participated in a genetic study that included a haplotype analysis on the human leucocyte antigen (HLA) region [13]. The 136 cases in this case–control study [10] were estimated to comprise 76–92% of all eligible cases. Controls were selected from the roll of voters for compulsory political elections. For each case, two control subjects were selected randomly and matched to the index case on sex and birth year. For the 136 cases included in the study, 272 eligible controls participated with a response rate of 76% [10]. The project received ethical approval from the Human Research Ethics Committee of the Royal Hobart Hospital and written consent was obtained from cases and controls.

Measurements

Cases and controls were interviewed between March 1999 and June 2001 by two research assistants; detailed information is provided elsewhere [10]. The standardized verbal questionnaire included questions on siblings' dates of birth and residence in the same house as the subject, past sun exposure over the life course, smoking history, illness history, whether the subject had been breastfed and sociodemographic characteristics such as level of education. Medical history, obtained by interviewer using standardized questions and protocol, included a section on 'Have you had or do you have any of the following conditions?'. Among the disorders listed, options included 'asthma diagnosed by a doctor' and 'hayfever'. If a positive response was obtained, participants were then asked the age of onset and whether the condition had been present in the 12 months prior to interview. Skin type was assessed using a spectrophotometer to assess melanin density at the upper inner arm. Skin type was classified as fair if the melanin density was less than 2% [10]. Blood samples were taken and serological IgG antibody titres to viral antigens were obtained. These included the following herpes viral antigens: Epstein–Barr virus (EBV) nucleic acid (EBNA) [(IgG enzyme-linked immunosorbent assay (ELISA); PanBio, Brisbane, Australia] and viral capsid antigens (VCA) (IgG ELISA; PanBio), cytomegalovirus (CMV) (AxSYM IgG microparticle enzyme immunoassay; Abbott Laboratories, Abbott Park, IL, USA) and herpes simplex virus 1 (HSV1) antigens (HerpeSelect IgG enzyme-linked immunosorbent assay; Focus Technologies, Cypress, CA, USA).

Seropositivity was defined using laboratory reference standards to separate positive from equivocal or negative results. For HSV1 IgG, we also examined the laboratory classification for a high positive result because the validity of very high titres against actual antibody levels has not been established clearly [HerpeSelect IgG ELISA documentation; Focus Technologies]. Case and control blood samples were collected and stored in an identical manner and analysed in a single batch and the laboratory staff were blind to case or control status.

Statistical methods

We examined the association between sibling exposure and these outcomes with an emphasis on the first 6 years of participant (index) life. The window of early life to age 6 was selected because intrahousehold sibling effects could be expected to be stronger before the age of regular school attendance: immune responses mature during the preschool years [14] and the primary exposure to a number of the herpes viruses occurs in this period. Because preliminary data analysis revealed that both sibling number and interbirth interval was important, we calculated cumulative days of exposure to a sibling to simultaneously account for sibling number and the interbirth interval between each sibling and the index [8]. The date of birth of the index and each sibling was used to calculate the number of days of sibling contact; that is, the days the index had spent before age 6 with a sibling in the same home. We calculated cumulative total sibling-years of exposure by age 6, regardless of sibling age by summing the younger and older sibling exposure. For older siblings, all elder siblings were assumed to have spent a full 6 years in contact with the index during the first 6 years of index life. The sibling-days tally for each sibling of the index was then summed and converted to years to provide cumulative total sibling years. Three subjects provided only partial sibling data and were excluded from analysis. For twins ($n = 7$), co-twins were counted as a younger sibling with an interbirth interval of zero.

Here, the term 'asthma' refers to doctor-diagnosed asthma prior to age-of-onset symptoms for MS or corresponding age for controls matched to that case. The term 'hayfever' refers to hayfever prior to age of onset symptoms for MS or corresponding age for controls matched to that case. High total sibling exposure is 6 or more years of cumulative exposure in the first 6 years of life. We focused on asthma prior to MS onset for two reasons. First, the objective was to examine how asthma predisposed to MS. Secondly, asthma diagnosed after MS onset could reflect disease-related [15] or medication-related [16] changes in immune activity or

Table 1. Atopic disease history for multiple sclerosis (MS) cases compared to controls.

Disorder	Cases % (*n*/*N*)	Controls % (*n*/*N*)	Matched OR (95% CI) for MS	*P*-value
Asthma ever	25·0 (34/136)	16·9 (46/272)	1·67 (1·00, 2·80)	0·05
Current asthma	12·5 (17/136)	8·1 (22/272)	1·59 (0·82, 3·05)	0·15
Asthma prior to MS onset	20·6 (28/136)	11·8 (32/272)	1·97 (1·10, 3·51)	0·02
Asthma prior to age 6	11·0 (15/136)	5·2 (14/272)	2·39 (1·08, 5·27)	0·03
Asthma from age 6 to MS onset	9·6 (13/136)	6·6 (18/272)	1·47 (0·67, 3·24)	0·29
Asthma at or after MS onset	4·4 (6/136)	5·2 (14/272)	0·84 (0·30, 2·34)	0·75
Hayfever prior to MS onset	28·7 (39/136)	27·3 (74/271)	1·07 (0·67, 1·71)	0·78
Hayfever at or after MS onset	8·8 (12/136)	8·5 (23/271)	1·05 (0·49, 2·24)	0·90
Hayfever ever	37·5 (51/136)	36·3 (98/270)	1·05 (0·68, 1·62)	0·83
Allergy ever	16·2 (22/136)	12·5 (34/272)	1·36 (0·75, 2·47)	0·30

increased surveillance with higher levels of medical care. For breastfeeding, 'don't know' answers were replaced with proxy reports for 40 subjects, providing data on 354 subjects. Matched odds ratios (OR) estimates of relative risk of being diagnosed with asthma prior to MS onset (for cases) or prior to attaining the age of MS onset of their matched case (for controls) and other atopic disorders were obtained by conditional logistic regression. Unless stated otherwise, adjusted odds ratios were additionally adjusted for smoking ever, sun exposure in winter at ages 6–10, skin type and education level. Tests of trend of ordered categorical variables were undertaken by testing the statistical significance of the coefficient of a linear predictor formed by assigning consecutive integer scores to the categories in ascending or descending order. We examined whether the asthma–MS patterns differed by early life sibling exposure. We tested for interaction by using the log likelihood ratio test to compare the reduction in deviance obtained by adding a product term [17]. Tests for an interaction often use a different significance level that $P = 0{\cdot}05$ [18]. Here, interaction values with $P < 0{\cdot}1$ were considered to indicate significance. Logistic regression was used to examine the association between sibling exposure and asthma among controls. Linear regression was used to examine the association between sibling exposure and $\log_2$-transformed viral titres. Analyses were conducted using STATA (Statacorp 2005: Statistical Software, release 9; College Station, TX, USA).

Results

The characteristics of cases and controls were as follows. Females were 68% ($n = 92$) of cases and 68% (184) of controls. The mean ± s.d. age in years was 43·5 ± 9·3 for cases and 43·6 ± 9·2 for controls. Almost all cases (96%; $n = 131$) and controls (95%; $n = 258$) were born in Tasmania. For MS cases, the mean ± s.d. age at diagnosis was 34·6 ± 9·1 years and mean ± s.d. age at first symptoms was 31·0 ± 9·1 years. MS cases were more likely ($P = 0{\cdot}02$) than controls to have asthma which began before age of onset of MS symptoms compared to the corresponding age for controls (Table 1). We investigated this further and found that most of the increased risk associated with asthma prior to MS was related to asthma that had an onset before, not after, age 6 (Table 1).

We then examined how cumulative sibling exposure by age 6 contributed to the finding of increased asthma history among MS cases at MS onset (OR 1·97, 95% CI: 1·10, 3·51). The association between asthma prior to onset and MS differed depending on whether there was early life exposure to any younger siblings (OR 0·90, 95% CI: 0·37, 2·13) or not (OR 5·03, 95% CI: 1·83, 13·85; difference in effect, $P = 0{\cdot}01$) (Table 2). This significant interaction persisted after further adjustment for older or total siblings. The interaction also persisted after further adjustment for smoking ever, education level, winter sun exposure at ages 6–10 and skin type. There was no evidence that the association between asthma and MS varied by older ($P = 0{\cdot}78$) or total ($P = 0{\cdot}64$) sibling exposure. After accounting for younger sibling exposure and the attenuation of MS risk by younger sibling exposure, a higher MS risk for those with asthma was no longer evident.

We then examined the combined effect of asthma and younger sibling exposure on the risk of MS. Compared to those without asthma and younger sibling exposure by age 6, those with asthma and no younger sibling contact had an OR

Table 2. The association between asthma and multiple sclerosis varies by any younger sibling exposure by age 6.

Asthma	OR (95% CI) for MS among those with any younger sibling exposure by age 6	OR (95% CI) for MS among those with no younger sibling exposure by age 6	Difference in the asthma–MS association by any younger sibling exposure by age 6
Asthma before MS onset	0·90 (0·37, 2·13)	5·03 (1·83, 13·85)	0·01
Asthma prior to age 6	1·00 (0·28, 3·48)	5·25 (1·44, 19·15)	0·07
Asthma from age 6 to MS onset	0·81 (0·26, 2·54)	4·24 (0·87, 20·71)	0·1

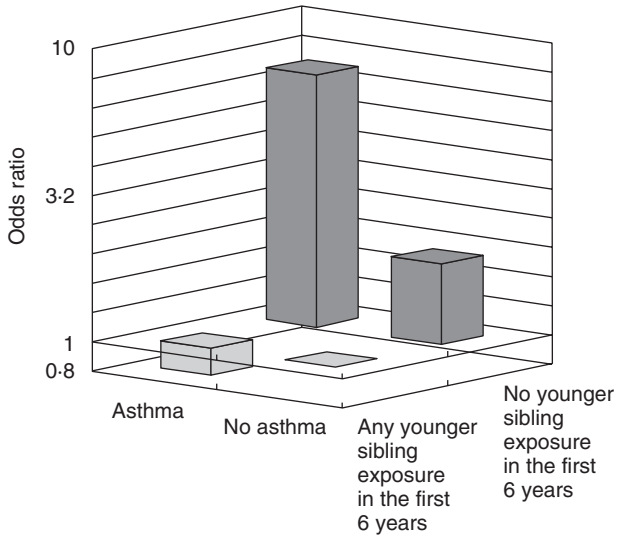

Fig. 1. Risk for multiple sclerosis by combinations of asthma and younger sibling exposure. Note: the association between asthma prior to age of onset and multiple sclerosis is potentiated by no younger sibling exposure (difference in effect; $P = 0{\cdot}04$).

of 7·22 (95% CI: 2·52, 20·65) for MS (Fig. 1). The observed interaction did not depend on the cut-off used to define younger sibling exposure. For example, we examined younger sibling exposure in cumulative years. Again, for those with less than 1 year of younger sibling exposure, asthma was strongly associated with MS ($P = 0{\cdot}007$), but each additional year of younger sibling exposure attenuated the asthma–MS association ($P = 0{\cdot}02$). Thus, these findings were not dependent upon the choice of cut-off for reduced younger sibling exposure.

To test the robustness of these findings for atopic disease more generally, we examined the associations with hayfever. Hayfever prior to MS onset was not associated with MS (OR 1·07, 95% CI: 0·67, 1·71). Similar patterns with effect modification by younger sibling exposure were found. That is, the association between hayfever and MS was stronger for those with no younger sibling exposure (OR 1·61, 95% CI: 0·72, 3·60) compared to those with younger sibling exposure by age 6 (OR 0·56, 95% CI: 0·24, 1·32; difference in effect $P = 0{\cdot}08$). We then examined combined risk. Compared to those without hayfever but with younger sibling exposure by age 6 [OR 1·00 (reference)], those with no younger sibling contact and no hayfever and had an adjusted OR (AOR) of 1·88 (1·06, 3·32) compared to an AOR of 3·02 (1·35, 6·78) for those with no younger sibling contact and hayfever (Fig. 2).

Examination of the association between cumulative sibling exposure by age 6 and asthma among controls

The median age of diagnosis of asthma among controls was 6 years (interquartile range, 3–19 years), much lower than the median age of onset of the first MS symptom (32 years, interquartile range, 25–38 years). Among controls, more than 6 years of total sibling exposure by age 6 was associated with a reduced risk of asthma [AOR 0·44 (0·20,0·99)]. The separate independent contributions of older and younger siblings could not be determined, due partly to the smaller sample size. We investigated asthma by age of onset to determine if there was any reverse causality, with early onset asthma in the index altering subsequent birth spacing and thus reducing early life sibling exposure. This did not appear to be the case.

That is, total, older or younger siblings were not associated with asthma with onset under age 6 (OR 0·88, $P = 0{\cdot}84$; OR 1·00, $P = 0{\cdot}99$; OR 1·12, $P = 0{\cdot}84$, respectively) but asthma onset from age 6 to time of the MS case onset (OR 0·32, $P = 0{\cdot}02$; OR 0·42, $P = 0{\cdot}08$; OR 1·40, $P = 0{\cdot}41$, respectively). This suggests that the sibling exposure–asthma associations did not reflect reverse causality, with a delay or reduction in sibling number by age 6 due to subsequent larger birth spacing due to early onset asthma in the index child.

Sibling exposure, MS, asthma and herpes viral serology

To understand more clearly how early life younger sibling exposure may be influencing risk, we examined how sibling exposure was related to viral serological indices among controls. Increasing years of younger sibling exposure tended to be associated with lower EBNA IgG ($P = 0{\cdot}54$) and viral capsid antigen (VCA) IgG ($P = 0{\cdot}34$) titres and significantly higher HSV1 IgG levels. For each 13 (95% CI 8, 43) years of increasing younger sibling exposure (equivalent to three younger siblings, born at short intervals after the index

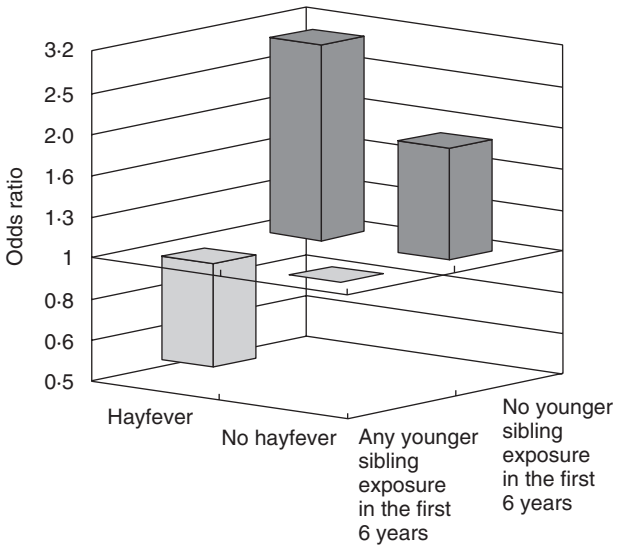

Fig. 2. Risk for multiple sclerosis by combinations of hayfever and younger sibling exposure. Note: the association between hayfever prior to age of onset and multiple sclerosis is potentiated by no younger sibling exposure (difference in effect; $P = 0{\cdot}08$).

Table 3. Age- and sex-adjusted odds ratios for herpes viral serology indices and (1) multiple sclerosis (MS) (2) asthma among controls.

	Matched OR (95% CI) for MS	*P*-value	Age- and sex-adjusted OR (95% CI) for asthma among controls	*P*-value
CMV IgG seropositivity	0·79 (0·50, 1·23)	0·29	1·25 (0·68, 2·29)	0·47
HSV1 IgG seropositivity	0·59 (0·37, 0·96)	0·03	1·32 (0·65, 2·65)	0·44
VCA IgG seropositivity	5·64 (2·00, 15·94)	0·001	1·89 (0·64, 5·60)	0·25
EBNA IgG seropositivity	28·00 (3·81, 205·67)	0·001	1·57 (0·58, 4·23)	0·37
CMV IgG titre (per doubling of titre)	0·93 (0·87, 1·00)	0·049	1·02 (0·93, 1·12)	0·64
HSV1 IgG titre (per doubling of titre)	0·94 (0·83, 1·07)	0·35	1·08 (0·90, 1·29)	0·78
VCA IgG titre (per doubling of titre)	1·82 (1·30, 2·57)	< 0·001	1·11 (0·81, 1·52)	0·50
EBNA IgG titre (per doubling of titre)	5·90 (3·08, 11·30)	< 0·001	1·26 (0·91, 1·73)	0·15

child), the HSV1 IgG level doubled; $P = 0{\cdot}005$) and with increasing years of younger sibling exposure the likelihood of being seropositive ($P = 0{\cdot}05$) or being classified as having high HSV1 IgG levels ($P = 0{\cdot}03$) increased. No younger siblings by age 6 was associated with a lower likelihood of seropositivity to all four antigens [97·3% (142/146) *versus* 100% (248/248), Fisher's two-sided exact *P*-value = 0·02]. Table 3 shows that a seropositive response or higher titre of IgG antibodies to EBV antigens was associated strongly with MS, as reported previously [8]. Examining the four viral antigens in the same model, seropositivity to EBNA IgG was associated positively ($P = 0{\cdot}004$) with MS but seropositivity to HSV1 IgG was inversely related ($P = 0{\cdot}04$). The latter persisted after adjustment for the higher composite EBV IgG titres [8] among MS cases. We examined the likelihood of asthma among controls. For asthma, seropositivity or IgG level for any individual viral antigen listed was not associated with asthma (Table 3). However, seropositivity to all four antigens was associated with a reduced risk of asthma (OR 0·12, 95% CI: 0·02, 0·90).

Discussion

In this population-based study, we observed inverse associations between higher sibling exposure and both a Th1-type disorder (MS) and a Th2-type disorder (asthma). People with MS were more likely to have asthma prior to MS onset, but further examination revealed that the asthma–MS association was attenuated by exposure to younger siblings in early life. The nature and timing of these effects appears complex. Overall, the findings are more supportive of an immunoregulatory model for the hygiene hypothesis rather than an immune deviation model in which increased sibling exposure deviates the immune response from a Th2 to Th1 bias. Defects in control of the immune response by T regulatory cells have been found in both MS [19] and allergic airways disease [20]. The immunoregulatory model is ecologically coherent with the increase of both Th1- and Th2-related immune disorders in western societies.

Strengths of this study include the opportunity to examine both asthma and MS in a population-based case–control study with detailed data on cumulative sibling exposure in early life. A life-course perspective was taken by using asthma cases incident prior to MS. Further, age at onset data were available, excluding that the differing asthma rate among people with MS reflected only post-disease ascertainment. The study sample was not large, but many of the associations observed were high in magnitude and significant. Disease classification was less precise for asthma than MS. However, doctor-diagnosed asthma was recorded, reducing the problem of self report [21] and the prevalence of current doctor-diagnosed asthma was similar to the 10–12% reported in the 2002 National Health Survey [22]. The interaction between asthma, younger sibling exposure and MS was also found for hayfever, a related atopic disease. Any non-differential misclassification of asthma should have biased results towards the null, yet clear patterns for sibling exposure and asthma were found.

The association between asthma and MS has differed across studies. While some studies have reported higher asthma prevalence among those with MS [23,24], others have reported similar [25] or reduced [15,26–28] atopic disease among those with MS. However, some of these reports were based on MS cases attending hospital clinics [26,28] or general practice [27], and presenting MS cases may not represent all cases arising from the source population. This may be a problem particularly if severe MS cases were more likely to be included, as atopic disease may be associated with milder disease course for MS [29]. Further, it has not always been established clearly that the control group has an asthma prevalence rate no higher than the general population, regardless of presentation to medical care. It is difficult to establish to what extent such inverse associations reflect disease-related [15] or medication-related [16] changes in immune activity. To exclude this issue, we focused on asthma development before MS development, using a life course perspective. Using this approach, asthma was over-represented among people with MS. However, a new determinant of the heterogeneity in the association between asthma and MS was identified, with the asthma–MS association potentiated by no younger sibling exposure in the first 6 years of life.

Although many studies have shown a protective effect of increased sibling number on allergic disease development the mechanism of such an effect is unclear. There is a paucity of evidence that sibling exposure can influence the immune response in later life [3]. Previously, we reported from this study that among controls, exposure was associated with a reduced risk of composite EBV IgG levels and infectious mononucleosis [8]. Here, higher younger sibling exposure was associated with higher HSV1 IgG titres and being seropositive to all four viral antigens among controls. These observations provide some evidence sibling exposure in early life may influence the viral immune response pattern in adulthood, although we could not control for subject's recent exposure to children. It is possible that early life exposure to viruses influences the development of both asthma and MS. Seropositivity in adult life to several infections that can be acquired in early life have been inversely associated with allergic respiratory tract disease [3]. Our finding of a strong inverse association between seropositivity to all four viral antigens and asthma is consistent with the findings from the Third National Health and Nutrition Examination Survey, where seronegativity to multiple antigens was associated with an OR of 2·0 for asthma [30]. The lack of association between individual viral antigen responses and asthma could reflect either misclassification due to sampling in adulthood or that key viruses were not examined. It is also consistent with past work – the inverse association between sibling number and atopy has not been accounted for by any specific past viral infection [3], leading to suggestions, it has been postulated, that it is the general microbial environment encountered in early postnatal life that is important in atopic disease development [3].

In contrast, for the Th1-related disorder of MS, prospective studies indicate a probable aetiological link to more specific responses to the herpes group viruses such as EBV and HHV6 [31–33]. In this study, EBNA or EBV VCA IgG seropositivity or titre was associated strongly with MS [8]. Here, HSV1 IgG seropositivity was related inversely to MS. A previous study of early onset MS also found MS to be positively associated with EBV seropositivity and negatively associated with HSV1 IgG seropositivity, consistent with a possible adverse effect of EBV infection if other potentially beneficial infections have not occurred [34]. The reasons for the contrasting pattern for HSV1 seropositivity and EBV seropositivity are not known. It is possible that this could relate to the finding that EBV has cross-reactivity to myelin [35]. If, as accumulating evidence suggests, disordered immunity to EBV infection is related causally to MS [36], it may be that past immunity to other herpes viruses, such as HSV1, assists the host immunity to control or respond to EBV infection more effectively. This has also been suggested in relation to the finding that increased CMV antibody responses are associated with reduced progression of MS [37].

The differences we found in MS and asthma with regard to viral serology and sibling patterns (total sibling exposure associated with a reduced risk of asthma among controls, and a greater protective effect for MS exerted by younger siblings rather than total siblings for MS) suggest that although exposure to infectious agents through sibling exposure may influence both disorders, the particular type and way in which infections which influence these disorders are likely to differ. For example, the contribution of initial infection (by an older sibling) may be more beneficial for asthma than MS, where the additional opportunities provided by younger siblings for repeated boosting and refining [38,39] of an established immune response may be particularly required. However, there are also common mechanisms which could influence both disorders.

For example, microbial exposure such as lactobacilli [40] or infections [41] can induce T regulatory cells and IL-10, an immunomodulatory cytokine which can reduce both Th1 and Th2 inflammation [41]. If these early life common determinants have not occurred, then the child may have an enhanced immuno-inflammatory profile, of which asthma could be one manifestation. The interaction we observed between asthma, younger sibling exposure and MS suggests that for the child with asthma, including asthma before age 6, the possible beneficial effect of younger sibling exposure during the first 6 years may be particularly required to prevent MS onset.

In conclusion, the incidence of doctor-diagnosed asthma over the subject's lifetime by the time of MS onset was significantly higher in MS cases than for the corresponding age of controls and this related to early life sibling exposure, with the magnitude of the asthma–MS association increasing with a reduction in younger sibling exposure. Links between early life sibling exposure, the immune response to herpes group viral antigens in adulthood and both diseases were found, suggesting that early life viral infections may play a role in the protection against both disorders. These findings support the concept of an immunoregulatory role for siblings and sibling-related infection during early life.

Acknowledgements

We thank the participants and Trish Groom and Jane Pittaway for conducting the interviews, Natasha Newton for administrative support and data entry, Sue Sawbridge and Tim Albion for the development and management of the database, John Carlin for statistical advice, the Tasmanian Multiple Sclerosis Society for assisting with the recruitment of volunteers and H. Butkueven, A. Hughes, B. Drulovis and S. Sjieka, who were involved with the clinical diagnosis. This project was supported with funding from the National Health and Medical Research Council of Australia, the Australian Rotary Health Research Fund and MS Australia. I. van der Mei was supported by the Cooperative Research Centre for Discovery of Genes for Common Human Diseases (gene-CRC), and T. J. Kilpatrick was a Senior NHMRC Research Fellow. The gene-CRC was established and is supported by

the Australian Government's Cooperative Research Centres programme. Serological assays were funded by the Canberra Hospital Private Practice Fund. The funding sources had no role in the study design, data collection, data analysis or interpretation, or writing of this report. The correspondence had full access to all the data in the study and the authors only had final responsibility for the decision to submit for publication.

References

1 Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 2002; **347**:911–20.
2 Strachan DP. Hay fever, hygiene, and household size. BMJ 1989; **299**:1259–60.
3 Strachan DP. Family size, infection and atopy: the first decade of the 'hygiene hypothesis'. Thorax 2000; **55** (Suppl. 1):S2–10.
4 Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. J Allergy Clin Immunol 2004; **113**:395–400.
5 Tedeschi A, Airaghi L. Common risk factors in type 1 diabetes and asthma. Lancet 2001; **357**:1622.
6 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.
7 Montgomery SM, Lambe M, Olsson T, Ekbom A. Parental age, family size, and risk of multiple sclerosis. Epidemiology 2004; **15**:717–23.
8 Ponsonby AL, Van Der Mei I, Dwyer T *et al.* Exposure to infant siblings during early life and risk of multiple sclerosis. JAMA 2005; **293**:463–9.
9 Sadovnick AD, Yee IM, Ebers GC. Multiple sclerosis and birth order: a longitudinal cohort study. Lancet Neurol 2005; **4**:611–17.
10 van der Mei IA, Ponsonby AL, Dwyer T *et al.* Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case–control study. BMJ 2003; **327**:316.
11 Paty DW, Oger JJ, Kastrukoff LF *et al.* MRI in the diagnosis of MS. a prospective study with comparison of clinical evaluation, evoked potentials, oligoclonal banding, and CT. Neurology 1988; **38**:180–5.
12 Poser CM, Paty DW, Scheinberg L *et al.* New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983; **13**:227–31.
13 Rubio JP, Bahlo M, Butzkueven H *et al.* Genetic dissection of the human leukocyte antigen region by use of haplotypes of Tasmanians with multiple sclerosis. Am J Hum Genet 2002; **70**:1125–37.
14 Holt PG. A potential vaccine strategy for asthma and allied atopic diseases during early childhood. Lancet 1994; **344**:456–8.
15 Tang L, Benjaponpitak S, DeKruyff RH, Umetsu DT. Reduced prevalence of allergic disease in patients with multiple sclerosis is associated with enhanced IL-12 production. J Allergy Clin Immunol 1998; **102**:428–35.
16 Farina C, Weber MS, Meinl E, Wekerle H, Hohlfeld R. Glatiramer acetate in multiple sclerosis. update on potential mechanisms of action. Lancet Neurol 2005; **4**:567–75.
17 Hosmer DW Jr. Applied logistic regression, 2nd edn. New York, NY: John Wiley & Sons, 2000.
18 Greenland S. Modeling and variable selection in epidemiologic analysis. Am J Public Health 1989; **79**:340–9.
19 Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. J Exp Med 2004; **199**:971–9.
20 Ling EM, Smith T, Nguyen XD *et al.* Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet 2004; **363**:608–15.
21 Westergaard T, Rostgaard K, Wohlfahrt J, Andersen PK, Aaby P, Melbye M. Sibship characteristics and risk of allergic rhinitis and asthma. Am J Epidemiol 2005; **162**:125–32.
22 Australian Bureau of Statistics. National health survey: summary of results. Canberra: Australian Bureau of Statistics, 2002.
23 Airaghi L, Tedeschi A. Results of the US Third National Health and Nutrition Examination Survey support the hygiene hypothesis. J Allergy Clin Immunol 2004; **113**:179–80; author reply 180.
24 Edwards LJ, Constantinescu CS. A prospective study of conditions associated with multiple sclerosis in a cohort of 658 consecutive outpatients attending a multiple sclerosis clinic. Mult Scler 2004; **10**:575–81.
25 Solaro C, Messmer Uccelli M, Guglieri P *et al.* Prevalence of atopy in multiple sclerosis patients: a case–control study. J Neurol 2001; **248**:239–40.
26 Oro AS, Guarino TJ, Driver R, Steinman L, Umetsu DT. Regulation of disease susceptibility. decreased prevalence of IgE-mediated allergic disease in patients with multiple sclerosis. J Allergy Clin Immunol 1996; **97**:1402–8.
27 Tremlett HL, Evans J, Wiles CM, Luscombe DK. Asthma and multiple sclerosis: an inverse association in a case–control general practice population. Q J Med 2002; **95**:753–6.
28 Neukirch F, Lyon-Caen O, Clanet M, Bousquet J, Feingold J, Druet P. Asthma, nasal allergies, and multiple sclerosis. J Allergy Clin Immunol 1997; **99**:270–1.
29 Kira J, Kawano Y, Yamasaki K. Multiple sclerosis with mite antigen-specific IgE. J Neurol Sci 1998; **157**:138–42.
30 Matricardi PM, Rosmini F, Panetta V, Ferrigno L, Bonini S. Hay fever and asthma in relation to markers of infection in the United States. J Allergy Clin Immunol 2002; **110**:381–7.
31 Ascherio A, Munger KL, Lennette ET *et al.* Epstein–Barr virus antibodies and risk of multiple sclerosis: a prospective study. JAMA 2001; **286**:3083–8.
32 Villoslada P, Juste C, Tintore M *et al.* The immune response against herpesvirus is more prominent in the early stages of MS. Neurology 2003; **60**:1944–8.
33 Levin LI, Munger KL, Rubertone MV *et al.* Temporal relationship between elevation of Epstein–Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. JAMA 2005; **293**:2496–500.
34 Alotaibi S, Kennedy J, Tellier R, Stephens D, Banwell B. Epstein–Barr virus in pediatric multiple sclerosis. JAMA 2004; **291**:1875–9.
35 Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell 1995; **80**:695–705.
36 Christensen T. The role of EBV in MS pathogenesis. Int MS J 2006; **13**:52–7 [Review].
37 Zivadinov R, Nasuelli D, Tommasi MA *et al.* Positivity of cytomegalovirus antibodies predicts a better clinical and radiological outcome in multiple sclerosis patients. Neurol Res 2006; **28**:262–9.
38 Adams CL, Macleod MK, Milner-White JE, Aitken R, Garside P, Stott DI. Complete analysis of the B-cell response to a protein antigen, from *in vivo* germinal centre formation to 3-D modelling of affinity maturation. Immunology 2003; **108**:274–87.

39 Wedderburn LR, Patel A, Varsani H, Woo P. The developing human immune system: T-cell receptor repertoire of children and young adults shows a wide discrepancy in the frequency of persistent oligoclonal T-cell expansions. Immunology 2001; **102**:301–9.

40 Smits HH, Engering A, van der Kleij D *et al.* Selective probiotic bacteria induce IL-10-producing regulatory T cells *in vitro* by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. J Allergy Clin Immunol 2005; **115**:1260–7.

41 Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Immunol 2001; **1**:69–75.