CHILDHOOD BCG VACCINATION AND THE RISK OF ASTHMA IN ADULTS

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ABSTRACT

Abstract

Asthma is a heterogeneous disorder, and the prevalence is increasing worldwide. The BCG vaccine is hypothesized to lower the risk of asthma by modulating specific aspects of T-cell mediated immunity (termed Th1 and Th2). We conducted a case-control study to determine the relationship between BCG vaccine given to children born and raised in Quebec and asthma in adult subjects. 93 case subjects with a clinical diagnosis of asthma and 118 control subjects without asthma answered a standardized questionnaire, and BCG vaccination status was verified in a central registry. After adjusting for potential confounders, vaccination with BCG after the age of one was associated with a reduced odds of adult-onset asthma (odds ratio: 0.3 (0.1-0.98)), and specifically of adult-onset atopic asthma (odds ratio: 0.2 (0.1-0.9)). These results suggest a critical time frame in immune system maturation during which administration of BCG vaccine may lower the risk of adult-onset asthma.

Résumé

L'asthme est une maladie hétérogène de plus en plus répandue mondialement. Le vaccin BCG est réputé réduire le risque d'asthme en modulant certains aspects immunitaires du lymphocyte de type T (Th1 et Th2). Notre étude cas-témoin a examiné la relation entre le vaccin BCG administré aux enfants nés et vivants au Québec et l'asthme à l'âge adulte. 93 sujets avec un diagnostic d'asthme et 118 sujets témoins sans asthme ont complété un questionaire standardisé et le statut de vaccination BCG fut vérifié au fichier central. Après ajustement des variables confondantes, nous avons conclu que le vaccin BCG, administré après l'âge de 1 an, est associé à une incidence réduite de l'asthme à l'âge adulte (OR: 0.3 (0.1-0.98)), plus spécifiquement de l'asthme atopique à l'âge adulte (OR: 0.2 (0.1-0.9)). Ces résultats suggèrent que pour potentiellement réduire le risque de développer l'asthme à l'âge adulte, le vaccin BCG doive être administré à un moment critique du processus de maturation du système immunitaire.

LIST OF KEY WORDS/ABBREVIATIONS

KEY WORDS

ASTHMA

ATOPY

Mycobacterium tuberculosis

ABREVIATIONS

BCG	BACILLE CALMETTE-GUERIN
CD4+, - 8+	CLUSTER OF DIFFERENTIATION 4+, - 8+
DLCO	DIFFUSION CAPACITY FOR CARBON MONOXIDE
ECRHS	EUROPEAN COMMUNITY RESPIRATORY HEALTH SURVEY
FEV1	FORCED EXPIRATORY VOLUME IN ONE SECOND
FVC	FORCED VITAL CAPACITY
IFN-γ	INTERFERON-GAMMA
IgG, -E	IMMUNOGLOBULIN "G", "E"
IL- 4, -5 etc..	INTERLEUKIN 4, -5, etc...
ISAAC	INTERNATIONAL STUDY OF ASTHMA AND ALLERGIES IN CHILDHOOD
SES	SOCIOECONOMIC STATUS
SPT	Skin Prick Test
TH1	TYPE 1 T-HELPER CELL
TH2	TYPE 2 T-HELPER CELL
TNF-β	TUMOUR NECROSIS FACTOR BETA
TST	TUBERCULIN SKIN TEST
TU	TUBERCULIN UNIT

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CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 OVERVIEW

Asthma is a chronic inflammatory disorder of the airways, causing recurrent episodes of wheezing, breathlessness, chest tightness, and cough associated with variable airflow obstruction, which is reversible spontaneously or with treatment. This inflammation also causes increased airways hyperresponsiveness in response to a wide variety of provoking stimuli (1). Although it has no standardized clinical definition, epidemiologic surveys repeated in the same populations have demonstrated an increase over time in the prevalence of self-reported wheeze and physician-diagnosed asthma (2). Prevalence is highest in economically developed countries, and lower in developing countries (3). It has been hypothesized that because children raised in affluent societies are less likely to be exposed to infectious diseases, their cell-mediated immune response may consequently be polarized towards a Type-2 T-helper cell lymphocyte (Th2) phenotype. Because this subtype is responsible for mediating allergic inflammation, among children who are genetically predisposed, their risk of allergic sensitization and development of asthma may be increased (4).

1.2 CLINICAL ASPECTS OF ASTHMA

1.2.1 Clinical Manifestations

The most common symptoms of asthma include wheezing, breathlessness, and cough. Wheezing is usually perceived by the subject as either an audible sound or sensation in the chest related to breathing, most often during expiration. Most subjects recognize breathlessness from asthma as distinct from that induced by exertion, and describe it as difficulty moving air into and out of the chest or chest "tightness" (5). Cough related to asthma usually occurs as abrupt repeated paroxysms, often waking the subject at night or in the early morning, and occasionally may be the sole manifestation of asthma (6).

These symptoms typically vary over time and may evolve abruptly, or slowly over

days to weeks, and may be provoked by a variety of stimuli. External environmental agents such as trees, grasses, or pollens may cause symptoms during defined periods of the year. By contrast, perennial symptoms are more likely to be provoked by indoor agents such as dust mites. Other stimuli include exercise, cold air, food substances, viruses, as well as a variety of agents found in the work environment (7).

The physical exam may be entirely normal, given the episodic nature of symptoms. At other times, findings during an episode of asthma reflect either airway narrowing, increased respiratory effort, imbalance of ventilation and perfusion, and increased distension of the lungs. Wheezing is caused by high velocity turbulent airflow through narrowed airways, and is usually noted on expiration, though it may be present on inspiration as well. Variation in the quality of wheezing reflects non-uniformity of airflow. Loudness and pitch of wheeze correlate with objective measures of peak expiratory flow, though wheezing may be absent in very mild or very severe airway obstruction. With increasing obstruction, the expiratory phase of respiration becomes longer, accessory muscles of respiration are used, and the patient prefers to sit or stand. Chest shape is usually normal in mild and intermittent asthma, but may become permanently "barrel-shaped" in those with persistent and severe airflow obstruction. Cyanosis and tachycardia normally parallel increasing airflow obstruction. Pulsus paradoxus, an inspiratory fall in systolic blood pressure of more than 10mmHg may be noted, but is an unreliable measure of the severity of airflow obstruction. Jugular venous distension during expiration may be noted, and reflects raised intrathoracic pressure (8).

Non-pulmonary manifestations associated with asthma may include eczema, sinusitis, rhinitis, and nasal polyps if asthma is a manifestation of an atopic state (see Section 1.2.3: "Pathophysiology").

1.2.2 Diagnosis

A diagnosis of asthma may be suspected on the basis of characteristic symptoms, but security in the diagnosis requires an objective demonstration of airflow limitation. Airflow limitation may be documented on the basis of reductions in peak expiratory flow,

or in the ratio of forced expiratory volume in one second (FEV1) to forced vital capacity (FVC). The utility of peak expiratory flow to detect airflow limitation is poor, since the test is highly dependent on patient effort, and variability of this measure within individuals may be as high as 30% (9). The FEV1 has been validated as a measure of airways obstruction by its close correlation with pathologic scores of airway disease (10), and has good reproducibility, with an intra-subject coefficient of variation of 3-5% (9). It is more sensitive to airway obstruction than auscultation or symptoms elicited by history or standardized questionnaires; furthermore, reduction in the FEV1/FVC ratio adjusts for the lung volume dependence of airflow. Reference values for these measures have been developed by Crapo (11), and Knudson (12), as well as from subjects participating in the third National Health and Nutrition Examination Survey conducted in the United States (13).

Once airflow limitation has been documented, assessment of its reversibility in response to bronchodilator administration is required. Although there is no universally accepted definition of what constitutes reversibility, the current criteria for a response consistent with a diagnosis of asthma is an increase in FEV1 or FVC of at least 12% and at least 200ml (14). Increments of less than 8% (or less than 150ml) are likely to be within measurement variability (15). Previous criteria were more stringent, requiring an increase in FEV1 of 15%, and while this is considered indicative of significant reversible airway obstruction, the cutoff is an arbitrary one, and lacks sensitivity for detecting asthma. A level of 12%, while sensitive, is not as specific and therefore does not discriminate between asthma and other forms of chronic airway obstruction, such as emphysema and chronic bronchitis, since there may be overlap between these patient groups in their degree of airway reversibility.

Use of bronchodilator-induced reversibility for diagnosing asthma is controversial, however, because of the high false-negative rate: the relationship between degree of baseline airways obstruction and percentage change in baseline is bell-shaped, with the largest mean response among asthmatics with moderate obstruction. Subjects with severe baseline obstruction, who have substantial airway secretions and edema are

not as likely to respond quickly to inhaled bronchodilators. Similarly, subjects with only a mild degree of baseline airways obstruction do not require a substantial bronchodilator response before they reach their maximal FEV1. Some subjects with asthma may similarly have no airway obstruction at the time of the test and be considered normal. Bronchodilator-induced reversibility is therefore unlikely to be adequately sensitive as a sole diagnostic criterion in these subject groups (15).

Airway responsiveness to methacholine or histamine (bronchoprovocation testing, methacholine-, or histamine-challenge testing) is used clinically to aid in the diagnosis of asthma, especially among subjects with normal baseline spirometry. Methacholine challenge testing results are correlated with objective markers of airway inflammation, as well as subjective symptoms of wheezing. It has a sensitivity of approximately 95% among subjects with physician-diagnosed asthma. The false-positive rate is greater than 5% if the same cutoff for a positive test is used in a general population sample to diagnose asthma, due to the high prevalence of increased airway reactivity among subjects with allergic rhinitis, smoking-related and other lung disease, and congestive heart failure, as well as the ability of the test to detect subclinical asthma. A small percentage of normal subjects (asymptomatic individuals) will have a positive test; these individuals are either truly normal, or have poor subjective perception of their airflow limitation. Conversely, subjects with typical asthma symptoms and a negative test result may be symptomatic on the basis of vocal cord dysfunction, or airways obstruction due to a central airway tumor, polyp, or foreign body (15).

Some subjects with asthma will experience symptoms related to bronchoconstriction only in relation to exercise. In these individuals, bronchoconstriction is induced by exercise on a treadmill and is detected via serial FEV1 measurements taken after cessation of exercise. A fall in baseline FEV1 of 15% or more after exercise is felt to be consistent with a diagnosis of exercise-induced asthma (16).

Other physiologic tests, such as measurement of diffusion capacity for carbon monoxide (DLCO), are helpful in distinguishing obstructive lung disease due to asthma

from that due to emphysema. In the latter condition, the DLCO is reduced, while in asthma it is usually normal or increased (15).

1.2.3 Pathophysiology

Asthma is characterized by multiple changes in the airway including mucus plugging, epithelial cell shedding, basement membrane thickening, vessel engorgement and angiogenesis, smooth muscle hypertrophy and hyperplasia, and inflammatory cell infiltration. The pathogenesis consists of inflammatory and remodeling components (17).

A number of cell types are implicated in the inflammatory component of asthma, and include eosinophils, mast cells, and CD4+ T helper lymphocytes. Neutrophil infiltration occurs during exacerbations and in the late response to allergen challenge (18). Dendritic cells are responsible for antigen presentation (19) and antigen cross-linking by immunoglobulin E (IgE) subsequently occurs, causing mast cell degranulation. Mast cell responses are responsible for the acute asthmatic response and contribute as well to airway remodeling in chronic asthma (20).

T lymphocytes play a critical role in the coordination of the inflammatory response in asthma. T-helper lymphocytes differentiate into two main subtypes-- Th1 and Th2-- each of which produces a distinct set of cytokines (21). Th1 cells produce interferon gamma, and are the main effectors of phagocyte defense whereas Th2 cells produce interleukins 4, 5, 9, 10 and 13. Interleukins 4 and 13 induce IgE and IgG4 production, while interleukin 5 enhances eosinophil differentiation and activation (22). Furthermore, atopic diseases such as asthma are characterized by a high expression of Th2 cytokines (23).

Atopy is an individual predisposition to develop an IgE mediated immune response against environmental allergens such as house dust mites, pets, and pollen (24). Both genetic and environmental factors interact to predispose an individual to atopic conditions such as rhinitis and conjunctivitis, dermatitis, and asthma. While most atopic subjects develop rhinitis, atopy can be clinically silent and detectable only by observing

the presence of specific IgE levels in the patient's serum or by skin prick testing (SPT) (25). The latter test involves subcutaneous injection on the volar surface of a subject's forearm of common allergens in the subject's community. These may include, but are not limited to, dust mite, cockroach, cat, dog, horse, mold and other antigens. Histamine and saline are injected as positive and negative controls, respectively, and a skin prick test is positive if the wheal induced by antigen injection is at least 3mm in diameter (mean of vertical and horizontal diameters) after 15 minutes, and is larger than the positive control. Most epidemiologic studies determining the atopic status of subjects define atopy as either at least one positive SPT or a single positive serum specific IgE to one of several inhaled allergens, or both (26).

The extent to which the development of asthma is attributable to atopy has been recently estimated in a meta- analysis (27). Among children, the proportion of asthma cases attributable to atopy (calculated as the population attributable risk), varies from 25% to 63% with a weighted mean of 38%. Among adults, the proportion varies from 8% to 55% with a weighted mean of 37%. These calculated proportions were based on studies which defined atopy as a single positive skin prick test. It has been suggested, however, that total serum IgE provides an overall estimate of the atopic component in asthma (28). When studies in adults using this definition of atopy are considered, the proportion of cases attributable to atopy vary from less than 0% (an inverse association) to 80% with a weighted mean of 33% (27). Data from Burrows (29) has shown that the proportion of asthma cases attributable to atopy varies with the cut-off point of total serum IgE level defining atopy, and ranges from 13% when serum IgE level greater than 640 IU/ml defines a subject as atopic, to 67% when a cut-off of 5IU/ml is used. Many studies using total serum IgE as a marker of atopy use a cut-off level of 100IU/ml.

While some studies therefore show an association between asthma and atopy, most do not (27), and so the observed association between total serum IgE levels (or other measures of atopy) and asthma may not necessarily be causal. This suggests that consideration of a subject's atopic status in relation to their potential to develop asthma may not be critical in the design and analysis of epidemiologic studies on asthma.

1.2.4 Clinical Phenotypes of Asthma

In addition to a wide variety of provoking stimuli, there are a variety of clinical phenotypes of asthma described among both children and adults. These may reflect a broad spectrum of manifestations of the same disorder through the life cycle, or may represent distinct disorders with different triggers and pathophysiologic mechanisms.

Among children, Martinez et. al. have studied over 1000 subjects enrolled as neonates since 1980 in the Tucson Children's Respiratory Study (30) to study the interrelationships between a large number of risk factors, acute lower respiratory tract illness during the first 3 years of life, and the development of asthma in later childhood and young adult life. Data from this study has demonstrated that there are 3 main syndromes of wheezing and asthma in children (31). These include: 1.Transient infant wheezing, characterized by wheezing only during the first 3 years of life, and not subsequently. This syndrome may be due to impaired lung function independent of the effects of lower respiratory tract infection, and of maternal smoking in pregnancy. Furthermore, children with this syndrome are no more likely to have a family history of asthma nor allergic disease than children who do not wheeze during the first 3 years of life. 2. Non-atopic wheezing, in which children who have an episode of respiratory syncitial virus-lower respiratory tract infection (RSV-LRTI) in early life are more likely to wheeze beyond the age of 3 compared to children not similarly affected. These children are more likely than their peers to wheeze up to the age of 13, though not beyond, and it is believed that this risk is related to alteration in airway tone, though whether this is due to the early life RSV-LRTI or is present at birth cannot been determined. 3. Atopic wheezing, which is characterized by persistence of symptoms into adolescence and is related to the development of allergic sensitization to a variety of antigens. Severity of wheezing as well as level of allergic sensitization in this subgroup is related to whether symptoms begin early (before the age of 3) or late (after the age of 3). In addition to these 3 main subgroups, this study has also distinguished between recurrent childhood cough in the absence of wheeze, and that associated with wheeze. It is believed that the latter subtype is more likely to represent asthma in that it is associated with family allergic history, wheezing LRTIs in early life, and high IgE levels at 6 years of

age.

Asthma persisting from childhood into adulthood is considered different from asthma starting in adulthood, and less is known regarding the natural history of the latter (32). The types of adult-onset asthma are based on etiologic factors and include:
1. Asthma associated with aspirin sensitivity, characterized by the development of chronic hyperplastic rhinosinusitis, nasal polyps, and asthma attacks after ingestion of aspirin and other non-steroidal anti-inflammatory drugs. 2. Asthma developing as a consequence of severe respiratory infection, usually with *Chlamydia* and *Mycoplasma Pneumonia*. 3. Asthma developing as a consequence of workplace exposures via immunologic mechanisms (both IgE and non-IgE mediated), and non-immunologic mechanisms.

1.3 EPIDEMIOLOGY OF ASTHMA

1.3.1 Methodological Issues

Epidemiologic surveys attempt to identify subjects with asthma on the basis of responses to questions about symptoms. Symptoms are not specific for the diagnosis, however, and questionnaires are usually validated against results of clinical or physiologic evaluations (a physician diagnosis of asthma or non-specific bronchial challenge test, respectively). Several questionnaires have been developed and modified over time to assess subjects with asthma, and have been evaluated for their validity and reproducibility. The 1984 International Union Against Tuberculosis and Lung Disease (IUATLD) questionnaire (33) was developed to assess subjects for asthma symptoms, and was the result of modification and refinement of previous questionnaires such as the Medical Research Council (34), American Thoracic Society (35), and Tucscon asthma study questionnaires (36). A review of studies determining the validity of asthma questionnaires (37) has shown that questions about "self reported asthma", when validated in relation to bronchial challenge testing, had a mean sensitivity of 36% (7%-80%), and specificity of 94% (74%-100%). The questionnaire item regarding a "physician diagnosis of asthma" had a mean specificity of 99%. When validated against a

clinical diagnosis of asthma, the mean sensitivity of a self-report of asthma was 68% (48%-100%), while the mean specificity was 94% (78%-100%). It has been determined that the questionnaire items with the best sensitivity and specificity in relation to a physician diagnosis of asthma and bronchial hyperresponsiveness are those which ask the subject if he or she "ever had asthma", "ever had asthma diagnosed by a physician", and "has experienced wheezing in the previous 12 months". Use of a clinical diagnosis of asthma as a "gold standard" against which questionnaires are validated is problematic, as clinical diagnoses will not detect mild subclinical asthma. Use of bronchial hyperresponsiveness as a "gold standard" is similarly problematic, as many subjects with bronchial hyperreactivity do not have respiratory symptoms. Clinical diagnoses and measurement of airway hyperreactivity will therefore underestimate the specificity and sensitivity of survey questionnaires, respectively (37). While most epidemiologic surveys only use questionnaires to identify subjects with asthma, pulmonary function test results, if available, can help to confirm that subjects selected by questionnaire have an objective measure of this disease.

With respect to atopy, few studies have validated the use of screening questionnaires for inclusion of atopic adult subjects in population studies on inhalent allergies and/or asthma. Nielsen et. al. validated a questionnaire among 1600 subjects aged 15-69 years (38) using skin prick tests as the reference standard. Odds ratios for the various screening questions in this study varied between 1.5 and 12. Up to 40% of atopic subjects, however, were not identified by screening questionnaires alone (39).

1.3.2 Evidence for the Increasing Prevalence of Asthma

Despite these methodological issues, numerous studies suggest that the prevalence of asthma is increasing worldwide; Peat et. al. (40) surveyed the prevalence of asthma in 1982 and 1992 in two Australian towns with different climates (Wagga Wagga and Belmont), using a self-administered questionnaire completed by parents of 8-10 year–old children recruited from a random sampling of primary schools. There were 769 and 718 children participating in 1982, and 795 and 873 children in 1992 in Wagga Wagga and Belmont, respectively. Standardized questions included those regarding symptoms of

wheeze, previous diagnosis of asthma by a physician, and previous drug therapy for asthma, as well as questions regarding allergic symptoms (hay fever, eczema). Children also underwent spirometry, histamine bronchoprovocation, and skin prick testing on both occasions. Significant increases were noted between 1982 and 1992 in the prevalence of diagnosed asthma, use of medications for asthma, and episodes of wheeze in the last twelve months in both towns. Prevalence of positive responses to these questions was 10% in 1982, and increased to 30% in 1992. There was an increase in both towns in the proportion of subjects having greater than four "attacks of wheeze" per year (3% in 1982 and 15% in 1992). While the prevalence of allergic symptoms and skin test positivity did not increase significantly in either town between both surveys, the prevalence of airway hyperactivity increased twofold in Belmont to 19.8%, and 1.4 fold to 18.1% in Wagga Wagga. While the parallel increase in symptoms of wheeze, diagnosis of asthma, and drug therapy for asthma suggest an increase in diagnostic labeling practice, they do not explain the increase in objective measures of airway hyperreactivity, nor the associated increase in prevalence of subjects having a higher number of "attacks of wheeze" per year. Although there was no noted increase in the prevalence of subjects genetically predisposed to atopic symptoms or allergen sensitization as measured by skin prick testing, the authors suggested that an explanation for the increase in the prevalence of airway hyperreactivity may have been due to the increase in dust mite load in the homes measured on both occasions.

Rona et. al. (41), using data from the UK National Study of Health and Growth, examined the prevalence of asthma symptoms in 1982 and 1992 among 5-11 year-old children from 22 districts of England, and 14 districts in Scotland. Data from this survey included questions regarding attacks of asthma in the last 12 months, as well as occasional and persistent symptoms of wheeze. Over 5000 subjects in England and 3000 subjects in Scotland were surveyed on both occasions. Over this 10 year period, a sharp increase in the prevalence of "attacks of asthma in the last 12 months" was noted (3% overall in 1982 compared with 8% in 1992). The authors noted that while this may have been due to changes in diagnostic labeling, the increase in the prevalence of "persistent wheeze" suggests a true increase in the prevalence of asthma.

In adults, Laor et.al. (42) used the national database of conscripts of the Israeli Defense Force to examine the prevalence of asthma in 17 and 18 year-olds. 443186 subjects (262836 male and 180350 female) were classified according to birth year as well as ethnic group (European, Israeli, Asian, and North African) and region of residence. All subjects underwent a standard medical evaluation during which they were asked if they had ever had asthma, had recurrent wheeze, nocturnal cough, or wheeze after exertion. Subjects who responded affirmatively were referred for further evaluation by a pulmonologist, and underwent spirometry and exercise bronchoprovocation testing. Asthma was diagnosed if the subject regularly used medication to control symptoms, if one or more symptoms were experienced in the 3-year period before the exam, or if the subject's FEV1 was less than 70% predicted, or decreased more than 10% after exercise. Overall, the prevalence of asthma was 26.5/1000 among males and 21.4/1000 among females. Asthma was more prevalent in males in most subgroups, and in almost all ethnic and regional subgroups, the prevalence was higher among subjects who were born later, suggesting an increase in prevalence over time.

While these studies suggest an increase in the prevalence of asthma over time, use of different diagnostic criteria has made meaningful comparisons between different studies difficult. Furthermore, as these studies were repeated within similar populations over time in order to demonstrate increasing prevalence, it has been difficult to clearly demonstrate which risk factors might be responsible for the increase. Comparisons of asthma prevalence between different populations in different geographic regions are required in order to determine potential reasons for the increase in asthma prevalence: risk factors related to differences in geography, environment, or lifestyle are more likely to emerge than when studies are repeated in similar populations over time.

1.3.3 International Prevalence Studies of Asthma

In order to address these issues, the International Study of Asthma and Allergies in Childhood (ISAAC) study was devised. This study was designed to measure the prevalence and severity of asthma, rhinitis, and eczema symptoms and diagnoses in 6-7 and 13-14 year old children living in different geographic centers, and to make

comparisons within and between countries (43). The study comprised three phases, including the use of core written as well as video questionnaires (phase 1) to assess the prevalence and severity of asthma and allergic disease in defined populations. Data on etiological factors (phase 2 of the ISAAC study) of asthma and allergy, is currently being collected and analyzed. Each centre in the study enrolled at least 3000 subjects from a random sample of at least 10 schools, thereby providing sufficient statistical power to detect small differences in prevalence (less than 5%) in wheezing, rhinitis, or eczema between centres. There were 463 801 children aged 13-14 years (the age group studied by all participating centers) enrolled in 155 collaborating centers in 56 countries. The 12 month prevalence of asthma (self-report of symptoms) ranged from 1.6% to 36.8% between these different countries. In addition to significant variation in asthma prevalence between countries, there was significant variation between centres within the same country among those countries with more than one participating centre. A similar range of prevalence was noted for symptoms of allergic rhinoconjunctivitis as well as atopic eczema, and significant correlations were noted between the prevalence of these different disorders in countries from Western Europe, the UK, and the United States. The highest noted prevalence of asthma was from centers in the UK, New Zealand, Australia, and Ireland (25%-35%). Intermediate prevalence was noted from centres in North, Central, and South America (10%-20%), and the lowest prevalence was reported from Eastern Europe, China, India, and Indonesia (2%-9%).

Similarly, the European Community Respiratory Health Survey (ECRHS) (44) is a continuing multi-centre survey which began in 1990, of the prevalence, determinants, and management of asthma in adults in 48 centers in 22 countries--several from the European Community, as well as India, the United States, Australia, New Zealand, Estonia, and Algeria. The study uses a questionnaire constructed from pre-existing questionnaires previously validated in multinational studies, as well as standardized measures of allergy, and bronchial responsiveness. In each participating centre, 1500 subjects of both sexes were selected-- from sampling frames of a total population of 150000 people -- to complete a "screening" questionnaire (Phase I of the study). Results of the first implementation of the survey revealed the prevalence of asthma symptoms to

be generally lower in northern, central and southern Europe (12%-20%), and higher in Britain, New Zealand, Australia, and the United States (>25%). Wide variation in the prevalence of asthma symptoms was noted, however, between different countries, different centers in different countries using the same language, and different centers in the same country using the same language. Furthermore, asthma and allergic symptom (allergic rhinitis and eczema) prevalence rates in the ISAAC and ECRHS have been shown to correlate well by country and centre (45).

Both of these studies have demonstrated a high prevalence of reported asthma symptoms in English-speaking countries (ie: the UK, Australia, New Zealand, and Canada). The ISAAC study also measured similar asthma prevalence using a video-based questionnaire (demonstrating asthma symptoms), in order to ensure that differences in reported prevalence were not a function of written questionnaire translation. It is still possible, however, that some of the measured differences were due to cultural and/or linguistic differences in interpretation of symptoms, or differences in diagnostic and treatment practices between countries, which might have influenced response rates. As discussed (see Section 1.2.2: "Diagnosis"), bronchial provocation testing provides a physiologic measure of airways hyperresponsiveness, and can be correlated with symptom prevalence measures from epidemiologic studies. Manfreda et. al. (46) used the phase I protocol of the ECRHS in six cities in Canada and demonstrated wide variation in the 12-month period prevalence of asthma symptoms (eg: 21.9% in Montreal, and 35.2% in Halifax), with higher rates noted in women compared with men. Using the phase II protocol of the ECRHS, these same authors also demonstrated wide variation in the prevalence of bronchial hyperresponsiveness among the six Canadian sites (equivalent to the range of variation noted in the ECRHS), lack of significant correlation of bronchial hyperresponsiveness with asthma symptoms by questionnaire response, and greater bronchial hyperresponsiveness among women than men. Furthermore, the geographic variability measured with respect to prevalence of asthma symptoms and bronchial hyperresponsiveness could not be explained by differences between sites in gender distribution, level of FEV1, smoking, or atopy as defined by positive skin prick tests (47).

The following conclusions have therefore been drawn from these two large-scale epidemiologic studies: 1. Among non-English speaking European countries, both studies have demonstrated a high prevalence of asthma in Western Europe, with a gradient toward lower prevalence in Eastern and Southern European countries. 2. ISAAC demonstrated a higher prevalence of asthma in Latin America countries compared with Spain, in contrast to the usual findings that more economically affluent countries have higher prevalence. 3. In the ISAAC study, developing countries in Asia (ie: China and Taiwan) had lower asthma prevalence, than more affluent Asian regions (ie: Singapore, Japan, and Hong Kong). In this regard, a striking contrast was noted between Hong Kong and Guangzhou, two cities sharing geographic, linguistic and ethnic climates, but different in that Guangzhou is far less affluent than Hong Kong. The 12-month prevalence of wheeze in Hong Kong was 10%, compared with 2% in Guangzhou. These data currently provide the best evidence that geographical differences in asthma prevalence are significant, and not the result of differences in methodologies

1.3.4 An Inverse Correlation With Infectious Diseases

An increase in the incidence of allergic conditions (asthma, rhinitis, and atopic dermatitis) in developed countries over the past three decades (48) has occurred in association with a decrease in the incidence of many infectious diseases as a result of antibiotics, vaccination, and improved hygiene and socio-economic conditions. Specifically, the incidence of tuberculosis, rheumatic fever, measles, and mumps in the United States, and Hepatitis A in France decreased between 1950-2000 (49), and intestinal colonization with gram-negative bacteria occurs later in developed compared with less-developed countries (50).

A geographic north-south gradient in the prevalence of asthma has been described in both ISAAC (43) and ECRHS (44), roughly corresponding to a similar, though opposite gradient in the prevalence of the previously mentioned infectious diseases. The differences are unlikely to be explained solely on the basis of genetic differences between populations, as significant changes in genotypes are unlikely to have occurred over the time period that the prevalence of allergic conditions such as asthma have been noted to

increase. It is unlikely that differences in diagnostic practice or access to health services can fully explain the north-south gradient, as developed countries in southern Europe (such as Greece) have a lower prevalence of asthma and allergic disease compared with the UK as noted in both ISAAC and ECRHS. The role of environmental exposures such as infectious agents should therefore be considered in the increasing prevalence of asthma. Based on these observations, Strachan proposed the "hygiene hypothesis" (51), noting that there was an inverse relationship between the risk of allergic rhinitis and birth order and the size of the family. He proposed that infections within households in early childhood might play a role in preventing allergic rhinitis.

1.4 IMMUNOLOGICAL BASIS FOR EPIDEMIOLOGIC TRENDS

Specific mechanisms by which infectious disease may influence the development of allergic disease are hypothesized to relate to modulation of immune responses generated by a subset of T-helper cells designated Th1 and Th2. As previously discussed (see Section 1.2.3: "Pathophysiology"), Th1 cells are responsible for the production of interleukin-2 (IL-2), interferon-gamma (IFN-γ), and TNF-beta (TNF-β) without interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-9 (IL-9) and interleukin-13 (IL-13) production. Th1 cells interact with B cells to produce IgG1 and IgG3, and activate phagocytic cells and CD-8 T-cells, thereby mediating cell-mediated and cytotoxic T-cell responses. Th2 cells, by contrast, produce IL-4,-5,-9, and 13 in the absence of IFN-γ and TNF-β production. These interleukin cytokines are responsible for affecting production of key elements of the atopic state (IgE, IgG4, mast cell and eosinophilic activation and differentiation) as well as inhibiting phagocytic cell function (52). In humans, the immune system is skewed at birth to spontaneous Th2 activation, in order to establish maternal tolerance of fetal implantation, as Th-1 cytokines can induce spontaneous abortion (53). Subsequent helper T-cell activation during immune system maturation varies between a Th1 and Th2 pole, depending on the predominance of cytokines produced in relation to type and level of respective immunologic exposures (25). There is furthermore a reciprocal relationship between these T-helper cell cytokine profiles, with stimulation of Th1 cytokine production antagonizing Th2 cell development and cytokine production and vice-versa (52).

Several in vitro experiments have demonstrated that the administration of microbial products including bacterial oligodeoxynucleotides containing CpG motifs can alter Th2 polarized responses towards a Th1 response (54). In in vivo experiments, inhibition of specific IgE production, pulmonary eosinophilia and airway hyperresponsiveness has been inhibited in ovalbumin-sensitized mice by pretreatment with bacterial deoxynucleotides (55) lactobacillus (56), as well as BCG (Bacille Calmette-Guerin, see Section 1.6, "The Role of BCG") (57). These data suggest that the immunologic basis for the "hygiene hypothesis" discussed above may be due to a relative lack of exposure in the developed world to microbial products which are capable of polarizing the immune system to a Th1 predominant cytokine profile. This lack of Th1 promotion thereby favours the continued expression of the Th2 profile, leading to an increased expression of clinical allergy in genetically susceptible, allergen-sensitized hosts.

1.5 EVIDENCE FOR THE ROLE OF INFECTIOUS AGENTS:

Several studies support the role of infectious agents in modulating the Th1/Th2 response, and the clinical expression of atopy. A longitudinal study of children in Guinea-Bissau (58) affected by an epidemic of measles before most had been immunized, demonstrated a significantly reduced prevalence of positive allergy skin-prick tests among those who were infected during the epidemic compared to those who were offered post-epidemic immunization. These data are consistent with the hypothesis that measles infection protects against the development of atopy via stimulation of Th1 cells. Alternative explanations are that large numbers of atopic subjects were either "selected out" of the study due to mortality from measles infection, or that atopic subjects are naturally protected from contracting measles.

A cross-sectional retrospective study of 1659 air force trainees in Italy (59) demonstrated that 26.7% were positive for Hepatitis A antibody. Atopy was less common among seropositive compared with seronegative subjects when defined as: 1. One or more positive allergy skin prick tests (21.9% vs. 30.2%, $P<0.001$). 2. Polysensitisation (sensitive to three or more allergens) (2.7% vs. 6.4% , $P<0.01$). 3. High specific IgE

concentration (9.7% vs. 18.4%, P<0.00005) and 4. Lifetime prevalence of allergic rhinitis, asthma, or both (8.4% vs. 16.7%, P<0.001). Hepatitis A seropositivity remained inversely associated with atopy after adjusting for father's education, number of older siblings, and area of residence. Similar relationships have been noted in a survey of Hepatitis A seropositivity in a general population sample of San Marino, Italy (60) as well as in a case-control study of the relationship of childhood infections with adult-onset atopy and wheeze in Aberdeen, Scotland (61).

A study of infants in Sweden and Estonia demonstrated significant differences in the types of faecal bacteria between atopic and non-atopic infants in both countries: non-atopic infants were more often colonized with Lactobacilli, while atopic infants were colonized with a higher proportion of coliforms and *S. aureus* bacteria (62). While the numbers of subjects in this study were small, the data support the hypothesis of immune modulation by infectious agents, as some strains of lactobacilli are known to induce production of IFN-γ (63), involved in regulating Th2 cell development and cytokine production.

Several studies have similarly demonstrated reduced expression of atopy among children raised on farms compared with their peers raised in non-farming rural environments. Ernst and Cormier (64) examined the prevalence of asthma and allergy symptoms as measured by the ECRHS questionnaire, atopy via skin prick testing to common farm and household allergens, and lung function by spirometry as well as bronchial hyperreactivity by methacholine challenge testing among 1199 subjects aged 12-19 from 14 secondary schools in rural Quebec. 802 subjects were raised on a farm, while 397 subjects had never been exposed to farming environments in their lifetime. Separate analyses were performed among subjects who had intermittent exposure to farming in their lifetime to ensure that study results were not secondary to these subjects being selected out of the farming environment because of allergic or other health conditions. Prevalence of wheeze in the last 12 months (24.7% vs. 17.1% p=.002), airway hyperresponsiveness (17.4% vs..12.9% p=.035), and skin test positivity to common inhaled allergens (53.4% vs. 40.8% p=.001), were more common among subjects who

had never been raised on a farm as compared to subjects from a farming environment. Current smoking was more common among subjects from a non-farming environment (21.4% vs. 12.7% p=.001), but after adjusting for gender, smoking, and number of siblings, the odds of current wheeze, airway hyperresponsiveness, and atopy in relation to being raised on a farm were 0.72 (0.56-0.99), 0.8 (0.56-0.87), and 0.62 (0.48-0.80), respectively. Furthermore, changing the definition of atopy to require sensitization to an indoor allergen (cat, dog, dust mite) in contrast to either indoor or farm allergen, did not alter the protective effect of the farm environment on the development of atopy. These results support the concept that a multitude of exposures found in the farming environment may support the development of Th1 mediated immunity and protect against the development of atopy. These results are consistent with other studies (65-67) of the relationship of farming environments to the development of atopy. Potential candidate exposures conferring a protective effect have been recently examined (68), and it is hypothesized that early exposure to bacterial endotoxin found among livestock in stables may protect against the development of atopy.

Mycobacterium tuberculosis is known to be a potent stimulus to the development of Th1 immunity (69-70). von Mutius et. al. (71) conducted an ecologic study to examine the relationship between tuberculosis notification rates and prevalence of allergic and asthma symptoms. Data on asthma and allergy symptoms was obtained from 13 and 14 year-old children from countries participating in the ISAAC study (43), which were considered to have valid TB notification rates (72). The study included 235 477 children in 85 centers from 23 countries. Annual tuberculosis notification rates for the time period when participating children would have been infants were used. Regression analysis was performed for the prevalence of asthma and allergy symptoms by centre against tuberculosis notification rates, adjusted for gross national product (a surrogate for confounders related to differences in lifestyle and exposure risks for tuberculosis and asthma). Tuberculosis notification rates were significantly inversely associated with 3 of 4 of the questions regarding asthma (correlation coefficient: -0.187, p=.024 for "ever had wheeze", -0.131, p=.001 for "ever had asthma" and -0.091, p=.018 for "wheeze at rest in the last 12 months").

While these studies support the role of infectious agents as an explanation of

Strachan's "hygiene hypothesis", the data are not consistent, as several other studies do not support the role of infectious agents in the changing prevalence of atopic disease (73-75).

1.6 THE ROLE OF BCG

The Bacille Calmette-Guerin (BCG) vaccine is a live, attenuated vaccine against human tuberculosis developed in France by Calmette and Guerin in 1921 from bovine tubercle bacilli (*M. bovis*) (76). Several different strains of BCG vaccine exist as a result of distribution of BCG lots in 1924 to various countries for local preparation of vaccine. There is large variability in the degree of protection imparted by the BCG vaccine against pulmonary tuberculosis, with estimates from clinical trials varying from 0% to 80% (77). This variability has been attributed to strain variation in BCG preparations, genetic or nutritional differences between populations, as well as exposure to a variety species of environmental mycobacteria other than *M. tuberculosis* (78). The vaccine is currently administered before the age of two years to as many as 80% of children born in developing countries because of its demonstrated ability to prevent disseminated tuberculosis as well as tuberculous meningitis in children (79).

Development of delayed type hypersensitivity to the antigens of *M. tuberculosis* as result of infection is measured by an individual's ability to develop an indurated reaction to the intracutaneous injection of purified protein derivative (the tuberculin skin test, or TST), which is a culture filtrate of *M. tuberculosis* (80). The standard test consists of the intracutaneous injection (Mantoux's method) of 5 tuberculin units (5TU) on the volar surface of the forearm, and the site of injection is examined 48-72 hours later to measure the diameter of induration that develops (81). In many areas of the world, non-tuberculous mycobacteria present in the environment can also induce some degree of sensitization to tuberculin, though the reactions induced by non-tuberculous mycobacteria are generally smaller than those caused by infection with *M. tuberculosis* (82). BCG vaccination can similarly induce sensitization to tuberculin, and a previous study in Montreal schoolchildren and young adults showed that the size of the reaction is dependent on the age at which BCG was administered, with a greater proportion of larger reactions ($\geq$10mm) occurring among subjects who received BCG after the age of one

(83). A murine model of antigen-induced bronchial hyperreactivity and airway eosinophilia (two key hallmarks of asthma) which produces high levels of immunoglobulin E (IgE) has been previously developed using ovalbumin-sensitized mice (84). Named BP-2 ("Bons Producteurs-2"), studies have shown that administration of BCG vaccine to newborn BP2 mice blocks allergic inflammation and bronchial hyperresponsiveness in response to ovalbumin (85). IgE production in response to aerosolized antigen can be inhibited by pre-immunization with *M. bovis* or *M. vaccae* (86). These 2 studies suggest that administration of BCG vaccine in early life could induce a Th-1 predominant immune response, thereby preventing the development of clinical manifestations of allergy.

Several studies have examined the relationship between BCG vaccination in childhood and the development of allergy and asthma. Shirakawa et. al. (87), examined the relationship between delayed type hypersensitivity and asthma and allergy symptoms as well as serum total and antigen-specific IgE among 867 12-13 year-olds in Southern Honshu, Japan, where BCG vaccine is routinely administered at birth and again at 6 and 12 years of age if tuberculin skin testing is negative (<10mm of induration). The odds of atopy and asthma given a positive tuberculin skin test at 6 years of age were 0.5 (0.29-0.83) and 0 .31 (0.22-0.45) respectively. Odds of atopy and asthma given conversion of the tuberculin skin test to positive between 6 and 12 years of age were 0.43 (0.25-0.83) and 0.42 (0.24-0.56) respectively.

While these results support the hypothesis that BCG vaccination in childhood may prevent the development of atopy by inducing development of a Th1 immune state, other studies have produced variable results. Alm et. al. (88) performed a study involving 216 subjects 3-7 years of age who received BCG vaccine in infancy, and 358 age-matched subjects who were not vaccinated. Both groups had atopic heredity, and no difference was observed between the groups in prevalence of asthma symptoms (10% vs. 12%, respectively) nor in prevalence of atopy as measured by skin prick testing or serum specific IgE level (29% vs. 24%, respectively). By contrast, Aaby et. al. (89) in a cross-sectional survey of 400 children 3-14 years of age, noted a reduced odds of atopy as measured by allergen-specific skin prick tests among subjects who received the BCG

vaccine (adjusted odds 0.19 (0.06-0.51)). The protective of BCG in the study by Aaby et. al. was more pronounced if vaccination occurred in the first week of life.

Reasons for variability of results between studies include differences in BCG strain used, differences in environmental exposures (to non-tuberculous mycobacteria), and differences in genetic backgrounds between populations (all subjects in the study by Alm. et. al. had atopic heredity, potentially attenuating any protective effect of BCG). Furthermore, differences in age at vaccination between studies may have influenced study results if there was a critical time point in immune system maturation during which promotion of a Th1 versus a Th2 profile occurred.

Most studies examining the relationship between BCG vaccination and the development of allergic disease and asthma have been conducted in children, when atopy plays an important role in the pathogenesis of asthma. As previously discussed (see Section 1.2.4: "Clinical Phenotypes of Asthma"), the pathogenesis of asthma in adults may be very different, with atopy playing less of a role than in children. How BCG vaccine, administered in infancy or early childhood may affect the subsequent development of asthma in either childhood or adulthood is currently unknown. We hypothesized, however, that adult subjects with asthma would be less likely to have received the BCG vaccine in childhood compared with similar adult subjects without asthma.

1.7 RATIONALE FOR EXAMINING THE BCG HYPOTHESIS IN QUEBEC:

Montreal, Quebec was an ideal locale within which to explore this hypothesis. Between 1926 and 1949, BCG vaccination was given in the Province of Quebec as an experimental procedure, to newborns, who received three doses of 0.01g of oral vaccine, over a two-day interval. This program was formally extended to schoolchildren up to the 11th grade inclusive in 1949 (90), with vaccination administered by the scarification method instead of orally (four scarifications of 1cm each for newborns, and six scarifications of 1cm for others). BCG fluid at a concentration of 60mg/ml was used. Vaccination was offered free of charge to newborns, preschool children, and schoolchildren, and tuberculin skin tests and BCG vaccinations were performed by

specially trained mobile teams working in close cooperation with the staff of local health departments, health units, and school authorities under the supervision of the Institute of Microbiology and Hygiene of the University of Montreal (now known as the Armand Frappier Institute). Tuberculin skin testing under this program was performed in Quebec using the Cuti test, a tuberculin skin test using a purified protein derivative which was a culture filtrate of BCG. Upon request, nurses, students, and known or suspected tuberculosis contacts were tested and vaccinated by members of the mobile teams. Priority for testing and vaccination was given to known or suspected contacts, but otherwise, the only criterion for testing and vaccination was informed consent from the individual or his/her parents. Many individuals were re-vaccinated under this program. The coverage rate for vaccination procedures averaged 50-60%, being higher in rural than in urban areas, with approximately 100000 vaccinations given annually between 1948 and 1976. Vaccination rates varied between 10% and 80% in different districts of greater Montreal depending in large part on policies and practice of the local public health authorities (90).

With the exception of newborns, every candidate for vaccination was subject to a BCG scarification test (to exclude subjects who were currently infected with tuberculosis). Using a transverse wheal diameter of 2mm or less as the criterion by which subjects were selected for vaccination, it is unlikely that any individuals with true mycobacterial infection were vaccinated.

Each test, vaccination, and re-vaccination was recorded on the subject's file card in a central BCG record system kept at the Institut Armand Frappier. The file card contained complete information on the subject (full name, date of birth, father's name), such that retrieval of the vaccination record of a given individual was easy and reliable.

1.8 SUMMARY

Asthma is a heterogeneous disorder with distinct presentations and risk factors for onset in childhood and adulthood (31-32). Although there is no standardized clinical definition, numerous studies have demonstrated an increase in the prevalence of asthma over time (40-42). Large-scale studies in both children and adults using standardized definitions have demonstrated wide variation in asthma prevalence between countries and between regions within the same country (43-44). Prevalence is higher in economically developed countries in North America and Western Europe, and lower in less-developed countries in Eastern Europe and Asia. A geographic Northwest-Southeast gradient of asthma prevalence exists, and a similar gradient has been observed for a number of environmental factors, most notably infectious diseases such as tuberculosis (49). These infections are hypothesized to lower the risk of asthma by stimulating the T-helper cells of the immune system in early childhood to differentiate towards a Th1 profile, thereby regulating production of Th2 cells and their cytokines and the development of an atopic state, which is a risk factor for asthma in childhood (51). The BCG vaccine is similarly a potent stimulus of Th1 cell differentiation and cytokine production (85-86). BCG was administered during a public health campaign in the Province of Quebec, and within a population with a low prevalence of infection with tuberculous and non-tuberculous mycobacteria (90). As vaccine coverage varied from 10%-80%, the opportunity was present to determine the effect of BCG vaccine on asthma in a population at low risk for tuberculous infection.

CHAPTER TWO: STUDY OBJECTIVES AND DEFINITIONS

2.1 PRINCIPAL STUDY OBJECTIVE

To compare the proportion of subjects who received BCG vaccination in childhood among adult subjects with asthma, with the proportion of subjects who received BCG vaccination in childhood among adult subjects without asthma.

2.2 SECONDARY STUDY OBJECTIVES

1. To determine the relationship between childhood BCG vaccination and the age of onset of asthma (childhood versus adulthood).
2. To determine the relationship between age at vaccination with BCG (infancy versus childhood), and the age of onset of asthma (childhood versus adulthood).
3. To compare the prevalence of BCG vaccination among atopic subjects with asthma with the prevalence of BCG vaccination among non-atopic subjects without asthma.
4. To determine the relationship between age at vaccination with BCG (infancy versus childhood) and the age of onset of asthma (childhood versus adulthood) among atopic subjects with asthma and non-atopic subjects without asthma.

2.3 HYPOTHESIS TESTED

Adult subjects diagnosed with asthma at the Montreal Chest Institute and Montreal General Hospital Asthma Clinics were less likely to have received BCG vaccination in childhood than a similar group of adult control subjects selected from the same hospitals.

2.4 MAIN DETERMINANT MEASURES:

1. BCG vaccination: subjects were classified as having received BCG vaccine if this was documented in the central registry of the Institut Armand Frappier.
2. Proportion of BCG vaccination among subjects diagnosed with asthma at the asthma clinics of the Montreal Chest Institute and Montreal General Hospital between January 1, 1996 and December 31, 1997, inclusive (proportion of vaccinated cases).
3. Proportion of BCG vaccination among control subjects presenting on selected dates to

outpatient orthopaedic departments within the McGill University Health Centre between December 12, 1997 and March 31, 1998 (proportion of vaccinated controls).

2.5 DEFINITIONS

1. BCG Vaccination: vaccination with a live attenuated strain of *Mycobacterium Bovis* bacillus, to prevent the progression of tuberculosis infection to disease. For the purpose of this study, only subjects with documentation of BCG vaccination in the central registry of l'Institut Armand- Frappier were considered to have been vaccinated.
2. Asthma: a disease characterized by episodes of variable airflow limitation, and clinical features of wheeze, cough, and shortness of breath that improve spontaneously or in response to medication. Case subjects were considered to have asthma on the basis of a formal clinical evaluation by a respirologist (see Section 3.2.3 C. "Definition of Case and Control Subjects"). The study excluded control subjects who answered "yes" to question number 14, "Have you ever had asthma?" of the study questionnaire (see Appendix 1, p. A-5).
3. Atopy: a state characterized by prior IgE production to allergen in a genetically susceptible individual, with the generation of a characteristic physiological response upon subsequent re-exposure to allergen. In this study, a subject was considered to be atopic if they answered "yes" to any one of 4 questions regarding allergic symptoms (excluding the question regarding eczema symptoms), in the study questionnaire (see Section 3.2.3 C "Definition of Case and Control Subjects").
4. Childhood onset asthma: a case subject was considered to have childhood-onset asthma if they responded on the study questionnaire that they had their first attack of asthma before the age of 18 (see question 14.2, Appendix 1, p. A-5).
5. Adult-onset asthma: a case subject was considered to have adult-onset asthma if they responded on the study questionnaire that they had their first attack of asthma at 18 years of age or older (see question 14.2, Appendix 1, p. A-5).
6. Vaccination with BCG in infancy: a subject was considered to have been vaccinated in infancy if records from the central registry of l'Institut Armand- Frappier confirmed their date of first BCG vaccination to have been before or at the age of 1.
7. Vaccination with BCG in childhood: a subject was considered to have been vaccinated

in childhood if records from the central registry of l'Institut Armand-Frappier confirmed their date of first BCG vaccination to have been after the age of 1.

CHAPTER THREE: STUDY DESIGN AND METHODS

3.1 STUDY DESIGN AND RATIONALE:

This was a case-control study in which potential case-subjects were identified on the basis of clinical and pulmonary function test criteria for the diagnosis of asthma, and were mailed a study questionnaire. Potential control subjects were identified in outpatient clinics, and were recruited onsite where the study questionnaire was administered. Control subjects who responded that they were previously diagnosed with asthma were excluded from the study. Childhood BCG vaccination status was subsequently confirmed for both case and control subjects on the basis of records in the central registry of the Institut Armand Frappier.

The case-control design offered a number of advantages in testing the study hypothesis:

1. Less time was required to implement the study than for a prospective cohort design. The annual incidence of asthma is less than 2% (91-92); therefore, a prospective cohort design would have required observing study subjects for many years after the time of BCG vaccination, until development and diagnosis of asthma.

2. Fewer subjects were required to detect a given risk difference between study groups than for a prospective cohort design. The low annual incidence of asthma would have required a large investment in resources following large numbers of study subjects--many of whom would likely have remained free of the disease-- if a prospective cohort design had been implemented. Using a case-control approach, sample sizes required for detecting pre-determined differences in vaccination prevalence between study groups were computed, and appropriate numbers of potential case and control subjects were recruited.

3. An experimental study using BCG vaccine would have been ethically unacceptable, since this would have exposed subjects to potential vaccine side effects

without conferring an appreciably lower risk of tuberculosis (as subjects were from a geographic area with a low incidence of tuberculosis). As BCG vaccine was administered between the 1940s to 1970s to a large number of subjects in accordance with previous public health practice in the Province of Quebec (90), the opportunity was available to explore the study hypothesis without compromising research ethics.

4.The study avoided bias in the assessment of exposure inherent in most case-control studies: pre-existing records of exposure status (vaccination records of l' Institut Armand Frappier) were used, avoiding potential differential recall between case and control subjects. Furthermore, selection bias was avoided in that evidence of a vaccination scar was not the criterion by which subjects were deemed to have received BCG vaccine, as in other studies (120-121) (124-125) .

A hospital-based case-control study was chosen to ensure diagnostic validity of the case series, as cases were subjected to a rigorous evaluation (ie: diagnosis was confirmed by a respirologist and by pulmonary function testing).

The control group consisted of age-restricted subjects presenting to outpatient clinics of the McGill University Health Center (M.U.H.C.). Inclusion of control subjects in the study was based on a negative response to the question, "Have you ever had asthma?"(see question 14, Appendix 1, p. A-5); therefore, clinics selected for recruitment of these subjects were those in which presenting patients would have a high likelihood of being free of asthma in order to avoid potential recruitment of a large number of ineligible or misclassified control subjects.

Orthopaedic clinics were selected since these assess patients with acute conditions (trauma related to work or leisure activity), not potentially associated with asthma or related diagnoses. A family medicine clinic, by contrast, potentially would have attracted a large proportion of patients presenting for respiratory problems, who might respond affirmatively to questions about asthma. Patients presenting to orthopaedic clinics were likely to have an equivalent risk of exposure to BCG vaccine as the case subjects, as they were relatively young, and more likely to have been born between 1949 and 1979 (the

period of the BCG vaccination campaign in the Province of Quebec).

The orthopaedic clinic of the Montreal General Hospital was the main source from which control patients were recruited. It was chosen with permission of members of the orthopaedic department for the following reasons: 1. The clinic serves large numbers of patients referred for follow-up care from the emergency room of the hospital; 2. These patients often wait a considerable amount of time (1.5 to 2 hours) before being evaluated, and it was believed that they would be more willing to participate and have sufficient time to read an explanation of the study, grant informed consent, and complete the study questionnaire; 3. Since July 1996, as part of restructuring initiatives within the McGill teaching hospital community, the majority of orthopaedic patients are assessed at the Montreal General Hospital. A limited amount of follow-up care occurs at the Royal Victoria Hospital, and a few control subjects were recruited from there as well. These clinics were therefore considered to be the "orthopaedic clinic" of the MUHC. Similarly, the asthma clinics of the Montreal General Hospital and Montreal Chest Institute were considered the overall "asthma clinic" of the MUHC, since there was no designated asthma clinic at the Royal Victoria Hospital.

The McGill Sports Medicine Clinic was also included as a site for control subject recruitment, since it functions as an affiliate of the Montreal General Hospital orthopaedic clinic.

The difference in asthma prevalence by gender (46) resulted in recruitment of a higher number of females than males among the cases. By contrast, control subjects recruited from the Montreal General Hospital orthopaedic clinic were more likely to be male. Other clinics were subsequently considered for recruitment of subjects in order to obtain similar numbers of subjects by gender.

The McGill University Dental Clinic of the Montreal General Hospital was evaluated as a possible site from which to recruit additional female control subjects, since it serves a young patient population with conditions unrelated to asthma (eg:dental

problems). This clinic was not selected, however, because: 1. The majority of patients were allophones, so that the potential number of Quebec-born female subjects was small. 2. Those patients presenting to the oral/maxillofacial section of the clinic were referred for very specialized care relating to temporomandibular joint problems, and likely were drawn from a highly referred population, very different from the case subjects.

Similarly, recruitment of female subjects from obstetric clinics would not have been appropriate, since pregnancy induces hormonal and anatomical changes causing more frequent respiratory symptoms than in the non-pregnant population (93). Such patients, because of their greater contact with the health profession due to pregnancy, may have been more likely to have been diagnosed with asthma than other potential control subjects, and therefore were not considered appropriate study subjects.

Because of the limitations in recruiting adequate numbers of female controls, the alternative of recruiting more male case subjects was chosen. These subjects were recruited prospectively on-site at the Montreal Chest Institute asthma clinic, in an attempt to obtain equal numbers of subjects by gender. This occurred only after recruitment of subjects from the Montreal General and Montreal Chest Institute asthma clinics was complete. Subjects were not directly approached by the study investigator, but were made aware of the study by clinic staff, and were given the option of approaching the study investigator on-site if they wished to participate.

Ideally, control subjects should have been similar to case subjects with regard to potential for exposure to BCG vaccination. It is possible that differences in potential for BCG vaccination were related to differences in socio-economic status. This is because the early period of the BCG vaccination campaign was characterized by mass vaccination of children by public health departments without consideration given to social class. The latter part of the campaign, however, was characterized by targeted vaccination of children in schools located in poorer neighbourhoods. It is possible, then, that for some subjects, potential for vaccination may have been related to poorer socio-economic status. This was assessed by examining each study subject's current occupation as a proxy for

socioeconomic status, and controlling for this variable in the analysis.

3.2 METHODS

3.2.1 CASE SOURCE:

Potential case subjects were selected via review of pulmonary function test records of the Montreal Chest Institute, a 100-bed hospital specializing in the treatment of respiratory diseases. The hospital is a member of the McGill University Health Centre (MUHC), and managed by the same administration.

Selection was similarly made from pulmonary function test records of the asthma clinic of the Montreal General Hospital, a 521-bed hospital which is also a teaching hospital of the MUHC. Both hospitals serve as referral centres for patients residing in Greater Montreal. Patients referred to both asthma clinics routinely receive a comprehensive evaluation in order to establish or confirm a diagnosis of asthma, including an asthma history questionnaire, as well as complete pulmonary function testing. In both clinics, potential case subjects were referred for pulmonary function testing by a respirologist because of a clinical evaluation consistent with a diagnosis of asthma. The ultimate criteria for recruitment of case subjects, therefore, was on the basis of pulmonary function test results.

3.2.2 CONTROL SOURCE

Control subjects were prospectively recruited from among patients presenting to the following orthopaedic clinics for the treatment of "acute" orthopaedic conditions (eg: bone fracture, ligament sprain): the Montreal General Hospital, the McGill Sports Medicine Clinic, and the Royal Victoria Hospital.

3.2.3 STUDY POPULATION

A. Inclusion Criteria:

Subjects were eligible for the study if they were born in the Province of Quebec between 1949 and 1979. They were also required to be fluent in either English or French,

and to provide informed consent to participate. Restriction of the study to the 1949-1979 birth cohort was implemented because this was the period during which the Quebec government implemented a province-wide mass BCG vaccination campaign. Restriction by place of birth in Quebec was implemented in order to ensure availability and uniformity of exposure data (ie: BCG vaccine was most likely to have been administered in infancy or early childhood, and reliable data on BCG vaccine administration in Quebec has been kept in a currently accessible central registry at the Institut Armand Frappier).

B. Exclusion Criteria:

No formal exclusion criteria were implemented.

C. Definition of Case and Control Subjects

1. **Case**: A case was defined according to the following **clinical** definition for asthma which was used as criteria to select them for recruitment:

i.) the subject was referred for pulmonary function testing by a respirologist in order to confirm a clinical diagnosis of asthma.

ii.)pulmonary function testing (PFT) revealed any of the following: an improvement of at least 12 percent in FEV1 20 minutes after bronchodilator administration, **or** a 20 percent week-to -week variability in FEV1, **or** demonstration of airway hyper-responsiveness by a fall in FEV1 of greater than or equal to 20% upon bronchial challenge with 8mg/ml or less of methacholine (PC20$\leq$ 8mg/ml).

Atopy was defined on the basis of a positive response to questions regarding the following conditions: 1. Nasal allergy or rhinitis. 2. Pet allergy. 3. Shortness of breath when ingesting medication. and 4. Previous vaccination for allergy (see Appendix 1, questions 15, and 19-21 pp. A5-A6). While eczema is a manifestation of an atopic state, and is associated with asthma (see Section 1.2.1 "Clinical Manifestations"), the question regarding eczema was not regarded as a reliable indicator of a subject's atopic status based on previous survey studies in adults (see Section 5.3 "Limitations of the Study").

2.**Control** : A subject was classified as a control if study eligibility criteria were met, the

subject presented to the orthopaedic clinic of either the Montreal General Hospital, Royal Victoria Hospital, or McGill Sports Medicine Clinic for treatment of an acute orthopaedic condition (such as bone fracture or ligament sprain), and the subject did not have asthma based on his/her response to question 14 of the study questionnaire (see Appendix 1, p. A-5).

3.2.4 RECRUITING PROCEDURES

Potentially eligible case subjects were identified from pulmonary function records of the asthma clinics at the Montreal General Hospital and Montreal Chest Institute of patients presenting during the years 1996 and 1997. Potential cases were identified for recruitment if they were born between 1949 and 1979 inclusive, and met the case definition for the diagnosis of asthma.

Place of birth was not noted in the hospital record; therefore, cases and controls who responded on the study questionnaire that they were born outside of the Province of Quebec were not included in the study.

Case subjects were recruited by mailed invitation, as the Research Ethics Board of the M.U.H.C. prohibited direct contact of patients with study investigators on site for the purpose of study recruitment. Each subject received a short cover letter signed by his/her treating respirologist briefly explaining why he/she was contacted, the purpose of the study, and an invitation to participate, or to contact the respirologist or study investigators for further information. This cover letter was accompanied by a letter from the study investigators explaining the purpose and methods of the study in detail, as well as telephone numbers at which the investigators could be reached if the subject had further questions. The subject also received a study questionnaire regarding risk factors for asthma and TB exposure, and a consent form authorizing the investigators to confirm the subject's BCG vaccination status with l'Institut Armand Frappier. The subject was asked to indicate his/her interest in participation by completing the consent form and questionnaire and mailing them in the provided pre-addressed, pre-stamped envelope to the McGill University Respiratory Epidemiology Unit.

If the subject did not respond within two weeks, a second attempt was made to recruit him or her via mailed invitation. No further attempt was made to contact potential case subjects who did not respond to the second invitation letter and questionnaire.

Control subjects presenting to their respective clinic appointments were approached by the investigator if they met the study age criteria (as determined by information in the patient's clinic chart), and were asked if they were born in the Province of Quebec, in order to further clarify eligibility. Subjects were told why they were approached, and the purpose of the study as well as consent for verification of BCG vaccination status were explained. They were then asked if they would be interested in participating. Interested subjects were provided with the letter explaining the study in greater detail, a consent form, study questionnaire, and pencil. Participating subjects completed and returned the questionnaire on site. The investigator was available to answer any questions the subject might have.

3.2.5 DATA COLLECTION:

Data was collected via a standardized questionnaire (see Appendix 1) consisting of selected questions from the European Commission Respiratory Health Survey Questionnaire (94). This questionnaire was modified such that only those questions pertaining to confounding variables of interest to this study were included. Selected questions from the Risk of Tuberculosis in Health Care Workers (RTHCW) baseline questionnaire were appended to the selected ECRHSQ questions. The RTHCW questionnaire had previously been used and validated (95).

i) Confounding variables and effect modifiers:

Questions regarding factors which might confound or modify the relationship between previous BCG vaccination and the development of asthma included: 1.Previous occupational exposure to dust/smoke/fumes. 2. Age when first diagnosed with asthma (among cases). 3. Presence of pets in the home. 4. Family history of asthma. 5. Smoking history. 6. Current occupation and industry. 7. Previous TB disease, and antituberculous medication usage. 8. Foreign travel. 9. Previous tuberculin skin test, and the result if

known.

ii) BCG Vaccination:

The subject's name, date of birth, and the name of the subject's father, were matched to the records in the central BCG vaccination registry of l'Institut Armand Frappier (IAF). Matching was performed by Armand Frappier personnel who were blinded to the case and control status of the study subjects. Vaccination was recorded as '0', '1', '2' or '3', depending on the number of times the subject received the BCG vaccine. Date at each vaccination was also recorded in order to determine the age at which the subject received BCG.

iii) Socio-Economic Status:

Socio-economic status (SES) was derived from questionnaire responses regarding the subject's current occupation and his/her parents' occupation(s) in childhood. This information was used to identify corresponding codes of the Canadian Classification and Dictionary of Occupations (96). These codes were then converted into childhood and current socio-economic status (SES) scores for the subject based on income and education level for each occupation from tables developed by Blishen and his colleagues (97). Analyses were performed using the subject's current SES score as well as an "average" SES score which combined both childhood and current SES scores.

3.2.6 SAMPLE SIZE CONSIDERATIONS

Two factors were considered in determining an appropriate sample size: the baseline proportion of BCG vaccination in the study population, and the detectable difference in BCG vaccination proportions between subjects with and without asthma.

It was estimated that in the Province of Quebec, BCG vaccination coverage during the 1949-1979 period varied between 10% and 80% of the population among the various health districts (90).

The following formula (98) was used to calculate the sample size n required to

detect a specific BCG proportion p with margin of error m at a 95% confidence level, using a two-sided test of significance:

$$n = \frac{p(1-p)(Z\alpha/2)^2}{m^2}$$

Where $Z\alpha/2$ is the two-sided value of the 5% point of the normal distribution. The following sample sizes were therefore calculated.

TABLE A : SAMPLE SIZE REQUIRED TO ESTIMATE BCG VACCINATION PROPORTION IN THE STUDY POPULATION:

Estimated Prevalence	Margin of Error	Sample Size
10%	±1%	3457
	±2%	864
	±3%	384
45%	±3%	1056
	±6%	264
	±7%	194
80%	±3%	683
	±4%	384
	±6%	171

An overall proportion of vaccination of 45% in the study population was assumed (this was calculated as an average of the highest and lowest vaccination rates in the Province of Quebec).

The null hypothesis (H_o) was: the proportion of BCG vaccination among subjects

with asthma is equal to the proportion of BCG vaccination among control subjects without asthma.

The alternative hypothesis (H_A) was: the proportion of BCG vaccination in subjects with asthma is less than the proportion of BCG vaccination among control subjects without asthma.

Assuming the null hypothesis was correct, the proportion of BCG vaccination should have been equal in both groups of subjects, which should have therefore been equal to the overall proportion of BCG vaccination among all subjects (ie: the estimate of 45% calculated above).

Having set the Type I error level as α, and the Type II error level as β, the following formula (99) was used to estimate the sample size required for the study:

$$(P_0Q_0+P_1Q_1)\,(Z1-\alpha/2-\,Z1-\beta)^2\,/\,(P_1-P_0)^2$$

Where P_0 denotes the proportion of exposure among controls and P_1 denotes the proportion of exposure among cases. Q_0 and Q_1 are derived, from subtraction from 1, of P_0 and P_1, respectively. $Z1-\alpha/2$ is the value of the standard normal distribution corresponding to a two-sided test at a particular alpha level, while $Z1-\beta$ is the value of the standard normal distribution, corresponding to a given level of power.

TABLE B: SAMPLE SIZE REQUIRED TO DETECT DIFFERENCE IN BCG VACCINATION PREVALENCE BETWEEN SUBJECTS WITH ASTHMA AND SUBJECTS WITHOUT ASTHMA (TYPE I ERROR SET IN ALL INSTANCES AT 0.05) {BCGV: BCG vaccination; a+/-: subjects with/without asthma}

Power	Difference	Prev. of BCGV(a+)	Prev. BCGV(a-)	N1	Total
80%	0.1	0.35	0.45	372	744
70%	0.1	0.35	0.45	315	630
60%	0.1	0.35	0.45	232	464
80%	0.15	0.3	0.45	159	318
70%	0.15	0.3	0.45	123	246
60%	0.15	0.3	0.45	99	198
80%	**0.2**	**0.25**	**0.45**	**85**	**170**
70%	0.2	0.25	0.45	66	132
60%	0.2	0.25	0.45	53	106

A total of 170 subjects was required (Table B) to detect a difference in BCG vaccination proportions of 20% between the two groups (asthmatics and non-asthmatics) with 80% power. Assuming a 10% non-participation rate among case and control subjects, and correcting for an assumed 10% prevalence of asthma and 30% prevalence of atopy (which is potentially associated with asthma), among controls, the study required 221 eligible subjects.

3.2.7 ANALYSIS

Data entry was performed by a trained technician using dBASE (© data Based Intelligence Inc.), and subsequently converted to SAS (© by SAS Institute, Cary, NC, USA) format using DBMS/Copy (© Conceptual Software Inc.) Verification of data entry was provided by both an investigator and a second data entry technician to ensure reliability of entry. Blishen index (97) entries for each subject's occupation were verified in a similar manner. BCG vaccination status data was subsequently entered in the data set

according to the records provided by the Institut Armand Frappier.

Data for cases and controls were compared using the student's t-test for means, or the χ^2 test for comparison of proportions, where appropriate. The calculated measure of association between the outcome (asthma) and exposure (BCG vaccination) variables was the odds ratio. Associations of dichotomous variables with the outcome (asthma) or exposure (BCG vaccination) variables were examined in order to assess for potential confounding. A p-value of 0.25 was selected as a threshold below which differences between study groups by a given variable were considered potentially significant. Variables showing potentially significant associations, and those known to be biologically associated with the outcome or exposure variable were entered in a logistic regression model (96) assessing the odds of developing asthma given prior vaccination with BCG. The crude odds of asthma given previous BCG vaccination was calculated for different levels (yes/no) of selected covariates in order to assess potential effect modification.

Beginning with the full model, the covariate with the largest p-value was removed, and regression analysis was performed with the remaining covariates. This process was repeated with this and other susbequently generated models. At each step, the effect of removal of the covariate on the subsequent point estimate and confidence interval of the exposure variable (BCG) in the remaining model was assessed (see Section 4.5, "Logistic Regression Analysis", and Table 11, p. 79). If there was no significant effect on the point estimate, and if the removed covariate was not felt to have a unique biological association with either the exposure or outcome variables, it was left out of the model.

The outcome variable (asthma), was modeled restricting asthma respectively to onset in childhood and onset in adulthood. Further outcomes assessed included concurrent atopy and asthma, and this was further subdivided into onset in childhood or adulthood, for a total of six different outcome variables.

BCG vaccination, the determinant variable, was used in the multivariate model as

one of the following: 1. a dichotomous variable indicating the absence or presence of vaccination. 2. a categorical variable: no vaccination, vaccination in infancy (between 0 and 1 year of age), or vaccination in childhood (after the age of one).

Twelve models (six outcome variables combined with two determinant variables) were therefore constructed to examine the relationship between BCG vaccination and odds of asthma.

All analyses were performed with SAS version 8.1.

3.2 ETHICAL CONSIDERATIONS

This was an observational study to document the prevalence of BCG vaccination among subjects diagnosed with asthma and similar subjects without asthma. Approval of the Research Ethics Board of the M.U.H.C was sought and obtained (see p. A-11, Appendix). The study imposed neither experimental therapy nor hospitalization on its participants. Neither blood products nor other biological specimens were procured or handled during the study; so that there was no risk to individuals involved in its implementation, nor to members of the general public.

The subject's right to privacy was respected at all times: initial contact with potential subjects was solely by written letter, and subjects were given freedom to respond to or reject the invitation to participation. No attempt was made to contact subjects by telephone unless they indicated an interest in participation and authorized study personnel to contact them. All subjects were assured that they would not be discriminated against even if they refused participation, or withdrew from the study before completion of required study procedures.

The purpose of the study was explained to the subject in his/her own language, and if they were interested in participation, they were requested to provide informed consent, including consent to verification of BCG vaccination status in the vaccination registry of l'Institut Armand Frappier, and to complete a study questionnaire. All data was

entered into two computer files: a master file containing identifying information, including names, address, date of birth, and a study identification number; and a second file containing clinical, questionnaire, BCG vaccination status data, and the study identification number as the **only** subject identifier. Once results of the questionnaire, BCG status, and clinical data were obtained and matched with the appropriate identifying information in the master file, the master file was placed under lock and key.

CHAPTER FOUR: ANALYSIS

4.1 RECRUITMENT AND PARTICIPATION

433 case subjects were invited to participate via mailed questionnaire, while 173 control subjects were approached in the orthopedic clinics of the MUHC (Figure 1). 75 of the 433 questionnaires mailed to prospective case subjects were returned because the subject no longer resided at the current mailing address. Of the remaining 358, 98 were completed and returned.

Among prospective controls, a total of 173 subjects were approached during their scheduled appointment in the orthopedic clinic. 20 of these subjects did not participate, leaving 153 who completed the questionnaire. The final participation rate among case and control subjects was therefore 27% and 88%, respectively. 5 case subjects and 11 control subjects were excluded because they were born after 1979, and so would have been born after the BCG vaccination campaign was over. 24 control subjects were excluded because of a positive response to question #14 ("Have you ever had asthma?") of the study questionnaire (see Appendix 1, p. A-5). This left 93 eligible cases and 118 eligible controls who participated.

4.2 DEMOGRAPHIC CHARACTERISTICS AND CRUDE ANALYSES

As seen in Table 1, 83 of 93 (89%), asthma cases were atopic as assessed by a positive response to one of four questions regarding allergic symptoms. Of non-asthmatic control subjects, 84 out of 118 (71%), were non-atopic. Among case subjects, 50 (54%) stated that their first attack of asthma occurred before the age of 18. These subjects were therefore considered to have childhood-onset asthma, while the remainder were considered to have adult-onset asthma. The majority of case subjects were female, while the majority of control subjects were male. Case subjects were older than control subjects (34 vs. 30 years), although age range as well as standard deviation were similar in the two groups of subjects. There was no significant difference between case and control groups in the proportion of subjects who received the BCG vaccine, nor were there significant differences between these groups with respect to age at vaccination.

Among case subjects, there were no significant differences between atopic and non-atopic subjects by BCG vaccination status, nor by age at which vaccination was administered (Table 2); furthermore, no significant differences in characteristics were noted between case subjects whose onset of asthma was in childhood compared with cases whose onset of asthma was in adulthood (Table 3).

With respect to control subjects, there were no significant differences in characteristics between those who were atopic, and those who were non-atopic, and although atopic subjects were more likely to have received BCG vaccine, there was no significant difference between the groups with respect to the age at which vaccination was given (Table 4) .

As shown in Table 5, crude estimates and 95% confidence intervals are given for the odds of having asthma (all subtypes included), atopic asthma, childhood-onset asthma, and adult-onset asthma given BCG vaccination at any time, and specifically vaccination in infancy, and after the age of one (Table 5). While the crude estimates were not statistically significant, the data suggest a reduced odds of adult-onset asthma associated with BCG vaccination given after the age of one.

4.3 COMPARISON OF STUDY GROUPS BY CONFOUNDERS

Atopic and non-atopic case and control subjects were compared by potential confounding variables, as shown in Table 6a. Each question of the questionnaire (potential confounder) was answered by greater than 90% of the study subjects. A p-value of 0.25 was considered the threshold below which differences between groups were considered potentially significant in considering variables for inclusion in subsequent multivariable logistic regression analysis as described by Hosmer and Lemeshow (100). Over 95% of subjects in the study answered "no" to questions regarding previous diagnosis and treatment of active tuberculosis, and of the 16% of subjects who traveled overseas, none had traveled to a country where tuberculosis was endemic (data not shown). Responses to questions from the Risk of Tuberculosis in Health Care Workers questionnaire were therefore not included in subsequent analyses, as the factors

concerned were not felt to constitute significant confounders.

Control subjects were somewhat more likely to be current pet owners, to be current smokers, and to have attended daycare in childhood. Case subjects by contrast, were more likely to have had a mother or sibling with asthma, and to have sustained a serious respiratory infection in childhood. Although case subjects were also more likely to have experienced allergic symptoms, to have work-induced respiratory symptoms, and to have changed jobs because of respiratory symptoms, these were felt to reflect differences in variables which are highly correlated with asthma, but are not confounders per se. They were therefore not considered in subsequent development of the logistic regression model.

No significant differences were noted between cases and controls in terms of the proportion of subjects vaccinated with BCG, nor the proportion vaccinated in infancy nor childhood. As well, there were no significant differences between the groups by socio-economic status as measured by the Blishen index (97); however, 16 control subjects in the series were university students, for which there was no assigned numeric value according to the Blishen index. 3 control subjects were unemployed, and one control subject did not respond to the question concerning occupation. A value of 70 was assigned to each subject that was a university student, corresponding to the value in the Blishen index assigned to secondary school teachers, which was felt to be a reasonable representation of the "average socioeconomic status" of most university students. Because the socio-economic status of the other 4 subjects could not be determined when the questionnaire was administered, these subjects were given a Blishen index value (44.75), which was an arithmetic average of values of all other subjects participating in the study. When these 20 subjects were excluded from the analysis, asthmatic case subjects were noted to be of a slightly higher socio-economic status than non asthmatics (mean Blishen index values of 46.2 and 42.0, respectively).

When atopic asthmatic cases were compared with non-atopic non-asthmatic control subjects (Table 6b), the findings were similar. Potentially significant differences were also noted in the proportion of subjects vaccinated with BCG in infancy ($p=.047$).

All subjects were similarly compared according to potential confounding variables by their BCG vaccination status (Table 7a). Subjects who received BCG vaccine were somewhat more likely to have had a father who smoked as well as had asthma, and to have had at least one sibling. They were also more likely to be currently employed. Subjects who did not receive BCG vaccine were, by contrast, more likely to have attended daycare in childhood, and to have reported exposure at work to vapours or fumes. Subjects who received BCG vaccine were of a slightly higher socio-economic status as measured by the Blishen index, even after excluding the 20 subjects who were assigned Blishen values as described above.

When the analysis was restricted to atopic asthmatic subjects and non-atopic non-asthmatic control subjects (Table 7b), those who received BCG vaccine were somewhat more likely to have had a sibling, while those who did not receive BCG vaccine were somewhat more likely to have been exposed at work to vapours and fumes.

4.4 ASSESSMENT OF EFFECT MODIFICATION

Crude estimates and 95% confidence intervals for the odds of having asthma are given for each group of subjects answering "yes" or "no" to specific questions of the study questionnaire (Table 8). These questions were surrogates for exposure variables which play a role in the pathogenesis of asthma, and were therefore chosen in order to assess modification of the effect of previous BCG vaccination by different exposures. The crude estimates of the odds ratios for males and females were not very different from the overall crude estimate for the odds of asthma given in Table 5, and the respective odds ratios for males and females were not significantly different from one another, suggesting that there was no significant modification of the effect of BCG vaccination by gender. Among subjects who stated that they attended daycare in childhood, the odds of asthma given previous BCG vaccination were 2.1, compared with 0.8 for those that did not attend daycare in childhood, suggesting the possible presence of effect modification by childhood daycare attendance. Similar effects were noted among subjects who stated they had a mother with asthma (OR= 2.9 vs. 1.0) and among subjects who denied that their father smoked when they were children (OR= 2.2 vs 0.8).

Table 9 is a listing of all variables which were felt to be potential confounders of the relationship between BCG vaccination and asthma based on reasonable biological hypotheses, as well as the analysis described in Tables 6 and 7.

4.5 LOGISTIC REGRESSION ANALYSIS

Logistic regression models were constructed beginning with a "full" model (Table 10), comprising all variables listed in Table 9, as well as BCG vaccination. All variables in the model were dichotomous categorical variables, with the exception of age and Blishen index, which were continuous variables. Point estimates and 95% confidence limits for the odds ratios for these variables are given. Variables significantly associated with an increased odds of asthma included older age, having a serious respiratory infection in childhood, and having a sibling with asthma. Apart from male gender, no other variable was significantly associated with a reduced odds of asthma.

The point estimate for BCG, as well as the point estimate of each variable in the model and its confidence limits just before removal from the model are shown in Table 11. The columns "Effect of BCG (Adjusted Odds)", and the adjacent column "95% Confidence Interval (BCG)" illustrate the point estimate and confidence limits of BCG vaccination respectively, with sequential removal of each variable from the model according to its p-value (least significant to most significant) in stepwise logistic regression analysis. No significant change in the point estimate for the BCG variable was noted with sequential removal of each variable from the model. The best model which was felt to explain the relationship between BCG vaccination while controlling for the potential effect of confounding variables was:

Asthma= Age+ Gender + BCG Vaccination + Childhood respiratory infection + Sibling With Asthma.

Table 12 illustrates various analyses using this final model. All analyses were performed including the 20 subjects for whom Blishen values could not be objectively assigned. When the analyses were performed excluding these 20 subjects, no difference in

point estimates of variables was observed in any analysis (data not shown), and these subjects were therefore included in the final analyses.

Model 1 included all subjects in the study. Data was complete for 209 (91 cases and 118 controls) of 211 subjects. The outcome assessed was the risk of developing asthma given previous BCG vaccination. The measure of association used in this and all subsequent models was the odds ratio. An increase in age by one year was associated with a slightly increased odds of asthma, while male gender was associated with a reduced odds. Both a history of a serious childhood respiratory infection, and a history of having a sibling with asthma were associated with an increased odds of having asthma. After adjustment for the effect of these variables, BCG vaccination appeared to be associated with a reduced odds of asthma, although not significantly.

When BCG vaccination was modeled as two levels-- vaccination in infancy (0-1year), or vaccination in childhood (>1year), vaccination with BCG in childhood was associated with a reduced odds of asthma (OR=0.5), as seen in model 2.

A history of childhood respiratory infection and having a sibling with asthma were associated with an increased odds of childhood-onset asthma (model 3). Case subjects who responded that their diagnosis of asthma was on or after the age of 18 were considered to have adult -onset asthma, and were excluded from the case series in this analysis. When BCG vaccination was modeled as two levels as previously described, vaccination in infancy was associated with an increased odds for childhood-onset asthma, while vaccination in childhood was associated with a reduced odds, though neither effect was statistically significant (model 4).

Previous BCG vaccination was associated with a reduced odds of adult-onset asthma (OR=0.5), while "childhood respiratory infection" and "sibling with asthma" were both associated with an increased odds of having adult-onset asthma, as shown in model 5. Case subjects who met the definition of childhood-onset asthma were excluded from the case series in this analysis. When BCG vaccination was modeled as two levels,

(model 6), odds of adult-onset asthma were reduced given both infancy and childhood vaccinations (ORs 0.8 and 0.3, respectively), though only the effect of childhood vaccination was statistically significant (CI: 0.1-0.9, p=0.02). Similar to model 5, the effects of a previous childhood respiratory infection as well as having a sibling with asthma were associated with an increased odds of having adult-onset asthma.

The analysis in models 7-12 was similar to that of models 1-6, with the exception that it was restricted to atopic asthmatic cases and non-atopic non-asthmatic controls in order to assess the relationship of BCG vaccination with atopic asthma.

Data was complete for 82 case subjects and 84 control subjects in model 7. Similar to model 1, age was associated with an increased odds of atopic asthma, while male gender was associated with a reduced odds. A history of a serious respiratory infection in childhood, as well as having had a sibling with asthma were both associated with an increased odds of atopic asthma. In contrast to model 1, previous BCG vaccination was not associated with a reduced odds of atopic asthma.

The effects of BCG vaccination in infancy, as well as in childhood (model 8), were similar to those seen in model 2, with BCG vaccination in infancy associated with an increased odds of atopic asthma, and childhood BCG vaccination associated with a reduced odds.

When the case series was restricted to subjects whose diagnosis of asthma was in childhood, BCG vaccination was associated with an increased odds of developing childhood-onset atopic asthma, though the effect was not statistically significant (model 9). The direction of effect of the variables "serious childhood respiratory infection" as well as "having a sibling with asthma" was similar to that noted in model 3.

BCG vaccination given in infancy was associated with an increased odds of having childhood-onset atopic asthma (model 10), while vaccination in childhood was no longer associated with a reduced odds, in contrast to model 4.

Model 11 was similar to model 5 in the magnitude of effect noted for the association between BCG vaccination and adult-onset atopic asthma. In model 12, where BCG vaccination was modeled as two levels, vaccination in childhood was associated with a reduced odds of adult-onset atopic asthma (OR=0.2), and the effect was statistically significant (CI=0.1-0.9).

Table 13 provides a summary of the various logistic regression models in Table 12. Vaccination with BCG after the age of one was associated with a reduced odds of adult-onset asthma overall, and specifically a reduced odds of adult-onset atopic asthma.

CHAPTER FIVE: DISCUSSION

5.1 OVERVIEW:

In this case control study, the relationship between previous BCG vaccination and the presence of asthma in adult subjects was assessed. The overall prevalence of BCG vaccination did not differ significantly between the two groups (37% for cases vs. 34% for controls). Subgroup analysis revealed that vaccination with BCG was associated with an increased crude odds of childhood-onset asthma, and a reduced crude odds of adult-onset asthma. After adjusting for confounding factors in a multivariate analysis, BCG vaccination was associated with a somewhat reduced odds of having asthma overall. Vaccination with BCG after the age of one was associated with a significantly reduced odds of adult-onset asthma, particularly among those asthmatic subjects with atopy. The protective effect of BCG vaccination was observed despite the presence of possible sub-clinical asthma among a small proportion of control subjects (see Section 5.2 "Strengths of the Study").

5.2 STRENGTHS OF THE STUDY:

The study had a number of strengths, the first of which was the use of a rigorous case definition. The majority of previous studies examining the relationship between BCG vaccination and asthma used responses to questions regarding a diagnosis of asthma or asthma symptoms as the sole criteria by which subjects were defined as having asthma or not, without clinical evaluation (see Table 14, pp.87-90). In the current study, all case subjects had been evaluated by a respirologist and had formal pulmonary function testing; all met strict clinical criteria for the definition of asthma, in addition to having provided appropriate responses to previously validated questions regarding asthma symptoms and a previous diagnosis of asthma. It is therefore highly unlikely that case subjects were misclassified.

Control subjects did not have the same rigorous evaluation to ensure that they did not have asthma; however, the mean specificity of questions about self-reported asthma, when validated against bronchial challenge testing and clinical diagnoses of asthma is

greater than 90%, while the mean sensitivity is 36% in several studies (37). If the prevalence of asthma in the population of potential controls was as high as 10%, this would mean that these questions have a negative predictive value of 93%.

While control subjects were therefore unlikely to have had asthma, and less than 5% of control subjects reported using an inhaled or oral medication or visiting the emergency room in the last 12 months because of a breathing problem, 23% experienced an attack of wheezing in the last 12 months, 13% experienced chest tightness, 6% experienced shortness of breath during the day, and 15% experienced it with effort. These data suggest that a significant proportion of these subjects may have had subclinical asthma; therefore, the effect of potential misclassification of these control subjects was assessed by analyzing the data after excluding them. No changes in measures of association between BCG vaccination and asthma were noted either in crude analysis, or after controlling for the effects of confounders in logistic regression analysis (data not shown). This suggests that even if misclassification of control subjects occurred, it had no effect on the study results. Moreover, if misclassification of controls by disease status occurred, it was likely random, and not related to exposure status, as BCG vaccination was administered in Quebec in infancy or early childhood; therefore, vaccination status would not have been recalled by the subject or inquired on by any physician who may have evaluated these subjects for asthma.

Data on exposure was uniform for both cases and controls and was reliable, having been extracted from a central registry, the accuracy of which has been previously validated (83). Exposure data was therefore not vulnerable to recall bias typical of many case-control studies, nor was exposure data vulnerable to observation bias, as personnel extracting data on vaccination status were blinded to the disease status of the study subjects. If misclassification of exposure status occurred, it was unlikely to have been on the basis of disease status (and therefore non-differential).

Several previous studies examining the relationship between BCG vaccination and the development of atopy or asthma used school health records or the presence of a scar as evidence of vaccination. As many as 25% of vaccinated persons may fail to develop a

scar (101). Floyd et. al. (102) assessed the sensitivity of BCG scar reading in Northern Malawi among individuals aged > 3 months who were recruited into a BCG vaccine trial between 1986 and 1989 (n=43722), and of infants vaccinated in health centres between 1989 and 1991 (n=3430). Both groups of subjects were among all residents of the district (some of whom were not enrolled in the trial or infant vaccination program) examined by blinded personnel for presence and size of BCG scars in subsequent years. Sensitivity of scar reading was > or = 93%, for those individuals enrolled in the trial who were between 3 months and 60 years of age at first BCG vaccination, and a substantial proportion (data not provided) of these subjects were followed up to 11 years after vaccination. No significant relationship was observed among these individuals between percentage of individuals by age at BCG vaccination still showing a scar and time since BCG vaccination. By contrast, there was no significant difference between subjects who received BCG vaccine at less than 1 month of age and subjects receiving BCG vaccine at 1-4 months of age with respect to proportion of subjects with a scar at 7-12 months after vaccination (90% in both groups had a scar); however, at 37-48 months post vaccination, 84% of the subjects vaccinated at 1-4months of age had a scar, while only 76% of those less than 1 month had a scar ($p<.001$).

The authors concluded that while a BCG vaccination scar is a highly sensitive indicator of vaccination status when the vaccine is given at over 3 months of age, it may not be when vaccinations are given within 1 month of birth. Since most vaccinations in the world are given soon after birth, this low sensitivity may have led to underestimation of BCG vaccination prevalence in studies which used the presence of a scar as evidence of BCG vaccination. This misclassification of BCG vaccination status would therefore have led to attenuation of any observed association between BCG vacciantion and asthma.

Age at which subjects received BCG vaccination in the current study was reliable, as this information was also obtained from a central provincial registry allowing for assessment of the relationship between age at vaccination and development of asthma. The majority of previous studies (see Table 14, pp. 87-90) have not specifically examined

this relationship and it is potentially important, as there may be critical time points in immune system development at which vaccination with BCG could be more or less beneficial in modulating the Th1/Th2 response. In assessing the effect of previous BCG vaccination on tuberculin reactivity among 1510 Quebec-born subjects, Menzies et. al. (83) noted a higher prevalence of tuberculin reactions among subjects vaccinated after infancy compared with those vaccinated in infancy (20% vs. 8%, respectively). Furthermore, among the 469 subjects vaccinated after infancy, tuberculin reactivity was related to age when vaccinated, but not to the interval between vaccination and tuberculin testing or repeat vaccination, suggesting a critical time frame in immune system development during which some subjects may be capable of being sensitized to the effects of BCG vaccine.

A final strength was the use of a questionnaire which had been previously validated and used in other large scale studies of the epidemiology of asthma in adults in many different populations (44)(46). In addition to having valid and reliable data concerning potential confounders and effect modifiers of the BCG vaccination-asthma relationship, the age at which a subject had their first attack of asthma was obtained. Few studies have examined the relationship between childhood BCG vaccination and asthma among adult subjects (103-104), and none has specifically examined the relationship between BCG vaccination and the age at which adult subjects had the onset of their disease. Most studies have examined the relationship between childhood BCG vaccination and the development of childhood atopic disease.

5.3 LIMITATIONS OF THE STUDY

Despite these strengths, there were a number of limitations to the study, which included: 1. A significant difference in participation rates between study groups. 2. Lack of objective measures of atopic status 3.Heterogeneity of the outcome measure (asthma) 4.Potential inadequate control of confounding, and 5.Use of hospital-based subjects.

Participation rates differed significantly between case and control subjects (27% and 88%, respectively); the prevalence of BCG vaccination among cases may not reflect

the true prevalence, if this was significantly different among non-participant cases. Formal consent was required in order to obtain information from the Institut Armand-Frappier regarding subjects' BCG vaccination status, so this could not be verified for non-respondents. The difference in participation rates may have been due to inherent differences between the groups, possibly leading to biased measures of association between previous BCG vaccination and asthma.

Definite conclusions, however, cannot be drawn regarding non-response: while these subjects may have truly refused participation, it is also possible that a certain proportion no longer resided at the mailing address, and the current occupant simply did not return the questionnaire. As ethical guidelines prohibited direct contact with prospective case subjects, the specific reasons why these questionnaires were not completed could not be determined.

The diagnosis of atopy among cases and controls was based on responses to one of 4 questions regarding atopic symptoms, and not on the basis of allergy skin prick testing or specific serum IgE antibody-- the reference standards used in most studies of atopy (26). Among cases, atopic status was not confirmed on the basis of skin prick testing nor specific serum IgE levels, since these tests were not routinely performed in all of the respiratory medicine clinics of the MUHC at the time of the study.

Based on questionnaire response, 89% of case subjects were atopic, while the proportion of control subjects who were atopic was 29%. The measured prevalence of atopy among control subjects was similar to that noted in population-based studies measuring the prevalence of atopy in adult subjects similar in age to those in the current study (105-108). In these studies, however, atopy was defined by at least one positive allergy skin prick testing, and the prevalence ranged from 21% to 45%. Moreover, the prevalence of atopy, defined as a single positive allergy skin prick test among 2788 subjects in a 6 city survey in Canada (47) varied from 52% to 66% (Dr. Jure Manfreda, personnal communication), suggesting that the prevalence of atopy among controls in the current study may have been underestimated. Furthermore, the prevalence of atopy

among controls in the current study was underestimated because control subjects who responded "yes" to the question, "Have you ever had asthma?" were excluded from the analysis. Given the association of asthma and atopy, exclusion of these subjects would have contributed to the underestimation of atopy prevalence among controls.

Screening questionnaires which define adult subjects as atopic have not been extensively validated. Lakwijk et.al. (39) validated a questionnaire on allergic symptoms against allergen specific and total serum IgE levels in 175 pregnant women in the Netherlands. The authors did not validate each questionnaire response with the antigen-specific IgE. Rather, they reported the sensitivity and specificity of groups of questions validated against total and specific IgE to any one of 5 antigens assessed in the questionnaire. The sensitivity and specificity of a positive response to one or more of 5 questions (regarding allergy to house dust, dust mite, pets, and hay fever) validated against antigen-specific IgE was 55%, and 87%, respectively. When validated against total serum IgE, sensitivity and specificity of a positive response to one or more questions was 53% and 77%, respectively.

In the same study (39), of 76 subjects who provided a history of eczema, only 9 had elevated specific IgE levels; it was concluded that a history of eczema was not a reliable indicator of atopy. In the current study, 67 subjects (42 cases and 25 controls) provided a positive response to the question regarding eczema symptoms (50% of the 135 subjects who provided at least one positive response to one of 5 questions regarding allergic history). Among these subjects, 18 (1 case and 17 control subjects) provided a positive response to the question regarding eczema symptoms as their sole allergic history. These subjects were classified as non-atopic, leaving 117 subjects (83 cases and 34 controls) who were atopic on the basis of a positive response to one of the remaining 4 questions regarding allergic history.

Because of the relatively low sensitivity of these questions, atopic prevalence may have been underestimated among control subjects; moreover, although the questions have relatively high specificity, if the true population prevalence of atopy is 50%, as suggested

by the data of Manfreda et.al. (47), the negative predictive value of these questions would be 67%. This suggests that atopy prevalence was potentially underestimated among controls.

Inadequate control of confounding factors such as age, gender, or clinical characteristics may have resulted in an inability to demonstrate a significant difference in BCG vaccination rates. Restriction by year of birth ensured that the groups were similar with respect to age, although mean age of cases was greater than that of controls. The difference, however, was not large enough to suggest that these two groups belonged to different birth cohorts with different risks of being vaccinated with BCG as a result of differences in public health practice over time. The age difference, moreover, was not large enough to correspond to a time period over which significant changes in the clinical diagnosis of asthma would have taken place, leading to differences in risk of asthma diagnosis between these two groups. Age was therefore unlikely to have been a significant confounder of the BCG-asthma relationship.

The majority of case subjects were women, consistent with previous studies (46)(91), demonstrating a higher prevalence of asthma among women than among men between the ages of 30-50. By contrast, 56% of the control subjects were men; however, stratification by gender showed there were no significant differences in the estimated odds ratios between men and women of the relationship between BCG vaccination and asthma (Table 8). This suggests that gender was not a significant confounder of this relationship. These results are to be expected, as previous public health practice should not have targeted males and females differently for BCG vaccination, so that gender would not be expected *a priori* to be a confounder. It is furthermore unknown how gender might modify the effect of BCG vaccination on the development of asthma. While there are different hormonal environments at puberty and adulthood, in males and females, it is unknown how these would interact with BCG vaccination given in infancy or childhood, or modulate the Th1/Th2 response.

Potential confounding by socioeconomic status was controlled by converting the subject's occupation to a socioeconomic score as formulated by Blishen (97). Measures

of socioeconomic status, however, may vary over time (109), and it is unknown whether a better alternative would be to use the occupation of a subject's parents to obtain a childhood socioeconomic score, and to combine this with the subject's current socioeconomic score to obtain an "average" score.

Other possible measures of socioeconomic status are proxy measures, or "area-based measures" of socioeconomic status and include average house value, percentage of residents with a high-school or university degree, median income, and other measures of socioeconomic status of census tracts or enumeration areas (neighbourhood) within which a study subject resides. These measures, however, have been found to correlate poorly with individual measures of socioeconomic status such as the Blishen score described above (109).

Use of hospital-based subjects, as opposed to subjects from the community, may also have influenced the results of the current study. Case subjects with asthma in this study are likely to have been referred for evaluation by a specialist physician at the MUHC, while most control subjects came to the orthopedic clinic as a follow-up to their initial presentation in the emergency room of the MUHC. The referral base of these two groups is therefore likely to have been different. The control group was more likely representative of the immediate catchment area of the MUHC, while the case series was likely drawn from a wider geographic area. Although BCG vaccination was part of a provincial public health campaign (90), vaccination rates varied from 10-80%, depending on the practice of local public health departments. The difference in vaccination rates between these groups may therefore relate to differences in vaccination rates between the different population bases from which the two groups were drawn (see Section 5.4 "Sources of Bias" below).

5.4 SOURCES OF BIAS:

Potential sources of bias included bias in the ascertainment and selection of cases and controls, ascertainment of exposure status, misclassification with respect to disease or exposure status, and bias in the assessment of the cause-effect relationship of exposure and outcome.

i) Ascertainment and selection bias:

Potential differential surveillance, diagnosis, referral, or selection of subjects into the study based on exposure status may have led to biased estimates of relative risk.

It is unlikely that subjects previously given BCG vaccination received less (or more) surveillance for medical conditions (such as asthma) than subjects who did not receive the vaccine, since previously there was no proposed relationship between vaccination and the risk of asthma. Vaccination with BCG, exposure to TB, and the development of asthma, may have been associated with factors such as socio-economic status (SES); however, one cannot predict how such factors vary with SES, nor how SES varies with health-services utilization and consequent differential surveillance for asthma. In order to determine a valid estimate of the relative risk, SES was controlled for in the analysis using the subject's current occupation as a surrogate marker for socio-economic status.

With regard to diagnosis, knowledge of a patient's vaccination status is unlikely to have played a role in establishing a subject's case status, as the diagnosis of asthma was based on a typical constellation of symptoms and objective measures of airflow obstruction, without regard to previous vaccination history. Study questionnaire criteria used in defining case status included positive responses to questions about a previous asthma diagnosis and subject self-reporting of symptoms, and asthma diagnosis is unlikely to have differed between exposed and unexposed subjects, nor along other axes potentially associated with the exposure (such as SES), and therefore is unlikely to have biased the case definition process.

As discussed in Schlessleman's Case-Control Studies (110), biases related to differential referral patterns between cases and controls potentially exist in a hospital-based case-control study. Physician and self-referral are the main selective factors influencing the composition of the final case-control series. While cases and controls should be chosen in a manner that ensures equal probability of selection of exposed and

unexposed subjects, a biased selection of one group (cases or controls) can be compensated by an equally biased selection of the other. With respect to the present study, an examination of sampling proportions s_1 to s_4 of four cells in a hypothetical 2x2 table with population frequencies A,B,C,and D, is required in order to assess potential referral bias:

	ASTHMA+	ASTHMA-
BCG+	S1 (A)	S2 (B)
BCG-	S3 (C)	S4 (D)

Absence of bias requires that $s_1 s_4/s_2 s_3=1$, or that, as previously noted, the "biasing factor", k, for selection of subjects on the basis of exposure is known and equal between cases and controls. This would allow for an unbiased estimate of the odds-ratio, as, for example, $s_1=ks_3$ and $s_2=ks_4$ so that $s_1 s_4/s_2 s_3=1$. In the present study, the comparison between cases and controls with regard to referral on the basis of exposure is along the axis of the different clinic settings (or medical conditions). Control subjects are more likely to have been self-referred to the orthopedic clinics of the MUHC, given the relatively acute nature of their condition (bone fracture, ligament sprain). Self-referral would therefore have been directly related to the catchment area of the hospital. By contrast, cases of asthma recruited via clinic records of the Montreal General and Montreal Chest Institute are more likely to have been physician-referred cases. The "bias factor" k, by which subjects were referred to the study on the basis of exposure, may therefore have been unequal between cases and controls, and have resulted in a biased estimate of the odds ratio. It is difficult to determine, however, whether the effect of this biasing factor should have been to attenuate or inflate calculated measures of association between BCG vaccination and asthma

The use of prevalent cases may have been a potential source of selection bias: these may have represented a sub-group with an exposure history which was not representative of the total population of asthma cases; in this regard, differential participation rates between cases and controls may relate to inherent differences between these groups and also may have biased study results.

ii) Ascertainment of exposure status: Assessment of exposure status is unlikely to have been biased, as it was determined by records in the vaccination database, and was not dependent on subject recall. Subjects were required to complete a self-administered questionnaire, so that interviewer bias did not play a role in potential differential assessment of confounding variables. Case subjects, however, are more likely to have had better recall about past exposure to potential allergens, family atopic history, or work exposures than control subjects, so that recall bias may have played a role in the assessment of potential confounding variables or effect modifiers.

iii) Misclassification: Estimates of relative risk would have been attenuated if errors in determination of case or control status occurred equally for exposed and unexposed individuals. Similarly, attenuation of the relative risk estimate would have occurred if errors in the determination of exposure status were made irrespective of case and control status. In this study, potential misclassification of controls was a function of potential lack of specificity of the questionnaire for asthma. This is not expected to have correlated with exposure status, so that misclassification may be regarded as non-differential. Similarly, as exposure status for all subjects was determined from the same database, errors in its classification are expected to have been equal for both cases and controls.

iv) Bias in assessment of cause-effect relationship: While it is generally recommended that cases used in a case-control study be incident cases ascertained over a time period pre-determined by the investigator, the use of prevalent cases in the current study did not lead to confusion regarding the cause-effect relationship of the exposure (vaccination) and outcome (asthma), since for more than 80% of recipients in Quebec, as well as

among cases and controls in the current study, vaccination is known to have occurred in infancy or early childhood (90), likely well before a diagnosis of asthma was established.

5.4 IMPLICATIONS OF THE STUDY:

The results of the current study have several implications: 1. The effect of BCG vaccination may be different depending on the age at which a subject is vaccinated, and this may be related to critical events in immune system development which determine commitment to either a Th1 or Th2 phenotype. 2. The different effects of BCG vaccination may relate to differences in subtypes of asthma (child- or adult-onset) each with different pathophysiologic mechanisms and risk factors. 3. The role of atopic predisposition in the clinical expression of these different asthma subtypes may or may not be influenced by BCG vaccination.

While there was no significant association between BCG vaccination at any age and asthma with onset at any age in the current study, vaccination after infancy was associated with a reduced odds of onset of asthma in adulthood, and specifically a reduced odds of onset of atopic asthma in adulthood. While these findings were derived from a subanalysis, the results are nevertheless interesting because the effect was observed despite the possible presence of subclinical asthma in some of the control subjects Few studies have specifically examined the effects of BCG vaccination in relation to age at which it was administered. Menzies et. al.(83) examined the effect on tuberculin reactivity of BCG vaccine given 10 to 25 years earlier among 4629 schoolchildren and young adults in Montreal. Of these, 1,511 (33%) had been vaccinated, 66% of whom were vaccinated once only in infancy, 23% of whom had been vaccinated once but after infancy, and 11% of whom had been vaccinated twice. Among those vaccinated in infancy, 7.9% had significant tuberculin reactions, compared with 18% among those vaccinated between 1 and 5 yr of age, and 25.4% among those vaccinated after the age of 5 (p less than 0.001). These results suggest that there may be a different effect of BCG vaccination on tuberculin reactivity at different ages of the vaccine recipient. Although tuberculin reactivity is not necessarily synonymous with acquisition of cell-mediated immunity to tuberculosis (111)(112), it is a measure of delayed-type

hypersensitivity, which is one aspect of T-cell mediated immunity. The findings of the study by Menzies et. al. therefore suggest there may be other aspects of the immune system whose modulation depends on the age at which BCG vaccination occurs.

Table 14 summarizes the majority of studies published in the English language (one study (128) is from the Beijing arm of ISAAC (43) phase II and was published in Chinese), which have examined the relationship between BCG vaccination (or tuberculin skin test response), and asthma (or atopy). Section A includes 7 studies which specifically examined the relationship between BCG vaccination and atopy. All studies were performed either in children or young adolescents, and as atopy plays a greater role in the pathogenesis of asthma in children compared with adults (see Section 1.2.4, "Clinical Phenotypes of Asthma), asthma was regarded as a manifestation of atopic disease in these studies, rather than as a distinct end-point.

Among the studies listed in Section A, three specifically examined the effect of age at which BCG was administered in relation to the development of atopy. Aaby et. al. (89), conducted a cross-sectional survey in 1994 among 400 subjects aged 3-14 in Guinea-Bissau, West Africa, who had documented measles during an epidemic in 1991. A lower prevalence of atopy (defined as any positive skin prick test) was measured among 271 children who had documented evidence of previous BCG vaccination, compared with 53 children who had neither vaccine documents nor a BCG scar [adjusted OR: 0.2 (0.1;0.6)]. The protective effect was greater the earlier the vaccine was administered, with the greatest effect occurring when BCG was given in the first week of life[OR:0.1(0.04;0.41)].

Two other studies listed in Section A of Table 14 found no difference in the effect of BCG vaccination by age at which it was administered. Krause et. al. (113) compared the prevalence of atopy (defined as specific serum IgE positivity) among 1065 children 8-16 years of age in Greenland who had been born before 1990, when BCG vaccine was routinely given as part of public health practice, with that among 510 children who did not receive BCG, and who were born after 1990, when routine BCG administration was

discontinued. No significant difference in the prevalence of atopy was observed between vaccinated and unvaccinated children (16.2% vs 12.2%), and while subjects who received BCG were older, the adjusted odds ratio for atopy given BCG vaccination was 1.03 (0.7-1.5). Furthermore, the odds ratios for atopy were calculated for the 1065 BCG vaccinated children according to age at which the subject was vaccinated (0->181 days). The odds of atopy was not associated with age at vaccination (p value for heterogeneity of the odds ratio= 0.2).

Similarly, Annus et. al. (114) surveyed 979 Estonian schoolchildren aged 10-11 years according to methods of Phase II of the ISAAC study (115). 643 of these children underwent skin prick testing and 717 children had been vaccinated with BCG, of which 638 (89%) received the vaccine at 3-4 days of age, 42 (6%) between 1 and 11 months, and 37 (5%) at 1 year or older. These children were periodically tested in subsequent years for tuberculin responses via tuberculin skin testing. 101 subjects (29%) of 343 subjects who were tuberculin skin tested at age 10-11 were re-vaccinated with BCG between 7-8 years of age, though data on revaccination with BCG was not provided for the other subjects. Nevertheless the prevalence of atopy, adjusted for sex, age, and number of BCG vaccinations, was not significantly different between subjects who received BCG vaccination before 1 month of age and those who were vaccinated after 1 month (data not provided).

The study by Aaby et. al (89) is therefore the only one of those listed in Section A of Table 14 demonstrating a protective effect of BCG on the development of atopy. The discrepancy with the results of Krause et. al. and Annus et. al. noted above may be due to differences in baseline prevalence of atopy in the populations studied, as the prevalence of atopy in the study of Aaby et. al. was between 21% and 40%, while the prevalence in the other studies in Section A was 12%-15% or less. A lower baseline prevalence may therefore have not allowed detection of a protective effect of BCG, and specifically a protective effect by age at vaccination. Alternatively, other factors not measured in this study may have been responsible for the findings of Aaby et. al., such as high levels of IgE (induced by helminth infestation), which block mast cell receptors for allergen-

specific IgE (116).

The different effects of BCG vaccination observed in the current study may have been due to heterogeneity of the case series. There were 93 subjects with asthma, of whom 50 had their first attack in childhood, while 43 had their first attack in adulthood. Adult-onset asthma is known to differ from childhood-onset asthma in a number of ways: 1.The role of genetic predisposition is not as clear. 2. Atopy is not as strong a risk factor. 3. Trigger factors are different than in childhood-onset asthma and include aspirin, persistent *chlamydia* and *mycoplasma* respiratory infection, and immunologic and non-immunologic exposures in the workplace (32).

Only two studies listed in Table 14 used adult subjects. Bager et. al.(104) (Section B, Table 14) conducted a cross-sectional study among 2176 pregnant women between 21 and 46 years of age participating in the Danish National Birth Cohort Study. BCG vaccination status was obtained from school health records, atopy by serum specific IgE, and allergic rhinitis and asthma symptoms by telephone interviews. 1622 women had received BCG vaccination in accordance with the Danish TB Eradication Program, which established voluntary BCG vaccination for school age children with a negative tuberculin skin test. The majority of these women (n= 717) were vaccinated at age 7 at school entry, though the age at BCG vaccination for the rest varied from 0-15years. No association was found between age at BCG vaccination and prevalence of atopy, nor with prevalence of allergic rhinitis nor asthma, even after adjusting for birth cohort, sibship size, age of the woman's mother at birth, and social class. When specifically comparing vaccinated subjects with unvaccinated subjects whose parents had not refused BCG vaccination when they were children, however, childhood BCG vaccination was associated with a reduced odds of asthma (OR=0.5 (0.3-0.9)). This effect was not significant, however, when additional adjustments were made for smallpox and pertussis vaccinations.

It is possible that an overall protective effect of BCG vaccination could not be detected in this study since it was conducted among pregnant women, and pregnancy is a predominantly Th2 state (see Section 1.4: "Immunological Basis for Epidemiologic Trends"); furthermore, pregnant women may be more likely to be diagnosed with

conditions such as allergic rhinitis and asthma than their non-pregnant counterparts both because of their greater contact with health care providers, and because hormonal and physiologic changes occurring during pregnancy lead to a greater occurrence of both rhinitis and asthma symptoms (93). Nevertheless, the protective effect of childhood BCG vaccination against asthma in adulthood measured by Bager et. al.in a subanalysis is consistent with the findings of the current study.

By contrast, the study by Jentoft et. al. (103) (Section D, Table 14) is the only other one examining the relationship between BCG vaccination and asthma in adult subjects. In this study, however, tuberculin reactivity was used as a surrogate of protective immunity induced by previous BCG vaccination. No association was observed between tuberculin reactivity and asthma symptoms, level of FEV1, nor bronchial hyperresponsiveness among 386 adult subjects participating in the ECRHS (44) who received BCG vaccination at age 14. A protective effect by age at vaccination could not be observed as in the current study, as all subjects received BCG at a uniform age. It is possible that an overall protective effect was not detected because commitment towards a Th1 or Th2 pathway in the developing immune system may occur in infancy or early childhood, well before the age of 14. Weir et. al. (117) have examined the relationship between IFN-gamma (a Th1 cytokine) and tuberculin skin test responses to a variety of purified protein derivatives from different species of mycobacteria among 424 UK schoolchildren 13-15 years of age prior to vaccination with BCG. While there was a strong association between tuberculin skin test grade and the in vitro IFN-gamma response to tuberculin, there was considerable variation in IFN-gamma responses within tuberculin skin test categories, as well as considerable overlap in IFN-gamma responses between categories, indicating discordance between these two responses. Similar results have been noted among subjects in Malawi (118). These results also support findings from animal models (119) and illustrate the difficulty in drawing definitive conclusions, about the effect of BCG vaccination on the Th1/Th2 cytokine profile and its relationship to atopy and asthma, from studies which have used tuberculin skin testing as a marker of the effectiveness of the BCG vaccine.

Several studies (87)(120-123) have examined the relationship between a positive tuberculin skin test and atopy, and are summarized in Section C of Table 14. While the initial study by Shirakawa et. al.(87) observed an inverse relationship between positive tuberculin skin tests and atopy among 12-13 year olds in Southern Honshu, Japan, these findings were not replicated in the subsequent studies listed. The explanation may relate to the use of the tuberculin skin test as a valid measure of a Th1 response induced by BCG as discussed. Alternatively, other environmental factors which influence immune system maturation such as exposure to non-tuberculous mycobacteria, parasite exposure, and the effect of repeat BCG vaccination after a negative tuberculin skin test (not controlled for in the study by Shirakawa) need to be accounted for.

BCG vaccination was associated with a reduced odds of atopic asthma among adult-onset asthmatics, while no significant association was observed among childhood-onset atopic asthmatics. This finding may be due to differences in the relative contributions of atopic predisposition between childhood-onset and adult-onset asthma, and may explain why the majority of studies examining the relationship of BCG vaccination on atopic disease in children have not shown an effect. The study by Alm et. al. (88) (Section A, Table 14), is the only one to specifically examine the role of atopic heredity in the BCG-atopy relationship. This was a retrospective cohort study of 216 children 3-8 years of age with atopic heredity, who received BCG vaccination when they were younger than 6 months, and 358 age-matched controls who had not been vaccinated. Both groups were assessed for atopic history and clinical signs of atopic disease. All children also underwent skin-prick testing (SPT) and serum was analysed for allergen-specific IgE antibodies. 77 (36%) children in the BCG group and 145 (41%) in the control group had a positive history or clinical signs of atopic disease. In the vaccinated group, 26 (12%) children had one or more positive SPT, and 61 (31%) had circulating allergen-specific IgE antibodies, whereas in the control group, the numbers were 35 (10%) and 84 (27%) respectively. Atopy was confirmed by serology in parents of almost two-thirds of the children in each group. Other risk factors for atopic disease were evenly distributed between the two groups. These results suggest that BCG vaccination may be unable to prevent the development of atopy in children who are genetically predisposed to its

development.

By contrast, the studies by Marks et. al.(124) and daCunha et. al. (125) (Table 14, Section B), conducted in somewhat older subjects, demonstrated a protective effect of BCG vaccination among subjects with an atopic predisposition. Marks et. al. conducted a retrospective cohort study among 751 children of South-East Asian heritage aged 7-14 in Sydney, Australia. There were 309 BCG vaccinated subjects and 442 non-BCG vaccinated subjects in the cohort. While the BCG vaccinated subjects did not have a lower prevalence of allergic sensitization, among subjects with a family history of rhinitis or eczema, BCG vaccination was associated with a lower prevalence of current asthma, defined as recent wheeze (within the last 12 months), and a positive methacholine challenge test suggesting airway hyperresponsiveness. Similarly, in a cross-sectional study conducted among 12-16 year old subjects in Brazil, daCunha et. al. (125) measured a lower prevalence of "ever having asthma" among subjects with "allergy and sneezing" (adjusted odds ratio 0.6(0.4-0.9). This study was nested in a randomized trial studying the effectiveness of BCG re-vaccination at school entry, and 504 of the 1089 subjects classified as having received BCG in childhood were vaccinated a second time, while 220 of 523 subjects who were never vaccinated in childhood were vaccinated at school entry. Furthermore, childhood BCG vaccination in this district in Brazil was administered between 0 and 4 years of age (defined as "neonatal" vaccination in the article) but age of vaccination was not specifically addressed in the analysis.

These data suggest that while atopic predisposition may prevent modulation of the Th1/Th2 response by BCG vaccination in early life, its role in the evolution of childhood-onset asthma and pathogenesis of adult-onset asthma may be very different. BCG vaccination may therefore exert a protective effect on the development of asthma even in subjects with an atopic predisposition, though this may be dependent on when the vaccine is administered.

5.5 CONCLUSION

This hospital-based case control study was designed to test the hypothesis that subjects with asthma would be less likely to have received the BCG vaccine in childhood than subjects without asthma. Although vaccination rates were not different

overall, BCG vaccination given after the age of one was associated with a reduced odds of adult-onset asthma as well as a reduced odds of atopic adult-onset asthma. By contrast, BCG vaccination, whether administered in infancy or childhood, was not associated with a reduced odds of childhood-onset asthma. These findings illustrate the complexity of the relationship between the Th1/Th2 profile, the development and establishment of an atopic state, and the subsequent development of asthma as either a manifestation of an underlying atopic state, or a clinical syndrome unrelated to it.

Previous studies examining the relationship between BCG vaccination and asthma have yielded conflicting results. This may be due to a failure to adequately define homogenous subgroups based on specific asthma subtypes, each of which may have different risk factors (such as atopy) with varying importance to asthma pathogenesis (see 1.2.4 "Clinical Phenotypes of Asthma"). In this regard, the few studies which have been conducted in adults did not specifically address the age of onset of asthma symptoms, which may therefore have distinguished specific asthma subtypes. Other factors contributing to variation in results between previous studies include: 1. Differences in BCG vaccine strain and dose used between studies, which may have been responsible for eliciting immune responses of different magnitude among study subjects 2. Variation between studies in age at which subjects were vaccinated, which may represent different points in immune system maturation, and therefore different points at which vaccination with BCG may or may not effect a change in Th1/Th2 profile.

Our study specifically distinguished subjects with asthma on the basis of the age at which their symptoms first began, as well as the age at which BCG vaccine was administered, thereby demonstrating that vaccination with BCG after the age of one was associated with a reduced odds of adult-onset asthma. This finding is consistent with the only other study (104) that examined the effect of age at BCG vaccination on asthma in an adult population. In that study, BCG vaccination in childhood was associated with a reduced odds of asthma in adults, although the age of onset of asthma was not studied.

A number of factors need to be considered when estimating the effect of BCG

vaccination given in childhood on the development of asthma. These include consideration of dose of BCG vaccination, strain of BCG vaccine, as well as age at which BCG vaccination occurred. Despite the complexity of the potential relationships among these factors, our data suggest that age at BCG vaccination is an important factor in modulation of the immune system, and that administration of BCG after the age of one may prevent the development of asthma in adulthood. Future studies confirming this effect could potentially impact public health policy in providing a rationale to promote vaccination with BCG of children at risk of developing asthma in adulthood.

FIGURE 1: RECRUITMENT PROCEDURES AND DETERMINATION OF ELIGIBILITY

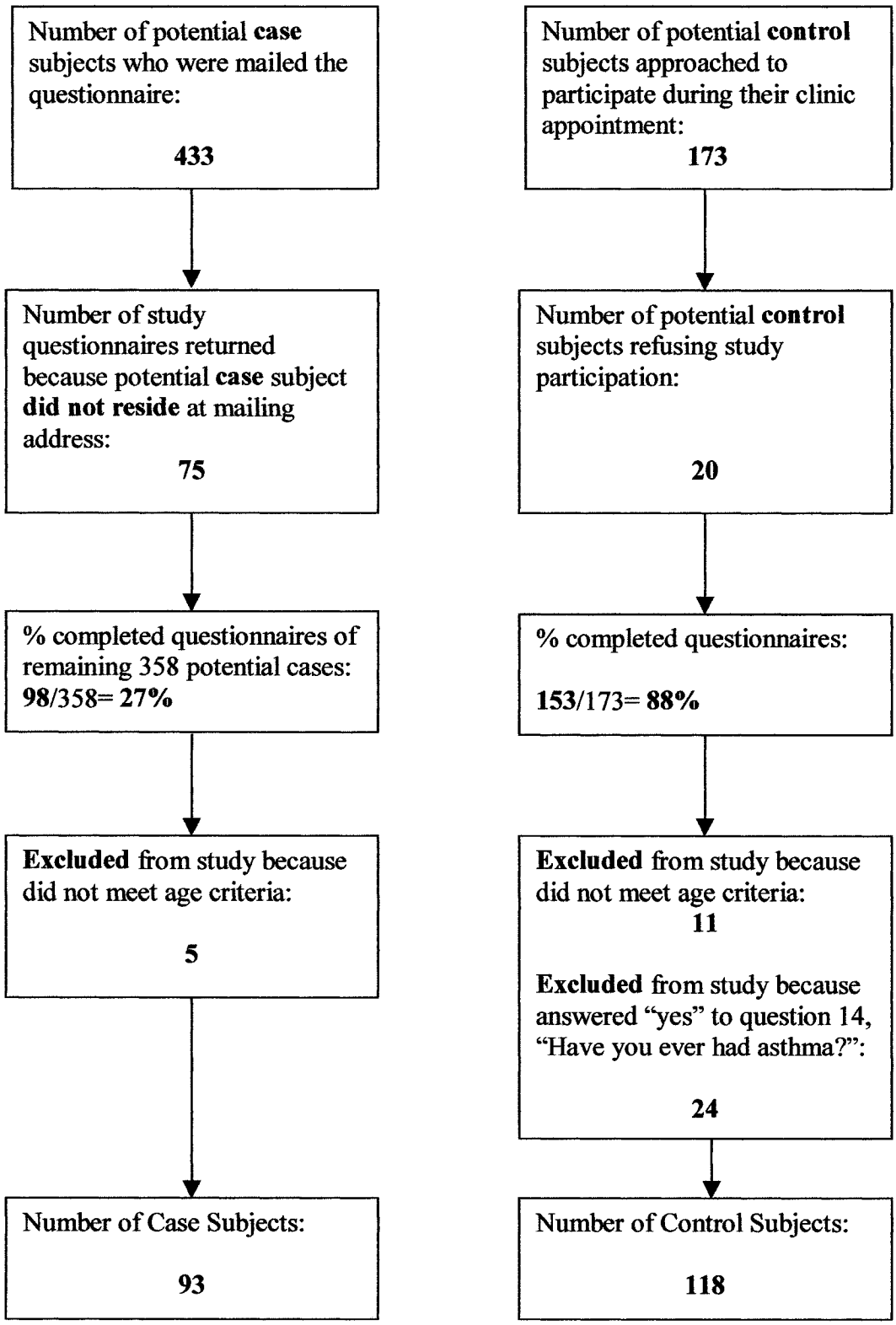

TABLE 1: PARTICIPATION BY STUDY SUBJECTS

	ASTHMATIC CASES	NON-ASTHMATIC CONTROLS	P-Value
Participants	93	118	
Childhood-Onset Asthmatic Cases: N (%)	50 (54)	-	
Adult-Onset Asthmatic Cases: N (%)	43 (46)	-	
Atopic: N (%)	83 (89)	34 (29)	<.001
Non-atopic: N (%)	10 (11)	84 (71)	
Male: N (%)	37 (40)	66 (56)	.02
Female: N (%)	56 (60)	52 (44)	
Age: Mean (SD)	34 (8.3)	30 (7.4)	.001
Range	18-48	18-47	
Documented BCG Vaccination: N (%)	34 (37)	40 (34)	.69
In infancy (age 0-1): N (%)	18 (19)	16 (14)	.26
In childhood (age>1): N (%)	16 (18)	24 (20)	.56

TABLE 2: CHARACTERISTICS OF CASES BY ATOPIC STATUS

	Atopic Asthmatic Cases (83)	Non-Atopic Asthmatic Cases (10)	P-Value
Male: N (%)	34 (41)	3 (30)	.5
Female: N (%)	49 (59)	7 (70)	.5
Age: Mean (SD)	34 (8.3)	30 (8.3)	.18
Documented BCG Vaccination: N (%)	31 (37)	3 (30)	.65
-In infancy (age 0-1) : N (%)	17 (20)	1 (10)	.43
- In childhood (age >1) : N (%)	14 (17)	2 (20)	.80

TABLE 3: CHARACTERISTICS OF CASES BY ASTHMA SUBTYPE

	Childhood-Onset Asthma Cases (50)	Adult-Onset Asthma Cases (43)	P-Value
Male: N (%)	21 (42)	27 (63)	.64
Female: N (%)	29 (58)	16 (37)	.64
Mean Age (SD)	33(8.4)	35 (8.2)	.30
Documented BCG Vaccination: N (%)	21 (42)	13 (30)	.24
-in infancy (0-1yr) : N (%)	11 (22)	7 (16)	.49
-in childhood (>1yr) : N (%)	10 (20)	6 (14)	.44

TABLE 4: CHARACTERISTICS OF CONTROLS BY ATOPIC STATUS

	Atopic Non-Asthmatic Controls (34)	Non-Atopic Non-Asthmatic Controls (84)	P-Value
Male: N (%)	18 (53)	48 (57)	.68
Female: N (%)	16 (47)	36 (43)	.68
Age: Mean (SD)	31 (8)	29 (7)	.19
Documented BCG Vaccination: N (%)	16 (44)	24 (28)	.05
-In infancy (age 0-1) : N (%)	8 (22)	8 (9)	.07
- In childhood (age >1) : N (%)	8 (22)	16 (19)	.58

TABLE 5: CRUDE ODDS OF ASTHMA GIVEN PREVIOUS BCG VACCINATION

Asthma Subtype	BCG Vaccination	Crude Odds (95% CI)
All Asthma*	All BCG Vaccination**	1.1 (0.6-2.0)
All Asthma	Vaccination (Age 0-1)	1.5 (0.7-3.2)
All Asthma	Vaccination (Age >1)	0.9 (0.4-1.8)
Atopic Asthma•	All BCG Vaccination**	1.5 (0.8-2.9)
Atopic Asthma•	Vaccination (Age 0-1)	2.5 (1.0-6.1)
Atopic Asthma•	Vaccination (Age >1)	1.0 (0.5-2.3)
Childhood-Onset Asthma+	All BCG Vaccination**	1.4 (0.7-2.8)
Childhood-Onset Asthma+	Vaccination (Age 0-1)	1.9 (0.8-4.4)
Childhood-Onset Asthma+	Vaccination (Age >1)	1.1 (0.5-2.6)
Adult-Onset Asthma++	All BCG Vaccination**	0.8 (0.4-1.8)
Adult-Onset Asthma++	Vaccination (Age 0-1)	1.1 (0.4-3.3)
Adult-Onset Asthma++	Vaccination (Age >1)	0.7 (0.2-1.8)

* "All Asthma" included subjects with childhood-onset and adult-onset asthma. Control group included both atopic and non-atopic subjects.

* * "All BCG Vaccination" included all subjects receiving BCG vaccination (regardless of age at vaccination).

•Analysis included atopic asthmatic subjects as cases, and **non-atopic** non-asthmatic subjects as controls.

+ Analysis included case subjects whose onset of asthma was before the age of 18. Control group included both atopic and non-atopic subjects.

++ Analysis included case subjects whose onset of asthma was at age 18 or older. Control group included both atopic and non-atopic subjects.

TABLE 6a: Assessment of Potential Confounding: Association of Potential Confounders With Outcome

HISTORY/EXPOSURE	ASTHMATICS	NON-ASTHMATICS	P Value
Number of Subjects	**93**	**118**	
Age: Mean (SD)	34 (8.3)	30 (7.4)	
Male	37	66	
Female	56	52	
Nasal Allergies	71%	24%	.001
Eczema/Skin allergies	47%	21%	.001
Currently Own a Pet	40%	48%	.24
Pet in Childhood	65%	70%	.4
Pet Allergy	71%	12%	.001
Medication-Induced Respiratory Symptoms	20%	3%	.001
Vaccinated for Allergy	34%	7%	.001
Father Smoked	67%	68%	.88
Father had Asthma	7%	6%	.87
Mother Smoked	41%	47%	.45
Mother had Asthma	15%	6%	.04
Sibling(s)	94%	93%	.72
Sibling(s) had Asthma	31%	16%	.01
Daycare Attendance	24%	32%	.19
Childhood Respiratory Infection	14%	3%	.002
Currently Employed	74%	64%	.16
Work-Induced Respiratory Symptoms	49%	7%	.001
Changed Job Because of Breathing Symptoms	17%	3%	.001
Exposure at Work to Vapours/Fumes	53%	46%	.31
Ever Smoked	50%	46%	.6
Current Smoker	31%	59%	.003
Stopped/Reduced Smoking	72%	63%	.33
BCG Vaccination	36%	34%	.67
-Infancy Vaccination	19%	14%	.26
-Childhood Vaccination	17%	20%	.56
Blishen Index (mean) *	46.6	44.3	.23

* When 20 subjects whose occupation was unclassifiable were excluded from analysis, values for asthmatics and non-asthmatics were 46.2 and 42.0, respectively (p=.035, see Section 4.3, "Comparison of Study Groups by Confounders" pp.42-44).

Table 6b: Assessment of Potential Confounding: Association of Potential Confounders With Outcome, Comparing Atopic Cases With Non-Atopic Controls

HISTORY/EXPOSURE	ATOPIC ASTHMATICS	NON ATOPIC/ NON ASTHMATICS	P Value
Number of Subjects	**83**	**84**	
Age: Mean (SD)	34 (8.3)	29 (7.0)	
Male	34	48	
Female	49	36	
Nasal Allergies	80%	0%	.001
Eczema/Skin allergies	49%	23%	.001
Currently Own a Pet	37%	47%	.21
Pet in Childhood	63%	70%	.30
Pet Allergy	79%	0%	.001
Medication-Induced Respiratory Symptoms	22%	0%	.001
Vaccinated for Allergy	37%	0%	.001
Father Smoked	67%	68%	.91
Father had Asthma	8%	5%	.52
Mother Smoked	39%	50%	.14
Mother had Asthma	15%	7%	.13
Sibling(s)	94%	92%	.59
Sibling(s) had Asthma	32%	12%	.002
Daycare Attendance	22%	35%	.07
Childhood Respiratory Infection	16%	2%	.003
Currently Employed	70%	64%	.41
Work-Induced Respiratory Symptoms	48%	7%	.001
Changed Job Because of Breathing Symptoms	18%	4%	.002
Exposure at Work to Vapours/Fumes	50%	47%	.70
Ever Smoked	49%	42%	.36
Current Smoker	29%	58%	.007
Stopped/Reduced Smoking	75%	56%	.08
BCG Vaccination	37%	29%	.23
-Infancy Vaccination	20%	10%	.047
-Childhood Vaccination	17%	19%	.71
Blishen Index (mean) *	46.1	45.9	.94

When 20 subjects whose occupation was unclassifiable were excluded from analysis, values for asthmatics and non-asthmatics were 45.6 and 44.1, respectively (p=.10, see Section 4.3, "Comparison of Study Groups by Confounders" pp.42-44).

TABLE 7a: Assessment of Potential Confounding: Association of Potential Confounders With Exposure

HISTORY/EXPOSURE	BCG Vaccinated	Not BCG Vaccinated	P Value
Number of Subjects	**74**	**137**	
Age: Mean (SD)	36(6.4)	29 (8.0)	
Male	40	63	
Female	34	74	
Nasal Allergies	55%	39%	.02
Eczema/Skin allergies	34%	32%	.77
Currently Own a Pet	41%	46%	.42
Pet in Childhood	70%	66%	.57
Pet Allergy	49%	32%	.02
Medication-Induced Respiratory Symptoms	6%	13%	.11
Vaccinated for Allergy	22%	17%	.44
Atopic	64%	51%	.08
Father Smoked	77%	63%	.05
Father had Asthma	10%	5%	.19
Mother Smoked	42%	46%	.61
Mother had Asthma	12%	8%	.38
Sibling(s)	97%	92%	.12
Sibling(s) had Asthma	22%	23%	.82
Daycare Attendance	22%	32%	.10
Childhood Respiratory Infection	7%	8%	.74
Currently Employed	74%	65%	.17
Work-Induced Respiratory Symptoms	24%	26%	.71
Changed Job Because of Breathing Symptoms	10%	9%	.87
Exposure at Work to Vapours/Fumes	43%	53%	.19
Ever Smoked	57%	43%	.06
Current Smoker	46%	46%	1.0
Stopped/Reduced Smoking	69%	65%	.67
Blishen Index (mean) *	48.4	43.8	.02

* When 20 subjects whose occupation was unclassifiable were excluded from analysis, values for asthmatics and non-asthmatics were 47.4 and 42.7, respectively (p=.035, see Section 4.3, "Comparison of Study Groups by Confounders" pp.42-44).

Table 7b: Assessment of Potential Confounding: Association of Potential Confounders With Exposure Among Atopic Asthmatics and Non-Atopic Non-Asthmatics

HISTORY/EXPOSURE	BCG Vaccinated	Not BCG Vaccinated	P Value
Number of Subjects	**55**	**112**	
Age: Mean (SD)	36 (6.6)	30 (8.1)	
Male	27	55	
Female	28	57	
Nasal Allergies	51%	34%	.04
Eczema/Skin allergies	38%	35%	.69
Currently Own a Pet	42%	42%	1.0
Pet in Childhood	65%	67%	.85
Pet Allergy	49%	34%	.07
Medication-Induced Respiratory Symptoms	6%	14%	.13
Vaccinated for Allergy	22%	17%	.43
Atopic	56%	46%	.23
Father Smoked	72%	64%	.32
Father had Asthma	7%	6%	.70
Mother Smoked	42%	45%	.66
Mother had Asthma	15%	9%	.29
Sibling(s)	96%	91%	.20
Sibling(s) had Asthma	22%	22%	1.0
Daycare Attendance	24%	31%	.35
Childhood Respiratory Infection	9%	9%	1.0
Currently Employed	71%	65%	.48
Work-Induced Respiratory Symptoms	26%	27%	.93
Changed Job Because of Breathing Symptoms	11%	11%	1.0
Exposure at Work to Vapours/Fumes	42%	52%	.22
Ever Smoked	51%	42%	.30
Current Smoker	36%	45%	.41
Stopped/Reduced Smoking	67%	65%	.91
Blishen Index (mean) *	49.6	44.5	.04

* When 20 subjects whose occupation was unclassifiable were excluded from analysis, values for asthmatics and non-asthmatics were 48.0 and 43.4, respectively (p=.035 see Section 4.3, "Comparison of Study Groups by Confounders" pp.42-44).

TABLE 8: ASSESSMENT OF EFFECT MODIFICATION BY DIFFERENT COVARIATES

Question or Covariate	Subjects Answering "Yes" Crude Odds (95%CI) of Asthma Given Previous BCG Vaccination	Subjects Answering "No" Crude Odds (95%CI) of Asthma Given Previous BCG Vaccination
Gender (Yes=Male, No=Female)	1.1 (0.5-2.5) N=103	1.3 (0.6-2.9) N=108
Daycare Attendance in Childhood	**2.1 (0.6-6.7) N=59**	**0.8 (.4-1.6) N=148**
Owned Pet in Childhood	1.3 (0.7-2.6) N=143	0.8 (0.3-2.3) N=68
Ever Smoked	0.8 (0.4-1.8) N=100	1.6 (0.7-3.6) N=109
Serious Childhood Respiratory Infection	0.9 (0.1-12.9) N=16	1.0 (0.5-1.8) N=184
Mother Had Asthma	**2.9 (0.9-21) N=20**	**1.0 (0.6-1.9) N=183**
Sibling Had Asthma	0.6 (0.2-1.9) N=47	1.4 (0.8-3.0) N=148
Father Smoked	**0.8 (0.4-1.7) N=138**	**2.2 (0.7-6.7) N=65**
Atopy	0.7 (0.3-1.5) N=117	1.1 (0.3-4.5) N=94

Note: bold type indicates potentially significant effect modification by given question/covariate

Table 9: Potential Confounders of Relationship Between BCG Vaccination and Asthma:

1. Age
2. Gender
3. Serious Childhood Respiratory Infection
4. Sibling With Asthma
5. Maternal Asthma
6. Father Smoked
7. History of Smoking
8. Daycare Attendance in Childhood
9. Current Employment
10. Paternal Asthma
11. Exposure to Vapours or Fumes at Work
12. Current Pet Ownership
13. Blishen Index Score

TABLE 10: FULL LOGISTIC REGRESSION MODEL: (table includes point estimate and confidence intervals for all independent variables included in a logistic regression model examining the odds of having asthma adjusted for the effect of other covariates in the model) (case: control= 81:104)

Variable	Point Estimate	95% Confidence Limits
BCG Vaccination	0.9	0.4 , 1.8
Older Age•	**1.1**	**1.03 , 1.14**
Male Gender	**0.4**	**0.2 , 0.7**
-- Serious Childhood Respiratory Infection	**4.6**	**1.03 , 20.5**
- Sibling Asthma	**3.2**	**1.4 , 7.2**
--Maternal Asthma	1.6	0.5 , 5.1
-Father Smoked	0.7	0.3 , 1.3
-History of Smoking	1.0	0.5 , 2.0
- Childhood Daycare Attendance	0.9	0.4 , 1.8
-Current Employment	1.4	0.7 , 2.8
-Paternal Asthma	1.1	0.3 , 4.3
-Exposure to Vapours or Fumes at Work	1.7	0.8 , 3.6
Current Pet Ownership	0.7	0.4 , 1.4
Blishen Index Score† ‡	0.99	.97 , 1.02

• Calculated estimate of effect is per 1 year increment in age

† Calculated estimate of effect is per 1 unit increment in score

‡ Analysis includes 20 subjects with unclassifiable Blishen scores who were assigned average values (see Section 4.3, "Comparison of Study Groups by Confounders", p. 42)

Table 11: Change in Estimate of Effect of BCG Vaccination With Sequential Removal of Different Confounding Variables From Full Model :

Model	Point Estimate (variable*)	95% Confidence Interval (variable*)	Effect of BCG (Adjusted Odds)	95% Confidence Interval (BCG)
Smoking	1.0	(0.5 , 2.0)	0.9	(0.4 , 1.8)
-Paternal Asthma	1.1	(0.3 , 2.3)	0.9	(0.4 , 1.8)
-Childhood Daycare Attendance	0.8	(0.4 , 1.8)	0.8	(0.4 , 1.8)
- Current Employment	1.3	(0.6 , 2.7)	0.9	(0.4 , 1.8)
- Blishen Score	0.99	(0.97-1.02)	0.8	(0.4 , 1.8)
-Maternal Asthma	1.6	(0.5 , 5.0)	0.8	(0.4 , 1.7)
-Current Pet Ownership	0.7	(0.4 , 1.4)	0.7	(0.4 , 1.5)
-Father Smoked	0.7	(0.3 , 1.3)	0.8	(0.4 , 1.6)
-Exposure to Vapours or Fumes at Work	1.8	(0.9 , 3.4)	0.8	(0.4 , 1.6)
-Sibling Asthma	2.7	(1.3 , 5.7)	0.8	(0.4 , 1.5)
-Male Gender	0.5	(0.3 , 0.8)	0.8	(0.4 , 1.5)
-Serious Childhood Respiratory Infection	7.6	(2.0 , 29.1)	0.7	(0.4 , 1.4)
-Older Age (per year)	1.1	(1.03 ,1.11)	0.7	(0.4 , 1.4)

*Values for variable within model **before** removal; variables were sequentially removed according to p-value in logistic regression analysis (stepwise removal of least significant to most significant variables). The full model therefore included "smoking" plus all other variables. Smoking was then removed and the analysis performed with the remaining variables. "Paternal asthma" was then removed and all subsequent analyses were performed in similar fashion. BCG variable was left in model in **all** analyses.

TABLE 12: FINAL MODELS

Model 1: **ALL** subjects (atopic/non-atopic cases, and atopic /non-atopic controls). Outcome is **asthma**. Case:control = 91:118.

Variable	Adjusted Odds Ratios	95% Confidence Limits
Older Age	1.1	1.04, 1.14
Male Gender	0.5	0.2, 0.8
BCG Vaccination	0.8	0.4, 1.5
Serious Respiratory Infection in Childhood	9.5	2.3, 38.7
Sibling With Asthma	2.7	1.3, 5.7

Model 2: **ALL** subjects. Outcome is **asthma**. BCG modeled as **age** at vaccination (infancy vs. childhood). Case:control = 91:118.

Variable	Adjusted Odds Ratios	95% Confidence Limits
Older Age	1.1	1.04, 1.14
Male Gender	0.5	0.3, 0.9
BCG Vaccination in Infancy (0-1yr)	1.1	0.5, 2.6
BCG Vaccination in Childhood (>1 yr)	0.5	0.2, 1.2
Serious Respiratory Infection in Childhood	9.6	2.4, 39.0
Sibling With Asthma	2.7	1.3, 5.6

Model 3: Outcome is **childhood-onset asthma** (adult-onset asthmatics are **excluded**). Case:control = 50:118.

Variable	Adjusted Odds Ratios	95% Confidence Limits
Older Age	1.06	1.01, 1.12
Male Gender	0.6	0.3, 1.3
BCG Vaccination	1.1	0.5, 2.6
Serious Respiratory Infection in Childhood	12.3	2.8, 55.1
Sibling With Asthma	4.1	1.8, 9.2

Model 4: Outcome is **childhood-onset asthma** (adult-onset asthmatics are **excluded**). BCG modeled as **age** at vaccination (infancy vs. childhood). Case: control = 50:118

Variable	Adjusted Odds Ratios	95% Confidence Limits
Older Age	1.07	1.01, 1.13
Male Gender	0.6	0.3, 1.3
BCG Vaccination in Infancy (0-1yr)	1.5	0.6, 4.1
BCG Vaccination in Childhood (>1 yr)	0.8	0.3, 2.4
Serious Respiratory Infection in Childhood	12.6	2.8, 55.9
Sibling With Asthma	4.0	1.8, 9.2

Model 5: Outcome is **adult-onset asthma** (child-onset asthmatics are **excluded**). Case:control = 41:118

Variable	Adjusted Odds Ratios	95% Confidence Limits
Older Age	1.12	1.06, 1.18
Male Gender	0.3	0.1, 0.8
BCG Vaccination	0.5	0.2, 1.2
Serious Respiratory Infection in Childhood	5.4	1.1, 28.0
Sibling With Asthma	1.5	0.6, 4.2

Model 6: Outcome is **adult-onset asthma** (child-onset asthmatics are **excluded**). BCG modeled as **age** at vaccination (infancy vs. childhood). Case:control = 41:118

Variable	Adjusted Odds Ratios	95% Confidence Limits
Older Age	1.12	1.06, 1.19
Male Gender	0.4	0.1, 0.8
BCG Vaccination in Infancy (0-1yr)	0.8	0.3, 2.3
BCG Vaccination in Childhood (>1 yr)	0.3	0.1, 0.9
Serious Respiratory Infection in Childhood	5.7	1.1, 29.7
Sibling With Asthma	1.6	0.6, 4.5

Model 7: **Atopic asthmatic case subjects and non-atopic non-asthmatic control subjects**. Outcome is **atopic asthma.** Case:control = 82:84

Variable	Adjusted Odds Ratios	95% Confidence Limits
Older Age	1.1	1.04, 1.15
Male Gender	0.5	0.3, 1.0
BCG Vaccination	1.0	0.5, 2.1
Serious Respiratory Infection in Childhood	12.4	2.3, 65.7
Sibling With Asthma	4.1	1.7, 10.0

Model 8: **Atopic asthmatic case subjects and non-atopic non-asthmatic control subjects**. Outcome is **atopic asthma**. BCG modeled as **age** at vaccination (infancy vs. childhood). Case:control = 82:84.

Variable	Adjusted Odds Ratios	Confidence Limits
Older Age	1.1	1.05, 1.16
Male Gender	0.5	0.3, 1.1
BCG Vaccination in Infancy (0-1yr)	1.8	0.7, 4.9
BCG Vaccination in Childhood (>1 yr)	0.5	0.2, 1.4
Serious Respiratory Infection in Childhood	12.5	2.4, 65.3
Sibling With Asthma	3.9	1.6, 9.6

Model 9: **Atopic asthmatic case subjects and non-atopic non-asthmatic control subjects**. Outcome is **childhood-onset atopic asthma** (adult-onset asthmatics **excluded**). Case:control = 47:84

Variable	Adjusted Odds Ratios	95% Confidence Limits
Older Age	1.06	1.0, 1.13
Male Gender	0.6	0.2, 1.3
BCG Vaccination	1.5	0.6, 3.7
Serious Respiratory Infection in Childhood	14.7	2.6, 84.4
Sibling With Asthma	5.8	2.2, 15.4

Model 10: **Atopic asthmatic case subjects and non-atopic non-asthmatic control subjects**. Outcome is **childhood-onset atopic asthma**. (adult-onset asthmatics **excluded)**. BCG modeled as **age** at vaccination (infancy vs. childhood). Case:control = 47:84

Variable	Odds Ratio	Confidence Limits
Older Age	1.067	1.004, 1.134
Male Gender	0.6	0.3, 1.4
BCG Vaccination in Infancy (0-1yr)	2.2	0.7, 7.0
BCG Vaccination in Childhood (>1 yr)	1.0	0.3, 3.1
Serious Respiratory Infection in Childhood	14.8	2.6, 83.3
Sibling With Asthma	5.4	2.0, 14.5

Model 11: **Atopic asthmatic case subjects and non-atopic non-asthmatic control subjects**. Outcome is **adult-onset atopic asthma**. (childhood-onset asthmatics **excluded)**. Case:control = 35:84.

Variable	Adjusted Odds Ratios	95% Confidence Limits
Older Age	1.14	1.07, 1.22
Male Gender	0.5	0.2, 1.2
BCG Vaccination	0.5	0.2, 1.5
Serious Respiratory Infection in Childhood	6.5	1.0, 43.0
Sibling With Asthma	2.3	0.7, 7.2

Model 12: **Atopic asthmatic case subjects and non-atopic non-asthmatic control subjects.** Outcome is **adult-onset atopic asthma.** (childhood-onset asthmatics **excluded)** BCG modeled as **age** at vaccination (infancy vs. childhood). Case:control = 35:84.

Variable	Adjusted Odds Ratios	95% Confidence Limits
Older Age	1.2	1.08, 1.24
Male Gender	0.5	0.2, 1.2
BCG Vaccination in Infancy (0-1yr)	1.2	0.4, 4.4
BCG Vaccination in Childhood (>1 yr)	0.2	0.1, 0.9
Serious Respiratory Infection in Childhood	7.14	1.04, 49.22
Sibling With Asthma	2.3	0.7, 7.4

TABLE 13: SUMMARY OF LOGISTIC REGRESSION MODELS:

MODEL	OUTCOME (CASE SUBJECTS)	CONTROL SUBJECTS	AGE AT BCG VACCINATION	ASSOCIATION OF BCG VAX WITH ASTHMA: ADJUSTED ODDS RATIO (95% CI)
1	All Asthma	All Controls	All Ages	0.8 (0.4, 1.5)
2	All Asthma	All Controls	Infancy (0-1yr)	1.1 (0.5, 2.6)
			Childhood (>1yr)	0.5 (0.2, 1.2)
3	Childhood-Onset Asthma	All Controls	All Ages	1.1 (0.5, 2.6)
4	Childhood-Onset Asthma	All Controls	Infancy (0-1yr)	1.5 (0.6, 4.1)
			Childhood (>1yr)	0.8 (.3, 2.4)
5	Adult-Onset Asthma	All Controls	All Ages	0.5 (0.2, 1.2)
6	**Adult-Onset Asthma**	All Controls	Infancy (0-1yr)	0.8 (0.3, 2.3)
			Childhood (>1yr)	**0.3 (0.1, .9)**
7	Atopic Asthma	Non-Atopic Controls	All Ages	1.0 (0.5, 2.1)
8	Atopic Asthma	Non-Atopic Controls	Infancy (0-1yr)	1.8 (0.7, 4.9)
			Childhood (>1yr)	0.5 (0.2, 1.4)
9	Childhood-Onset Atopic Asthma	Non-Atopic Controls	All Ages	1.5 (0.6, 3.7)
10	Childhood-Onset Atopic Asthma	Non-Atopic Controls	Infancy (0-1yr)	2.2 (0.7, 7.0)
			Childhood (>1yr)	1.0 (0.3, 3.1)
11	Adult-Onset Atopic Asthma	Non-Atopic Controls	All Ages	0.5 (0.2, 1.5)
12	**Adult-Onset Atopic Asthma**	Non-Atopic Controls	Infancy (0-1yr)	1.2 (0.4, 4.4)
		Non-Atopic Controls	**Childhood (>1yr)**	**0.2 (0.1, 0.9)**

TABLE 14: SUMMARY OF STUDIES EXAMINING THE RELATIONSHIP BETWEEN BCG VACCINATION /TUBERCULIN SKIN TESTING AND ASTHMA / ATOPY.

SECTION A: STUDIES WHICH EXAMINED BCG-ATOPY RELATIONSHIP

Author/Year/ Country	Design	N/Age	Asthma*	Atopy#	BCG confirmed by	BCG age at vaccination	TST+	Association
Alm, 1997 SWEDEN (88)	Retrospective cohort	574 (3-7 yo) -	Questionnaire+Clinical exam	+SPT and/or IgE	Hospital records and scar	< 6months Mean (17d):girls Mean (21d):boys	----------	RR Atopy: 1.21 RR Asthma: 0.83
Strannegard, 1998 SWEDEN (126)	Cross-sectional	802 (4-5y) 2138 (8-9y) 3557 (7-8y)	Questionnaire	Questionnaire	Questionnaire stating year of vaccination	< 1year	2 TU RT23	OR Atopy: 0.86
Aaby, 2000 GUINEA-BISSAU (89)	Cross-sectional	400 (3-14y)	----------	+SPT	Health card record or scar	Documented in 90/347; 64/347<6months 26/347>6 months	----------	Adjusted OR Atopy: 0.19
Gruber, 2002 GERMANY (127)	Cross-sectional	38808 (mean age 6y)	Questionnaire	Questionnaire	Vaccination Document (rarely used scar if document missing)	"usually given in neonatal period"	----------	Adjusted Odds of : Atopic dermatitis: .99 Bronchial asthma: 0.85 Hay Fever: 0.97
Krause, 2003 GREENLAND (113)	Cross-sectional	1575 (8-16y)	----------	IgE	Health center records (vaccination protocols or hospital records)	926/1062 within 1 month. 53: 1-6months 83: > 6months		Adjusted Odds of Atopy = 1.03
Ma Y, (Chinese), 2003 CHINA (128)	Cross-sectional	1863 (13-14 y)	ISAAC Questionnaire	SPT testing	Scar	Not given in abstract		Comparison of mean BCG scar diameters between atopic and non-atopic subjects
Annus, 2004 ESTONIA (114)	Cross-sectional	979 (10-11y)	ISAAC Questionnaire	+ SPT	School medical records	638/717: <1m 42/717: 1-11m 37/717: >1yr	2 TU	OR Atopy (comparing Vaccination<1m vs >1m)

* "Questionnaire" refers to use of positive response to questions such as "ever had asthma" or "wheeze in the last 12 months". More detailed evaluation (clinical exam, lung function test) noted if described in study.
# Atopy defined by positive allergen skin prick test (SPT) or allergen specific or high serum IgE level
+ TST: tuberculin skin test; TU: tuberculin unit

TABLE 14: SUMMARY OF STUDIES EXAMINING THE RELATIONSHIP BETWEEN BCG VACCINATION/TUBERCULIN SKIN TESTING AND ASTHMA/ATOPY.

SECTION B: STUDIES WHICH EXAMINED BCG-ASTHMA RELATIONSHIP

Author/Year/ Country	Design	N/Age	Asthma	Atopy	BCG confirmed by	BCG Age at vaccination	TST	Association
Sarhino, 2000 BRAZIL (129)	Case-control	264 (2-9y)	Case: By clinical assessment Control: By parental assessment	----------	scar	95% of popn vaccinated at birth	-------- --	OR=0.42 (odds of asthma given BCG scar>5mm)
Bager, 2003 DENMARK (104)	Cross-sectional	2176 (21-46y)	Questionnaire	IgE	School health records	0d-15y; 718/1653 were vaccinated at 7y.		Odds of asthma (vaccination age 1vs. age 7)= 1.71 **Childhood bcg was asssoc with Odds of asthma of 0.53 (.3-.95) comparing with unvaxd women whose parents had not refused vax**
Marks, 2003 AUSTRALIA (124)	Retrospective cohort	751 (7-14y)	Questionnaire and positive methacholine test	SPT +ve	Health record or presence of scar	<8 weeks	10 TU	RR Atopy 0.97 RR Asthma 1.23
daCunha, 2004 BRAZIL (125)	Cross-sectional	1612 (12-16y)	Modified ISAAC Questionnaire	Questionnaire	Scar	0-4y		Adjusted OR for asthma among atopics :.58 (.36-.92)

* "Questionnaire" refers to use of positive response to questions such as "ever had asthma" or "wheeze in the last 12 months". More detailed evaluation (clinical exam, lung function test) noted if described in study.
# Atopy defined by positive allergen skin prick test (SPT) or allergen specific or high serum IgE level
+ TST: tuberculin skin test; TU: tuberculin unit

TABLE 14: SUMMARY OF STUDIES EXAMINING THE RELATIONSHIP BETWEEN BCG VACCINATION/TUBERCULIN SKIN TESTING AND ASTHMA/ATOPY.

SECTION C: STUDIES WHICH EXAMINED TUBERCULIN SKIN TEST AND ATOPY RELATIONSHIP

Author/Year/ Country	Design	N/Age	Asthma	Atopy	BCG confirmed by	BCG Age at vaccination	TST	Association
Shirakawa, 1997 JAPAN (87)	Cross sectional	867 (12-13y)	Questionnaire	IgE	Health records	0, 6y, 12y (re-vax if tst-ve)	Not specified	(OR for atopy) TST +0 to 6y : .5 (atop) TST + 6-12y: .43 (atop)
Yilmaz, 2000 TURKEY (120)	Cross-sectional	736 (3-16y)	Clinical evaluation	+SPT and/or IgE	Scar or vaccine record	All children vaxd 0, 6y, 12 y;	5TU	Difference in mean TST response (atopic vs non-atopic)
Wong, 2001 HONG KONG (121)	Cross-sectional	2201 (10y)	ISAAC Questionnaire	+SPT	Immunization record and scar	Within 1-2d of birth. Re-vax at 7-10y if TST-	1 TU RT23	Adjusted OR for Atopy: 0.98
Ota, 2003 THE GAMBIA (122)	Cross-sectional	507 (8-12 y)	Clinical evaluation	+SPT	scar	Not specified	#TU not specified	OR for atopy: 1.01
Nuhoglu, 2004 TURKEY (123)	Cross-sectional	252 (3-9y)	ATS criteria	+SPT	Not Specified	All subjects vaxd in newborn period	5TU	OR for atopy: 0.73

* "Questionnaire" refers to use of positive response to questions such as "ever had asthma" or "wheeze in the last 12 months". More detailed evaluation (clinical exam, lung function test) noted if described in study.
# Atopy defined by positive allergen skin prick test (SPT) or allergen specific or high serum IgE level
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TABLE 14: SUMMARY OF STUDIES EXAMINING THE RELATIONSHIP BETWEEN BCG VACCINATION /TUBERCULIN SKIN TESTING AND ASTHMA/ATOPY.

SECTION D: STUDIES WHICH EXAMINED TUBERCULIN SKIN TEST AND ASTHMA RELATIONSHIP

Author/Year/ Country	Design	Age/N	Asthma	Atopy	BCG confirmed by	BCG Age at vaccination	TST	Association
Jentoft, 2002 NORWAY (103)	Cross-sectional	386 (20-44y)	Not defined; subjects answered ECRHS Questionnaire, underwent spirometry and methacholine challenge testing	----------	School health record	14y	Epi-Pirquet test	Adjusted odds of +ve tst per 10% loss of fev1: .91 (.73-1.14), per increase of 1 resp sx: 1 (.8-1.18)

* "Questionnaire" refers to use of positive response to questions such as "ever had asthma" or "wheeze in the last 12 months". More detailed evaluation (clinical exam, lung function test) noted if described in study.
# Atopy defined by positive allergen skin prick test (SPT) or allergen specific or high serum IgE level
+ TST: tuberculin skin test; TU: tuberculin unit

BIBLIOGRAPHY

1. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. NHLBI/WHO Workshop Report. US National Institutes of Health, 1995.

2. Magnus P, Jaakola JK. "Secular trends in the occurrence of asthma among children and young adults: critical appraisal of repeated cross-sectional surveys". *BMJ.* 1997; 314: 1795- 99.

3. Bjorksten B, Dumitrascu D, Foucard T, et al. "Prevalence of childhood asthma, rhinitis, and eczema in Scandinavia and Eastern Europe". *Eur. Respir. J.* 1998; 12: 432-37.

4. Strachan D. "Family size, infection and atopy: the first decade of the 'hygiene hypothesis'". *Thorax.* 2000; 55: (Suppl.1): S2-S10.

5. Li JTC, O'Connell EJ. "Clinical evaluation of asthma" *Ann. Allergy Asthma Immunol.*1996; 76:1-14.

6. Glauser FL. "Variant asthma" *Ann. Allergy.* 1972; 30: 457.

7. Murray JF, Nadel JA, Mason RJ, Boushey HA. Textbook of Respiratory Medicine. WB Saunders Company. New York. 2000. Vol. 2: p.1266.

8. Brewis RAL, Corrin B, Geddes DM, Gibson GJ. Respiratory Medicine 2nd ed.: Vol. 2 WB Saunders Co. London. 1995. p.1180.

9. Enright PL, Johnson LR, Connett JE, Voelker H, Buist AS. "Spirometry in the Lung Health Study: 1. Methods and quality control" *Am. Rev. Respir. Dis.* 1991; 143:1215-23.

10. Hogg JC, Macklem PT, Thurlbeck WM. "Site and nature of airway obstruction in chronic obstructive lung disease" *NEJM.* 1968; 278:1355-60.

11. Crapo RO, Morris AH, Gardner RM. "Reference spirometric values using techniques and equipment that meet ATS recommendations" *Am. Rev. Respir. Dis.* 1981; 123(6): 659-64.

12. Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. "Changes in the normal maximal expiratory flow-volume curve with growth and aging" *Am. Rev. Respir. Dis.* 1983; 127(6): 725-34.

13. Hankinson JL, Odencrantz JR, Fedan KB. "Spirometric reference values from a sample of the general US population" *AJRCCM.* 1999; 159(1): 179-87.

14. Becklake M, Crapo RO, Buist AS, et al. "Lung function testing: Selection of reference values and interpretive strategies. Official statement of the American Thoracic Society" *Am Rev. Respir Dis.*1991; 144: 1202-18.

15. Enright PL, Lebowitz, MD Cockroft DW. "Physiologic measures: Pulmonary function tests" *AJRCCM.* 1994; (Supplement Part 2: Asthma outcome measures): S9-S18.

16. McFadden ER and Gilbert IA. "Exercise-induced asthma" *NEJM.* 1994; 330(19): 1362-7.

17. Hogg JC. "Pathology of asthma" *J. Allergy Clin. Immunol.* 1993; 92: 1-5.

18. Fahy JV, Kim KW, Lin J, Bousley H. "Prominent neutrophilic inflammation in sputum from patients with asthma exacerbations" *J. Allergy Clin. Immunol.* 1995;95: 843-52.

19. Jahnsen FL, Moloney ED, Hogan T, Upham JW, Burke CM, Holt PG. "Rapid dendritic cell recruitment to the bronchial mucosa of patients with atopic asthma in response to local allergen challenge" *Thorax.* 2001; 56:823-26.

20. Williams CM, Galli SJ "Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice" *J. Exp. Med.* 2000; 192: 455-62.

21. Mossman T, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. "Two types of murine helper T-cell clones. Definition according to profiles of lymphokine activities and secreted proteins" *J. Immunol.* 1986; 136: 2348-57.

22. Mossman T, Coffman R. "Two types of mouse helper T-cell clones. Implications for immune regulation" *Immunol. Today.* 1987; 8: 223-27.

23. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM et. al. "Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma" *NEJM* .1992; 326: 298-304.

24. Ishizaka K. "Mechanisms of reaginic hypersensitivity" *Clin. Allergy.* 1971; 1: 9-24.

25. El Biaze M, Boniface S, Koscher V, Mamessier E, et al "T cell activation, from atopy to asthma : more a paradox than a paradigm" *Allergy.* 2003; 58: 844-53.

26. Pepys J. Clinical Aspects of Immunology 3rd edn. Blackwell Scientific Publications. Oxford. 1975: 877-902.

27. Pearce N, Pekkanen J., Beasley R. "How much asthma is really attributable to atopy?" *Thorax.* 1999; 54: 268-72.

28. Burrows B, Martinez FD, Halonen M, et. al. "Association of asthma with serum IgE levels and skin-test reactivity to allergens" *NEJM.* 1989; 320: 271-7.

29. Burrows B, Halonen M, Lebowitz MD, et. al. "The relationship of serum immunoglobulin E, allergy skin tests, and smoking to respiratory disorders" *J. Allergy Clin. Immunol.* 1982;70: 199-204.

30. Taussig LM, Wright AL, Harrison HR, Ray CG. "The Tucson Children's Respiratory Study. I. Design and implementation of a prospective study of acute and chronic respiratory illness in children" *Am. J. Epidemiol.* 1989; 129: 1219-31.

31. Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. "Tucson Children's Respiratory Study: 1980 to present" *J. Allergy Clin. Immunol.* 2003; 111: 661-75.

32. Bel EH. "Clinical phenotypes of asthma" *Curr. Opin. Pulm. Med.* 2003; 10: 44-50.

33. Burney PGJ, Chinn S, Britton JR, Tattersfield AE, Papacosta AO. "What symptoms predict the bronchial response to histamine? Evaluation in a community survey of the bronchial symptoms questionnaire (1984) of the IUATLD" *Eur. J. Resp. Dis.*1989;18: 165-73.

34. Medical Research Council. "Standardized questionnaires of respiratory symptoms" *BMJ.* 1960; 2: 1665.

35. Ferris BG. "Recommended respiratory disease questionnaires for use with adults and children in epidemiological research: epidemiology standardization project" *Am Rev. Resp Dis.* 1976.

36. Lebowitz MD, Burrows B. "Comparison of questionnaires: the BMRC and NHLI Respiratory Questionnaire and a new self-completion questionnaire" *Am. Rev. Respir. Dis.* 1976; 113: 627-35.

37. Toren K, Brisman J, Jarvholm B. "Assessment of asthma by questionnaire" *Chest.* 1993;104: 600-08.

38. Nielsen NH, Dirksen A, Madsen F. "Can subjects with a positive allergen skin test be selected by a short questionnaire? The Glostrup Allergy Study, Denmark" *Allergy.* 1993;48: 319-26.

39. Lakwijk N, Van Strien RT, Doekes G, Brunekreef B, Gerritsen J. "Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women" *Clin. Exp.Allergy.* 1998;28: 454-58.

40. Peat JK, van den Berg RH, green WF, Mellis CM, Leeder SR, Woolcock AJ. "Changing prevalence of asthma in Australian children" *BMJ.* 1994;308: 591-6.

41. Rona RJ, Chinn S, Burney PGJ. "Trends in the prevalence of asthma in Scottish and English primary school children 1982-92" *Thorax.*1995;50: 992-3.

42. Laor A, Cohen L, Danon YL. "Effects of time, sex, ethnic origin, and area of residence on prevalence of asthma in Israeli adolescents" *BMJ.* 1993;307:841-4.

43. Beasley R et. al. "Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC" *Lancet.* 1998; 351:1225-32.

44. Burney P, Chinn S, Jarvis D, Luczynska C, Lai E et. al. "Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS)" *Eur. Resp. J.* 1996;9: 687-95.

45. Pearce N, Sunyer J, Cheng S, Bjorksten B, et. al. "Comparison of asthma prevalence in the ISAAC and the ECRHS" *Eur. Resp. J.* 2000; 16: 420-26.

46. Manfreda J, Becklake MR, Sears MR, Chan-Yeung M, et. al. "Prevalence of asthma symptoms among adults aged 20-44 years in Canada" *CMAJ.* 2001; 164(7): 995-1001.

47. Manfreda J, Sears MR, Becklake MR, Chan-Yeung M, et al. "Geographic and gender variability in the prevalence of bronchial responsiveness in Canada" *Chest.* 2004;125:1657-64.

48. Woolcock AJ, Peat JK. "Evidence for the increase in asthma worldwide. CIBA Found. Symp" 1997; 206:122-34.

49. Bach JF. "The effect of infections on susceptibility to autoimmune and allergic diseases" *NEJM.* 2002; 347(12): 911-20.

50. Adelberth I, Carlsson B, de Man P, et al. "Intestinal colonization with Enterobacteriaceae in Pakistani and Swedish hospital delivered infants" *Acta Paediatr. Scand.* 1991;80: 602-10.

51. Strachan DP. "Hay fever, hygiene and household size" *BMJ.* 1989; 299: 1259-60.

52. Abbas AK, Murphy K, Sher A. "Functional diversity of helper T lymphocytes" *Nature.* 1996;383: 787-93.

53. Raghupathy R. "Th1-type immunity is incompatible with successful pregnancy" *Immunol. Today* 1997; 18: 478-82.

54. Brugnolo F, Sampognoro S, Fintoni T. "The novel synthetic immune response modifier R-848 (Resiquimod) has a powerful effect on the in vitro switching of allergen-specific CD4+ Th2 lymphocytes into IFN-gamma producing cells" *J. Allergy Clin. Immunol.* 2003;111: 380-88.

55. Kline JN, Waldschmidt TJ, Businga TR, Lemish JE, et. al. "Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma" *J. Immunol.* 1998; 160: 2555-59.

56. Murosaki S, Yamamoto Y, Ito K, Inokuchi T, et. al. . "Heat-killed Lactobacillus plantarum L-137 suppresses naturally fed antigen-specific IgE production by stimulation of IL-12 production in mice" *J. Allergy Clin. Immunol.* 1998; 102: 57-64.

57. Herz U, Gerhold K, Gruber C, Braun A, et. al. "BCG infection suppresses allergic sensitization and development of increased airway reactivity in an animal model" *J. Allergy Clin. Immunol.* 1998; 102: 867-74.

58. Shaheen SO, Aaby P, Hall AJ et. al. "Measles and atopy in Guinea-Bissau" *Lancet.* 1996; 347:1792-96.

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

60. Matricardi PM, Rosmini F, Rapicetta M, et. al. "Atopy, hygiene, and anthroposophic lifestyle" *Lancet.* 1999; 354:430.

61. Bodner C, Anderson WJ, Reid TS, et. al. "Childhood exposure to infection and risk of adult onset wheeze and atopy" *Thorax.* 2000; 55: 383-87.

62. Bjorksten B, Naaber P, Sepp E, Mikelsaar M. "The intestinal microflora in allergic Estonian and Swedish 2 -year-old children" *Clin. Exp. Allergy.* 1999; 29: 342-46.

63. Moreau M, Gaboriau-Routhiau V. "The absence of gut flora, the doses of antigen ingested, and aging affect the long-term peripheral tolerance induced by ovalbumin feeding in mice" *Res. Immunol.* 1996;147: 49-59.

64. Ernst P, Cormier Y. "Relative scarcity of asthma and atopy among rural adolescents raised on a farm" *AJRCCM.* 2000; 161:1563-66.

65. Braun-Fahrlander C, Gassner M, Grize L, et. al. "Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and

Respiratory Symptoms with Respect to Air Pollution" *Clin. Exp. Allergy* 1999; 29:28-34.

66. Leynaert B, Neukirch C, Jarvis D, et.al. "Does living on a farm during childhood protect against asthma, allergic rhinitis, and atopy in adulthood?" *AJRCCM* 2001; 164:1829-34.

67. Horak F, Studnicka M, Gartner C, et. al. "Parental farming protects against atopy: longtitudinal evidence involving skin prick tests" *Clin. Exp. Allergy* 2002; 32: 1155-1159.

68. Braun-Fahrlander C. "Environmental exposure to endotoxin and other microbial products and the decreased risk of childhood atopy: evaluating developments since April 2002" *Curr.Opin. Allergy Clin. Immunol.* 3: 325-29.

69. Orme IM, Andersen P, Boom WH. "T cell response to *Mycobacterium tuberculosis*" *J. Infect. Dis.* 1993;167: 1481-97.

70. Schluger NW, Rom WN. "The host immune response to tuberculosis" *AJRCCM.* 1998; 157: 679-91.

71. von Mutius E, Pearce N, Beasley R, Cheng S., et. al. "International patterns of tuberculosis and the prevalence of symptoms of asthma, rhinitis, and eczema" *Thorax.* 2000; 55: 449-453.

72. Styblo K, Rouillon A. "Estimated global incidence of smear-positive pulmonary tuberculosis. Unreliability of officially reported figures on tuberculosis" *Bull. Int. Union Tuberc. Lung Dis.* 1981; 56: 118-26.

73. Cohet C, Cheng S, MacDonald C, et al " Infections, medication use, and the prevalence of symptoms of asthma, rhinitis, and eczema in childhood" *J. Epidemiol Comm. Health.* 2004; 58(10):852-7.

74. Cullinan P, Harris JM, et al "Can early infection explain the sibling effect in adult atopy?" *Eur Respir J.* 2003; 22(6):956-61

75. Nja F, Nystad W, Hetlevik O et al "Airway infection in infancy and the presence of allergy and asthma in school-age children" *Arch. Dis. Child* 2003; 88(7):566-9.

76. Calmette A. "Preventive vaccination against tuberculosis with BCG" *Proc. R. Soc. Med.* 1931;24: 85-94.

77. Comstock GW. "Identification of an effective vaccine against tuberculosis" *Am Rev. Respir Dis.* 1988; 138: 479-80.

78. Fine PE. "Variation in protection by BCG: implications of and for heterologous immunity" *Lancet* 1995; 346: 1339-45.

79. Clemens JD, Chuong JH, Feinstein AR. "The BCG controversy: A methodological and statistical reappraisal" *JAMA.* 1983; 249: 362-69.

80. Chan J, Kaufman SHE. "Immune mechanisms of protection". In Bloom (ed): Tuberculosis: Pathogenesis, Protection and Control. American Society for Microbiology Press. 1994: 389-415.

81. Murray JF, Nadel JA, Mason RJ, Boushey HA. Textbook of Respiratory Medicine. WB Saunders Company. New York. 2000. Vol 1:1059-1060.

82. Murray JF, Nadel JA, Mason RJ, Boushey HA. Textbook of Respiratory Medicine. WB Saunders Company. New York. 2000. Vol 1:1060.

83. Menzies R., Vissandjee B. "Effect of Bacille Calmette-Guerin vaccination on tuberculin reactivity" *Am. Rev. Respir. Dis.* 1992; 145: 621-625.

84. Eum SY, Harle S, Lefort J et al "Eosinophil recruitment into the respiratory epithelium following antigenic challenge in hyper-IgE mice is accompanied by IL-5-dependent bronchial hyperresponsiveness". *Proc Natl Acad. Sci.* 1995; 92 (26): 12290-94.

85. Nahori MA, Lagranderie M, Lefort J et al. "Effect of *Mycobacterium bovis* BCG on the development of allergic inflammation and bronchial hyperresponsiveness in hyper-IgE BP2 mice vaccinated as newborns" *Vaccine* 2001; 19: 1484-95.

86. Tukenmez F, Bahceciler NN, Barlan IB, Basaran MM. "Effect of pre-immunization by killed *Mycobacteria bovis* and *vaccae* on immunoglobulin E response in ovalbumin-sensitized newborn mice" *Pediatr. Allergy Immunol.* 1997; 8: 53-8.

87. Shirakawa T, Enomoto T, Shimazu S, Hopkin J. "The inverse association between tuberculin responses and atopic disorder" *Science.* 1997; 275: 77-79.

88. Alm JS, Lilja G, Pershagen G, Scheynius A. "Early BCG vaccination and development of atopy" *Lancet.* 1997; 350: 400-03.

89. Aaby P, Shaheen SO, Heyes CB et. al. "Early BCG vaccination and reduction in atopy in Guinea-Bissau" *Clin. Exp. Allergy.* 2000; 30: 644-50.

90. Frappier A. et. al. "BCG vaccination and pulmonary tuberculosis in Quebec" *CMAJ.* 1971; 105: 707-10.

91. Dodge RR, Burrows B. "The prevalence and incidence of asthma and asthma-like symptoms in a general population sample" *Am Rev. Respir. Dis.* 1980; 122:567-75.

92. McWhorter WP, Polis MA, Kaslow RA. "Occurrence, predictors, and consequences of adult asthma in NHANESI and follow-up survey" *Am. Rev. Respir. Dis.* 1989; 139:721-24.

93. Pereira A Krieger BP. "Pulmonary complications of pregnancy" *Clinics in Chest Medicine.* 2004; 25(2):299-310.

94. Burney PGJ, Luczynska C, Chinn S, Jarvis D. "The European community respiratory health survey" *Eur. Respir. J.* 1994;7:954-60.

95. Schwartzman K, Loo V, Pasztor J, Menzies D. "Tuberculosis infection among health care workers in Montreal" *AJRCCM* 1996; 154 (4 pt.1):1006-12.

96. Statistics Canada (Standards Division). *Standard Occupational Classification* (1980). Ottawa; Cat 12-565E.

97. Blishen BR, Carroll WK, Moore C. "The 1981 socioeconomic index for occupations in Canada" *Rev. Soc. Anth.* 1987;24:465-88.

98. Colton J. Statistics in Medicine. Little, Brown and Co. Boston. 1974: p. 160.

99. Hennekens CH and Buring JE. Epidemiology in Medicine. Little, Brown and Co. Boston. 1987: p. 261.

100. Hosmer D., Lemeshow S. Applied Logistic Regression. Wiley and Sons. New York:1989.

101. Grindulis MI, Baynham MID, Scott PH, Thompson RA, Wharton BA. "Tuberculin response two years after BCG vaccination" *Arch. Dis. Child.* 1984; 59:614-9.

102. Floyd S, Ponnighaus JM, Bliss L et. al. "BCG scars in northern Malawi: sensitivity and repeatability of scar reading, and factors affecting scar size" *Int. J. Tubercl.Lung Dis.* 4(12):1133-42, 2000.

103. Jentoft HF, Omenaas E, Eide GE, Gulsvik A. "Absence of a relationship between tuberculin reactivity and asthmatic symptoms, level of FEV1 and bronchial hyperresponsiveness in BCG vaccinated young adults" *Allergy.* 2002;57: 336-340.

104. Bager P, Rostgaard K, Nielsen NM et. al. "Age at bacille Calmette-Guerin vaccination and risk of allergy and asthma" *Clin Exp. Allergy.* 2003; 33:1512-17.

105. Mensinga TT, Schouten JP, Rijcken B et. al. "The relationship of eosinophilia and positive skin test reactivity to respiratory symptom prevalence in a community-based population study" J *Allergy Clin. Immunol.* 1990; 86:99-107.

106. Wuthrich B, Schindler C, Leuenberger P, Ackermann-Liebrich U, and the SAPALDIA team. "Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA study)" *Int. Arch. Allergy Immunol.* 1995; 106: 149-56.

107. Settipane RJ, Hagy GW, Settipane GA. "Long-term risk factors for developing asthma and allergic rhinitis: a 23 year follow-up of college students" *Allergy Proc.* 1994; 15:21-5.

108. Bodner CH, Ross S, Little J et. al. "Risk facors for adult-onset wheeze: a case-control study" *AJRCCM.* 1998; 157: 35-42.

109. Demissie K, Hanley JA, Menzies D, Joseph L, Ernst P. "Agreement in measuring socio-economic status: area-based versus individual measures" *Chr.Dis.Canada.* 2000; 21(1):1-7.

110. Schlessleman J. Case-Control Studies: Design, Conduct, Analysis. Oxford University Press. New York. 1982.

111. Hart PD'A, Sutherland I, Thomas J. "The immunity conferred by effective BCG and whole bacillus vaccines, in relation to individual variations in tuberculin sensitivity and to technical variations in the vaccines" *Tubercle* 1967; 48:201-10.

112. Fine PEM, Sterne JAC, Ponnighaus JM, Rees RJW. "Delayed-type hypersensitivity, mycobacterial vaccines, and protective immunity" *Lancet.* 1994; 344:1245-49.

113. Krause TG, Hviid A, Koch A et. al. "BCG vaccination and risk of atopy" *JAMA.* 2003; 289(8): 1012-15.

114. Annus T, Montgomery SM, Riikjarv MA, Bjorksten B. "Atopic disorders among Estonian schoolchildren in relation to tuberculin reactivity and the age at BCG vaccination" *Allergy* 2004; 59:1068-73.

115. Riikjarv MA, Annus T, Braback L, et. al. "Similar prevalence of respiratory symptoms and atopy in Estonian schoolchildren with changing lifestyle over 4 years" *Eur. Respir. J.* 2000; 16:86-90.

116. Godfrey RC, Gradidge CF. "Allergic sensitization of human lung fragments prevented by saturation of IgE binding sites" *Nature.* 1976: 259:484-86.

117. Weir RE, Fine PE, Nazareth B, Floyd S, et. al. "Interferon-γ and skin test responses of schoolchildren in southeast England to purified protein derivatives from *Mycobacterium tuberculosis* and other species of mycobacteria" *Clin Exp. Immunol.* 2003; 134:285-294.

118. BlackGF, Fine PEM, Warndorff DK et. al. "Relationship between IFN-γ and skin test responsiveness to *Mycobacterium tuberculosis* PPD in healthy, non-BCG vaccinated young adults in Northern Malawi" *Int. J. Tub. Lung Dis.* 2001 5: 664-72.

119. Orme IM, Collins FM. "Adoptive protection of the *Mycobacterium tuberculosis*-infected lung. Dissociation between cells that passively transfer protective immunity and those that transfer delayed-type hypersensitivity to tuberculin" *Cell Immunol.*1984; 84: 113-20.

120. Yilmaz M, Bingol G, Altintas D, Kendirli SG. "Correlation between atopic diseases and tuberculin responses" *Allergy.* 2000; 55:664-67.

121. Wong GWK, Hui DSC, Tam CM et. al. "Asthma, atopy and tuberculin responses in Chinese schoolchildren in Hong Kong" *Thorax.* 2001; 56:770-73.

122. Ota MOC, Van der Sande MAB, Walraven GEL et. al. "Absence of association between delayed type hypersensitivity to tuberculin and atopy in children in the Gambia" *Clin. Exp. Allergy.* 2003; 33: 731-36.

123. Nuhoglu Y, Nuhoglu C, Ozcay S. "The association between delayed type hypersensitivity reaction to *Mycobacterium tuberculosis* and atopy in asthmatic children" *Allergol. et Immunolopathol.* 2003; 31(1):14-7.

124. Marks GB, Ng K, Zhou J et. al. "The effect of neonatal BCG vaccination on atopy and asthma at age 7-14: an historical cohort study in a community with a very low prevalence of tuberculosis infection and a high prevalence of atopic disease" *J. Allergy Clin. Immunol.* 2003;111:541-49.

125. daCunha SS, Cruz AA, Dourado I et. al. "Lower prevalence of asthma in adolescents with symptoms of rhinitis that received neonatal BCG" *Allergy.* 2004; 59:857-62.

126. Strannegard I-L, Larson L-O, Wennergren G, Strannegard O. "Prevalence of allergy in children in relation to prior BCG vaccination and infection with atypical mycobacteria" *Allergy.* 1998; 53:249-54.

127. Gruber C, Meinlschmidt G, Bergmann R, Wahn U, Stark K. "Is early BCG vaccination associated with less atopic disease? An epidemiological study in German preschool children with different ethnic backgrounds" *Ped. Allergy Immunol.* 2002; 13:177-81.

128. Ma Y, Li XF, Zhao J, Wong GW et. al. "Relationships between the diameters of bacille Calmette-Guerin scars and asthma, atopy in urban and rural Beijing children" *Zhonghua Jie He He Hu Xi Za Zhi* 2003; 26(9):526-30.

129. Sarinho E, Schor D, Veloso M, Lima M. "BCG scar diameter and asthma: a case-control study" *J. Allergy Clin. Immunol.* 2000; 106:1199-2000.

BCG VACCINATION AND ASTHMA A CASE-CONTROL STUDY:

QUESTIONNAIRE

Conducted By :
The Montreal Chest Institute
The Montreal General Hospital
McGill University

Most of these questions ask you to provide a "yes" or "no" answer. You should provide ONLY ONE answer to these questions by circling either a "yes" or a "no". Other questions ask you to provide a written response. If you are unsure about what to answer, LEAVE THE QUESTION BLANK.

SECTION ONE:

We'll begin with some background information about yourself. Please fill in the blank spaces with a written response.

1. Last name:_____ First name:_____

2. Father's last name:_____ Father's first name:_____

3. Address: No:_____ Street:_____ Apt:_____
   City:_____ Postal Code:_____

4. Telephone number: Home:_____ At work (local):_____

5. Date of birth: Day:____ Month:____ Year:19___

6. Gender (circle one): Male Female

7. Country of birth:_____

   If born in Canada, which province:_____

   If not born in Canada, in what year did you move to Canada? 19___

SECTION TWO:

This set of questions will ask you about a variety of symptoms you may have experienced in the past, or are still experiencing. Circle one response only for "yes/no" questions. Provide a written response in the blank spaces If unsure, leave the question blank.

Wheeze and tightness in the chest

1. Have you had wheeze or whistling in your chest at any time in the last 12 months? No Yes

2. Have you woken up with a feeling of tightness in your chest or difficulty in breathing at any time in the last 12 months? No Yes

Shortness of breath

3. Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last 12 months? No Yes

Study of Effect of BCG Vaccination on Risk of Asthma

Consent to Participate

I agree to participate in the BCG-Risk of Asthma study. In agreeing to participate, I understand that:

1. Study personnel may review my medical file at this hospital to gather necessary information about my condition.

2. Study personnel may review my BCG vaccination record at the Institut Armand-Frappier.

3. The Research Ethics Committees of the Montreal General Hospital and Montreal Chest Institute may review research records in order to ensure that this study conforms to their standards for research involving human subjects.

4. A questionnaire will be administered.

5. All results and information obtained will remain strictly confidential.

6. I can refuse to participate or withdraw from this study at any time without any change in my care from my usual doctor.

I have read and understood this consent form. I have had the opportunity to ask questions about this research study, and these questions have been answered to my satisfaction. I agree to participate in this study. By signing my agreement to participate, I understand that I do not give up any of my legal rights.

Signed:_____ **Date:**_____

Name (Print):_____

Witness (if available):_____ **Investigator (if available):**_____

Name (Print):_____ **Name (Print):**_____

4. Have you had an attack of shortness of breath that came on following strenuous activity at any time in the last 12 months? No Yes

5. Have you been woken by an attack of shortness of breath at any time in the last 12 months? No Yes

6. Have you used any inhaled medicines to help your breathing at any time in the last 12 months?

No Yes

6.1 IF "YES" PLEASE SPECIFY:_____

7. Have you used any pills, capsules, tablets or medicines (other than inhaled medicines) to help your breathing at any time in the last 12 months?

No Yes

7.1. IF "YES" PLEASE SPECIFY:_____

8. Have you visited a hospital emergency room because of breathing problems in the last 12 months?

No Yes

Cough and phlegm from the chest

9. Have you been woken by an attack of coughing at any time in the last 12 months? No Yes

10. Do you usually cough first thing in the morning in the winter? No Yes

11. Do you usually cough during the day, or at night, during the winter? No Yes

If "No" go to Question 12, if "Yes":

11.1 Do you cough like this on most days for as much as 3 months each year? No Yes

12. Do you usually bring up phlegm from your chest first thing in the morning in the winter? No Yes

13. Do you usually bring up phlegm from your chest during the day, or at night, in the winter? No Yes

If "No", go to Question 14, if "Yes":

13.1 Do you bring up phlegm like this on most days for as much as 3 months each year? No Yes

Asthma

14. Have you ever had asthma? No Yes

If "No", go to Question 15, if "Yes":

14.1 Was this confirmed by a doctor? No Yes

14.2 How old were you when you had your first attack? Age in Years:____

14.3 How old were you when you had your most recent attack of asthma? Age inYears:____

14.4 Have you had an attack of asthma in the last 12 months? No Yes

14.5 Are you currently taking any medicines (including inhalers, aerosols or tablets) for asthma? No Yes

Other conditions

15. Do you have any nasal allergies, including "hay fever"? No Yes

16. Have you ever had eczema or any kind of skin allergy? No Yes

17. Do you have any pets? No Yes

IF "YES" SPECIFY:_____

18. When you were a child did you keep any pets? No Yes

IF "YES", SPECIFY:_____

19. Are you allergic to pets? No Yes

IF "YES", SPECIFY:_____

20. Have you ever had any difficulty with your breathing after taking medicines? No Yes

IF "NO" GO TO QUESTION 21, IF YES:
20.1 Which medicine(s)?_____

21. Have you ever been vaccinated **for allergy** No Yes

at any time in your life?

SECTION THREE:
Now, some questions about your family. Circle **only one** response for "yes/no"questions. Provide a **written response** in the blank spaces. If unsure, **leave the question blank**.

22. Did your father ever smoke regularly during your childhood? No Yes

23. Did your father ever have asthma? No Yes

24. What was your father's occupation when you were a child?_____

25. Did your mother ever smoke regularly during your childhood? No Yes

26. Did your mother ever have asthma? No Yes

27. What was your mother's occupation when you were a child?_____

28. Do/Did you have any brothers or sisters? No Yes

IF NO BROTHERS OR SISTERS, GO TO QUESTION 29, OTHERWISE:

28.1 Did any of your brothers or sisters ever have asthma? No Yes

29. Did you go to a daycare, school or creche with other children before the age of 5 years? No Yes

30. Did you have a serious respiratory infection before the age of 5 years? No Yes

SECTION FOUR

Now, we're going to ask some questions about your work. Circle **only one** response for "yes/no"questions. Provide a **written response** in the blank spaces. If unsure, **leave the question blank**.

31. Are you currently employed or self-employed? No Yes

32. What is or was your current or most recent job? (Be as precise as possible):_____

33. In what industry is/was this job?_____

34. Does work ever make your chest feel tight or wheezy? No Yes

35. Have you ever had to change or leave your job because it affected your breathing? No Yes

IF "NO", GO TO QUESTION 36, IF "YES":

35.1 What was this job? (Be as precise as possible)_____

36. Have you ever worked in a job which exposed you to vapours, gas, dust or fumes? No Yes

IF "NO", GO TO QUESTION 37, IF "YES":

36.1 What was this job? (Be as precise as possible): _____

SECTION FIVE

The next set of questions are about your smoking habits (if any). Again, this information is strictly confidential, and will not be shared with your doctor. Circle only one response for "yes/no"questions. Provide a written response in the blank spaces. If unsure, leave the question blank.

37. Have you ever smoked for as long as a year? No Yes

("Yes" means at least 20 packs of cigarettes or 12 oz (360 grams) of tobacco in a lifetime, or at least one cigarette per day or one cigar a week for one year)

IF "NO" GO TO QUESTION 38, IF "YES":

37.1 How old were you when you started smoking? Age in Years:_____

37.2 Do you now smoke (as of one month ago)? No Yes

IF "NO" GO TO QUESTION 37.3, IF "YES"

37.2.1 How much do you now smoke on average? Number per Day:_____

37.3 Have you stopped or cut down on smoking? No Yes

IF "NO" GO TO QUESTION 38, IF "YES":

37.3.1 How old were you when you stopped or cut down smoking? Age in Years:_____

37.3.2 On average of the entire time you smoked, (before you stopped or cut down) how much did you smoke?

Number per day:_____

SECTION SIX

Now, to finish, some questions about BCG vaccination and exposure to tuberculosis. Circle only one response for "yes/no"questions. Provide a written response in the blank spaces. If unsure, leave the question blank.

38. Have you ever received the BCG vaccination against tuberculosis? (In Quebec, this was given as scratches on the lower back) No Yes

IF "NO" GO TO QUESTION 39, IF "YES":

At what age did you last receive this vaccination? Age in Years _____

39. Have you ever had the tuberculin skin test (PPD, Mantoux, Tine)? No Yes

IF "NO" GO TO QUESTION 42 , IF "YES" COMPLETE QUESTIONS 40 AND 41

40. In what year was your most recent PPD skin test? Year: 19___

41. Were you told that your last test was positive or negative? positive negative (circle one)

Diagnosis and treatment

42. Have you ever lived in the same household as someone with active tuberculosis? (someone who was diagnosed and/or treated while you were living with them) No Yes

IF "YES", WHAT YEAR? Year: 19___

43. Have you ever been diagnosed to have active tuberculosis? No Yes

44. Have you ever taken medication for active tuberculosis? No Yes

45. Have you ever taken medication for prevention of tuberculosis (usually isoniazid INH)?

IF "YES" TO QUESTION 44 OR 45: What year? 19 ___

Foreign travel (outside of Canada and the U.S.)

We are interested in possible exposure to TB during foreign travel. For Canadian-born persons, travel refers to any time during your lifetime outside Canada and the U.S. For immigrants, travel refers to trips made since immigrating (moving) to Canada.

46. Have you ever spent more than three continuous months outside Canada? No Yes
Do not count time spent in the USA.

IF "NO", GO TO END OF QUESTIONNAIRE

IF "YES", WHERE WAS THE LAST TRIP?_____

47. If you could add up all the months you have spent on these trips, how much time would that be? Number of months:_____

Congratulations! You've reached the end of the questionnaire. Thank you for your participation.

HÔPITAL GÉNÉRAL DE MONTRÉAL
THE MONTREAL GENERAL HOSPITAL
1650 AVE CEDAR, MONTRÉAL, QUÉBEC H3G 1A4 (514) 937-6011

DATE OF APPROVAL
REC #
DEC 0 3 1997
MONTRÉAL GÉN. HOSP.
& MONTREAL GEN. HOSP.
RESEARCH INSTITUTE

December 3, 1997

Drs Dick Menzies & George Samuel
Department of Epidemiology &
Biostatistics
Room L10-417
Montreal General Hospital

RE: REC 97-051 entitled "Childhood BCG Vaccination and Risk of Atopic Asthma."

Dear Drs Menzies & Samuel:

We wish to acknowledge receipt of your modifications complying to the recommendations made by the Committee on October 14, 1997. We wish to inform you that approval for the above mentioned study and the revised consent forms has been provided on December 3, 1997, valid until November 3, 1998.

Please be reminded that our policies require the following information:

- **date of activation and completion of the study**
- **notification of any change to the protocol**
- **notification of any adverse events, drug reactions, or problems occurring during the course of the study**
- **an annual report (a short questionnaire will be sent to you in approximately 10 months from approval date)**
- **a reprint of article arising from the study**
- **a final report upon completion of the study**

Please be reminded that your study has not been approved for recruitment of minors or adults not competent to consent and that consent forms should disclose all reasonably foreseeable risks no matter how rare or minimal. Please note that patient recruitment and study procedures should follow recommendations found in the minutes (October 14, 1997) of the Research Ethics Committee meeting.

Good luck in your study.

Sincerely.

Dr. Denis Cournoyer, Chairman
MGH Research Ethics Committee

DC/eb

L'INSTITUT THORACIQUE DE MONTRÉAL
MONTREAL CHEST INSTITUTE

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Approved
for
97/12/22

December 22, 1997

Dr. Denis Roy
Director of Professional Services
Royal Victoria Hospital/Montreal Chest Institute
by Fax: 843-1490

RE: **Case control study of BCG vaccination and development of atopic asthma**

Dear Dr. Roy:

The above named study was recently approved by the Ethics Committee at the Montreal Chest Institute. Study investigators include myself as principal investigator, Dr. Ron Olivenstein at the Montreal Chest Institute, Dr. Pierre Ernst of the Montreal General Hospital, and Dr. George Samuel, a resident in community medicine who is conducting this research project as part of his master's thesis in the Department of Epidemiology and Biostatistics here at McGill University. As part of the study, we need access to medical patient records in order to identify patients potentially eligible for this study. According to the protocol considered and approved by the Ethics Committee, the investigators would review the charts, identify eligible patients, contact the treating physician who would then send out a letter on our behalf to ask their patients if they were interested in participating. Therefore, we would not directly contact the patients.

As in the past with all such studies, all information reviewed would remain strictly confidential. No patient identities or individual patient information will be revealed in any presentation of results. Furthermore, if patients do not respond to their physicians' letter, we will not contact the patients ourselves directly.

If you could give me your approval to allow us to access the records, I would be most grateful. If you need any further information, do not hesitate to contact me.

Yours very truly,

Dick Menzies, M.D.

McGill

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Fax: (514) 398-8981

March 19, 1998

Mme Maryse Odesse
Institut Armand Frappier
PO Box 100
531, boul. Des Prairies
Laval, Qc
H7N 4Z3

Dear Mme Odesse,

I enclose a list of subjects who have participated in a study we are conducting on the influence of BCG vaccination on the incidence of asthma. This case control study has been approved by the Montreal Chest Institute Research Centre Ethics Committee. The study is being conducted by Dr. George Samuel, a resident in community medicine and Masters student in the Department of Epidemiology and Biostatistics, under my supervision.

For each subject listed on the attached sheets, we have obtained signed informed consent that gives us permission to forward the information attached to you and request verification of BCG vaccination status. I attach a single copy of the consent form duly signed by one such individual on the list. In the interests of costs, and to reduce paper work for you, we have not made photocopies of all other consents, but I can assure you we have them on file and they are available for inspection by you or your personnel at any time.

As in the past, I would ask that you mark directly on the sheet whether BCG vaccination was given once, twice, or more often, and at what age. Also, if you have information regarding the Cuti test - could you indicate the date or year performed and result.

Also, as in the past, I would be willing to pay $6.50 per individual verified. Please send me an invoice when you have completed this list, I would be happy to ensure that payment is sent forthwith.

If you have any questions or need clarification, do not hesitate to contact me. I thank you in advance for your collaboration and help, as well as meticulous verification which has proved invaluable to us in the past.

Yours very truly,

Dick Menzies, MD

DM/so
Enclosure