

Exposure to Infant Siblings During Early Life and Risk of Multiple Sclerosis

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THE “HYGIENE HYPOTHESIS” PROPOSES that early life infections may down-regulate allergic and autoimmune disorders.¹ Having siblings may increase the number of early life infections. In fact, lack of contact with siblings has been associated with T_H2-related immune disorders and some T_H1-related disorders. For multiple sclerosis (MS), a T_H1-related autoimmune disease, the association with birth order has been inconsistent across studies²⁻⁴ and absent in others.⁵⁻⁷ However, birth order provides clearer information on the number of older siblings, and the independent influence of younger siblings has not been evaluated. Younger siblings may be important because infants provide a source of common viral infections. Reexposure to active viral infection is known to cause immune boosting with rising IgG titers in seropositive individuals.⁸ Also, repeated antigenic stimulation induces affinity maturation of the B-cell line⁹ and influences T-cell phenotype¹⁰ and T-cell receptor diversity.¹¹ This may be advantageous if the acquisition of a highly developed immune response to puta-

Context The “hygiene hypothesis” has implicated sibship as a marker of infection load during early life and suggests that exposure or reexposure to infections can influence the developing immune system. Viral infection has also been implicated in the pathogenesis of multiple sclerosis (MS).

Objectives To evaluate whether exposure to infant siblings in early life is associated with the risk of MS, and to explore the possible mechanism for any apparent protective effect, including altered Epstein-Barr virus (EBV) infection patterns.

Design, Setting, and Patients Population-based case-control study in Tasmania, Australia, from 1999 to 2001 based on 136 cases of magnetic resonance imaging-confirmed MS and 272 community controls, matched on sex and year of birth.

Main Outcome Measure Risk of MS by duration of contact with younger siblings aged less than 2 years in the first 6 years of life.

Results Increasing duration of contact with a younger sibling aged less than 2 years in the first 6 years of life was associated with reduced MS risk (adjusted odds ratios [AORs]: <1 infant-year, 1.00 [reference]; 1 to <3 infant-years, 0.57 [95% confidence interval {CI}, 0.33-0.98]; 3 to <5 infant-years, 0.40 [95% CI, 0.19-0.92]; ≥5 infant-years, 0.12 [95% CI, 0.02-0.88]; test for trend, *P* = .002). A history of exposure to infant siblings was associated with a reduced IgG response to EBV among controls. Controls with at least 1 infant-year contact had a reduced risk of infectious mononucleosis and a reduced risk of very high composite EBV IgG titers (AOR, 0.33; 95% CI, 0.11-0.98) compared with other controls. The inverse association between higher infant contact and MS was independent of EBV IgG titer.

Conclusion Higher infant sibling exposure in the first 6 years of life was associated with a reduced risk of MS, possibly by altering childhood infection patterns and related immune responses.

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tive viral triggers is required to prevent MS development in later life.

A protective role for early life infection in the development of MS is consistent with several features of MS, including the apparent recent increase in incidence that has accompanied a decline in childhood infection rates over time.¹ The suggestion that MS may result from the postponement of early life infection was first made in 1966.¹² Since then, several studies have shown MS to be associated with childhood infection of later onset, especially Epstein-Barr virus (EBV) infection. A Danish historical cohort reported infectious mononucleosis, a marker of late EBV

infection, to be associated with subsequent MS,¹³ and 2 recent cohort studies have reported strong associations between elevated EBV IgG antibodies and subsequent MS.^{14,15}

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See also Patient Page.

In addition to early life infection, other risk factors for MS have been identified. In prospective studies, cigarette smoking has been associated with higher MS risk¹⁶ and vitamin D supplementation with reduced risk.¹⁷ Higher levels of sunlight exposure have been associated with reduced MS risk, with an adjusted odds ratio (AOR) for higher sun exposure at ages 6 to 15 years and MS in a previous study of 0.31 (95% confidence interval [CI], 0.16-0.59).¹⁸ Sun exposure may modify the host immune response. For example, antigen-specific T-suppressor cells are induced by exposure to UV radiation.¹⁹

This report attempts to identify whether infant contact in early life is associated with MS in a case-control study. Further, we explored how any protective effect may operate in the context of previously identified factors related to MS risk, including elevated EBV antibodies^{14,15} and past sun exposure.¹⁸

METHODS

Participants

The source population consisted of residents younger than 60 years in Tasmania, Australia, with at least 1 grandparent who was born in Tasmania.¹⁸ Eligible cases had cerebral magnetic resonance imaging abnormalities consistent with MS²⁰ and clinically definite MS based on neurological review.²¹ To recruit cases, multiple population-based strategies were used, including information evenings at local MS societies and letters of invitation from neurologists to patients. Cases also participated in a genetic study that included a haplotype analysis on the human leukocyte antigen region.²² The 136 cases in this case-control study¹⁸ were estimated to include 76% to 92% of all eligible cases. Controls were selected from the roll of voters for compulsory political elections. For each case, 2 control subjects were randomly selected 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%.¹⁸ The project re-

ceived ethics 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 2 research assistants; detailed information is provided elsewhere.¹⁸ The standardized verbal questionnaire was designed to investigate the contribution of environmental factors, particularly sun exposure, on the development of MS. It included questions on number of siblings and dates of birth, whether the sibling lived 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. At the end of the interview, subjects were asked to nominate a proxy to recall aspects of the subject's childhood by telephone after receiving a mailed questionnaire. Overall, 84.1% (343/408) of subjects had a proxy who participated; these were most often the subject's mother (70.9% [243/343]).

Skin type was determined 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%.¹⁸

Blood samples were drawn and IgG antibody titers to EBV nuclear antigen (EBNA) and EBV capsid antigen (VCA) were determined by enzyme-linked immunosorbent assay (PANBIO, Brisbane, Australia). The sample absorbance was divided by the cutoff value and then multiplied by 100 to allow comparison with the prospective reports on EBV IgG and MS.^{14,15} Other enzyme-linked immunosorbent assays using a recombinant antigen have been found to have a 3% to 14% single-parameter discrepancy for positive VCA IgG compared with "gold standard" indirect immunofluorescence assay.²³ Case and control blood samples were collected and stored in an identical manner and analyzed in a single batch at Westmead Hospital, Sydney, Aus-

tralia. Laboratory staff members were blind to case or control status.

Statistical Methods

To determine whether our focus on younger siblings was justified, we first looked at risk of MS by birth order, number of siblings, number of older siblings, and number of younger siblings.

For each subject to age 6 years, we calculated cumulative infant-years of exposure to a younger sibling to simultaneously account for number of younger siblings and the interbirth interval between each younger sibling and the subject. The date of birth of the subject and each sibling was used to calculate the number of days of infant contact, that is, the days the subject had spent before age 6 years with a younger sibling aged less than 2 years in the same home. The infant-days tally for each sibling of the subject was summed and converted to years to provide cumulative total infant-years. The window of early life to age 6 was selected because intrahousehold effects could be expected to be stronger before the age of regular school attendance and immunomodulation may particularly occur at this stage.²⁴ The infancy period of the first 2 years was selected because primary infection with viruses that may influence the pathogenesis of MS, such as herpesviruses and enterovirus, commonly occur in the first 2 years.²⁵ In addition, we calculated cumulative total sibling-years of exposure by age 6 years, regardless of sibling age. Three subjects provided only partial sibling data.

Conditional logistic regression provided matched ORs for MS.²⁶ We adjusted for other factors using multivariable models. These factors included past smoking, low past sun exposure, and fair skin type, which were associated with MS in an earlier report from this study.¹⁸ An education level of high school certificate or higher was also adjusted for because education was associated with family size and birth order and of borderline significance with MS. 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 order.

To account for family characteristics such as genetic predisposition and socioeconomic status, we also compared, within case families, the MS cases with their own siblings. We examined the likelihood of a case having any younger siblings compared with a sibling of the case having any younger siblings using a generalized estimating equation model with a logit link with family group as a fixed effect.

Among cases, proportional hazards modeling was used to examine infant contact and age of onset of MS, adjusting for skin type.¹⁸

Because UV radiation can modify host immune responses to infection, we examined effect modification of the infant contact-MS association by childhood sun or outdoor exposure. We tested for interaction by using the log likelihood ratio test²⁶ to compare the reduction in deviance obtained by adding a product term.

Next, we examined factors related to infectious mononucleosis and elevated EBV antibody levels among healthy controls using logistic regression.²⁶ Cases were excluded from this analysis to remove any alterations to EBV antibody titers as a consequence of the active disease process or therapy. Because smoking was associated with higher EBV antibodies and a history of having been breastfed tended to be associated with a reduced risk of infectious mononucleosis, these factors were considered as confounders of the association between infant contact and either infectious mononucleosis or EBV IgG titers.

We then examined the association between a history of infectious mononucleosis and EBV antibody titers and MS. Our analyses indicated that risk of MS for increasing EBNA antibody concentrations rose more at higher VCA concentrations than at lower VCA concentrations. To present results as simply as possible but in a way that captured this feature, we divided the joint distribution of the EBV antibodies into

Table 1. Characteristics of the Cases and Controls

	Cases	Controls
Female/male ratio	92/44	184/88
Age, mean (SD), y	43.5 (9.3)	43.6 (9.2)
Born in Tasmania, % (n/N)	96.3 (131/136)	95.2 (258/271)
Living in Tasmania at the age of 10 y, % (n/N)	96.3 (131/136)	97.4 (265/272)
Age at diagnosis, mean (SD), y	34.6 (9.1)	
Age at first symptoms, mean (SD), y	31.0 (9.1)	
Duration of MS since diagnosis, mean (SD), y	9.4 (7.5)	
EDSS score, mean (SD)	3.5 (2.2)	
DRB1*1501-DQB1*0602 haplotype frequency, % (n/N)	53.7 (66/123)	

Abbreviations: EDDS, Expanded Disability Status Scale (range, 0-10); MS, multiple sclerosis.

4 composite categories: EBNA IgG (0-300 units) + VCA IgG (0-300 units); EBNA IgG (0-300 units) + VCA IgG (>300 units); EBNA IgG (>300 units) + VCA IgG (0-300 units); and EBNA IgG (>300 units) + VCA IgG (>300 units). To indicate the importance of the residual infant contact-MS association that was independent of EBV antibodies, we added dummy variables for composite EBV antibody categories to a model of infant contact and MS, controlling for education, smoking, sun exposure, and skin type.

Results with $P < .05$ are considered statistically significant. We made no adjustment for multiple testing but report all analyses²⁷ undertaken to allow readers to make formal adjustments if they desire. Analyses were conducted using STATA 8.0. (Statacorp 2003: Statistical Software: Release 8, College Station, Tex).

RESULTS

Participant characteristics are shown in TABLE 1. A total of 134 cases had a defined clinical course: 89 (66.4%) participants with relapsing-remitting MS, 35 (26.1%) with secondary progressive MS, and 10 (7.5%) with primary progressive MS. Overall, birth order was not associated with MS (OR, 1.06; 95% CI, 0.95-1.19 per increase in birth order), but total number of siblings tended to be associated with a reduced risk of MS (OR, 0.90; 95% CI, 0.81-1.00 per sibling). The inverse association between number of siblings and MS was due to younger, not older, siblings (TABLE 2). Number of older siblings was

not associated with a reduced risk of MS (OR, 1.06; 95% CI, 0.94-1.19 per sibling) (test for trend, $P = .58$). The lack of association between number of older siblings and MS remained after adjustment for number of younger siblings (AOR, 1.01; 95% CI, 0.88-1.14 per sibling). The strong inverse association between number of younger siblings and MS (OR, 0.77; 95% CI, 0.67-0.90 per sibling) persisted after adjustment for number of older siblings (AOR, 0.78; 95% CI, 0.66-0.91 per sibling).

Increasing number of younger siblings was strongly associated with a reduced risk of MS with a dose-response relation (test for trend, $P = .002$) (Table 2). We examined the interbirth interval between the subject and next younger sibling. The effect was of largest magnitude if the younger sibling was born within 2 years of the subject. No younger sibling effect was evident if the nearest younger sibling was born more than 6 years after the subject (Table 2). Increasing infant-years contact by age 6 years was strongly and inversely associated with MS (test for trend, $P = .002$) (Table 2). The findings were not altered by further individual adjustment for number of older siblings, total number of siblings, or a history of having been breastfed. One or more infant-years exposure by age 6 years compared with less than 1 infant-year was associated with an AOR of 0.46 (95% CI, 0.28-0.75; $P = .002$) for MS. Five or more infant-years exposure by age 6 years compared with less than 1 infant-year was associated with an AOR of 0.12 (95% CI, 0.02-0.88; $P = .009$) for MS (Table 2). In

contrast, total sibling exposure by age 6 years, regardless of sibling age, was not significantly associated with MS.

We also examined the contribution of noninfant sibling exposure by examining total sibling exposure after adjustment for infant-years exposure. The AORs for total sibling exposure were: less than 0.1 sibling-year, 1.00 (reference); 0.1 to 4 sibling-years, 1.02 (95% CI, 0.35-2.98); 4.1 to 6 sibling-years, 1.35 (95% CI, 0.56-3.26); 6.1 to 12 sibling-years, 0.86 (95% CI, 0.36-2.07), and more than 12 sibling-years, 0.86 (95% CI, 0.36-2.04) (test for trend, $P=.47$).

We studied case families by comparing each MS case directly with his/her own siblings within the family to account for family group characteristics such as genetic predisposition or socioeconomic status. Again, higher number of younger siblings was associated with a reduced risk of MS (OR, 0.78; 95% CI, 0.69-0.88 per younger sibling).

Among cases, higher infant contact in early childhood was associated with delayed MS onset. The adjusted hazard ratio for higher infant contact and age at MS onset was 0.91 (95% CI, 0.84-0.99) for each cumulative year of infant contact in the first 6 years of life.

We examined whether the apparent protective effect of having infant siblings was stronger among subjects with higher childhood sun exposure. The association between any younger sibling by age 6 years and MS was stronger for those with higher winter sun exposure in childhood (≥ 1 hour on winter nonschool days; AOR, 0.37; 95% CI, 0.22-0.64) compared with those with low winter sun exposure (AOR, 0.69; 95% CI, 0.20-2.41; difference in effect, $P=.37$) and for those who often had winter outdoor activity (AOR, 0.31; 95% CI, 0.18-0.55) compared with those who did not (AOR, 1.06; 95% CI, 0.36-3.14; difference in effect, $P=.05$).

We examined the associations between high infant contact in early life and EBV infection among controls. The FIGURE shows that among controls, higher infant contact in early life was inversely related to infectious mononucleosis and very high composite EBV antibody titers. Among controls, the AOR for at least 1 infant-year contact by age 6 years and very high EBV IgG titers was 0.33 (95% CI, 0.11-0.98). After adjustment for infant sibling exposure, total sibling contact during early life was not associated with the likelihood of having very high EBV IgG titers ($P=.76$) or past infectious mononucleosis ($P=.74$) among controls.

We then examined the association between EBV infection features and MS. Serological evidence of probable past EBV infection was virtually universal, with all (136/136) cases and 97% (252/261) of controls having a VCA IgG level over 20 units. The self-report of infectious mononucleosis was associated

Table 2. Association Between Number of Siblings, Interbirth Interval, and Multiple Sclerosis (MS)

	MS Cases, No. (%)	Controls, No. (%)	Odds Ratio (95% CI) for MS	P Value	Adjusted Odds Ratio (95% CI) for MS*	P Value
No. of older siblings						
0	48 (35.8)	97 (35.8)	1.00		1.00	
1	36 (26.9)	77 (28.4)	0.96 (0.56-1.63)	.87	0.98 (0.53-1.83)	.96
2	19 (14.2)	44 (16.2)	0.86 (0.47-1.61)	.65	0.87 (0.43-1.79)	.71
≥ 3	31 (23.3)	53 (19.6)	1.18 (0.67-2.07)	.56	1.27 (0.66-2.45)	.48
Test for trend P value			.69		.58	
No. of younger siblings						
0	58 (43.3)	74 (27.3)	1.00		1.00	
1	38 (28.4)	69 (25.5)	0.68 (0.39-1.18)	.17	0.70 (0.38-1.28)	.25
2	20 (14.9)	64 (23.6)	0.37 (0.20-0.70)	.002	0.33 (0.15-0.71)	.005
≥ 3	18 (13.4)	64 (23.6)	0.35 (0.18-0.66)	.001	0.34 (0.17-0.70)	.003
Test for trend P value			<.001		.001	
Interbirth interval between index child and younger sibling						
No younger sibling	58 (43.3)	74 (27.3)	1.00		1.00	
More than 6 y interbirth interval	11 (8.2)	12 (4.4)	1.17 (0.47-2.92)	.74	1.18 (0.44-3.18)	.72
2-6 y interbirth interval	40 (29.9)	93 (34.3)	0.53 (0.31-0.90)	.02	0.55 (0.30-1.00)	.05
<2 y interbirth interval	25 (18.7)	92 (34.0)	0.34 (0.19-0.60)	<.001	0.31 (0.16-0.61)	.001
Test for trend P value			.001		.001	
Cumulative infant-years exposure by age 6 y						
<1	75 (56.0)	99 (36.5)	1.00		1.00	
1-<3	40 (29.9)	98 (36.2)	0.54 (0.34-0.88)	.01	0.57 (0.33-0.98)	.04
3-<5	17 (12.7)	52 (19.2)	0.42 (0.22-0.80)	.008	0.40 (0.19-0.92)	.03
≥ 5	2 (1.5)	22 (8.1)	0.12 (0.03-0.53)	.005	0.12 (0.02-0.88)	.009
Test for trend P value			.001		.002	

Abbreviation: CI, confidence interval.

*Adjusted for education level (<high school certificate, \geq high school certificate), past history of smoking, higher sun exposure when aged 6 to 15 years (an average ≥ 2 -3 hours a day in summer weekends or holidays vs less), fair skin type (<2% melanin density) vs darker skin (2% melanin or more).

with higher composite EBV antibody titers among cases ($P = .03$) and controls ($P = .02$), consistent with a vigorous response to EBV infection. Cases or their proxies were more likely to report past infectious mononucleosis (AOR, 2.01; 95% CI, 1.11-3.62 and AOR, 4.38; 95% CI, 1.80-10.68, respectively) than controls. Cases had higher composite EBV antibody titers than controls (TABLE 3). After adjustment for EBV antibody titer, an increasing amount of contact with an infant during the first 6 years of life was still associated with reduced MS risk (AORs: <1 infant-year, 1.00 [reference]; 1 to <3 infant-years, 0.55 [95% CI, 0.30-1.02]; 3 to <5 infant-years, 0.53 [95% CI, 0.22-1.27]; ≥ 5 infant-years, 0.18 [95% CI, 0.04-0.73]; test for trend, $P = .006$).

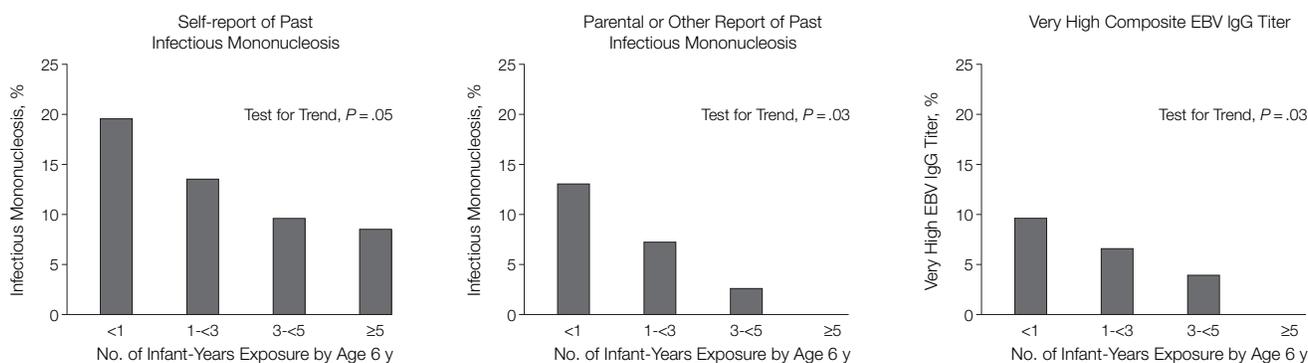
COMMENT

The inverse association between infant exposure and MS was strong with

highly statistically significant dose-response trends that persisted after adjustment for confounding. In addition, there was an effect of infant exposure on the age of MS onset. Nevertheless, given the potential limitations of the case-control study design, we thought it important to investigate the biological plausibility of the strong infant sibling effect. The finding that, among controls, high infant exposure reduces the risk of infectious mononucleosis and elevated EBV IgG levels is important, given that risk of infectious mononucleosis and elevated EBV IgG levels have been prospectively identified as MS risk factors.¹³⁻¹⁵ It is also reassuring that the protective effect for infant younger sibling exposure in contrast to any sibling exposure on MS is also observed when looking at the outcomes of EBV antibody levels or past infectious mononucleosis. However, causality cannot be established within a single case-control study.

The finding that the protective effect was not evident for younger siblings born more than 6 years after the index child may be due to either increased plasticity for immune modulation in early life²⁴ or the decline in importance of the infant sibling as an infection source after children begin school. The greater protective effect exerted by younger siblings compared with older siblings may result from additional opportunities for repeated boosting of an established immune response to an infection in the older child rather than only the initial acquisition of immunity. The boosting of immune responses against latent infections at certain stages of the life course may be important. The prevention of viral reactivation by child contact-induced boosting of immunity has been proposed to explain the striking protective effect of higher child-days contact against herpes zoster, a reactivation of latent varicella zoster infection.²⁸

Figure. Proportion of Controls With Past Infectious Mononucleosis or Very High Epstein-Barr Virus (EBV) IgG Titers by Infant Sibling Exposure in Early Life



The tests for trend have been adjusted for history of having been breastfed or ever smoked. Very high composite EBV IgG titer=(EBV nuclear antigen IgG>300 units+EBV capsid antigen IgG>300 units).

Table 3. Association Between Composite Epstein-Barr Virus (EBV) Antibody Titers and Multiple Sclerosis (MS)

Composite EBV IgG titers	Cases, No. (%)	Controls, No. (%)	Odds Ratio (95% CI) for MS	P Value	Adjusted Odds Ratio (95% CI) for MS*	P Value
Low (EBNA IgG [0-300 units] + VCA IgG [0-300 units])	41 (30.2)	147 (56.3)	1.00		1.00	
Moderate (EBNA IgG [0-300 units] + VCA IgG>300 units)	16 (11.8)	55 (21.1)	1.08 (0.54-2.17)	.83	0.94 (0.41- 2.15)	.89
High (EBNA IgG>300 units + VCA IgG [0-300 units])	41 (30.2)	42 (16.1)	3.32 (1.90-5.82)	<.001	3.77 (1.91- 7.47)	<.001
Very high (EBNA IgG>300 units + VCA IgG>300 units)	38 (27.9)	17 (6.5)	11.08 (4.76-25.77)	<.001	15.64 (5.42-45.10)	<.001
Test for trend P value			<.001		<.001	

Abbreviations: CI, confidence interval; EBNA, EBV nuclear antigen; VCA, EBV capsid antigen.

*Adjusted for education level (<high school certificate, \geq high school certificate), past history of smoking, higher sun exposure when aged 6 to 15 years (an average ≥ 2 -3 hours a day in summer weekends or holidays vs less), fair skin type (<2% melanin density) vs darker skin ($\geq 2\%$ melanin).

Infants differ significantly from older children as an infection source both in the type of infections and possibly transmission mechanisms with increased salivary contact in infants. Some infections such as herpesvirus and enterovirus are particularly common in infancy, and oral poliovirus vaccination has been demonstrated to influence immunity in those in close contact with the infants.²⁹ Human herpesvirus 6 (HHV-6) infection is acquired by child-to-child transmission at a very early age.³⁰ Human herpesvirus 6 and enteroviruses have been implicated in MS pathogenesis.^{31,32} Repeated exposures to such common infant infections may confer protection against any adverse autoimmunity triggering effect of these infectious agents in later life. Early onset MS patients are more likely to be seropositive to EBV (both the EBNA and VCA antigens) and less likely to be seropositive to herpes simplex virus type 1 compared with controls, which is consistent with a possible adverse effect of EBV infection if other “protective” early infections have not occurred.³³ In evolutionary terms, the natural sequencing of viral exposures in childhood may be important for human immune development. Correct immunity to later infection may be impaired if inadequate exposure to common, closely related infant infections has not occurred. Several herpesviruses are common in infancy and also closely related, with homologous protein segments and antigenic cross-reactivity.²⁵ Cytomegalovirus can cause both EBV and HHV-6 reactivation,^{34,35} and HHV-6 Variant A can activate the EBV genome.³⁶

Higher infant contact in early life was also associated with a reduced risk of elevated EBV antibodies or infectious mononucleosis among healthy controls. This strengthens the inference that the association between infant contact and MS may reflect a difference in early life infection exposure and/or subsequent immune response to infection. However, the apparent protective effect of early life infant contact on MS persisted after EBV antibody titers were taken into account.

Multiple sclerosis is mediated by self-reactive T cells that may be induced or expanded by viral or other agents. The generation of cytotoxic and potentially self-reactive T cells in response to EBV appears to occur more readily in adults than children.³⁷ Potential mechanisms of expansion of the self-reactive T-cell population include molecular mimicry and epitope spreading.³⁸⁻⁴⁰ Molecular mimicry is the process by which virus infection activates T cells that are cross-reactive with self-antigens. Epitope spreading is activation of self-reactive T cells by sequestered self-antigens released secondary to tissue destruction induced by virus-specific T cells. Potentially self-reactive T cells that mediate autoimmunity may increase throughout life and increasingly require postnatally matured T-regulatory cells to control them.⁴¹ Antigen-specific T-regulatory cells appear to require restimulation in the periphery by antigen-presenting dendritic cells in lymph nodes before converting to memory phenotype and adopting a suppressive function.⁴¹ Recurrent exposure to infant infection may assist the antigenic stimulation required for immunological maturation. Further, UV radiation exerts immunomodulation through various mechanisms, including the induction of T-regulatory cells.¹⁹ In this study, the inverse association between infant exposure and MS was significantly stronger in children with higher amounts of winter outdoor activity and was nearly 2-fold stronger in children with higher sun exposure. The latter finding did not reach statistical significance, partly because this study was underpowered to examine interaction effects. The finding of a possible potentiation effect of sun exposure on the beneficial effect of infant contact among children indicates that biological pathways common to UV radiation and infection, such as T-regulatory cell development, should be one focus for future work on underlying mechanisms. Personal exposure to UV radiation and infection may differ even among siblings in the same family. Such intrafamilial environmental

differences may explain the lack of contribution of a shared family environment in past work.⁴²

Although this is a retrospective study, the main exposure, early life exposure to a younger sibling aged less than 2 years, is not prone to recall bias. Case-control differences in recall could contribute to the association between infectious mononucleosis and MS, but not to the case-control difference in EBV antibody titers. Day care patterns were not studied, but day care attendance was relatively uncommon in Tasmania when these adults were children. Although selection bias is possible, participation rates were high and cases and controls were chosen from a common source population.

Higher infant contact in the first 6 years of life was associated with a reduced risk of MS and delayed age of onset. Further work is required to confirm this effect and elucidate underlying mechanisms. The finding that higher infant contact in early life was associated with reduced EBV antibody titers and a reduced likelihood of infectious mononucleosis among healthy controls strengthens the inference that infant contact in early life may alter childhood infection patterns and related immune responses and reduce the risk of MS.

Author Contributions: Dr Ponsonby had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Ponsonby, van der Mei, Dwyer, Simmons, Kilpatrick.

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