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Bacillus Calmette-Guérin (BCG) Vaccination in Infancy and Risk of Childhood Diabetes

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Abstract

Background: A narrow time window in infancy may be relevant for the aetiology of immune-mediated type 1 diabetes. We investigated whether a non-specific immune stimulation in the first year of life, as resulting from Bacillus Calmette-Guérin (BCG) vaccination, was associated with childhood diabetes.

Methods: Using data from a birth cohort assembled through linkage of administrative databases, 78 492 subjects born in 1974 were the object of the present analysis. Information was extracted from the birth, death, and BCG vaccination registries. Diabetes-related health services were obtained from administrative health databases (physician billing claims and hospitalisation data) until 1994. Subjects were classified as having diabetes according to two validated definitions: (1) ≥2 diabetes-related medical visits within 2 years or ≥1 hospitalisation for diabetes; and 2) ≥4 diabetes-related medical visits within 2 years. Cox proportional hazards regression was used to estimate adjusted hazard ratios (HR) and 95% confidence interval (CI), adjusted for potential confounders.

Results: Forty-four per cent of subjects were BCG vaccinated in the first year of life. According to the first and second definition, respectively, 293 (0.37%) and 230 (0.29%) subjects were classified as having diabetes. There was no association between BCG vaccination in the first year of life and risk of diabetes with either definition ($HR_{def1} = 0.92$, 95% CI 0.73, 1.17; $HR_{def2} = 1.04$, 95% CI 0.80, 1.37), and results did not differ by sex.

Conclusions: Given the potentially critical importance of the exposure window and paucity of studies addressing BCG vaccination timing in relation to diabetes risk, this question deserves further investigation.

Keywords: *Bacillus Calmette-Guérin, BCG, diabetes, birth cohort, health administrative databases.*

Diabetes is one of the most common life-threatening conditions among children and youth, and both incidence and prevalence are increasing globally.[1] Risk of type 1 diabetes, the most common form of diabetes in children, is influenced by non-modifiable determinants such as sex, age, race, ethnicity, and genetic susceptibility.[2] Several environmental factors in the pre-, peri-, and post-natal periods have been investigated including aspects of the gestational environment (e.g. maternal age, enterovirus infections),[3] infant feeding,[4] as well as childhood vaccinations and infections.[5] These factors could play a role in initiating autoimmunity or accelerating destruction of insulin-producing β-cells, but no environmental risk factors were definitively identified despite a wealth of research, signifying a need for better understanding of the aetiologic mechanisms involved. Environmental factors affecting the immune response may act during narrow time windows in early development.[3] Indeed, most children who will develop type 1 diabetes express islet autoantibodies before 2 to 4 years of age, suggesting that exposures preceding this period may be most relevant for aetiology.[3]

The Bacillus Calmette-Guérin (BCG) vaccine modulates the immune response, and has been used since the 1920s to immunise neonates, children, and adults against tuberculosis. Unlike most other vaccines which stimulate antibody production via Th2 lymphocytes, the BCG vaccine induces cell-mediated immunity through an effect on Th1, Th17 lymphocytes and regulatory T cells.[6–8] These three T cell types have been implicated in immune processes related to autoimmune diseases such as type 1 diabetes.[9] Overall, the mechanisms underlying BCG-related immunomodulation are complex and remain ill defined, but this vaccine displays important non-specific effects beyond tuberculosis protection.[10] It has been shown to

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increase the ability of the immune system to control infections other than tuberculosis,[11] but its impact on immune-mediated diseases and specifically on type 1 diabetes is unresolved. Infancy is an important time window for immune system maturation,[12] which further justifies specific consideration of early life exposure when assessing a potential effect of BCG vaccination, used as a marker of non-specific immune stimulation, on childhood diabetes occurrence.

BCG vaccination was shown to prevent type 1 diabetes development in non-obese diabetes-prone mice when administered before onset of pathological changes in pancreatic islets.[13,14] Conversely, observational epidemiological studies assessing the association between BCG vaccination and occurrence of type 1 diabetes suggested null results.[5,15–21] Altogether, it is difficult to reconcile the immunological, experimental, and epidemiological evidence. However, all but one[5] of the previous epidemiological studies lacked formal consideration of the timing of BCG vaccination, which may have resulted in misclassification of the relevant exposure and bias towards the null.

The present study uses information from a province-wide BCG vaccination programme held in the province of Québec (Canada) from 1949 to 1974, later linked to several administrative databases. In the resulting large population-based birth cohort, we aimed to determine whether a non-specific immune stimulation in the first year of life, as resulting from BCG vaccination, was associated with the occurrence of childhood diabetes.

Methods

Study subjects

The Québec Birth Cohort on Immunity and Health (QBCIH), a population-based birth cohort assembled through linkage of administrative databases, was used for the project described here. It comprises individuals born in the province of Québec (Canada) in 1974 at or after 32 weeks of gestation. Data linkage was successful for 81 496 subjects, representing 90.5% of eligible subjects.[22] Information was extracted from the birth and death registries (1974–1994), the 2010 Healthcare Registration File (universal public health system), as well as the Québec BCG Vaccination Registry. Health services relating to a diabetes diagnosis [International Classification of Diseases (ICD)-9: 250) were obtained from physician billing claims (1983–1994) and hospitalisation data (1987–1994).

The study was approved by the ethics committees at *Institut National de la Recherche Scientifique*, *Institut de la Statistique du Québec* and *Régie de l'Assurance Maladie du Québec* (RAMQ), as well as Québec's *Commission d'Accès à l'Information*.

Exposure definition

The exposure, based on the Québec BCG Vaccination Registry, was defined as having been vaccinated with BCG or not. By design, all vaccinated subjects were less than 1 year old at vaccination (born and vaccinated in 1974). Indeed, subjects vaccinated from 1975 onwards ($n = 2890$, 3.5% of the cohort) were excluded, since the province-wide BCG vaccination programme ended at the end of 1974.

Outcome definitions

Two outcome indicators were defined. Based on use of health services, cohort subjects were classified as having diabetes according to two validated definitions used in Canada. For the first definition, utilised by the former National Diabetes Surveillance System now referred to as the Canadian Chronic Diseases Surveillance System,[23] subjects who have had ≥2 medical visits within 2 years or ≥1 hospitalisation recorded as diabetes-related were identified as having diabetes. For the second definition, validated by Guttmann *et al.*[24] in Ontario (Canada) children, subjects were considered to have diabetes if they have had ≥4 diabetes-related medical visits within 2 years. Among diabetes cases, age at first occurrence of diabetes was defined as the earliest of either the age at first diabetes-related medical visit or hospitalisation. The time at risk was considered to be from 1 January 1983 (beginning of health administrative data availability) until occurrence of diabetes, death, or 31 December 1994, whichever came first.

Covariates

Information on potential confounders of the association between BCG vaccination and diabetes occurrence was extracted from the Birth Registry, as well as the medical services and hospitalisations databases. Information extracted from the Birth Registry included sex, birthweight for gestational age (BWGA)

according to a Canadian sex-specific reference,[25] number of older siblings (used as a surrogate measure of infections during childhood, suggested to be associated with type 1 diabetes),[5] parental age at childbirth, and parental place of birth. Subjects' postal code in 1991 obtained from the Healthcare Registration File was used to determine (1) area of residence using Canada Post's definition for mail delivery (urban or rural, based on the second character of postal code)[26] and (2) income (estimated by median household income from the 1991 Canadian census using the first three characters of subjects' postal code).

Data related to allergic diseases, also potentially associated with occurrence of type 1 diabetes,[27] were extracted from the RAMQ medical services claims and hospitalisation databases. These included asthma (ICD-9 code 493), rhinitis (ICD-9 code 477), eczema (ICD-9 code 692.9), allergic urticaria (ICD-9 code 708.0), and unspecified allergies (ICD-9 code 995.3, V15.0 and V07.1). Subjects were considered to have allergic diseases if they have had (1) ≥ 2 medical visits within 2 years or ≥1 hospitalisation for asthma[28] or (2) ≥ 1 health encounter (medical visit or hospitalisation) related to rhinitis, eczema, allergic urticaria, or unspecified allergies.

Statistical analyses

Among the 81 496 cohort subjects, 25 (0.03%) were deceased before the beginning of coverage of administrative health databases in 1983, 2890 (3.5%) were excluded because they received the BCG vaccine after the province-wide vaccination programme had ended, and BCG vaccination status could not be determined for 89 (0.1%) subjects. Analyses were thus conducted on the remaining 78 492 subjects.

Frequency distributions were produced for the exposure variable, covariates, and health services use for diabetes (medical visits, hospitalisations). The prevalence of diabetes according to the two definitions was calculated. Separate Cox proportional hazards regression analyses were carried out to estimate hazard ratios (HR) and 95% confidence interval (CI) for the effect of BCG vaccination on diabetes occurrence according to each of the two diabetes definitions. Models were adjusted for the following a priori covariates: sex, BWGA, number of older siblings, maternal age at childbirth, family income, area of residence, parental place of birth, and the presence of allergic diseases. An interaction term for sex and BCG vaccination was included in the models to determine whether the effect of vaccination on diabetes occurrence differed according to sex on a multiplicative scale.

There were missing values for some covariates, of which the 'number of older siblings' had the highest proportion (4.7%). Given the non-monotone missing pattern, multiple imputations by the Markov Chain Monte Carlo method were performed (five imputed data sets), and Cox proportional hazards regression analyses were done on the imputed data set.

As a sensitivity analysis, subjects with interruptions in provincial health insurance coverage between 1983 and 1994 were excluded from the analysis to ascertain whether a temporary lack of health coverage had an effect on risk estimates.

All analyses were carried out using SAS Enterprise Guide, version 5.1 (SAS Institute Inc., NC, USA).

Results

Characteristics of the study population are presented in Table 1. Fifty-one per cent of subjects were males, the vast majority of their parents were born in the province of Québec, and 68% were living in urban areas. Birthweight for gestational age was appropriate or large for most subjects. Half of the subjects had at least one older sibling. For the vast majority of subjects, maternal and paternal ages at childbirth were below 35 and 40 years, respectively. Forty-four per cent of subjects were BCG vaccinated within their first year of life.

Table 2 shows health services use for diabetes and the prevalence of diabetes among all subjects. Overall, 0.77% (606 subjects) had at least one medical visit and 0.22% (171 subjects) had at least one hospitalisation for diabetes. Applying the first definition of diabetes (≥2 medical visits within 2 years or ≥1 hospitalisation), a total of 293 (0.37%) subjects were classified as having diabetes. According to the second definition (≥4 medical visits within 2 years), prevalence was 0.29% (230 subjects). Thus, 63 subjects were defined as having diabetes according to the first, but not the second definition. For the vast majority of these subjects (76%), the first definition was satisfied based on the number of medical visits for diabetes. Over the follow-up, these 63 subjects had an average of 2.5 medical visits [standard deviation (SD) 1.5], 0.32 hospitalisations (SD 0.84), and first diabetes-related health

Table 1. Characteristics of subjects ($n = 78\,492$), the Québec Birth Cohort on Immunity and Health

Variables	*n*	%
Sex		
Males	40 128	51.1
Females	38 3596	48.9
Missing	*5*	*0.01*
Birthweight for gestational age		
Very small for gestational age	11 918	15.2
Small for gestational age	6522	8.3
Appropriate for gestational age	52 839	67.3
Large for gestational age	7196	9.2
Missing	*17*	*0.02*
Older siblings		
0	34 860	44.4
1	24 582	31.3
>1	15 397	19.6
Missing	*3653*	*4.7*
Maternal age at childbirth (years)		
<35	72 380	92.2
≥35	4907	6.3
Missing	*1205*	*1.5*
Paternal age at childbirth (years)		
<40	71 467	91.1
≥40	3919	5.0
Missing	*3635*	*4.0*
Maternal birthplace		
In Québec	66 998	85.4
Outside Québec	9800	12.5
Missing	*1694*	*2.2*
Paternal birthplace		
In Québec	64 409	82.1
Outside Québec	10 448	13.3
Missing	*3635*	*4.6*
Area of residence in 1991[a]		
Rural	22 317	28.4
Urban	53 490	68.2
Missing	*2685*	*3.4*
Family income[b] ($/year)		
≤35 333	19 277	24.6
35 334–40 667	18 133	23.1
40 668–47 289	18 730	23.9
≥47 290	19 066	24.3
Missing	*3286*	*4.2*
Presence of allergic disease		
No	46 264	58.9
Yes	32 228	41.1
BCG vaccination status		
Not vaccinated	43 628	55.6
Vaccinated <1 year of age	34 864	44.4

[a]Determined using the second character of subjects' residential postal codes (RAMQ, 1991; 0: rural, ≠ 0: urban).
[b]Estimated by 'median family income' from the 1991 Canadian census using the first three characters of subjects' residential postal codes (RAMQ).

Table 2. Health services use for diabetes and prevalence of diabetes, the Québec Birth Cohort on Immunity and Health

	Overall ($n = 78\,492$)	
	n	%
Diabetes-related medical visits		
0	77 886	99.23
≥1	606	0.77
0	77 886	99.23
1	319	0.41
2	33	0.04
≥3	254	0.32
Diabetes-related hospitalisations		
0	78 321	99.78
≥1	171	0.22
0	78 321	99.78
1	90	0.11
2	35	0.04
≥3	46	0.06
Prevalence of diabetes		
≥2 medical visits in 2 years or ≥1 hospitalisation[a]	293	0.37
≥4 medical visits in 2 years[b]	230	0.29

[a]First definition, used by the Canadian Chronic Diseases Surveillance System[23]
[b]Second definition, validated by Guttmann *et al*.[24]

encounter occurring at 17 years (SD 3.0). Irrespective of case definition, diabetes prevalence did not differ by sex (data not shown).

As shown in Table 3, no association was found between BCG vaccination within the first year of life and diabetes, using either the first or second definition. The adjusted HRs were 0.92 (95% CI 0.73, 1.17) and 1.04 (95% CI 0.80, 1.37), respectively. Results did not differ according to sex (*P*-values for interaction terms between BCG vaccination status and sex were 0.29 and 0.71 in models with the first and second definitions of diabetes, respectively).

With respect to assessing the effect of interruptions in health coverage on risk estimates, only 4.0% among the 78 492 subjects had at least one interruption between 1983 and 1994 (cumulative median length of interruption = 1247 days). Cox regression models carried out on the sample of 75 333 subjects with uninterrupted coverage showed similar results to those based on the full analytical sample. The adjusted HRs for the association between BCG vaccination in the first year of life and childhood diabetes were

Table 3. Crude and adjusted hazard ratios for the association between BCG vaccination and diabetes, the Québec Birth Cohort on Immunity and Health ($n = 78\,492$)

Diabetes definition Exposure definition	Diabetes, *n*	Follow-up, person-years	Crude HR (95% CI)	Adjusted HR[a] (95% CI)
≥2 medical visits in 2 years or ≥1 hospitalisation				
BCG vaccination status				
Not vaccinated	163	521 928	Reference	Reference
Vaccinated <1 year old	130	417 041	1.00 (0.79, 1.26)	0.92 (0.73, 1.17)
≥4 medical visits in 2 years				
BCG vaccination status				
Not vaccinated	122	522 065	Reference	Reference
Vaccinated <1 year old	108	417 114	1.11 (0.86, 1.44)	1.04 (0.80, 1.37)

[a]Models were adjusted for sex, birthweight for gestational age, number of older siblings, maternal age at child birth, census-based family income, area of residence, parental place of birth, and presence of concomitant allergies.

0.89 (95% CI 0.70, 1.14) and 1.02 (95% CI 0.77, 1.33) using the first and the second diabetes definitions, respectively.

Comment

A unique contribution of our study is the focus on an effect of BCG vaccination during a specific time window, the first year of life, highly relevant for type 1 diabetes aetiology, and during which the BCG vaccine can plausibly influence maturation of the immune response. In this large population-based birth cohort followed until age 20, we observed no effect of BCG vaccination within the first year of life on the occurrence of childhood diabetes.

To our knowledge, the impact of the timing of BCG vaccination on incidence of childhood diabetes was only assessed in one previous study, led by the EURODIAB Substudy 2 Study Group.[5] This was a large case–control study combining data from seven study centres in Europe and including 900 diabetics and 2302 controls. The authors found no evidence of early BCG vaccination being associated with diabetes but did not report the precise time periods of exposure considered.[5] In our study, we specifically assessed the association between childhood diabetes and BCG vaccination in the first year of life but did not compare with the effect of vaccination at older ages. Indeed, subjects who were older when vaccinated also happened to receive the vaccine after the province-wide programme when it was only offered to those considered at higher risk for tuberculosis, impeding a valid direct comparison of different ages at vaccination.

The effect of BCG vaccination on type 1 diabetes, without formal consideration for timing of exposure, was assessed in several studies. Examining the exposure patterns and results described in these studies may provide indirect evidence on an effect of age at vaccination. One study was ecological, set in Sweden where the policy was to offer the vaccine to newborns.[16] Others include prospective cohorts with short follow-up periods (2–5 years) among offspring of parents with type 1 diabetes set in Germany where babies were vaccinated within 6 weeks of birth.[17,18] There were also case–control studies set in: (1) Sweden (policy changed over the course of the study: BCG vaccine was part of general vaccination programme until 1975, after which only newborns at risk vaccinated);[15] (2) the UK[20] (policy was to offer vaccine to those at high risk for tuberculosis);[29] or (3) Canada (vaccine offered to newborns and schoolchildren – authors reported that 77% of vaccinated subjects received the BCG before age 1).[19] No association between BCG vaccination and diabetes was observed in these studies including those in which most vaccinated subjects might be presumed to have been vaccinated in infancy,[16–19] although Parent *et al*. suggested that vaccination at birth may have resulted in delayed diabetes onset when compared with non-vaccination.[19] The lack of formal analysis of age at vaccination or alternatively presentation of age distribution at vaccination in these studies precludes us from drawing definitive conclusions. Furthermore, only three of these studies presented highly desirable methodological features such as the use of vaccination records and official diabetes registries, as well as a follow-up until adolescence.[5,15,19]

In our study population, BCG vaccination was free but not mandatory. The policy was to target newborns, school-aged children upon acceptance of school boards, and high-risk individuals for tuberculosis. We have investigated vaccination rates and found that they differed greatly by region,[30] likely due to local availability of human and financial resources, as well as acceptance of the vaccine by both health professionals and the population. We also found that vaccination was related to gestational age, birthweight, parental age, parental birthplace, and income level.[22] These and other factors potentially associated with diabetes were considered as covariates in our current analyses. There might have been additional factors related to BCG vaccination that we did not consider, and some might well be related to diabetes risk. Although unlikely given the wealth of factors already considered and the paucity of established risk factors for childhood diabetes, residual confounding (negative or positive) by unknown factors cannot be entirely excluded.

The selection of definitions for identifying diabetes cases using health administrative data was based on sensitivity and specificity estimated in the Canadian population. Both definitions were validated in a population of Ontario children.[24] The first definition (≥2 medical services in 2 years or ≥ 1 hospitalisation for diabetes) had a sensitivity of 1.00 and a specificity of 0.94, while respective values for the second definition (≥4 medical services for diabetes in 2 years) were 0.83 and 0.99.[24] The number of false positives is minimised with the second definition at the expense of false negatives. The first definition was also selected because it is nationally used for diabetes surveillance.[23] In addition, considering hospitalisations, as in the first definition, was in line with clinical practice during the study period (1980s and 1990s). At that time in Québec, it was customary to hospitalise children and adolescents newly diagnosed with type 1 diabetes to provide the necessary skills and knowledge to understand and manage this condition (i.e. blood glucose monitoring and insulin injections). After applying both definitions, the pediatric endocrinologist in our team (LL) examined patterns of health services use among 63 subjects classified as having diabetes with the first definition but not with the second one. Based on these patterns, we agreed that their diabetes status was equivocal, and reported results from both definitions which led to the same conclusion of an absence of association.

Upon comparison, our estimates of diabetes prevalence for the first and second definitions (0.37% and 0.29%) were slightly higher than the 0.30% obtained in Canada (2001/02)[23] and 0.18% in Ontario (1994)[24] with the first and second definitions, respectively. Our estimates were based on 20-year olds in 1994, whereas the comparison estimates were age and sex standardised to the Canadian population under 20 years. Diabetes prevalence is thus expected to be higher in our study since it includes older subjects.

We could not use definitions of diabetes that took into account prescription drug information (e.g. in Canada, Vanderloo *et al*.[31]) as these data were only available for the most socioeconomically disadvantaged subjects, a very small proportion of the QBCIH cohort. However, using prescription drug information in addition to physician claims and hospitalisations for the identification of pediatric diabetes cases in Manitoba (Canada) did not improve sensitivity or specificity.[32] This suggests that the impact of not considering such data is minor.

Due to the specific time availability of the administrative databases, there were periods for which we did not have medical information. The Medical Services Claims database is available from January 1983, and the hospitalisation database from April 1987. As a result, incident cases of diabetes between 1974 and 1982 could not be identified due to the lack of coverage. Similarly, the lack of data on hospitalisations until 1987 may have delayed identification for some cases. Nevertheless, we were able to capture these as prevalent cases from 1983 onwards, as children with diabetes usually visit their physicians on a regular basis, and lifetime follow-up is necessary. This implies that the 20-year follow-up allowed us to identify all, or nearly all, prevalent cases of diabetes diagnosed before the age of 9 as they would have led to medical visits and/or hospitalisations. However, if BCG vaccination at birth resulted in a temporary boost of immune functions and slightly delayed diabetes development as suggested by Parent *et al*.,[19] the lack of documentation of medical visits before age 9 and hospitalisations before age 13 in our study could have prevented us from observing such an effect.

In the present study, the outcome of interest is childhood diabetes. The information available in administrative health databases did not allow distinguishing between type 1 and type 2 diabetes (ICD-9 code: 250), but study subjects born in 1974 were followed-up until age 20 in 1994, a time period during

which the prevalence of type 2 diabetes in youth was very low. The current estimates in our setting suggest that <5% of all pediatric diabetes is type 2.[33] While there is no precise estimate to which we can refer for the period covered by our study, we know that it was even lower at the time. This suggests that the vast majority of diabetes cases identified in our study were in fact of type 1. Although not impossible, it is highly unlikely that such a proportion of type 2 diabetes among the identified cases would have importantly biased our results towards an absence of association.

This study is unique in the sense of addressing a hypothesis on the effects of non-specific stimulation of the immune function resulting from BCG vaccination on the development of childhood diabetes, in a population with non-mandatory BCG vaccination yet presenting a large enough proportion of vaccinated newborns. The fact that individual records of BCG vaccination for the entire province were available, that data quality was excellent, and that records could successfully be linked to other administrative health databases[34] were other advantages of the study. Moreover, reporting of diagnoses by physicians is expected to be complete as every family with a child who has diabetes residing in the Province of Québec is eligible for a family allowance supplement under the *Régie des Rentes du Québec*, a programme that was first initiated in 1980.[35]

There is an inherent benefit in studying the role of non-specific stimulation of the immune function among children born in Québec, Canada, in the 1970s. This population was largely constituted of individuals of French descent, characterised by a relatively homogeneous genetic pool as compared with many other populations. This homogeneity facilitates the observation of an association between exposure and outcome in the event that genetic background modulates this association.

Conclusions

In conclusion, we observed no association between BCG vaccination in the first year of life, a marker of non-specific immune stimulation, and occurrence of childhood diabetes. Given that our study was one of the very few in which a specific time window of vaccination was considered, this area deserves further investigation in large or pooled studies with valid measurements of both exposure and outcome, as well as accurate documentation of potential confounders. Such studies may ultimately contribute to a better understanding of the early life aetiologic mechanisms involved in childhood diabetes.

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