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Immunogenicity and Safety of Influenza Vaccination in Children with Inflammatory Bowel Disease

by

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Abstract

Objective: To evaluate the immunogenicity and safety of influenza vaccination in children with inflammatory bowel disease (IBD).

Methods: Sixty IBD subjects and 53 controls completed the study. Immunogenicity was measured by immunogenic response (≥fourfold increase from pre-immunization to post-immunization hemagglutination-inhibition titers) and serologic protection (post-immunization hemagglutination-inhibition titer ≥40).

Results: Seventy percent, 72%, and 53% of IBD subjects developed an immunogenic response to H3N2, H1N1, and B strains, respectively; similarly, 95%, 98%, and 85% developed serologic protection, respectively. For the B strain, IBD subjects were less likely to mount an immunogenic response compared to controls, and immunosuppressed subjects were less likely to develop serologic protection compared to nonimmunosuppressed subjects with IBD. In the majority, the vaccine appeared to be well tolerated.

Conclusion: Though children with IBD develop appropriate immunogenicity to the influenza vaccine A strains, the response to the B strain appears to be impaired, especially with use of immunosuppressive therapy.

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Symbol	Definition
CI	Confidence Interval

Chapter One: Purpose

The purpose of this study was to evaluate the immunogenicity and safety of influenza vaccination in children with inflammatory bowel disease.

Chapter Two: Rationale

Inflammatory bowel disease is a chronic immune-mediated condition of the gastrointestinal tract resulting from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host (1). Inflammatory bowel disease affects 70 per 100,000 children with 4.7 new cases per 100,000 children per year in North America and Western Europe, the regions of highest prevalence (2,3).

The cornerstone of management for inflammatory bowel disease is medical therapy. The armamentarium of medical therapy, including immunosuppressive therapies, continues to expand. Increasingly, children with inflammatory bowel disease require more potent medical therapy, such as immunosuppressive therapy. A major concern about the use of immunosuppressive therapy is the increased risk for more frequent and severe infections. Furthermore, intercurrent infections may exacerbate inflammatory bowel disease activity.

Therefore, protection with vaccines against vaccine-preventable diseases is important in children with inflammatory bowel disease. However, immunosuppression from medical therapy or immune dysregulation from inflammatory bowel disease itself may influence the adequacy of immune response to vaccines and the ability to develop clinical protection from disease. In addition, due to the inherent immune dysregulation in inflammatory bowel disease, vaccines may exacerbate disease activity. This study will therefore evaluate the immunogenicity and safety of influenza vaccination in children with inflammatory bowel disease.

Chapter Three: Literature Review

3.1 Inflammatory Bowel Disease

3.1.1 Classification of Inflammatory Bowel Disease

Inflammatory bowel disease is broadly classified into three types: ulcerative colitis, Crohn's disease, and indeterminate colitis.

Ulcerative colitis was first described by Wilks in 1859 (4). In ulcerative colitis, inflammation is classically limited to the colon and is usually continuous, starting at the rectum. Approximately 80% of children with ulcerative colitis have pancolitis with inflammation extending proximal to the splenic flexure or involving the entire colon (5,6). The endoscopic features of ulcerative colitis include ulcers, erythema, loss of vascular pattern, friability, spontaneous bleeding, and pseudopolyps (7). Histologically, inflammation in ulcerative colitis is confined to the mucosa; other histologic features include crypt distortion, crypt abscesses, goblet cell depletion, and rarely mucin granulomas (7). Individuals with ulcerative colitis may also have other histological features such as inflammation of the ileum or stomach, periappendiceal inflammation, patchy distribution, and relative rectal sparing at the time of diagnosis; the presence of these features does not exclude a diagnosis of ulcerative colitis (7).

Crohn's disease was initially described by Crohn, Ginzberg, and Oppenheimer in 1932, although case reports of the same clinical and pathologic condition were published as early as the nineteenth century (8). In contrast to ulcerative colitis, Crohn's disease affects any region of the gastrointestinal tract and is characteristically segmental with areas of sparing throughout the gastrointestinal tract. Approximately 35 to 40% of individuals with Crohn's disease will have disease limited to the ileum and cecum, 30 to

40% will have disease limited to the small intestine, and 15 to 25% will have only colonic disease (9). Compared to ulcerative colitis, inflammation in Crohn's disease affects all layers of the bowel. Gross inspection of the bowel in well-established Crohn's disease demonstrates marked wall thickening from chronic transmural inflammation accompanied by luminal narrowing. Because of transmural inflammation, bowel loops may become matted together, fistulae may develop from extension of inflammation through the serosa into adjacent structures, and strictures may form. The appearance of "creeping fat" with fat extension over the serosal surface of the bowel may also be present on gross inspection. The endoscopic features of Crohn's disease include apthous, stellate, or linear ulcers, cobblestoning, and skip areas of normal mucosa (7). The microscopic features of Crohn's disease include transmural inflammation, granulomas, and skip areas of normal uninflamed mucosa (7).

A diagnosis of indeterminate colitis is based on endoscopic, histologic, and radiologic findings when the criteria for either Crohn's disease or ulcerative colitis cannot be definitively established. There are no definitive criteria for diagnosing indeterminate colitis. However, a working group for the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition suggested that an individual may be given a putative diagnosis of indeterminate colitis if inflammation is limited to the colon and features inconsistent with the diagnosis of ulcerative colitis are present (7). These features include colitis with a normal rectum on endoscopy and histology (absolute rectal sparing), mild ileitis with features atypical for backwash ileitis (eg. ileal apthous ulcers), microscopic ileitis with colitis limited to the left colon, severe focal gastritis, pancolitis with anal fissures or anal tags, and colitis with growth failure.

3.1.2 Clinical Manifestations of Inflammatory Bowel Disease

Inflammatory bowel disease manifests during childhood or adolescence in up to 25% of cases (10). In Crohn's disease, the classic presentation involves abdominal pain, diarrhea, poor appetite, and weight loss. In comparison, the classic presentation of ulcerative colitis and indeterminate colitis involves bloody diarrhea, abdominal pain, and tenesmus. Approximately 25 to 35% of individuals with inflammatory bowel disease develop extraintestinal manifestations (11). The most common extraintestinal manifestations include arthropathy, liver abnormalities, and skin disorders. Other less common extraintestinal manifestations include eye disorders, thromboembolic disease, hematologic abnormalities, pancreatic disorders, and bone disease.

3.1.3 Epidemiology of Inflammatory Bowel Disease

The incidence and prevalence of inflammatory bowel disease shows distinct geographic variations. Historically, inflammatory bowel disease has been most prevalent in North America, Northern Europe, and the United Kingdom. In these regions, the incidence and prevalence of inflammatory bowel disease are 4.7 per 100,000 children per year, and 70 per 100,000 children, respectively (2,3). The increases in incidence and prevalence of pediatric Crohn's disease have paralleled overall population trends (13,14). More recently, an increase in the incidence of inflammatory bowel disease, especially ulcerative colitis, has been observed in Asian Pacific countries including China, Korea, India, and Japan (15). Overall, individuals of Jewish descent appear particularly susceptible to inflammatory bowel disease (16).

3.1.4 Pathogenesis of Inflammatory Bowel Disease

The exact cause of inflammatory bowel disease is not known although evidence supports that inflammatory bowel disease results from a complex interplay between susceptibility genes, the environment, and the immune system; that is, an inappropriate inflammatory response to intestinal microbes occurs in a genetically susceptible host (1).

A genetic predisposition to inflammatory bowel disease is demonstrated by the noticeable variation in prevalence patterns among different ethnic groups and by evidence from family and twin studies (17). In addition, susceptibility genes are increasingly identified for Crohn's disease and ulcerative colitis (18).

Numerous environmental factors have been suspected to play a role in inflammatory bowel disease, including diet, cow's milk feeding in infancy, perinatal events, smoking, appendectomy, non-steroidal anti-inflammatory drugs, and oral contraceptives (19-24). However, robust evidence is lacking to support any of these factors aside from smoking as a risk factor in Crohn's disease and a protective factor in ulcerative colitis (19).

Immune dysregulation and intestinal microbial flora have also been implicated in the pathogenesis of inflammatory bowel disease and will be discussed in further detail below (Sections ***3.1.5*** and ***3.1.8***).

3.1.5 Immune Dysregulation in Inflammatory Bowel Disease

The immune system protects against potentially pathogenic organisms with multiple levels of defense of increasing specificity. The immune system is broadly divided into two complementary sets of defense mechanisms: the innate and adaptive immune systems.

Innate immunity is the first line of host defense and provides an immediate, quick response. The components of innate immunity include the epithelial barrier (skin and mucosa) with surface antimicrobial substances produced by epithelial cells; the complement system; and various blood cells that induce cytotoxicity or initiate phagocytosis and intracellular killing. The cells involved in innate immunity include resident cells (macrophages, dendritic cells, epithelial cells, mast cells) and infiltrating cells (neutrophils, natural killer cells, dendritic cells, monocytes). The specificity of an innate immune response is based on pattern recognition of molecules that are common to multiple microbes, but not present in the host. The innate immune system activates and instructs the adaptive immune system for antigen-specific T and B lymphocyte responses and the development of immunological memory.

In contrast to innate immunity, adaptive immunity provides a highly specific and long lasting form of protection. The adaptive immune system is designed to specifically recognize and differentiate a large number of molecules. A remarkable feature of the adaptive immune system is immunologic memory. Antigen stimulation activates lymphocytes, causing a clonal expansion of lymphocytes with production of long-lasting antigen-specific memory clones. Thus, when the same antigen is encountered in the future, immediate recognition and effective protection is provided by these memory cells, even with prolonged intervals between exposures. At future encounters with the antigen, both cellular and humoral responses are produced more rapidly than in the first encounter and more memory cells are generated.

Increasing evidence suggests that immune dysregulation is a major contributor to the pathogenesis of inflammatory bowel disease. Indeed, the hallmark of active

inflammatory bowel disease is a pronounced lamina propria infiltration of innate immune cells (neutrophils, macrophages, dendritic cells, and natural killer T cells) and adaptive immune cells (B and T lymphocytes).

Alterations in innate immunity have been described in inflammatory bowel disease. In inflammatory bowel disease, the physical barrier of the intestinal epithelium that prevents excessive entry of bacteria and other antigens from the intestinal lumen into the circulation may be compromised due to defective regulation of tight junctions and increased permeability of the paracellular space (25). In animal models of inflammatory bowel disease, other types of epithelial dysfunction leading to intestinal inflammation include defects in epithelial cell development or proliferation, barrier function, cell-matrix adhesion, endoplasmic reticulum stress, and epithelial restitution after injury (25-27). The abnormalities of the intestinal epithelium in inflammatory bowel disease may be a primary defect or the consequence of intestinal inflammation. Disruption of the intestinal epithelium may impact the relationship between the intestinal epithelium and intestinal microbial flora; certainly intestinal microbial flora has long been implicated in the pathogenesis of disease onset and relapse (28-30). In addition, numerous genetic mutations associated with alterations in innate immunity have been described in inflammatory bowel disease, including *NOD2/CARD15*, *ATG16L1*, and *IRGM* (1,18,31). The *NOD2* gene is expressed by epithelial cells, Paneth cells, macrophages, and dendritic cells (1). The gene encodes an intracellular pattern recognition receptor protein which recognizes specific ligands derived from bacterial components and consequently activates nuclear factor κ B and mitogen-activated protein kinase signalling pathways. This leads to the production of cytokines (eg. tumor necrosis factor and interleukin-1β) and

antimicrobial peptides (1). Polymorphisms in the *NOD2* gene are susceptibility factors in Crohn's disease (18). The *ATG16L1* and *IRGM* genes both encode proteins involved in autophagy, a mechanism for clearing intracellular components. Genetic mutations in both genes have been associated with Crohn's disease (31,32).

Immune dysregulation also occurs in the adaptive immune system in inflammatory bowel disease. Functional assays suggest that adaptive immunity involving CD4+ T cells is distorted in inflammatory bowel disease. Effector subgroups of helper T cells (Th1, Th2, and Th17) are vital for defense against pathogens and excessive entry of luminal micro-organisms, but proliferation and overactivity of these cells relative to the regulatory CD4+ T-cells can produce intestinal inflammation. In Crohn's disease, the Th1 and Th17 responses are exaggerated with increased intestinal mucosal production of the Th1 cytokines interferon-γ and tumor necrosis factor-α and the Th17 cytokine interleukin-17 (33,34). Polymorphisms of genes involved in the interleukin-23 pathway-Th 17 pathway have been associated with both Crohn's disease and ulcerative colitis (18). In addition, mucosal macrophages increase production of interleukin-12 and interleukin-18 in Crohn's disease. In ulcerative colitis, the Th2 response is exaggerated with increased intestinal production of the cytokine interleukin-17 (33,34).

3.1.6 Management of Inflammatory Bowel Disease

Currently, the main therapeutic objectives in inflammatory bowel disease are to induce symptomatic remission and to decrease mucosal inflammation. Though inflammatory bowel disease has long been considered a disease characterized by exacerbations and remissions, evidence now reveals that endoscopic and histologic lesions may persist despite resolution of symptoms and biochemical abnormalities

(35,36). Therefore, control of intestinal inflammation is included as an important therapeutic goal. The other therapeutic goals relevant to pediatric inflammatory bowel disease include optimization of growth, normalization of pubertal development, facilitation of normal social development, and avoidance of long-term disease-related complications. The different therapeutic modalities for pediatric inflammatory bowel disease include nutritional support, pharmacologic treatment, and surgery. Pharmacologic therapy for inflammatory bowel disease is based broadly on the following medications: sulfasalazine and 5-aminosalicylates, corticosteroids, antibiotics, immunomodulators, and biologics.

3.1.7 Immunosuppressive Therapy in Inflammatory Bowel Disease

In order to achieve the therapeutic objectives of symptomatic remission and controlling intestinal inflammation, physicians often use immunosuppressive medications. Management of inflammatory bowel disease with immunosuppressive medications was previously limited to corticosteroids. However, medical therapy has expanded over the past few decades to include a wide spectrum of more potent immunosuppressive medications. The benefits of immunosuppressive therapy beyond corticosteroids are to minimize corticosteroid use, induce and maintain long term remission, and prevent complications including growth failure and surgery. The medications used in inflammatory bowel disease considered to suppress the immune system are corticosteroids, azathioprine, 6-mercaptopurine, methotrexate, cyclosporine, tacrolimus, and biologic therapies (infliximab, adalimumab). These medications may be further classified according to their mechanisms of action (Table 1).

Table 1

Classification of Medical Therapy in Inflammatory Bowel Disease

Classification	**Type**	**Individual Medication Names**
Immunosuppressive	Systemic corticosteroid	Prednisone Prednisolone Methylprednisolone
	Immunomodulator	Azathioprine 6-mercaptopurine Methotrexate Cyclosporine Tacrolimus
	Anti-tumor necrosis factor-α biologic therapy	Infliximab Adalimumab
Non-immunosuppressive	No therapy	-
	Antibiotic	Metronidazole Ciprofloxacin
	5-Aminosalicylate	Mesalamine Sulfasalazine Balsalazide Olsalazine

Systemic corticosteroids are widely used for the induction of remission in both Crohn's disease and ulcerative colitis. Corticosteroids have broad anti-inflammatory and immunosuppressive effects that impair predominantly adaptive immunity, but also affect innate immunity as well. Some of these mechanisms include inhibition of nuclear factor κ B function; decreasing release of lymphocyte growth and activating factors such as interleukin-1, interleukin-2, interferon-γ, and interleukin-12; impairing chemotaxis of

neutrophils and monocytes; and inhibiting lymphocyte migration and proliferation (37,38).

Azathioprine is a prodrug that is metabolized to 6-mercaptopurine; azathioprine and 6-mercaptopurine are used in the maintenance of remission in both Crohn's disease and ulcerative colitis. The metabolites of 6-mercaptopurine impair the adaptive immune system by decreasing intracellular purine synthesis, which leads to reduced numbers of circulating B and T lymphocytes, immunoglobulin synthesis, and interleukin-2 secretion. In addition, a metabolite of 6-mercaptopurine modulates Rac1 activation, leading to apoptosis of T lymphocytes (39).

Methotrexate is a folic acid antagonist used in the induction and maintenance of remission of Crohn's disease. Methotrexate impairs adaptive immunity by inhibiting DNA synthesis, which leads to decreased lymphocyte number and function. It also inhibits synthesis of interleukin-1, interleukin-2, interleukin-6, and interleukin-8 (40).

Cyclosporine and tacrolimus are calcineurin inhibitors used primarily for induction of remission in ulcerative colitis. These agents impair adaptive immunity by preventing transcription of interleukin-2, which inhibits T-cell activation (41). Other T-cell cytokines blocked by cyclosporine and tacrolimus include interleukin-3, interleukin-4, interleukin-5, granulocyte macrophage colony-stimulating factor, and interferon-γ. Cyclosporin and tacrolimus also impair the innate immune system by inhibiting degranulation and transcriptional activation of genes encoding interleukin-3 and interleukin-5 by mast cells and basophils (42).

Infliximab and adalimumab are biologic agents used for induction and maintenance of remission in primarily Crohn's disease, but also in ulcerative colitis.

These agents are antibodies directed against tumor necrosis factor-α. They impair innate and adaptive immunity by binding and clearing tumor necrosis factor-α, inducing apoptosis of activated effector cells, and decreasing complement activation (43-45).

Because of the wide spectrum of immunosuppressive mechanisms for these medications, the extent and nature of immunosuppression due to medical therapy for inflammatory bowel disease patients may vary according to type of therapy.

3.1.8 Infections in Inflammatory Bowel Disease

Infections may contribute to the course of disease in inflammatory bowel, as both an initiating factor in the pathogenesis and etiology of inflammatory bowel disease and as a complication of inflammatory bowel disease itself or immunosuppressive treatment.

Intestinal microbial flora has long been implicated in the pathogenesis of inflammatory bowel disease as an important factor in initiation and relapse. In a study by Schumacher et al. of the initial presentation of 61 patients with inflammatory bowel disease, microbial agents were detected in 21% (28). The most common microbial agents included *Yersinia enterocolitica*, *Salmonella typhi*, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Clostridium difficile*, adenovirus, enterovirus, and cytomegalovirus. Relapses in inflammatory bowel disease have also been associated with superinfection with organisms such as *Clostridium difficile*, *Campylobacter jejuni, Salmonella typhimurium*, enteropathogenic *Escherichia coli, and* cytomegalovirus (29,30). In addition, symptomatic respiratory infections have been associated with 40 to 60% of relapses in inflammatory bowel disease (46-48).

Not only may infections contribute to the pathogenesis of inflammatory bowel disease, but they may also lead to serious complications in inflammatory bowel disease.

Individuals with inflammatory bowel disease may be at risk for increased frequency and severity of infections due to underlying disease, malnutrition, surgery, or immunosuppressive therapy (49,50). This is especially a concern with immunosuppressive therapy where the ability of the immune system to respond appropriately to an infection is impaired. Numerous case reports describe infectious complications during azathioprine or 6-mercaptopurine therapy for Crohn's disease including severe varicella zoster pneumonia, cutaneous varicella zoster complicated by esophagitis, mild hepatitis and colitis with cytomegalovirus, and fatal infectious mononucleosis from Epstein Barr virus (51-56). In infliximab therapy for Crohn's disease, case reports describe disseminated primary varicella, severe pneumococcal pneumonia, hepatitis B reactivation and subfulminant hepatitis, fatal staphylococcal sepsis, staphylococcal liver abscess, *Listeria monocytogenes* meningitis, and *Pneumocystis jiroveci* (carinii) pneumonia (57-64). To date, no reports have been published describing influenza infections or complications of influenza infection in inflammatory bowel disease.

3.1.9 Protection with Immunizations in Inflammatory Bowel Disease

Because of the multifactorial increased risk of infection in inflammatory bowel disease, protection against vaccine-preventable diseases is an important part of health care maintenance in inflammatory bowel disease. Guidelines for patients with inflammatory bowel disease propose that immunizations do not deviate from recommended schedules for the general population and counsel that most immunizations, except for live agent vaccines, may be safely administered in individuals with inflammatory bowel disease, even when immunosuppressed (65-67).

3.2 Influenza

3.2.1 Influenza Infection

Influenza is an acute, usually self-limited, febrile illness resulting from infection with the influenza virus. The initial predominantly systemic manifestations include fever, chills, headaches, myalgia, malaise, and anorexia. Respiratory involvement is common with symptoms of cough, severe pharyngeal pain, nasal obstruction and discharge, hoarseness, and dry or sore throat. The gastrointestinal symptoms of influenza infection include abdominal pain, nausea, and emesis. The most well recognized complications of influenza are primary viral pneumonia and secondary bacterial pneumonia.

Influenza causes outbreaks of varying severity almost every winter. The attack rates during outbreaks may be as high as 10 to 40% over a 5 to 6-week period with the highest attack rates occurring in children (68). Influenza outbreaks are frequently associated with excess morbidity and mortality; influenza accounts for approximately 20,000 deaths and 300,000 hospitalizations annually in the United States (69). In children, influenza accounts for a substantial number of outpatient visits and antibiotic courses (68). One of the factors contributing to the considerable impact of influenza in the general population is the malaise and consequent disability. A typical case of influenza, on average, has been estimated to be associated with 5 to 6 days of restricted activity, 3 to 4 days of disability, and about 3 days lost from work or school (70).

Individuals with specific high-risk medical conditions such as individuals with cardiovascular and pulmonary conditions; individuals who require regular medical care because of chronic metabolic disease, renal dysfunction, hemoglobinopathies; and

individuals with neurologic conditions and compromised handling of respiratory secretions are at increased risk for more severe disease (71).

Higher rates of influenza infection and complication are also suggested to occur among immunosuppressed populations (72). No reports have been published specifically focusing on frequency of influenza infection or complications in patients with inflammatory bowel disease or patients treated with immunosuppressive therapies for chronic immune-mediated conditions. However, in solid-organ transplantation recipients who are frequently on immunosuppressive medications, influenza infection has been reported to cause kidney and lung allograft rejection (73,74). In bone-marrow transplant recipients who also undergo immunosuppressive treatment regimens, the frequency of influenza infection during local epidemics was reported to be as high as 23 to 29% (75,76). Influenza infection may also have devastating consequences in bone-marrow transplant recipients with case fatality rates as high as 23% (77). Chemotherapy for treatment of malignancies also produces profound immunosuppression. Patients receiving chemotherapy for treatment of malignancies are at increased risk of more severe disease with case fatalities ranging from 11 to 33% (78-80). In patients with immunosuppression due to human immunodeficiency virus infection, influenza infection results in more severe and prolonged disease with high rates of hospital admission and mortality (81-84). In addition, immunosuppression with low CD4+ counts due to human immunodeficiency virus infection causes prolonged virus shedding which leads to unchecked replication and antiviral drug resistance (85-87).

Influenza viruses are classified into three types (A, B, and C virus) according to major antigenic differences. Influenza C rarely causes infections in humans. The other

important differences among the influenza viruses occur in genetic organization, structure, host, epidemiology, and clinical characteristics. The standard nomenclature for influenza viruses includes the influenza type, location of initial isolation, strain number, and year of isolation, followed by hemagglutinin and neuraminidase subtypes for influenza A viruses. Influenza A is further subdivided into subtypes based upon antigenic differences in the hemagglutinin and neuraminidase components, whereas influenza B is divided into lineages based upon the hemagglutinin component. Subtypes H1, H2 & H3 of influenza A viruses are the "seasonal influenza" subtypes associated with yearly outbreaks and pandemics. The influenza virus has two important surface glycoproteins - hemagglutinin and neuraminidase. Hemagglutinin plays a key role in virus cell entry by binding to cell surface receptors. In vitro binding of the influenza virus to red blood cells leads to hemagglutination; this can be observed as a layering of agglutinated red blood cells at the bottom of a tube or well. The hemagglutination-inhibition test measures the presence and quantity of antibodies directed against the hemagglutinin component of the influenza virus. In the hemagglutination-inhibition test, antibodies directed against hemagglutinin block the influenza virus from binding to red blood cells, thus inhibiting the hemagglutination reaction. The other surface glycoprotein neuraminidase is involved in the cleavage of sialic acid from the cell surface which allows the influenza virus to be released from the host cell membranes. This facilitates the release of progeny viruses and the spread of influenza from the host cell to uninfected surrounding cells. The influenza virus has the remarkable ability to modify the surface glycoproteins hemagglutinin and neuraminidase in a phenomenon known as antigenic variation. This permits the virus to evade a host immune response and allows for

reinfection due to nonrecognition by the host's immune system. Because of antigenic variation, influenza continues to be a major epidemic disease of humans.

Infection with the influenza virus elicits an immune response. The cytokines interleukin-6 and interferon-α peak early in the course of infection and contribute to symptom development and a host defense response against influenza. Tumor necrosis factor-α levels increase later as viral shedding and symptoms subside. During the latter days of the illness (days 4 to 6), interleukin-8 levels rise, correlating with the onset of lower respiratory tract symptoms (88). The development of influenza-specific cytotoxic T lymphocytes is important for clearance of the virus and recovery from illness; in individuals with T-lymphocyte immunodeficiency states, clearance of virus is delayed (89). Influenza infection induces production of antibodies to the surface glycoproteins hemagglutinin and neuraminidase. Antibodies against hemagglutinin neutralize infectivity of the virus, decrease the likelihood of influenza infection, and lessen the severity of disease if influenza infection occurs (90). Antibodies to neuraminidase reduce virus replication (91-93).

3.2.2 Influenza Vaccination

The influenza vaccine is an inactivated, multivalent vaccine containing surface antigens (hemagglutinin and neuraminidase) of virus strains produced in embryonated hen eggs. Each year, 1 to 2 strains of the vaccine are changed to account for antigenic variation in anticipation of the predominant influenza strains expected to circulate in North America in the upcoming winter. Immunity to these antigens, especially to hemagglutinin, reduces the likelihood of infection and lessens the severity of disease if infection occurs. Within 7 days after vaccination, there is an adaptive immune response

of increasing hemagglutination-inhibition antibody titers mediated by induction of cytotoxic T lymphocytes. The response peaks at approximately 2 to 4 weeks after vaccination (94).

An immunogenic response occurs when there is a four-fold or greater increase in hemagglutination-inhibition titer from pre-vaccination to post-vaccination; an immunogenic response is a marker of the ability of an individual's immune system to mount an appropriate response to the vaccine (95). Although no exact correlation exists, an individual is considered to have serologic protection against influenza if the post-vaccination hemagglutination-inhibition titer is 1:40 or greater (95). The serologic protection rate is the percentage of individuals with a post-vaccination hemagglutination-inhibition titer of 1:40 or greater. After influenza vaccination in healthy children, the immunogenic response rate ranges between 81 to 94% depending on influenza strain, and the serologic protection rate is 94 to 95% (96,97). Serologic conversion occurs when the hemagglutination-inhibition titer increases from negative pre-vaccination to 1:40 or greater post-vaccination. Other less commonly used methods of measuring response to the influenza vaccine include the neuraminidase inhibition test and the neutralization enzyme immunoassay.

Efficacy refers to the reduction in clinical outcomes specifically related to influenza, such as clinically-suspected cases and laboratory-confirmed influenza infections. In healthy children, a recent meta-analysis concluded that the efficacy of the influenza vaccine was 67% against laboratory-confirmed cases, and 36% against clinically-suspected cases (98). In adults less than 65 years old, influenza vaccine prevents influenza infection in 70 to 90% of individuals when there is a strong antigenic

match between the virus strains in circulation and those in the vaccine (99). Other factors affecting vaccine efficacy include age and immune competency of recipient, and previous exposure to influenza antigens.

3.2.2.1 Influenza Vaccination in Inflammatory Bowel Disease

The Public Health Agency of Canada recommends annual influenza immunization for those at risk of more severe complications of influenza infection (100). This includes children with inflammatory bowel disease on immunosuppressive therapy. Annual influenza immunization is also recommended for household contacts of these individuals. In addition, a recent review by Melmed recommends annual influenza immunization for all patients with inflammatory bowel disease (66). Despite these recommendations, influenza vaccine uptake is suboptimal in patients with inflammatory bowel disease. A survey of adults with inflammatory bowel disease reported that 72% of those at risk for influenza were not receiving regular influenza vaccinations; the most common reasons cited were lack of awareness of need, fear of side effects, and no specified reason (101).

The main concerns regarding vaccination of patients with inflammatory bowel disease on immunosuppressive therapy are the ability of a medically-suppressed immune system to mount an appropriate response to the vaccine and exacerbation of disease activity by stimulation of the immune system with a vaccine.

Two recent studies have evaluated the immunogenicity and safety of the influenza vaccine in children and young adults with inflammatory bowel disease. Mamula et al. compared the rate of serologic conversion (defined by change in hemagglutination-inhibition titer from a negative pre-vaccine titer to a post-vaccine titer of 1:40 or greater)

to the 2002-2004 inactivated influenza vaccines between 50 pediatric subjects with inflammatory bowel disease and 29 healthy pediatric controls (102). The proportion of subjects with inflammatory bowel disease who developed serologic conversion was similar to healthy controls for both of the influenza A strains (A/New Caledonia/20/99 [H1N1], A/Panama/2007/99 [H3N2]). In contrast, a lower proportion of subjects with inflammatory bowel disease developed serologic conversion to the B/Hong Kong/300/2001 strain (62%) compared to healthy controls (89%). Further subanalysis demonstrated that subjects with inflammatory bowel disease on combination infliximab and immunomodulatory therapy (corticosteroids, 6-mercaptopurine, or methotrexate) were less likely to develop serologic conversion to the A/New Caledonia/20/99 (H1N1) strain (63%) and B/Hong Kong/300/2001 strain (33%) compared to healthy controls (95% and 89%, respectively). The authors reported no serious adverse events or effect on inflammatory bowel disease clinical activity from influenza vaccination. The authors concluded that concomitant infliximab and immunomodulatory therapy decreases the probability of serologic conversion from influenza vaccination in pediatric subjects with inflammatory bowel disease.

A study by Lu et al. evaluated post-vaccine serologic protection (defined by hemagglutination-inhibition titer of 1:40 or greater) in 137 children and young adults with inflammatory bowel disease to the inactivated trivalent influenza vaccine for the 2007 influenza season (103). The authors demonstrated a high prevalence of serologic protection against the influenza A strains in the vaccine of 96% for A/Solomon Islands/3/2006 (H1N1) and 88% for A/Wisconsin/67/2005 (H3N2) regardless of use of immunosuppressive medications. In contrast, only 39% of subjects with inflammatory

bowel disease developed serologic protection against the B/Malaysia/2506/2004 strain. Subanalysis of subjects without serologic protection at baseline showed that those receiving anti-tumor necrosis factor-α therapy were less likely to develop serologic protection against the B/Malaysia/2506/2004 strain (14%) compared to nonimmunosuppressed patients (39%, p=0.03). The vaccine did not produce any serious adverse events though 2 subjects required hospitalization for inflammatory bowel disease flares in the period between the vaccination and the follow-up visit 3 to 9 weeks later. A limitation of the study was the lack of control subjects; only historical controls were used. Another limitation was the use of serologic protection as the primary outcome. Post-vaccine hemagglutination-inhibition titers are affected by pre-vaccine hemagglutination-inhibition titers (104). Therefore, post-vaccine serologic protection may have already been present pre-vaccination if an individual were previously exposed to the influenza strain by immunization or infection, or if there were cross-reactivity with a similar influenza strain exposed by immunization or infection. Though serologic protection is an important clinical outcome as a marker of protection against infection, evaluation of serologic protection may be limited because it does not assess the ability of the immune system to respond to the administered vaccine as individuals may already have developed serologic protection prior to vaccination.

These two studies demonstrate that patients with inflammatory bowel disease are able to develop high rates of serologic conversion and serologic protection against the A strains of the influenza vaccine (102,103). However, there may be a decreased ability to develop serologic conversion and serologic protection against the B strain of the influenza vaccine in patients with inflammatory bowel disease, especially those on more

potent immunosuppressive therapy such as biologic agents or a combination of biologic agents and other immunosuppressive therapies. The lessons in study design learned from these two studies are the importance of using a control group of children without inflammatory bowel disease or immunosuppression for comparison and selecting an outcome that measures the ability of the immune system to mount a response to the vaccine that is unaffected by pre-vaccination exposure to the virus.

Gelinck et al. evaluated serologic protection to influenza vaccination in 112 adults with rheumatoid arthritis or Crohn's disease on anti-tumor necrosis factor-α treatment and found no significant difference between the proportions with post-vaccine serologic protection when comparing subjects on anti-tumor necrosis factor-α treatment to healthy controls (105). However, the post-vaccination geometric mean antibody titers against the H3N2 and B strains of the influenza vaccine were lower in subjects on anti-tumor necrosis factor-α treatment compared to healthy controls (105).

3.2.2.2 Influenza Vaccination in Other Immune-mediated or Immunosuppressed Conditions

The immunogenicity and safety of the influenza vaccine has been evaluated in other chronic immune-mediated conditions and conditions of medical immunosuppression. Adults with rheumatoid arthritis had a lower rate of immunogenic response or serologic conversion to the influenza vaccine compared to healthy controls for the B strain of the influenza vaccine (67% versus 87%, respectively); response to vaccine was unaffected by the use of immunosuppressive therapies including prednisone, methotrexate, infliximab, or etanercept (106). In adults with systemic lupus erythematosus, only 75% of subjects mounted an immunogenic response to at least one

strain of the influenza vaccine; lower immunogenic response rates were present in subjects on prednisone or azathioprine (107). Another study also confirmed that adults with systemic lupus erythematosus were less likely to mount an immunogenic response compared to healthy controls; use of azathioprine again decreased the rate of immunogenic response in adults with systemic lupus erythematosus (108). The influenza vaccine did not exacerbate disease activity in adults with systemic lupus erythematosus or rheumatoid arthritis (106-108). Adult recipients of liver transplants were less likely to develop serologic protection all three strains of the influenza vaccine compared to healthy controls; however, serologic protection rates improved with administration of an additional dose of influenza vaccine (109). The vaccine was well tolerated in adult recipients of solid organ transplants with no effect on clinical disease or transplant rejection (109,110). In children with liver, kidney, or heart solid organ transplants, the immunogenic response or serologic protection rate to influenza vaccination ranged between 60 to 91%, depending on antigen; the vaccine was well tolerated with no increased allograft rejection (111-113).

3.3 Immunogenicity of Other Vaccines in Inflammatory Bowel Disease

Others studies of vaccines in inflammatory bowel disease demonstrate impaired adaptive immunity to vaccination, including impaired antibody responses to tetanus toxoid booster in adults with inflammatory bowel disease, and to cholera and salmonella vaccinations in adults with ulcerative colitis post-colectomy (114-117). A recent study by Melmed et al. demonstrated impaired immune response (defined by twofold or greater increase from pre-vaccination titers and $\geq$1 μg post-vaccination titer) to the pneumococcal polysaccharide vaccine in adults with inflammatory bowel disease on a

combination of anti-tumor necrosis factor-α and immunomodulatory therapies (118). In addition, impaired cellular immune response to numerous recall antigens has been shown in adults with Crohn's disease (119). Therefore, individuals with inflammatory bowel disease may have impaired immune responses to vaccines due to immune dysregulation inherent to inflammatory bowel disease or from immunosuppressive medications, and due to anatomical changes such as colectomy for oral vaccines.

3.4 Design Considerations

Multiple measures are available to evaluate the effectiveness of influenza vaccination. These may be based on hemagglutination-inhibition antibody levels (immunogenic response, serologic protection, serologic conversion, geometric mean titers), clinical protection against disease (efficacy), or other antibodies (neuraminidase inhibition test, neutralization enzyme immunoassay).

An immunogenic response is defined by a four-fold or greater increase in hemagglutination-inhibition antibody titers from pre-vaccine to post-vaccine (95). It is a very useful measure of the ability of the immune system to respond to the influenza vaccine. Serologic protection is an important marker of protection against infection. Although no exact correlation exists, serum hemagglutination-inhibition antibody titers of 1:40 or greater are associated with protection against infection (95). A limitation of serologic protection is the influence of factors aside from vaccination. The post-vaccination hemagglutination-inhibition titer is affected by the pre-vaccination hemagglutination-inhibition titer (104). For example, if a person has previously been vaccinated or infected by the same influenza strain, then serologic protection may be achieved prior to vaccination with the current influenza vaccine. In addition, influenza A

strains have strong cross-reactivity. Therefore, serologic protection may be achieved prior to vaccination despite no previous exposure to the specific vaccine strain if an individual were infected or vaccinated with a similar strain of influenza A. Serologic conversion is defined as a change in hemagglutination-inhibition titer from negative pre-vaccination to 1:40 or greater post vaccination. An advantage of serologic conversion is that it is not affected by pre-vaccination serologic protection; however use of serologic conversion reduces the available sample to only those individuals naïve to the antigen strains of the vaccine. The geometric mean titer is a quantitative measure of a group's central tendency of antibody level. It is calculated by determining the n^{th} root of the product of *n* antibody levels and it is often used to summarize data covering several orders of magnitude. Unlike an arithmetic mean, the geometric mean is less affected by very high or low values, which skew the arithmetic mean. The geometric mean titer may be determined for each group pre- and post-vaccination; the change in geometric mean titer may then be compared between differing groups (such as subjects with inflammatory bowel disease compared to sibling controls). However, use of geometric mean titers is limited by the lack of clinically meaningful absolute levels of geometric mean titers for a group or increase in geometric mean titer for a group from pre- to post-vaccination. Efficacy refers to the reduction in clinical outcomes specifically related to influenza, such as clinically-suspected cases and laboratory-confirmed influenza infections. It is a very clinically meaningful measure of the degree of clinical protection provided by vaccination. However, it requires a large sample size and long follow-up period during the influenza season for proper efficacy analysis.

The neuraminidase inhibition test measures antibodies directed against neuraminidase, another surface antigen of the influenza virus; however, antibodies against the hemagluttinin antigen are considered a better method of neutralizing infectivity of the virus and therefore a better correlate of protection against influenza infection (90,120). The neutralization enzyme immunoassay is a sensitive measure of neutralizing serum antibody titers to influenza that strongly correlates with hemagglutination-inhibition titers; however it's use is limited by intensive labor requirements and high costs (121,122).

In a study evaluating the immunogenicity of influenza vaccine in children with inflammatory bowel disease, a control group is valuable to provide a comparative point of reference of the immunogenicity in children without inflammatory bowel disease. It is important for the control group to be immunocompetent as an immunosuppressive medical condition or medication may affect the ability to generate an immune response to the influenza vaccination. The control group also should be limited to the same age range as children with inflammatory bowel disease to minimize other variability between the two groups. An option for a control group was non-immunosuppressed siblings of children with inflammatory bowel disease followed at the Alberta Children's Hospital Pediatric Gastroenterology Clinic. This would be a practical and convenient group as siblings are an accessible population and recruitment of subjects with inflammatory bowel disease may be enhanced if all family members were offered simultaneous vaccination. However, a drawback to recruitment of siblings is the requirement for phlebotomy to measure immune response in a population not typically needing blood tests. Another option for a control group was using pediatric studies of immunogenicity

for the recommended influenza vaccine reported to the Vaccines and Related Biological Products Advisory Committee from the Food and Drug Administration; however the process for obtaining this data would be more complex. Another option for a control group was selecting a population of children for whom the influenza vaccine is recommended annually and who also undergo regular phlebotomy along with frequent clinic assessments, such as children with cystic fibrosis. However, children with cystic fibrosis are frequently on multiple medications and prone to frequent infections. Therefore, the possibility of immune dysregulation in children with cystic fibrosis may affect the immunogenicity of the influenza vaccine. Another option is using pediatric patients followed at the Alberta Children's Hospital Pediatric Gastroenterology Clinic who do not have inflammatory bowel disease and are not immunosuppressed. However, as above, patients who do not require regular blood tests may be less interested in enrolment and patients with other chronic diseases such as celiac disease may have underlying immune dysregulation.

An open-label prospective design is useful for studies evaluating influenza vaccine immunogenicity as essentially only the current Public Health Agency of Canada-recommended seasonal influenza vaccine (consisting of 3 strains of influenza virus) is available during each influenza season. This negates the benefit of blinding participants or researchers as all subjects receive the same intervention.

Chapter Four: Objectives

Primary Objective

To estimate the proportion of children with inflammatory bowel disease who develop an immunogenic response, as defined by a four-fold or greater increase in hemagglutination-inhibition titers from pre-vaccine to post-vaccine, to each strain of the influenza vaccine.

Secondary Objective #1

Comparison of immunogenic response:

To determine if children with inflammatory bowel disease are less likely to develop an immunogenic response to each strain of the influenza vaccine compared to children without inflammatory bowel disease.

To determine if children with inflammatory bowel disease on immunosuppressive therapy are less likely to develop an immunogenic response to each strain of the influenza vaccine compared to children with inflammatory bowel disease not on immunosuppressive therapy.

Secondary Objective #2

To estimate the proportion of children with inflammatory bowel disease who develop serologic protection, as defined by post-vaccine hemagglutination-inhibition titers greater than or equal to 1:40, to each strain of the influenza vaccine.

Secondary Objective #3

Comparison of serologic protection:

To determine if children with inflammatory bowel disease are less likely to develop serologic protection to each strain of the influenza vaccine compared to children without inflammatory bowel disease.

To determine if children with inflammatory bowel disease on immunosuppressive therapy are less likely to develop serologic protection to each strain of the influenza vaccine compared to children with inflammatory bowel disease not on immunosuppressive therapy.

Secondary Objective #4

To evaluate the safety of influenza vaccination in children with inflammatory bowel disease with regards to inflammatory bowel disease activity by:

- Comparison of pre-vaccine and post-vaccine Pediatric Crohn's Disease Activity Index or Pediatric Ulcerative Colitis Activity Index scores.
- Comparison of pre-vaccine and post-vaccine inflammatory bowel disease-related laboratory parameters (hemoglobin, platelet count, erythrocyte sedimentation rate, c-reactive protein, albumin).
- Frequency of change in disease activity from inactive / mild disease to moderate / severe disease.
- Frequency of hospitalizations or surgeries related in inflammatory bowel disease in the 4 weeks following vaccination.

Secondary Objective #5

To evaluate the safety of influenza vaccination in children with inflammatory bowel disease with regards to frequency and type of adverse reactions.

Chapter Five: Methods

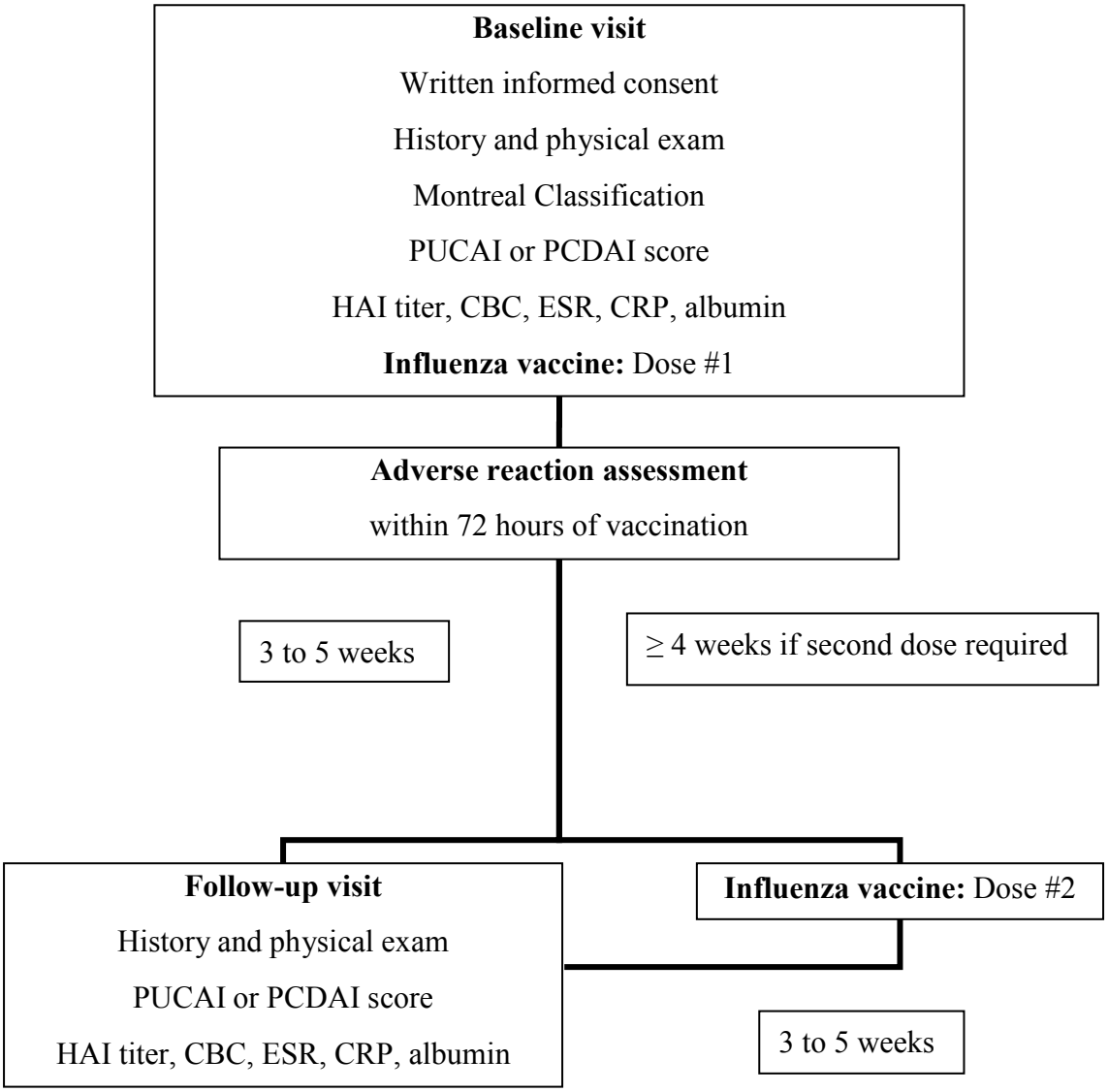
The study was submitted to the Conjoint Health Research Ethics Board of the Faculty of Medicine, University of Calgary and received ethical approval prior to initiation.

5.1 Study Design

The study was a prospective, open-label, cohort study. For the secondary objectives, when comparing immunogenic response or serologic protection to influenza vaccine between cohorts of subjects with inflammatory bowel disease and sibling controls, the exposure was having inflammatory bowel disease and the outcome was the proportion of subjects who developed an immunogenic response or serologic protection. When comparing immunogenic response or serologic protection to influenza vaccine between cohorts of immunosuppressed and non-immunosuppressed subjects with inflammatory bowel disease, the exposure was use of immunosuppressive therapy and the outcome was the proportion of subjects who developed an immunogenic response or serologic protection.

5.2 Study Conduct

Figure 1: Study Conduct for Subjects with Inflammatory Bowel Disease



PUCAI, Pediatric Ulcerative Colitis Activity Index; PCDAI, Pediatric Crohn's Disease Activity Index; HAI, hemagglutination inhibition; CBC, complete blood count; ESR, erythrocyte sedimentation rate; CRP, c-reactive protein

5.3 Subjects

For the inflammatory bowel disease cohort, the target population was patients with inflammatory bowel disease between 2 to 17 years of age. The accessible

population was eligible patients living in or near the City of Calgary followed by the Pediatric Gastroenterology clinic at the Alberta Children's Hospital who were identified between September 1, 2008 and January 1, 2009.

For the healthy control cohort, the target population was non-immunosuppressed individuals between 2 to 17 years of age. The accessible population was non-immunosuppressed children living in or near the City of Calgary who had siblings with inflammatory bowel disease followed by the Pediatric Gastroenterology clinic at the Alberta Children's Hospital. These subjects were identified between September 1, 2008 and January 1, 2009.

All subjects must have fulfilled the following inclusion and exclusion criteria.

Inclusion Criteria for Inflammatory Bowel Disease Cohort

1. Diagnosis of inflammatory bowel disease established by accepted criteria for endoscopy, histology, clinical course, radiology, and surgery (7)
2. Males or females between 2 to 17 years of age

Inclusion Criteria for Non-immunosuppressed Sibling Controls

1. Males or females between 2 to 17 years of age
2. Current good health status without any immunosuppressive medical condition or therapy

Exclusion Criteria

1. Anaphylactic reaction to previous dose of influenza vaccine
2. Known hypersensitivity to eggs or chicken or other components of influenza vaccine
3. Serious acute febrile illness

4. Previous severe lower respiratory symptoms within 24 hours of influenza vaccination, an apparent allergic reaction to the vaccine, or any other symptoms that raise concern regarding the safety of re-immunization
5. Already received current 2008 seasonal influenza vaccine
6. Parent or legal guardian unwilling or unable to provide signed informed consent

Sampling and Recruitment and Subject Selection Procedures

Subjects were selected through nonprobability, convenience sampling. Potential subjects were identified and recruited through outpatient Pediatric Gastroenterology clinic at the Alberta Children's Hospital. The Alberta Children's Hospital is the only tertiary care pediatric hospital providing care for children residing in Southern Alberta and Southeastern British Columbia. All children with inflammatory bowel disease living in Southern Alberta and Southeastern British Columbia are followed at the Pediatric Gastroenterology clinic at the Alberta Children's Hospital. All patients with inflammatory bowel disease attending the outpatient Pediatric Gastroenterology clinic at the Alberta Children's Hospital are asked to sign a "Consent to be Contacted" form if they are interested in participating in clinical research conducted by the members of the Gastroenterology division. All patients who had signed the consent form were contacted by mail, telephone, or in person at the time of a visit to the Alberta Children's Hospital (at the outpatient Pediatric Gastroenterology clinic, during an inpatient hospital admission, at the time of endoscopy, or at the time of infliximab infusion in the medical day treatment). All interested patients who appeared eligible were scheduled for a visit at the Alberta Children's Hospital in the outpatient Pediatric Gastroenterology clinic,

medical day treatment, inpatient ward, or day surgery units. At the visit, the nature of the study was reviewed in detail and informed consent was obtained (Appendix 1).

Classification of Subjects with Inflammatory Bowel Disease

All subjects with inflammatory bowel disease were classified into two groups based on whether their inflammatory bowel disease therapy included any immunosuppressive medications. The two groups were: non-immunosuppressed and immunosuppressed. The non-immunosuppressed group consisted of subjects on no medication, or receiving only antibiotics, 5-aminosalicylates, or probiotics at the time of vaccination. The immunosuppressed group consisted of subjects receiving any of the following medications at time of vaccination: systemic corticosteroids, azathioprine, 6-mercaptopurine, methotrexate, cyclosporine, tacrolimus, infliximab, or adalimumab. The immunosuppressed group was further subclassified into three groups based on type of immunosuppression. The three groups, by increasing immunosuppression, were: (1) systemic corticosteroids alone (prednisone, prednisolone, budesonide) (2) immunomodulators (azathioprine, 6-mercaptopurine, methotrexate, cyclosporine, tacrolimus) and (3) biologics (infliximab, adalimumab). Subjects on systemic corticosteroids alone were required to be on corticosteroids for at least 2 weeks prior to study enrolment. Subjects on more than one immunosuppressive therapy were classified according to the medication with the greatest immunosuppressive effect.

5.4 Study Protocol

Subjects were seen at the Alberta Children's Hospital outpatient Pediatric Gastroenterology clinic, medical day treatment, inpatient ward, or day surgery units.

Baseline visit

At the baseline visit for subjects with inflammatory bowel disease, subjects were assessed by a physician or nurse member of the research team. Demographic data and medical history were collected, including current and past inflammatory bowel disease-related medications and influenza vaccination history. A physical exam was performed focusing on the abdomen, extraintestinal manifestations of inflammatory bowel disease, height, and weight. Permission was obtained to access medical records from the outpatient Pediatric Gastroenterology clinic to confirm diagnosis, determine date of diagnosis, and define disease location and behaviour according to the Montreal Classification (123) (Section ***5.5.2***). Disease activity was evaluated by the Pediatric Crohn's Disease Activity Index for subjects with Crohn's disease (124,125) (Section ***5.5.3***), and the Pediatric Ulcerative Colitis Activity Index for subjects with ulcerative colitis or indeterminate colitis (126) (Section ***5.5.4***).

For subjects with inflammatory bowel disease and sibling controls, serum was collected by a laboratory technician or physician at baseline for pre-vaccination hemagglutination-inhibition titer. For subjects with inflammatory bowel disease, the following inflammatory bowel disease-related laboratory studies were also collected: complete blood count, erythrocyte sedimentation rate, c-reactive protein, and albumin.

Subsequently, the trivalent inactivated influenza vaccine (Fluviral, GlaxoSmithKline, Mississauga, Ontario, or Vaxigrip, Sanofi Pasteur, Toronto, Ontario) was administered intramuscularly by a physician or nurse member of the research team. The three influenza strains for the seasonal 2008 influenza vaccine were: A/Brisbane/59/2007 (H1N1)-like virus strain, A/Brisbane/10/2007 (H3N2)-like virus strain (Canadian vaccine contained A/Uruguay/71/2007), and B/Florida/4/2006 (B)-like

virus strain (from the B/Yamagata lineage). The H1N1 strain included in the 2008-09 vaccine was not the A/California/07/2009 (H1N1) pandemic strain. A standard 0.5 mL dose of vaccine contained 15 μg hemagglutinin of each strain. In accordance with recommendations by the Public Health Agency of Canada, all children < 9 years of age received Vaxigrip for thimerosal reduction and children ≥ 9 years of age received either Fluviral or Vaxigrip according to availability. In addition, all children < 9 years of age required two doses of vaccine given ≥ 4 weeks apart if they were receiving the influenza vaccine for the first time, or if they had received only one dose of influenza vaccine during the previous influenza season as their first dose (127). The vaccine was stored between 2 to 8°C in a refrigerator and administered intramuscularly for all participants into the deltoid muscle.

Adverse Reaction Assessment

All subjects were contacted by telephone at 72 hours post-vaccination by a physician or research assistant to document any adverse reactions to the influenza vaccine. An adverse reaction was defined as a response which is more severe than usual, or is unusual or unexpected in character according to the Adverse Event Reporting Criteria of Alberta Health and Wellness (128). The Adverse Event Reporting Criteria provides specific definitions for adverse reactions according to symptoms; these are broadly categorized into local reactions at injection site, neurological symptoms or diagnosis, and systemic reactions. The Adverse Event Reporting Criteria of Alberta Health and Wellness requires the report of all events temporarily related (i.e. related in time) to vaccination, with or without clear evidence of causality, and also requires that all reported adverse events following vaccination meet the specified criteria for each

category. The Adverse Events were submitted by paper forms to Alberta Health and Wellness and then a follow-up telephone call was made by Alberta Health and Wellness to the principal investigators.

Follow-Up Visit

Subjects returned for a follow-up visit between 3 to 5 weeks after influenza vaccination. For children who required a two-dose schedule, the follow-up visit occurred 3 to 5 weeks after the second dose of influenza vaccine. At the follow-up visit for subjects with inflammatory bowel disease, an assessment was conducted by a physician or nurse member of the research team including a medical history and physical examination. Inflammatory bowel disease-related laboratory studies (complete blood count, erythrocyte sedimentation rate, c-reactive protein, and albumin) were collected again. Disease activity was reassessed with the appropriate activity index (Pediatric Crohn's Disease Activity Index or Pediatric Ulcerative Colitis Activity Index).

In subjects with inflammatory bowel disease and sibling controls, a serum sample was collected by a laboratory technician or physician to measure post-vaccination hemagglutination-inhibition titer.

5.5 Measurements

5.5.1 Hemagglutination-Inhibition Titers

At the baseline and follow-up visit, serum samples were collected for hemagglutination-inhibition titers from all subjects. Serum samples were stored at -20°C at the Provincial Laboratory, Calgary, Alberta before and after testing.

Serum samples were tested against all three components contained in the 2008 vaccine. The laboratory technicians and virologist who performed the hemagglutination-

inhibition assay were blinded to the medical history of subjects, including classification as control or subject with inflammatory bowel disease, previous history of influenza vaccination, and immunosuppression status.

The hemagglutination-inhibition assay was performed according to standard methods described in the World Health Organization protocol (129). In brief, sera were treated with receptor-destroying enzyme using 4 hemagglutination-inhibition units of the appropriate influenza antigen and guinea-pig erythrocytes as the indicator. Doubling dilutions of the treated serum, starting at a 1:10 dilution, were titrated to determine the antibody endpoint. From this dilution, further two-fold dilutions were prepared (ie. 1:20, 1:40, 1:80, etc). The hemagglutination-inhibition titer was defined as the dilution factor of that dilution that still completely inhibited hemagglutination. For example, if a specific serum hemagglutination were completely inhibited for the dilutions 1:10, 1:20, 1:40 and 1:80, but not for the dilution 1:160, then the hemagglutination-inhibition titer was 1:80. Hemagglutination-inhibition titers are by definition conservative; they may underestimate the highest dilution that still completely inhibits hemagglutination and therefore underestimate the true titer. Baseline and post-vaccination samples were tested in parallel to assess rising titers. An immunogenic response was defined as samples with fourfold or greater differences in titer between pre-vaccination and post-vaccination sera. Serologic protection was defined as a hemagglutination-inhibition titer of 1:40 or greater.

5.5.2 Montreal Classification

The Montreal Classification is a well recognized, widely used, and accepted clinical classification system for Crohn's disease and ulcerative colitis (123). For Crohn's disease, the 3 categories are: age at diagnosis, location, and behaviour (Table 2).

For ulcerative colitis (and indeterminate colitis), the 2 categories are extent of disease and severity of disease (Table 3); however the Pediatric Ulcerative Colitis Activity Index was used to measure disease activity instead in this study.

Table 2

Montreal Classification for Crohn's disease

Age at diagnosis	A1	Below 16 y
	A2	Between 17 and 40 y
	A3	Above 40 y
Location	L1	Ileal
	L2	Colonic
	L3	Ileocolonic
	L4	Isolated upper disease*
Behaviour	B1	Non-stricturing, non-penetrating
	B2	Stricturing
	B3	Penetrating
	p	Perianal disease modifier†

*L4 is a modifier that can be added to L1–L3 when concomitant upper gastrointestinal disease is present.

†"p" is added to B1–B3 when concomitant perianal disease is present.

Table 3

Montreal Classification for Ulcerative Colitis

Extent	**Anatomy**	
E1	Ulcerative proctitis	Involvement limited to the rectum (that is, proximal extent of inflammation is distal to the rectosigmoid junction)
E2	Left sided UC (distal UC)	Involvement limited to a proportion of the colorectum distal to the splenic flexure
E3	Extensive UC (pancolitis)	Involvement extends proximal to the splenic flexure

UC, ulcerative colitis

5.5.3 Pediatric Crohn's Disease Activity Index

The Pediatric Crohn's Disease Activity Index is a widely recognized instrument used to measure severity of illness in children with Crohn's disease (Table 4). It was developed by a group of senior pediatric gastroenterologists at a research forum in April 1990, and initially validated in a cohort of 133 children and adolescents (124). The instrument is based on (a) subjective reporting of the degree of abdominal pain, stool pattern, and general well-being; (b) presence of extraintestinal manifestations, such as fever, arthritis, rash, and uveitis; (c) physical examination findings; (d) weight and height; and (e) laboratory parameters including serum hematocrit, erythrocyte sedimentation rate, and albumin. It is a valid and reliable instrument that has been further validated in a multicenter prospective study (125) and is commonly used in pediatric Crohn's disease studies. All of the components were obtained at the clinic visit. Disease activity was categorized as: inactive (<10 points), mild (11 to 29 points), moderate (30 to 44 points), and severe (≥45 points) (124,125). An increase in score of 15 points was considered clinically significant (124, 125).

Table 4

Pediatric Crohn's Disease Activity Index

Item					Points
History (Recall, 1 week)					
Abdominal pain	None				0
	Mild: Brief, does not interfere with activities				5
	Moderate / Severe: Daily, longer lasting, affects activities, nocturnal				10
Stools (per day)	0-1 liquid stools, no blood				0
	Up to 2 semi-formed with small blood, or 2-5 liquid				5
	Gross bleeding, or ≥ 6 liquid, or nocturnal diarrhea				10
Patient functioning, general well being	No limitation of activities, well				0
	Occasional difficulty in maintaining age-appropriate activities, below par				5
	Frequent limitation of activity, very poor				10
Examination					
Weight	Weight gain or voluntary weight stable / loss				0
	Involuntary weight stable, weight loss 1-9%				5
	Weight loss ≥ 10%				10
Height	Height velocity ≥ -1 SD				0
	Height velocity < -1SD, > -2 SD				5
	Height velocity ≤ -2 SD				10
Abdomen	No tenderness, no mass				0
	Tenderness, or mass without tenderness				5
	Tenderness, involuntary guarding, definite mass				10
Perirectal disease	None, asymptomatic tags				0
	1-2 indolent fistula, scant drainage, no tenderness				5
	Active fistula, drainage, tenderness, or abscess				10
Extra-intestinal manifestations (Fever ≥ 38.5°C for 3 days over past week, definite arthritis, uveitis, erythema nodosum, pyoderma gangrenosum)	None				0
	One				5
	Two or more				10
Laboratory					
Hematocrit (%)	<10 years	11-14 years (male)	11-19 years (female)	15-19 years (male)	
	≥ 33	≥ 35	≥ 34	≥ 37	0
	28-32	30-34	29-33	32-36	2.5
	< 28	< 30	< 29	< 32	5
ESR (mm/hr)	< 20				0

	20-50	2.5
	> 50	5
Albumin (g/dL)	≥ 3.5	0
	3.1-3.4	5
	≤ 3.0	10

SD, standard deviation; ESR, erythrocyte sedimentation rate;

5.5.4 Pediatric Ulcerative Colitis Activity Index

The Pediatric Ulcerative Colitis Activity Index is a noninvasive instrument used to measure disease activity in children with ulcerative colitis (126) (Table 5). The instrument was established by item selection by a Delphi group of 36 experts in pediatric inflammatory bowel disease. Item weighting was then performed by regression modeling with a prospective cohort of 157 children with ulcerative colitis. The instrument was validated in a separate prospective cohort of 48 children with ulcerative colitis undergoing complete colonoscopy and responsiveness was assessed at a follow-up visit of 75 children. The rigorously developed Pediatric Ulcerative Colitis Activity Index is a valid, highly reliable, and responsive instrument. It is based on the following items: abdominal pain, rectal bleeding, stool consistency, number of stools per 24 hours, nocturnal stools, and activity level. Each of the components was assessed at the clinic visit. Disease activity was categorized as: inactive (<10 points), mild (10 to 34 points), moderate (35 to 64 points), and severe (≥65 points) (126). An increase in score of 20 points was considered clinically significant (126).

Table 5

Pediatric Ulcerative Colitis Activity Index

Item		Points
Abdominal pain	No pain	0
	Pain can be ignored	5
	Pain cannot be ignored	10
Rectal bleeding	None	0
	Small amount only, < 50% of stools	10
	Small amount with most stools	20
	Large amount, > 50% of stool content	30
Stool consistency of most stools	Formed	0
	Partially formed	5
	Completely unformed	10
Number of stools per 24 hours	0-2	0
	3-5	5
	6-8	10
	> 8	15
Nocturnal stools (any episode causing awakening)	No	0
	Yes	10
Activity level	No limitation of activity	0
	Occasional limitation of activity	5
	Severe restricted activity	10

5.6 Sample Size

For the primary objective of estimating the proportion of children with inflammatory bowel disease who develop an immunogenic response (fourfold or greater increase in hemagglutination-inhibition titers from pre-vaccine to post-vaccine) to each vaccine strain, we attempted to recruit a sample size of 80 subjects with inflammatory bowel disease which would yield an estimate with a 95% confidence interval (CI) width of 0.21. This was based on an estimate of 0.72 derived from the range of reported

proportions developing an immunogenic response in studies on influenza vaccination in children with inflammatory bowel disease or organ transplant recipients (102,103,111-113). That is, the estimated proportion of subjects who developed an immunogenic response to the influenza vaccine would be 0.72, 95% CI 0.61 to 0.82. If we recruited less than 80 subjects with inflammatory bowel disease or the proportion with an immunogenic response were different, then the corresponding 95% CI widths would vary according to Table 6.

Table 6

95% Confidence Interval Width for Varying Sample Sizes and Proportions of Successes

Sample size	Successes	Proportion	95% Confidence Interval	95% Confidence Interval Width
80	64	0.80	0.70 - 0.88	0.18
	56	0.70	0.59 - 0.80	0.21
	48	0.60	0.48 - 0.71	0.23
	40	0.50	0.39 - 0.61	0.22
70	56	0.80	0.69 - 0.89	0.20
	49	0.70	0.58 - 0.80	0.22
	42	0.60	0.48 - 0.72	0.24
	35	0.50	0.38 - 0.62	0.24
60	48	0.80	0.68 - 0.89	0.21
	42	0.70	0.57 - 0.81	0.24
	36	0.60	0.47 - 0.72	0.25
	30	0.50	0.37 - 0.63	0.26

For the secondary objective of comparing the proportions that developed an immunogenic response to each strain between subjects with inflammatory bowel disease

and sibling controls, an estimated required sample size was calculated based on the assumption of a 0.23 difference between the proportion of subjects with inflammatory bowel disease (0.72) and the proportion of sibling control subjects (0.95) who develop an immunogenic response. Based on a power of 80% and an alpha of 0.05, 48 subjects would be required in each group to detect a difference of 0.23 between the groups. If the difference were less than expected, then the required sample sizes would vary according to Table 7 based on a power of 80% and an alpha of 0.05.

Table 7

Sample sizes for Varying Proportions of Subjects in Each Group Developing an Immunogenic Response

p_1 (Subjects with inflammatory bowel disease)	p_2 (Sibling control subjects)	Sample size ($n_1 = n_2$)
0.70	0.95	43
0.75	0.95	59
0.80	0.95	88
0.85	0.95	160
0.90	0.95	474

5.7 Statistical Analysis

All statistical tests of significance were two-sided with an alpha of 0.05. Analysis was performed using the statistical software Stata (Stata Corporation, College Station, Texas). For the secondary objectives, corrections for multiple comparisons were not made.

Characteristics of Participants

Demographic characteristics, medical history, previous influenza vaccination history, medications, disease activity, laboratory data, and Montreal Classification were

described for all subjects with inflammatory bowel disease. Comparisons for the above variables were made between the group of immunosuppressed subjects with inflammatory bowel disease and the group of nonimmunosuppressed subjects with inflammatory bowel disease. Demographic characteristics and previous influenza vaccination history were described for all sibling control subjects. Comparisons for the above variables were made between the group of subjects with inflammatory bowel disease and the group of sibling control subjects. For continuous variables, groups were compared using *t*-tests or Mann-Whitney U test. For dichotomous variables, groups were compared using X^2 test or Fisher's exact test.

An analyzable subject for the Primary Objective and Secondary Objectives #1, 2, and 3 was required to have both pre-vaccination and post-vaccination hemagglutination-inhibition antibody titers collected. An analyzable subject for Secondary Objective #4 was defined as any subject who participated in baseline and follow-up visits, and obtained pre-vaccination and post-vaccination inflammatory bowel disease-related laboratory investigations. An analyzable subject for Secondary Objective #5 was any subject who received the influenza vaccination.

Primary Objective

To estimate immunogenic response in children with inflammatory bowel disease, the proportion of subjects with inflammatory bowel disease who developed a four-fold or greater increase in hemagglutination-inhibition titers from pre-vaccine to post-vaccine was estimated along with the corresponding exact binomial 95% CI calculated by Stata. This was determined for each of the three antigen strains in the influenza vaccine.

Secondary Objective #1

To compare immunogenic response between children with inflammatory bowel disease and controls and between immunosuppressed and non-immunosuppressed children with inflammatory bowel disease, the proportion of subjects in each category who developed a four-fold or greater increase in hemagglutination-inhibition titers from pre-vaccine to post-vaccine to each vaccine strain was compared using the Fisher's exact test or X^2 test. The analysis comparing children with inflammatory bowel disease and controls was not a matched analysis.

Secondary Objective #2

To estimate serologic protection in children with inflammatory bowel disease, the proportion of subjects with inflammatory bowel disease with post-vaccine hemagglutination-inhibition titers greater than or equal to 1:40 was estimated along with the corresponding exact binomial 95% CI calculated. This was determined for each of the three antigen strains in the influenza vaccine.

Secondary Objective #3

To compare serologic protection between children with inflammatory bowel disease and controls and between immunosuppressed and non-immunosuppressed children with inflammatory bowel disease, the proportion of subjects in each category who developed post-vaccine hemagglutination-inhibition titers greater than or equal to 1:40 to each vaccine strain was compared using the Fisher's exact test or X^2 test. The analysis comparing children with inflammatory bowel disease and controls was not a matched analysis.

Secondary Objective #4

To assess the impact of influenza vaccination on inflammatory bowel disease activity, comparisons were made between pre-vaccine and post-vaccine Pediatric Crohn's Disease Activity Index scores or Pediatric Ulcerative Colitis Activity Index scores with paired t-tests or Wilcoxon signed-rank test. For subjects with incomplete data to calculate post-vaccine Pediatric Crohn's Disease Activity Index or Pediatric Ulcerative Colitis Activity Index scores, best and worst case scenarios were determined. The proportion of subjects who developed a clinically significant increase in Pediatric Crohn's Disease Activity Index (15 points) or Pediatric Ulcerative Colitis Activity Index scores (20 points) was estimated along with the corresponding 95% CI. The proportion of subjects who developed a change in disease activity from inactive / mild to moderate / severe and the proportion of subjects who required hospitalizations or surgeries related in inflammatory bowel disease in the 4 weeks following vaccination were estimated.

To assess the impact of influenza vaccination on inflammatory bowel disease-related laboratory parameters, pre- and post-vaccine levels of hemoglobin, platelet count, erythrocyte sedimentation rate, c-reactive protein, and albumin were compared using paired t-tests or Wilcoxon signed-rank test.

Secondary Objective #5

To assess for adverse reactions associated with the influenza vaccine, adverse reactions were described by frequency and type post-vaccination for all subjects.

Chapter Six: Results

6.1 Subjects

Subjects were recruited from September 2008 to January 2009. One hundred and sixty-four patients with inflammatory bowel disease were assessed for eligibility and contacted by telephone, mail, or in person at the Alberta Children's Hospital (Figure 2). One patient was excluded because of having already received the seasonal influenza vaccine. One patient was excluded because of a history of egg allergy. One hundred and one patients were excluded because of refusal to participate (by verbal communication over telephone or by no response to telephone calls or mailed invitation letter). Of these 101 patients, 51 resided outside of the City of Calgary. Where a reason for refusal was obtained, the reasons included: fear of bloodwork, unable to allocate time for clinic visits, lack of belief in effectiveness of influenza vaccination, and stable or unstable health status. Therefore, 61 subjects enrolled in the inflammatory bowel disease cohort and 55 subjects enrolled in the sibling control group.

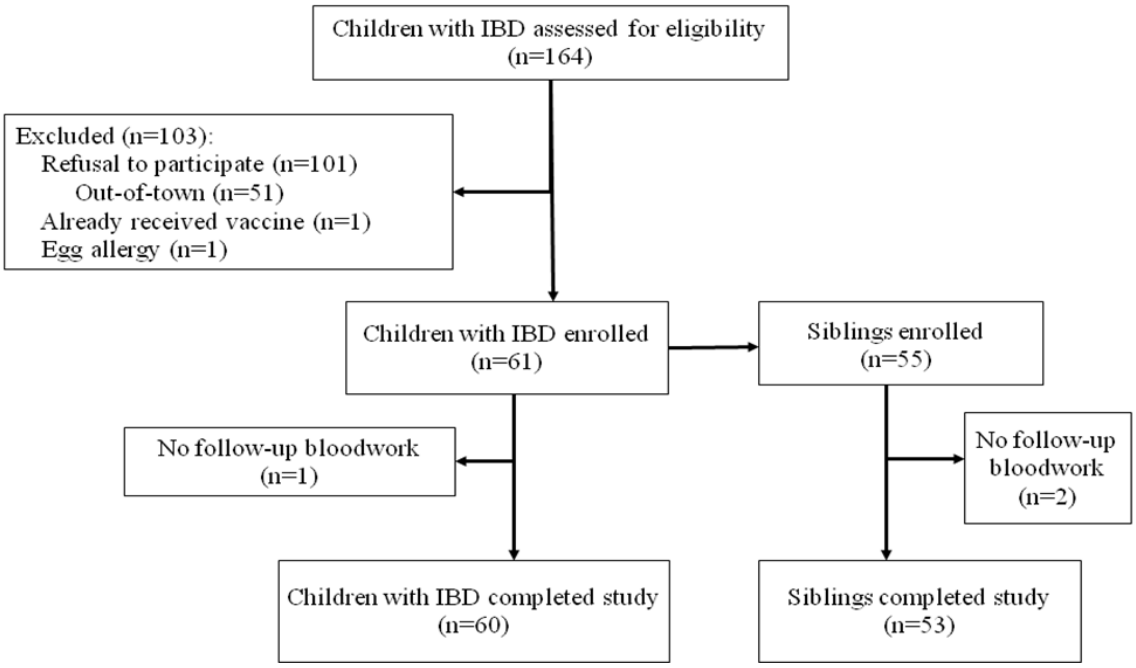
Overall, 60 of 61 subjects (98%) with inflammatory bowel disease and 53 of 55 sibling control subjects (96%) completed pre- and post-vaccination hemagglutination-inhibition antibody titers and are included in further analyses.

Demographic and clinical information was available for a portion of non-participants with inflammatory bowel disease who participated in other research studies at our center. Of 103 non-participants, 62 children (60%) were males and the median age was 14.5 years (minimum 3.2 years, maximum 17.9 years). The classification of inflammatory bowel disease was available for 91 non-participants; 54 children (59%) had Crohn's disease, 33 (36%) had ulcerative colitis, and 4 (4%) had indeterminate colitis.

The age at diagnosis was available for 84 non-participants; the median age at diagnosis was 10.9 years (minimum 0.6 years, maximum 17.2 years). For 34 non-participants with available vaccination records, only 9 children (26%) had documentation of ever receiving a prior influenza vaccination.

Thirty-seven subjects with inflammatory bowel disease had siblings enrolled in this study. Five subjects with inflammatory bowel disease had no eligible siblings. Three subjects with inflammatory bowel disease had eligible siblings who did not enrol in this study. The status of eligible siblings was not known for 16 subjects with inflammatory bowel disease.

Figure 2: Flow of Subjects



IBD, inflammatory bowel disease

Table 8 shows the characteristics of the 60 subjects with inflammatory bowel disease who completed the study with pre-vaccination and post-vaccination hemagglutination-inhibition titers. In the inflammatory bowel disease group, only 30 subjects (50%) with inflammatory bowel disease reported a history of prior influenza vaccination. The median age at enrolment was 15.4 years and only one subject with inflammatory bowel disease required two doses of influenza vaccine because of age less than 9 years and no previous history of influenza vaccination. The distribution of diagnoses included 26 subjects (43%) with Crohn's disease, 25 subjects (42%) with ulcerative colitis, and 10 subjects (17%) with indeterminate colitis. At enrolment and during the study, 42 subjects (70%) with inflammatory bowel disease were on immunosuppressive therapy, and 18 subjects (30%) were not on any immunosuppressive therapy. Of those subjects on immunosuppressive therapy, 2 were receiving systemic corticosteroids alone (for at least 1.5 months prior to study enrolment); 32 were receiving azathioprine, 6-mercaptopurine, or methotrexate; none were receiving cyclosporine or tacrolimus; and 8 were receiving biologic therapy. The distribution of diagnostic types of inflammatory bowel disease (Crohn's disease, ulcerative colitis, or indeterminate colitis) were different between immunosuppressed and nonimmunosuppressed subjects with inflammatory bowel disease (p=0.003); immunosuppressed subjects were more likely to have a diagnosis of Crohn's disease and non-immunosuppressed subjects were more likely to have a diagnosis of ulcerative colitis.

Table 8

Baseline Characteristics of Subjects with Inflammatory Bowel Disease

Characteristics	**All subjects with IBD n=60**	**Immuno-suppressed n=42**	**Nonimmuno-suppressed n=18**	***p****
Gender Male, n (%)	34 (57%)	24 (57%)	10 (56%)	0.9
Age [years, median] (Q1, Q3)				
At enrolment	15.4 (10.5, 16.4)	13.2 (10.4, 15.7)	15.2 (13.8, 17.2)	0.9
At diagnosis	11.7 (9.7, 14.7)	10.2 (12.2, 15.3)	11.1 (8.5, 14.3)	0.2
Previous influenza vaccination, n (%)	30 (50%)	14 (45%)	11 (61%)	0.4
Pre-vaccine serologic protection, n (%)				
H3N2	24 (50%)	14 (33%)	10 (56%)	0.1
H1N1	30 (50%)	24 (48%)	10 (56%)	0.6
B/Florida	22 (36%)	14 (33%)	8 (44%)	0.4
Diagnosis				
Crohn's disease	26 (43%)	23 (55%)	3 (17%)	0.003
Ulcerative colitis	24 (40%)	11 (26%)	13 (72%)	
Indeterminate colitis	10 (17%)	8 (14%)	2 (11%)	
Montreal classification				
Crohn's disease (n=26)				
Disease location				
L1 (terminal ileum)	2 (8%)	1 (4%)	1 (33%)	
L2 (colon)	9 (35%)	7 (30%)	2 (67%)	
L3 (ileocolon)	15 (58%)	15 (65%)	0	
L4 (isolated upper disease)	0	0	0	
Disease behaviour				
B1 (non-stricturing, non-penetrating)	22 (85%)	19(83%)	3 (100%)	
B2 (stricturing)	4 (15%)	4 (17%)	0	
B3 (penetrating)	0	0	0	
Perianal disease	13 (50%)	12 (52%)	1 (33%)	
UC or IC (n=34)				
Disease extent				
E1 (proctitis)	0	0	0	
E2 (left-sided colitis)	4 (11%)	3 (15%)	1 (7%)	
E3 (pancolitis)	31 (89%)	17 (85%)	14 (93%)	

Disease Activity Index, n (%)				
Inactive	32 (53%)	22 (52%)	10 (56%)	0.6
Mild	17 (28%)	13 (31%)	4 (22%)	
Moderate	9 (15%)	5 (12%)	4 (22%)	
Severe	2 (3%)	2 (5%)	0	

IBD, inflammatory bowel disease; UC, ulcerative colitis; IC, indeterminate colitis
*The reported p-values are the results of X^2 test, Fisher's exact test, or Mann-Whitney U test comparing the characteristic between immunosuppressed and nonimmunosuppressed children with inflammatory bowel disease.

Table 9 compares the baseline characteristics of inflammatory bowel disease subjects to the 53 sibling control subjects who completed the study with pre- and post-hemagglutination-inhibition antibody titers. Similar to subjects with inflammatory bowel disease, only 24 sibling control subjects (45%) reported a history of prior influenza vaccination. Subjects with inflammatory bowel disease were more likely to have pre-vaccine serologic protection (pre-vaccine hemagglutination-inhibition titer ≥ 1:40) against the B / Florida strain of the vaccine compared to sibling controls (36% vs 19%, p=0.04). Five sibling control subjects required two doses of influenza vaccine because of age less than 9 years and no previous history of influenza vaccination. Comparing sibling controls to subjects with inflammatory bowel disease, siblings were younger (median age at enrolment 12.3 years) than subjects with inflammatory bowel disease (median age at enrolment 15.4 years, p<0.0001).

Table 9

Comparison of Baseline Characteristics Between Subjects with Inflammatory Bowel Disease and Sibling Controls

Characteristics	IBD subjects n=60	Siblings n=53	*p**
Gender Male, n (%)	34 (57%)	34 (64%)	0.4
Age in years [median] (Q1, Q3)			
At enrolment	15.4 (10.5, 16.4)	12.3 (9.9, 13.9)	<0.0001
Previous influenza vaccination, n (%)	30 (50%)	24 (45%)	0.8
Pre-vaccine serologic protection, n (%)			
H3N2	24 (50%)	21 (40%)	0.3
H1N1	30 (50%)	28 (53%)	0.8
B / Florida	22 (36%)	10 (19%)	0.04

IBD, inflammatory bowel disease

* The reported p-values are the results of X^2 test, Fisher's exact test, or Mann-Whitney U test comparing the characteristic between children with inflammatory bowel disease and sibling controls.

6.2 Primary Objective: Immunogenic Response

The primary objective was to estimate the proportion of children with inflammatory bowel disease who developed an immunogenic response, as defined by a four-fold or greater increase in hemagglutination-inhibition titers from pre-vaccine to post-vaccine, for each strain of the influenza vaccine.

In subjects with inflammatory bowel disease, 70% (95% CI 57 - 81%), 72% (95% CI 59 - 83%), and 53% (95% CI 40 - 66%) achieved an immunogenic response to A/Brisbane/10/2007 (H3N2), A/Brisbane/59/2007 (H1N1), and B/Florida/4/2006 antigens, respectively, as shown in Figures 3A, 3B, and 3C. Though there was no statistically significant difference between the proportion of subjects with inflammatory

bowel disease who developed an immunogenic response to each antigen (p=0.09), there was a trend of decreased proportion of subjects with an immunogenic response to the B/Florida/4/2006 antigen as compared to the A/Brisbane/10/2007 (H3N2) and A/Brisbane/59/2007 (H1N1) antigens. This trend was also evident when subjects with inflammatory bowel disease were classified according to immunosuppression status.

Figures 3A, 3B, 3C: Immunogenic Response to Influenza Vaccine

Figure 3A: Influenza A/Brisbane/10/2007 (H3N2)

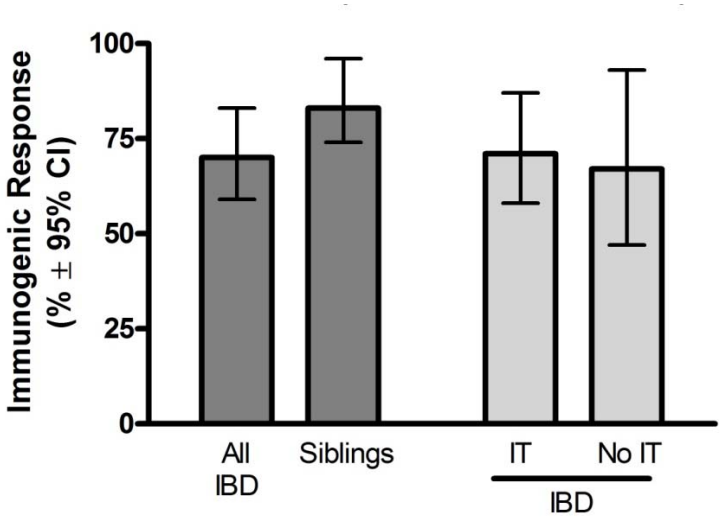


Figure 3B: Influenza A/Brisbane/59/2007 (H1N1)

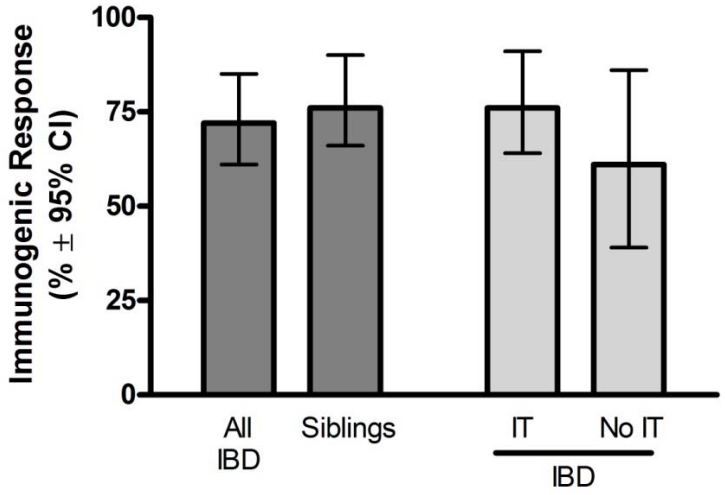
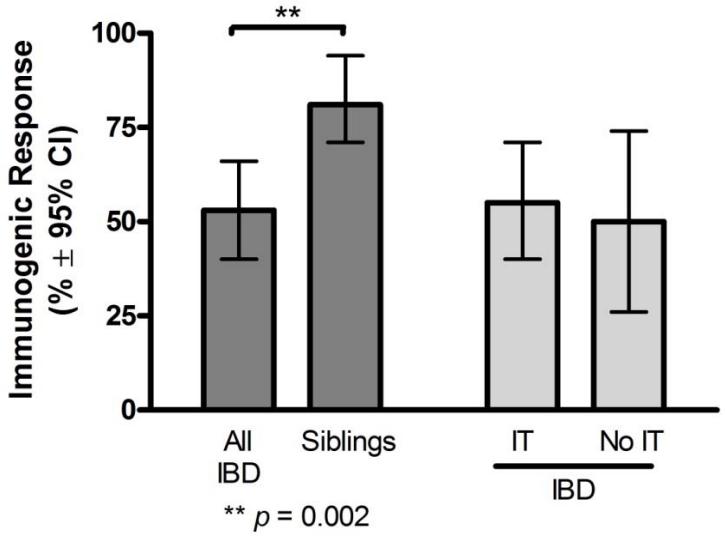


Figure 3C: Influenza B/Florida/4/2006



IBD, inflammatory bowel disease; IT, immunosuppressive therapy; CI, confidence interval

6.3 Secondary Objective #1: Comparison of Immunogenic Response

A secondary objective was to determine if children with inflammatory bowel disease were less likely to develop an immunogenic response compared to children without inflammatory bowel disease and if children with inflammatory bowel disease on

immunosuppressive therapy were less likely to develop an immunogenic response compared to children with inflammatory bowel disease not on immunosuppressive therapy.

There was no significant difference between immunogenic response in subjects with inflammatory bowel disease and sibling controls for A/Brisbane/10/2007 (H3N2) and A/Brisbane/59/2007 (H1N1) antigens, as shown in Figures 3A and 3B. However, a lower proportion of subjects with inflammatory bowel disease (53%, 95% CI 40 – 66%) developed an immunogenic response to B/Florida/4/2006 antigen compared to sibling controls (81%, 95% CI 68 – 91%, p=0.002), as shown in Figure 3C. There was no significant difference between the proportion developing immunogenic response in immunosuppressed and nonimmunosuppressed subjects with inflammatory bowel disease for all three influenza vaccine strains, as shown in Figures 3A, 3B, and 3C.

6.4 Secondary Objective #2: Serologic Protection

A secondary objective was to estimate the proportion of children with inflammatory bowel disease who developed serologic protection, as defined by post-vaccine hemagglutination-inhibition titers greater than or equal to 1:40, to each vaccine strain.

In subjects with inflammatory bowel disease, 95% (95% CI 86 – 99%), 98% (95% CI 91 – 100%), and 85% (95% CI 73 – 93%) achieved serologic protection against A/Brisbane/10/2007 (H3N2), A/Brisbane/59/2007 (H1N1), and B/Florida/4/2006 antigens, respectively, as shown in Figures 4A, 4B, and 4C. The proportion of subjects with inflammatory bowel disease who developed serologic protection to each antigen differed (p=0.02); subjects were more likely to develop serologic protection to

A/Brisbane/10/2007 (H3N2) and A/Brisbane/59/2007 (H1N1) than to B/Florida/4/2006. This difference was also present specifically for subjects with inflammatory bowel disease on immunosuppressive therapy (p=0.001).

6.5 Secondary Objective #3: Comparison of Serologic Protection

A secondary objective was to determine if children with inflammatory bowel disease were less likely to develop serologic protection compared to children without inflammatory bowel disease and if children with inflammatory bowel disease on immunosuppressive therapy were less likely to develop serologic protection compared to children with inflammatory bowel disease not on immunosuppressive therapy.

There was no significant difference in the proportion achieving serologic protection between subjects with inflammatory bowel disease and sibling controls for A/Brisbane/10/2007 (H3N2), A/Brisbane/59/2007 (H1N1), and B/Florida/4/2006 antigens, as shown in Figures 4A, 4B, and 4C. There was no significant difference in the proportion achieving serologic protection between immunosuppressed and nonimmunosuppressed subjects with inflammatory bowel disease for A/Brisbane/10/2007 (H3N2) and A/Brisbane/59/2007 (H1N1) antigens, as shown in Figures 4A and 4B. However, a lower proportion of immunosuppressed subjects with inflammatory bowel disease (79%, 95% CI 63 – 90%) achieved serologic protection to B/Florida/4/2006 antigen compared to nonimmunosuppressed subjects with inflammatory bowel disease (100%, 95% CI 90 – 100%, p=0.0.03), as shown in Figure 4C.

Figures 4A, 4B, 4C: Serologic Protection to Influenza Vaccine

Figure 4A: Influenza A/Brisbane/10/2007 (H3N2)

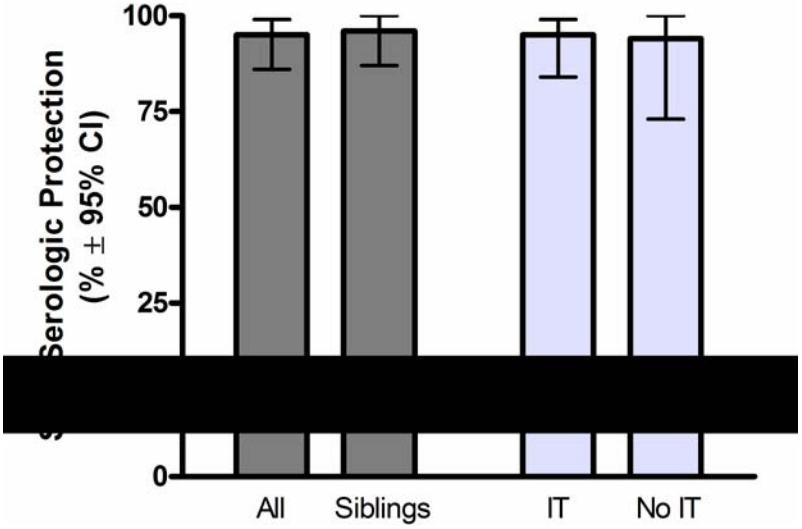


Figure 4B: Influenza A/Brisbane/59/2007 (H1N1)

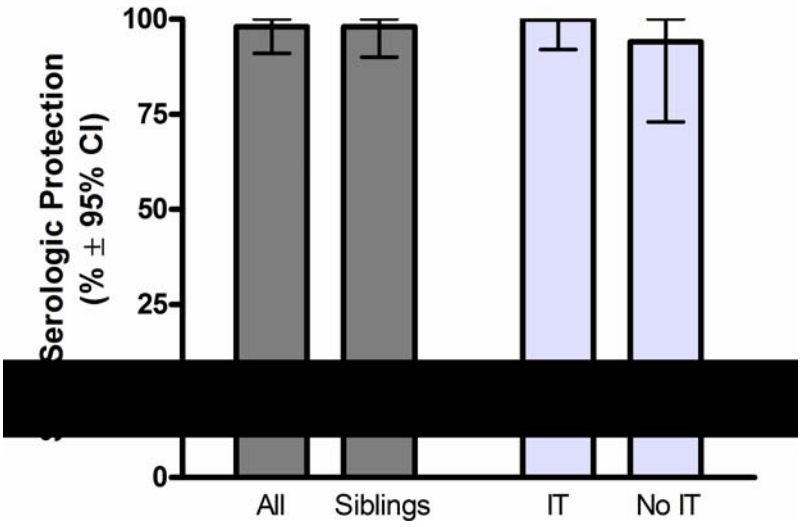
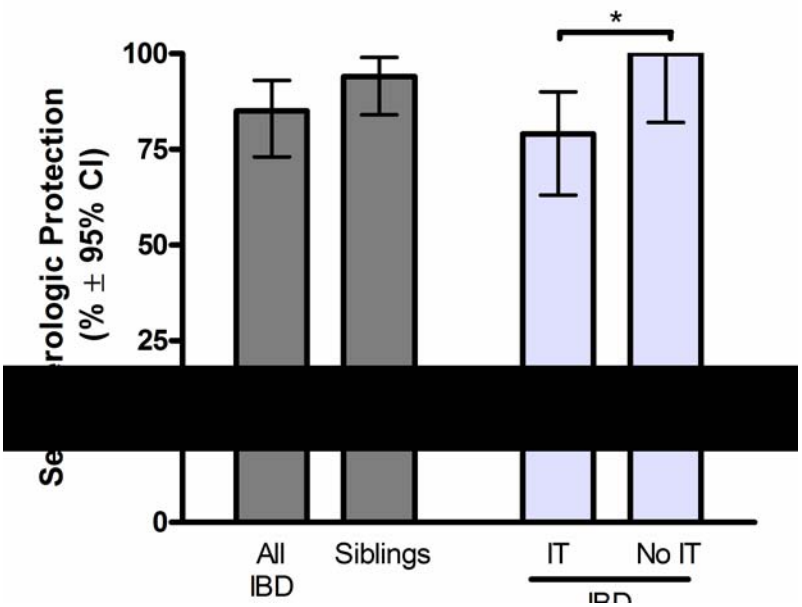


Figure 4C: Influenza B/Florida/4/2006



IBD, inflammatory bowel disease; IT, immunosuppressive therapy; CI, confidence interval

6.6 Secondary Objective #4: Impact of Influenza Vaccine on Disease Activity

A secondary objective was to evaluate the safety of influenza vaccination in children with inflammatory bowel disease with regards to disease activity.

In assessing for changes in disease activity categories, only 1 of 49 subjects with pre-vaccination inactive or mild disease activity had a change in disease activity to post-vaccination moderate or severe activity. This subject was also the only subject with inflammatory bowel disease to have a clinically significant increase in disease activity score (Pediatric Ulcerative Colitis Activity Index scores of 15 pre-vaccination and 35 post-vaccination). She was a 14 year old with pancolonic ulcerative colitis who was managed with an increased dose of prednisone and start of 5-aminosalicylate medication.

In assessing for complications of hospitalization or surgery related to inflammatory bowel disease post-vaccination during the 4 weeks following vaccination, no subjects with inflammatory bowel disease required hospitalization or surgery related to inflammatory bowel disease in the 4 weeks post-vaccination.

Pre-vaccination and post-vaccination Pediatric Crohn's Disease Activity Index scores were compared for subjects with Crohn's disease to assess the impact of influenza vaccination on disease activity. Five subjects with Crohn's disease were excluded for this comparison due to no follow-up physical exam (4 subjects) or no follow-up laboratory erythrocyte sedimentation rate level (1 subject). Of the remaining 21 subjects with Crohn's disease with complete pre-vaccination and post-vaccination Pediatric Crohn's Disease Activity Index scores, the median pre-vaccination and post-vaccination scores were both 10; there was no difference between pre-vaccination and post-vaccination scores (p=0.7) (Table 10). There was also no difference between pre-vaccination and post-vaccination scores for immunosuppressed and non-immunosuppressed subjects with Crohn's disease.

The following analysis includes the 5 subjects with Crohn's disease with incomplete post-vaccine Pediatric Crohn's Disease Activity Index scores. When the best-case scenario of no change in physical exam or erythrocyte sedimentation rate scores were assumed for these 5 subjects, the median pre-vaccination and post-vaccination scores were both 10; there was no difference between pre-vaccination and post-vaccination scores (p=0.7). When the worst-case scenario of maximum increase in physical exam or erythrocyte sedimentation rate categories were assumed for these 5

subjects, the median pre-vaccination and post-vaccination scores were both 10; again, there was no difference between pre-vaccination and post-vaccination scores (p=0.1).

Pre-vaccination and post-vaccination Pediatric Ulcerative Colitis Activity Index scores were compared for subjects with ulcerative colitis or indeterminate colitis to assess the impact of influenza vaccination on disease activity. All 33 subjects with ulcerative colitis or indeterminate colitis had complete pre-vaccine and post-vaccine Pediatric Ulcerative Colitis Activity Index scores. The median pre-vaccine score was 10 and the median post-vaccine score was 5; there was no significant difference between pre-vaccine and post-vaccine scores (p=0.08) (Table 10). For immunosuppressed subjects with ulcerative colitis or indeterminate colitis, there was also no significant difference between pre-vaccine and post-vaccine scores (p=0.5). For nonimmunosuppressed subjects with ulcerative colitis or indeterminate colitis, the median post-vaccine score of 0 was in fact lower than the median pre-vaccine score of 5 (p=0.03).

Table 10

Comparison of Pre-vaccine and Post-vaccine Activity Index Scores

	Pre-vaccine Activity Index Score	**Post-vaccine Activity Index Score**	***p****
Crohn's disease, median (Q1, Q3) n=21	10 (5, 15)	10 (5, 15)	0.7
Ulcerative colitis or Indeterminate colitis, median (Q1, Q3) n=33	10 (0, 25)	5 (0, 20)	0.08

Q1, first quartile; Q3, third quartile

*Comparison between pre-vaccine and post-vaccine activity index scores with Wilcoxon signed rank test.

Pre-vaccination and post-vaccination inflammatory bowel disease-related laboratory parameters were compared for subjects with inflammatory bowel disease. There was no significant difference between the pre-vaccine and post-vaccine levels of hemoglobin, platelet count, erythrocyte sedimentation rate, c-reactive protein, and albumin for subjects with inflammatory bowel disease (Table 11).

Table 11

Inflammatory Bowel Disease-related Laboratory Parameters

	Pre-vaccine level	**Post-vaccine level**	***p****
Hemoglobin (mg/dL), mean (95% CI)	132 (129 – 136)	133 (130 – 137)	0.3
Platelet count (x 10^9 /L) mean (95% CI)	333 (309 – 357)	344 (319 – 369)	0.2
Erythrocyte sedimentation rate, median (Q1, Q3)	9 (2, 20)	8 (3, 17)	0.8
C-reactive protein (mg/L) median (Q1, Q3)	1.6 (0.4, 6.7)	1.5 (0.5, 4.8)	0.9
Albumin (g/L) mean (95% CI)	39 (37 – 40)	39 (38 – 40)	0.6

*Comparison between pre-vaccine and post-vaccine levels with paired t-test or Wilcoxon signed rank test.

6.7 Secondary Objective #5: Adverse Reactions of Influenza Vaccine

A secondary objective was to evaluate the safety of influenza vaccination in children with inflammatory bowel disease with regards to frequency and type of adverse reactions.

Only 1 of 60 subjects with inflammatory bowel disease developed a reportable adverse reaction. A 15 year old male with ileocolonic Crohn's disease maintained on

adalimumab therapy with a previous history of pancreatitis on 2 occasions developed pancreatitis 84 hours post-vaccination. He presented with epigastric abdominal pain, nausea, vomiting, and anorexia along with elevated serum pancreatic enzyme levels. He required hospitalization for 4 days of supportive management. Because of his previous history of pancreatitis, the current episode of pancreatitis was not conclusively attributed to influenza vaccination. Since completion of this study, he has not had any subsequent episodes of pancreatitis.

No sibling control subjects developed any reportable adverse reactions to the influenza vaccine.

Chapter Seven: Discussion

7.1 Review of Findings

This study assessed the immunogenicity and safety of the influenza vaccination in children with inflammatory bowel disease.

With regards to the immunogenic response (four-fold or greater increase in hemagglutination-inhibition titers from pre-vaccine to post-vaccine), overall a high proportion of pediatric subjects with inflammatory bowel disease mounted an appropriate immunogenic response to the A/Brisbane/10/2007 (H3N2) and A/Brisbane/59/2007 (H1N1) strains of the influenza vaccine (70% and 72%, respectively); however, only 53% mounted an immunogenic response to the B/Florida/4/2006 antigen. Subjects with inflammatory bowel disease appeared to be able to mount a similar immunogenic response compared to sibling controls to the A/Brisbane/10/2007 (H3N2) and A/Brisbane/59/2007 (H1N1) strains; however, a lower proportion of subjects with inflammatory bowel disease (53%) appeared to develop an immunogenic response to B/Florida/4/2006 antigen compared to sibling controls (81%). The use of immunosuppressive medications did not appear to affect the proportion developing an immunogenic response to any of the influenza vaccine strains for subjects with inflammatory bowel disease. Comparison to the studies by Mamula et al. and Lu et al. for immunogenic response is not feasible as neither studies selected immunogenic response as an outcome (102,103).

For serologic protection (post-vaccine hemagglutination-inhibition titers greater than or equal to 1:40) against influenza, overall a high proportion of pediatric subjects with inflammatory bowel disease developed serologic protection to each antigen in the

influenza vaccine (95%, 98%, and 85% for A/Brisbane/10/2007 [H3N2], A/Brisbane/59/2007 [H1N1], and B/Florida/4/2006, respectively). In contrast, the study by Lu et al. showed that only 39% of subjects with inflammatory bowel disease developed serologic protection against the B/Malaysia/2506/2004 strain of the 2007 influenza vaccine compared to 96% and 88% for A/Solomon Islands/3/2006 (H1N1) and A/Wisconsin/67/2005 (H3N2), respectively (103). This may be explained by a higher proportion of subjects with inflammatory bowel disease in this study having pre-vaccine serologic protection against B/Florida/4/2006 (57%) compared to subjects with inflammatory bowel disease having pre-vaccine serologic protection against B/Malaysia/2506/2004 (14%) in the study by Lu et al (103). Similar proportions of subjects with inflammatory bowel disease appeared to develop serologic protection against influenza compared to sibling controls for all three strains in the influenza vaccine in this study. In the study by Mamula et al., there was no significant difference in the proportion with serologic protection for A/New Caledonia/20/99 (H1N1) and A/Panama/2004/99 (H3N2) antigens for the 2002 and 2003 influenza vaccines between subjects with inflammatory bowel disease and healthy controls; however a lower proportion of subjects with inflammatory bowel disease (64%) developed serologic protection to the B/Hong Kong/330/2001 antigen compared to healthy controls (90%) (102). Comparing immunosuppressed and nonimmunosuppressed subjects with inflammatory bowel disease in this study, there appeared to be no significant difference in the proportion with serologic protection for A/Brisbane/10/2007 (H3N2) and A/Brisbane/59/2007 (H1N1) antigens; however a lower proportion of immunosuppressed subjects with inflammatory bowel disease (79%) appeared to achieve serologic protection

to B/Florida/4/2006 antigen compared to nonimmunosuppressed subjects with inflammatory bowel disease (100%). Though this study was not designed to compare the proportion developing serologic protection between different groups of immunosuppressive medications due to small sample size, there appears to be a trend towards a lower proportion of subjects on biologic therapy with serologic protection against B/Florida/4/2006 antigen (63%) compared to subjects on immunomodulator therapy (azathioprine, 6-mercaptopurine, or methotrexate) (81%) or systemic corticosteroid therapy (100%). In the study by Mamula et al., subjects receiving a combination of infliximab (biologic) and other immunosuppressive therapy (corticosteroids, 6-mercaptopurine, or methotrexate) were less likely to develop serologic protection against A/New Caledonia/20/99 (H1N1) and B/Hong Kong/330/2001 compared to healthy controls (102). Because of the concern of hepatosplenic T-cell lymphoma with combination therapy (infliximab and either azathioprine or 6-mercaptopurine) which led to a Health Canada warning about the pediatric use of infliximab in July 2006, no subjects with inflammatory bowel disease in this study were on a combination of a biologic agent with another immunosuppressive medication (130). In the study by Lu et al., subanalysis of subjects with inflammatory bowel disease with baseline titers <1:40 showed that those receiving biologic anti-tumor necrosis factor-α therapy were less likely to develop serologic protection against B/Malaysia/2506/2004 (14%) compared to non-immunosuppressed subjects (39%) (103). The differing influenza vaccines for each influenza season used in the three studies limit the ability to make direct comparisons between studies. However, the results taken from the three studies suggest a decreased ability to mount serologic protection against the B strain of

the influenza vaccine in children with inflammatory bowel disease; this appears to be further affected if children are on immunosuppressive medications, especially more potent therapy such as biologic therapy or combination of a biologic agent with other immunosuppressive therapies. The B strain of influenza vaccines has been previously shown to be less immunogenic than the A strains in studies of influenza vaccination in healthy children (104,131). From this study and the study by Mamula et al., the immunogenicity of the B strain in the influenza vaccine appears to be further decreased compared to healthy controls in individuals with inflammatory bowel disease, especially with use of potent immunosuppressive therapy (102).

Hemagglutination-inhibition antibody titers may be affected by previous exposure to the same strains of influenza antigen. Review of data from the Public Health Agency of Canada of the influenza vaccine strains and circulating epidemic strains in the past 5 years reveal that the A/Brisbane/10/2007 (H3N2) and A/Brisbane/59/2007 (H1N1) antigens of the 2008 vaccine were amongst the most common circulating epidemic strains in the 2007 influenza season (132). B/Florida/4/2006 was not in any of the previous vaccines; however it was one of the more common circulating epidemic strains in the 2007 influenza season (Table 12). Influenza A strains tend to be more similar to each other and subjects may therefore be more primed to develop an immune response or serologic protection if there were a history of previous exposure to a similar influenza A strain by vaccination or infection. This may explain the higher rates of immune response and serologic protection against influenza A strains compared to influenza B. There is insufficient evidence at this time to demonstrate that decreased immunogenicity to the B

strain of the influenza vaccine results in greater susceptibility to influenza B infection during the influenza season.

Table 12

Influenza Strains of Previous Vaccines and Epidemics from 2003 to 2008

Influenza season (year)	Influenza vaccine strains	Circulating influenza strains identified by National Microbiology Laboratory
2008-09	• A/Brisbane/59/2007(H1N1) • A/Brisbane/10/2007(H3N2) • B/Florida/4/2006	• B/Malaysia/2506/2004 • A/California/07/2009 (H1N1) pandemic strain • A/Brisbane/59/2007 (H1N1) • B/Brisbane/60/2008 • A/Brisbane/10/2007 (H3N2) • B/Florida/4/2006
2007-08	• A/Solomon Islands/3/2006 (H1N1) • A/Wisconsin/67/2005 (H3N2) • B/Malaysia/2506/2004	• B/Florida/4/2006 • A/Solomon Islands/3/2006 (H1N1) • A/Brisbane/10/2007 (H3N2) • A/Brisbane/59/2007 (H1N1) • A/Wisconsin/67/2005 (H3N2) • B/Malaysia/2506/2004
2006-07	• A/New Caledonia /20/99 (H1N1) • Either A/Hiroshima/52/2005 (H3N2) or A/Wisconsin/67/2005 (H3N2) • B/Malaysia/2506/2004	• A/Wisconsin/67/2005 (H3N2) • A/New Caledonia/20/1999 (H1N1) • B/Malaysia/2506/2004 • B/Shanghai/361/2002
2005-06	• A/New Caledonia/20/99 (H1N1) • A/New York/55/2004 (H3N2) • B/Jiangsu/10/2003	• A/California/7/2004 (H3N2) • A/New Caledonia/20/1999 (H1N1) [similar to H1N1 and H3N2 strains of 2005-06 vaccine]

2004-05	• A/New Caledonia/20/99 (H1N1) • A/Wyoming/3/2003 (H3N2) • B/Jiangsu/10/2003	• A/Fujian/411/2002(H3N2) [similar to H3N2 strain of 2004-05 vaccine] • A/California/7/2004-like virus.
2003-04	• A/Panama/ 2007/99 (H3N2) • A/New Caledonia/20/99 (H1N1) • Either B/Hong Kong/330/2001 or B/Shangdong/7/97	• A/Fujian/411/2002 (H3N2) • A/Panama/2007/99 (H3N2) • A/New Caledonia/20/99-like (H1N1) • Influenza B [similar to B/Hong Kong/330/2001 strain for 2003-04 vaccine]

In this study, the influenza vaccine appeared to be well tolerated in the majority of subjects with inflammatory bowel disease. Only one subject developed a reportable adverse reaction of pancreatitis. However, because of the subject's previous history of pancreatitis, the current episode of pancreatitis was not conclusively caused by influenza vaccination. The studies by Mamula et al. and Lu et al. reported no serious adverse events (102,103).

In this study, of 49 subjects with inactive or mild pre-vaccination disease activity, only 1 had a change to moderate or severe post-vaccination disease activity. No subjects in this study required hospitalization or surgery related to inflammatory bowel disease in the 4 weeks post-vaccination. In the study by Lu et al., 6 of 146 subjects with inflammatory bowel disease required hospitalization for inflammatory bowel disease-related issues in the follow-up period; this included 4 patients with electively-scheduled colectomies and 2 patients with flares of inflammatory bowel disease (103). However, the authors report that none of these hospitalizations were felt to be related to influenza vaccination. In this study along with the studies by Mamula et al. and Lu et al., the

influenza vaccine did not appear to significantly worsen inflammatory bowel disease activity scores (102,103).

7.2 Threats to Validity and Limitations of the Study

Internal validity is the absence of bias or systematic error in a study (133). The evaluation of internal validity determines whether an estimate of the association between an exposure and an outcome of a sample is biased or systematically distorted from the true population value due to a defect in the study design or source of bias (134). Bias may be introduced by the methods employed to identify and recruit subjects (selection bias), the measurement of information on exposure or outcome (measurement bias), or by confounding. Confounding occurs when there is a mixing of the effect of the exposure on the outcome because of an association between the exposure with a third factor that influences the outcome (134).

Selection bias is a systematic error that results from the procedures used to select subjects and from factors that influence study participation; it occurs when the association between the exposure and outcome differs for those who participate and those who do not participate in the study (134). A defect may occur in the methods used to sample, select, or retain subjects in a study such that the probability of being selected into a study depends on both the exposure and outcome. For the Primary Objective and Secondary Objective #2 of estimating immunogenic response and serologic protection, respectively, in subjects with inflammatory bowel disease, there was no exposure or classification into exposed and non-exposed groups. For Secondary Objectives #1 and #3 of comparing immunogenic response and serologic protection, respectively, between groups of subjects with inflammatory bowel disease and sibling controls, the exposed

group was subjects with inflammatory bowel disease and the non-exposed group was sibling control subjects. There was no selection bias when comparing these two groups. Both groups had similar proportions with previous history of influenza vaccination (50% and 45% for subjects with inflammatory bowel disease and sibling controls, respectively). For Secondary Objectives #1 and #3 of comparing immunogenic response and serologic protection, respectively, between the groups of immunosuppressed and non-immunosuppressed subjects with inflammatory bowel disease, the exposed group was subjects on immunosuppressive therapy and the non-exposed group was subjects not on immunosuppressive therapy. There was no selection bias when comparing these two groups. Both groups had similar proportions with previous history of influenza vaccination (45% and 61% for immunosuppressed and non-immunosuppressed subjects, respectively). Though there were more immunosuppressed subjects (n=42) than non-immunosuppressed subjects (n=18) enrolled in the study, this reflects the general pattern of frequent use of immunosuppressive therapies in pediatric patients with inflammatory bowel disease. Overall, a high proportion of subjects with inflammatory bowel disease (98%) and controls (96%) completed the study with both pre-vaccination and post-vaccination hemagglutination-inhibition titers.

In this study, the larger target population for the inflammatory bowel disease cohort was patients with inflammatory bowel disease between 2 to 17 years of age. In comparison, the accessible population was those eligible patients living in or near the City of Calgary followed by the Pediatric Gastroenterology clinic at the Alberta Children's Hospital who were identified between September 1, 2008 and January 1, 2009. Subjects were selected through nonprobability, convenience sampling in that

potential subjects were identified and contacted by mail, telephone, or in person at the time of a visit to the Alberta Children's Hospital. To optimize enrolment, the influenza vaccine was offered to the entire family of patients with inflammatory bowel disease. Subjects were self-selected into this study. Self-selection may tend to yield a sample more likely to have had previous influenza vaccination and therefore have a high proportion with serologic protection due to cross-reactivity between influenza A strains; however only 50% of subjects with inflammatory bowel disease reported any prior influenza vaccination. In addition, a prior history of influenza vaccination should not affect the ability to mount an immunogenic response. The proportion of patients with a history of influenza vaccination among patients at the Pediatric Gastroenterology clinic with inflammatory bowel disease who did not participate in this study was unavailable. However, vaccination records were available from 34 non-participants; of these 34 children with inflammatory bowel disease, only 9 (26%) had documentation of ever receiving a prior influenza vaccination. In the literature, varying studies yield differing proportions; these studies are also limited by their study design. In a survey of adults with inflammatory bowel disease by Melmed et al., only 28% of subjects at risk for influenza reported receiving regular influenza vaccinations (101). In a study of children with inflammatory bowel disease by Lu et al., 73% of subjects reported receiving any previous influenza vaccine; however this higher proportion may be due to self-selection into the study sample (103). Self-selection may also tend to include subjects with more stable and milder disease activity. However, this study included a reasonable proportion of subjects with moderate to severe disease activity (19%). The distribution of disease activity in children with inflammatory bowel disease followed at the Pediatric

Gastroenterology clinic is not known, but can be expected to include all categories from inactive to severe, similar to disease activity of subjects with inflammatory bowel disease in this study. In this study, a large proportion of children with inflammatory bowel disease followed at the Pediatric Gastroenterology clinic who were excluded lived outside of the Calgary Health Region. Therefore, subjects in this study with inflammatory bowel disease and sibling controls differed from those who did not participate by being more localized to an urban setting rather than a rural setting. However, an urban residence compared to a rural residence should not affect the ability to generate an immunogenic response or serologic protection, exposure to immunosuppressive medications, disease activity, or adverse reactions from influenza vaccination.

External validity is the generalizability of the study findings to the wider population of interest (133). External validity may be assessed by comparing relevant characteristics of a study sample to the larger target population. In comparing the sample of subjects with inflammatory bowel disease in this study to the larger target population of children with inflammatory bowel disease, it is important to consider the following factors: previous influenza immunization (as discussed above), gender, diagnosis, age, disease classification, disease activity, and medication usage. For gender distribution, pediatric Crohn's disease is characterized by a male predominance which was found in this study (17 males and 9 females with Crohn's disease); in comparison, males and females are equally affected in pediatric ulcerative colitis which was also present in this study (12 males and 12 females with ulcerative colitis) (135). In pediatric inflammatory bowel disease, there is a preponderance of Crohn's disease compared to ulcerative colitis

(135). In this study, the distribution of subjects with Crohn's disease (26 subjects) compared to ulcerative colitis (24 subjects) was similar. This may be affected by the positively skewed age distribution of this study with a median age at enrolment of 15.3 years. Fear of bloodwork or immunization with intramuscular injections may have resulted in decreased enrolment of younger children into this study. Because Crohn's disease tends to have an earlier age predilection than ulcerative colitis (135), the older age distribution of subjects in this study may explain the equal distribution of Crohn's disease and ulcerative colitis in this study. Pediatric Crohn's disease tends to be ileocolonic in disease location and inflammatory in disease behaviour; similar findings were present in this study with 58% of subjects with Crohn's disease classified as ileocolonic in disease location, and 85% of subjects with Crohn's disease classified as inflammatory, non-penetrating, non-stricturing in disease behaviour (135). Pediatric ulcerative colitis demonstrates a predominance of pancolitis in disease extent which was also shown in this study with 89% of ulcerative colitis subjects classified with pancolitis. Though the majority of subjects with inflammatory bowel disease in this study had inactive or mild disease (81%), this study still included subjects with moderate to severe disease activity; in the target population of children with inflammatory bowel disease, disease activity may range across all categories of inactive to severe. Due to the high frequency of moderate to severe inflammatory bowel disease in children, immunosuppressive medications are frequently used in children (135). In this study, 42 subjects with inflammatory bowel disease were using at least one immunosuppressive medication at study enrolment and 18 subjects with inflammatory bowel disease were not on any immunosuppressive medications at study enrolment. Overall, the study sample

appears to be similar to the target population of children with inflammatory bowel disease. However, the older age distribution and more equal distribution between Crohn's disease and ulcerative colitis need to be taken into consideration. Only 3 of 60 subjects with inflammatory bowel disease were less than 9 years of age at study enrolment and children less than 2 years of age were excluded. An older age distribution should not affect the immunogenic response or post-vaccine serologic protection; however older subjects may be more likely to have previous exposure to influenza viruses through vaccination or infection and therefore more likely to have pre-vaccine serologic protection. Though Crohn's disease and ulcerative colitis have differing pathways of immune dysregulation, it is not clear at this time how these differences may affect immune response to vaccinations. Overall, this study has adequate external validity; the results from this study may be generalized to the larger target population of children over 9 years of age with inflammatory bowel disease.

Measurement bias can be minimized by having a clearly defined, objective outcome variable and by blinding the person measuring the outcome. In this study, the outcome for the Primary Objective was the proportion of subjects with inflammatory bowel disease who developed an immunogenic response to each strain of the influenza vaccine; an immunogenic response was defined as a four-fold or greater increase in hemagglutination-inhibition titers from pre-vaccine to post-vaccine. All pre-vaccine and post-vaccine serum samples were tested in parallel against all three components of the seasonal 2008 influenza vaccine with hemagglutination-inhibition assay performed at the Provincial Laboratory according to standard methods described in the World Health Organization protocol (129). To minimize measurement bias, the laboratory technicians

and virologist who performed the assays were blinded to the medical history of participants, including classification as subject with inflammatory bowel disease or sibling control, previous history of influenza vaccination, and immunosuppression status. It is important to note that standard hemagglutination-inhibition titers are a conservative method of measurement; they may underestimate the true titer by measuring the highest dilution that still completely inhibits hemagglutination. Thus this may result in underestimation of the proportion of subjects with inflammatory bowel disease who develop an immunogenic response or serologic protection to the influenza vaccine. However, in comparison between subjects with inflammatory bowel disease and sibling controls and between immunosuppressed and non-immunosuppressed subjects with inflammatory bowel disease, the effect of underestimation should impact all groups equally. In addition, the assay used in this study is considered standard methodology and has been used in previous studies evaluating influenza vaccine immunogenicity in other populations (96,97,102,103,106-113,120). Misclassification of the outcome (proportion developing an immunogenic response or serologic protection) because of underestimation of the hemagglutination-inhibition titer is unrelated to the exposure when comparing between groups (that is, between subjects with inflammatory bowel disease and siblings controls, and between immunosuppressed and non-immunosuppressed subjects with inflammatory bowel disease). Therefore this form of non-differential misclassification bias would produce estimates of effect that are diluted or closer to the null value than the actual effect.

For other sources of measurement, the distinction between the group of subjects with inflammatory bowel disease and the control group of healthy siblings was clearly

defined. Siblings were required to not have any known immunosuppressive medical conditions or be on any immunosuppressive medications. However, we recognize that an as yet undiagnosed immunodeficiency may have been present in any of the siblings. The etiology of inflammatory bowel disease involves genetic susceptibility; therefore, siblings of children with inflammatory bowel disease are at increased risk of developing inflammatory bowel disease. However, none of the siblings enrolled in the study were known to have manifestations of inflammatory bowel disease during the study. In addition, parents of children with inflammatory bowel disease would be more aware of symptoms and signs of inflammatory bowel disease and therefore perceptive of potential presentations of inflammatory bowel disease in siblings.

Another measurement is the classification of a subject with inflammatory bowel disease as immunosuppressed or non-immunosuppressed. No subjects changed from immunosuppressed to non-immunosuppressed status or vice versa during the study. Only 1 subject with inflammatory bowel disease changed from non-immunosuppressed to immunosuppressed status in the 1 month prior to starting this study; this subject began azathioprine 2 weeks prior. All other subjects remained in their immunosuppressed or non-immunosuppressed categories for at least 1 month prior to commencing this study. Given that this subject started an immunosuppressive medication 2 weeks prior to starting the study, there would be an adequate period of time for the medication to start impacting the immune system. For the 2 subjects with inflammatory bowel disease on systemic corticosteroids alone, the dose of prednisone used was at least 10 mg once daily.

To be a confounder, a factor must be associated with both the exposure and the outcome, and not be an intermediate variable between the exposure and outcome (134).

The strategies to control for confounding include stratification, randomization, restriction, matching, and modeling. Confounding can be minimized by measuring variables that may influence the outcome and assessing their influence in the analysis of the results.

In this study, a possible confounder may be the requirement of two doses of influenza vaccine in children < 9 years of age as these children required two doses of vaccine given ≥ 4 weeks apart if they were receiving the influenza vaccine for the first time, or if they had received only one dose of influenza vaccine during the previous influenza season as their first dose. However, only 1of 60 subjects with inflammatory bowel disease required two doses of influenza vaccine so it is unlikely that age and vaccine naivety was a confounder for subjects with inflammatory bowel disease. In comparison, 5 of 53 sibling control subjects required two doses of influenza vaccine. For siblings who only required one dose of influenza vaccine, 74%, 68%, and 74% developed an immunogenic response to A/Brisbane/10/2007 (H3N2), A/Brisbane/59/2007 (H1N1), and B/Florida/4/2006 antigens, respectively. In contrast, for siblings who required two doses of influenza vaccine, 100%, 80%, and 80% developed an immunogenic response to A/Brisbane/10/2007 (H3N2), A/Brisbane/59/2007 (H1N1), and B/Florida/4/2006 antigens, respectively. When the analysis is restricted to subjects receiving only one dose of influenza vaccine, comparison of the proportion with immunogenic response between subjects with inflammatory bowel disease and sibling controls yields the same results. That is, there is no difference between the proportion of subjects with inflammatory bowel disease and sibling controls who developed an immunogenic response to the two influenza A strains; however, a lower proportion of subjects with inflammatory bowel disease (51%) developed an immunogenic response to the influenza B strain compared to

sibling controls (81%, p=0.001). These results of the restricted analysis to subjects aged 9 years or older are similar to the results of the crude analysis; therefore, age is not a confounder.

Another potential confounder is previous history of influenza vaccination for Secondary Objective #1. Subjects' previous history of influenza vaccination may be influenced by health status (healthy control or diagnosis of inflammatory bowel disease) or by immunosuppressive medication use. In addition, a previous history of influenza vaccination may affect the immunogenic response as cross-reactivity may occur between similar strains of influenza virus. However, when the crude and stratified (by previous history of influenza vaccination) odds ratio of immunogenic response to each vaccine strain comparing immunosuppressed to non-immunosuppressed subjects with inflammatory bowel disease were calculated, the 95% CI of all odds ratios crossed one. Likewise, when the crude and stratified (by previous history of influenza vaccination) odds ratio of immunogenic response to each vaccine strain comparing subjects with inflammatory bowel disease to sibling controls were calculated, the 95% CI of all odds ratios crossed one. In addition, the proportion of subjects with previous influenza vaccination was similar across all groups (subjects with inflammatory bowel disease and sibling controls, and immunosuppressed and non-immunosuppressed subjects with inflammatory bowel disease). Therefore, previous influenza vaccination is not a confounder.

Another potential confounder for the effect of immunosuppressive medication use on immunogenic response or serologic protection (Secondary Objectives #1 and 3, respectively) is disease activity. Subjects with more active disease are more likely to be

on immunosuppressive medication. However, when the crude and stratified (by inactive / mild disease and moderate / severe disease) odds ratio of immunogenic response or serologic protection to each vaccine strain comparing immunosuppressed to non-immunosuppressed subjects with inflammatory bowel disease were calculated, the 95% CI of all odds ratios crossed one. In addition, there was no difference in the proportion of subjects with inactive or mild disease activity who developed an immunogenic response or serologic protection compared to those with moderate or severe disease activity.

In this study, the goal of enrolling 80 subjects with inflammatory bowel disease was not achieved. Instead, only 60 subjects with inflammatory bowel disease completed the study and the proportion who developed an immunogenic response ranged between 0.53 to 0.72, depending on the specific strain of influenza. However, there is not a marked variation in 95% CI widths for sample sizes of 60 subjects compared to 80 subjects (Table 6). In addition, the estimates from the sample of subjects with inflammatory bowel disease overall demonstrate adequate internal and external validity, as shown above. In this study, between 76% to 83% of sibling control subjects and 53% to 72% of subjects with inflammatory bowel disease developed an immunogenic response to the influenza vaccine (depending on vaccine strain). Table 7 shows the sample size requirement for various immunogenic response rates for subjects with inflammatory bowel disease and sibling controls. Where there was a statistically significant difference of proportions achieving immunogenic response comparing the subjects with inflammatory bowel disease (0.53, n=60) to sibling controls (0.81, n=53), the power to detect this difference was 0.85 based on an alpha of 0.05. For comparisons between specific therapies in inflammatory bowel disease, this study was not designed

nor powered to detect differences in immunogenic response between different groups of immunosuppressive medications. A limitation of this study is the potential effect of multiple comparisons for the secondary objectives; the multiple comparisons performed without any adjustment of the p value comparing immunogenic response or serologic protection between children with inflammatory bowel disease and sibling controls and between immunosuppressed and nonimmunosuppressed children with inflammatory bowel disease could lead to an increased risk of a difference between significant purely by chance.

In this study, a control group was used to determine the immunogenicity and safety of influenza vaccination in healthy non-immunosuppressed children. The control group consisted of non-immunosuppressed siblings of children with inflammatory bowel disease followed at the Alberta Children's Hospital. This control group was selected for convenience, along with ease of accessibility and recruitment. A disadvantage of using siblings is that immunodeficiencies may be inherited and therefore present in multiple members of a family. However, no siblings with known immunodeficiencies were enrolled in this study. In addition, the etiology of inflammatory bowel disease involves genetic susceptibility. However, no siblings were known to have clinical manifestations of inflammatory bowel disease at enrolment or during the study.

In this study, the measures of immunogenicity selected were immunogenic response (four-fold or greater increase in hemagglutination-inhibition antibody titers from pre-vaccine to post-vaccine) and serologic protection (hemagglutination-inhibition antibody titers of 1:40 or greater). These outcomes were selected because of their utility in measuring the ability of the immune system to respond to influenza vaccination and

the correlation as a marker for protection against disease. However, a limitation of this study is that the ideal outcome for a vaccine study is clinical protection from disease (that is, an efficacy study). Our ability to conduct an efficacy study assessing clinical protection from disease was limited by the requirement for a larger sample size and longer duration of follow-up.

7.3 Implications of the Study's Findings

The results of this study suggest that individuals with inflammatory bowel disease are able to mount an appropriate immune response and be serologically protected against the A strains of the influenza vaccine compared to sibling controls; however individuals with inflammatory bowel disease appear to be less likely to mount an appropriate immune response and be serologically protected against the B strain of the influenza vaccine, especially if on immunosuppressive therapy.

A potential future consideration for individuals with inflammatory bowel disease, especially those on immunosuppressive therapy, would be to administer two doses of influenza vaccine during an influenza season may to boost immune response and achieve serologic protection. Soesman et al. demonstrated that two doses of influenza vaccine improved hemagglutination-inhibition antibody titers in immunosuppressed adult liver transplant recipients with the proportion of subjects with serologic protection against all 3 influenza strains increasing from 68% after the first dose to 80% after the second dose of influenza vaccine administered 28 days later (109). Another potential strategy is to increase the amount of influenza B antigen in the vaccine to boost immune response and achieve serologic protection. A recent review on vaccination strategies for patients with inflammatory bowel disease on immunosuppressive therapy by Melmed recommends that

adequate immune response should be ascertained for patients requiring immunization while immunosuppressed whenever possible, and repeat dosing may be considered when immune response to immunization is insufficient (66). Though the Public Health Agency of Canada advises for close monitoring of immunocompromised individuals after vaccination and aggressive boosting to optimize magnitude and duration of vaccine-induced immunity, it currently reports insufficient evidence to recommend two doses of influenza vaccine during an influenza season to boost immunity in immunocompromised individuals (132).

The findings of this study demonstrate that the influenza vaccine is overall safe in individuals with inflammatory bowel disease and does not typically exacerbate inflammatory bowel disease activity.

The Public Health Agency of Canada recognizes the steadily growing population of immunocompromised people in Canada due to a variety of reasons, including subtle immunodeficiencies associated with chronic illnesses and the expanding spectrum of illnesses managed with immunomodulatory medications (136). The 7th Edition of the Canadian Immunization Guide advises that there are no contraindications to the use of inactivated vaccine in immunocompromised individuals and special attention should be paid to the completion of childhood immunizations, annual influenza immunization and pneumococcal vaccination (136). The Public Health Agency of Canada recommends that the optimal timing for administration of inactivated vaccines to immunocompromised individuals is ideally at least 14 days before the initiation of immunosuppressive therapy or at least 3 months after stopping immunosuppressive therapy (136). However, if immunosuppressive therapy cannot be stopped, inactivated vaccines should be given

when immunosuppressive therapy is at the lowest possible level. In addition, inactivated vaccines may be administered during immunosuppressive therapy for post-exposure or outbreak management. Therefore, the factors of intensity of immunosuppressive therapy, underlying disease, and necessity of vaccination all need to be taken into consideration for the timing of inactivated vaccinations for immunocompromised individuals. The Public Health Agency of Canada also counsels to consider the immunization environment broadly and therefore to vaccinate household contacts when appropriate (136).

7.4 Recommendations for Future Research

In this study, hemagglutination-inhibition antibody titers were used as a measure of the immune response to influenza. However, the ideal outcome for vaccine studies is clinical protection from disease. An efficacy study assessing clinical protection from disease though requires a larger sample size and longer duration of follow-up. Nevertheless, because of the superior merit of efficacy as a clinically meaningful outcome, a multi-center study on the efficacy of influenza vaccine in children with inflammatory bowel disease should be pursued.

The results of this study along with other studies of influenza vaccination in patients with inflammatory bowel disease demonstrate that patients with inflammatory bowel disease are less likely to mount an appropriate immune response and develop serologic protection against influenza B compared to controls without inflammatory bowel disease, especially if immunosuppressive therapy is also used (102,103). In addition, the results of these studies suggest patients on more potent immunosuppression (that is, biologic agents or a combination of a biologic agent with another immunosuppressive therapy) are less likely to develop serologic protection to the influenza B strain of the vaccine.

However, this study was not designed nor powered to detect differences in immunogenic response between different groups of immunosuppressive medications. A future multi-center study powered to detect differences between different types of immunosuppressive medications for immune response and serologic protection would be important. Soesman et al. demonstrated that two doses of influenza vaccine improve hemagglutination-inhibition antibody titers in immunosuppressed adult liver transplant recipients (109). Therefore, a future research study comparing the immune response or serologic protection from one versus two doses of influenza vaccine in subjects with inflammatory bowel disease is recommended.

Because patients with inflammatory bowel disease on immunosuppressive therapy are less likely to mount an appropriate immune response and develop serologic protection against influenza B compared to non-immunosuppressed patients, an important target group for influenza vaccination is patients with inflammatory bowel disease on biologic therapy, the most powerful class of medication for inflammatory bowel disease. The most commonly used biologic agent is infliximab, which is typically administered every 8 weeks by intravenous infusion. The optimal timing for influenza vaccine administration for a patient on infliximab therapy is not known. A future study comparing the immune response and serologic protection for subjects on infliximab administered the influenza vaccine at varying time points during their infliximab cycle is recommended. The study may compare subjects administered the influenza vaccine mid-way between the infusion dates (that is, 4 weeks after receiving infliximab) to those administered the vaccine shortly before receiving infliximab (that is, 1 week prior to the next infliximab dose). The study would be beneficial for determining the optimal timing

of the influenza vaccination to maximize immune response and serologic protection in patients with inflammatory bowel disease on infliximab therapy. A similar study by Elkayam et al. evaluating immunogenic response to influenza vaccine in adults with rheumatoid arthritis or ankylosing spondylitis on infliximab therapy showed that there was no difference in the proportions of subjects developing a response when comparing vaccination at time of infliximab infusion to vaccination 3 weeks post-infliximab infusion (137). However, subjects with rheumatoid arthritis on infliximab therapy vaccinated 3 weeks post-infusion had a lower increase in geometric mean titers. A study evaluating the optimal timing of influenza vaccination in inflammatory bowel disease is still important to perform as the disease state of inflammatory bowel disease is different than rheumatoid arthritis or ankylosing spondylitis; in addition, the current standard doses of infliximab used in inflammatory bowel disease are higher than the mean dose of 3 mg/kg used in the study by Elkayam et al (137).

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APPENDIX A: Consent Forms

Consent Form for Children with Inflammatory Bowel Disease

Study title: IMMUNOGENICITY AND SAFETY OF INFLUENZA VACCINATION IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE

Principal Investigator: Dr. Iwona Wrobel, Alberta Children's Hospital, (403) 955-7721
Co-Investigators: Dr. Jennifer deBruyn, Dr. Otto Vanderkooi, Dr. Jennifer Athayde, Dr. Robert Hilsden, Alberta Children's Hospital, (403) 955-7721

"You" may refer to you, your child, or your ward depending if you are a patient, parent, or legal guardian, respectively. You are being asked to give consent for yourself, your child, or your ward to take part in this research study. This consent form is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your child's participation will involve. Please inform us if you would like more detail about something mentioned here or information not included here. Take the time to read this carefully and to understand any accompanying information. You will receive a copy of this form.

BACKGROUND

Influenza (also known as the flu) is a common viral infection. It causes fever, headache, fatigue, and muscle aches. Also, sore throat, runny and stuffy nose, cough, red eyes, abdominal pain, nausea, and vomiting may occur. Health Canada recommends a yearly influenza vaccine (also known as the flu shot) for children at increased risk of severe complications from the flu or flu-associated clinic, emergency department, or hospital visits. This includes children with chronic illnesses (like inflammatory bowel disease). This also includes children on drugs that suppress their immune system. These drugs are used to control inflammatory bowel disease symptoms. They include azathioprine, methotrexate, and infliximab. Catching the flu may lead to worsening of inflammatory bowel disease symptoms. Also, children on drugs that suppress the immune system may be at increased risk of catching an infection and developing a more severe infection. Thus, it is important to give the flu shot to all children with inflammatory bowel disease. However, we do not yet know how much protection children on immune-suppressing drugs will develop. We are inviting 80 children with inflammatory bowel disease to join our study.

WHAT IS THE PURPOSE OF THE STUDY?

This study will look at how well the flu shot works in children with inflammatory bowel disease. We will collect a blood test for an immune response. We will also look at any potential vaccine-related side effects, follow inflammatory bowel disease activity, and follow any flu-like illnesses during the flu-season. If your child is on an immune-suppressing drug, we will record its type and dose to determine whether it affects immune response to vaccine, flu-like illnesses, inflammatory bowel disease activity, or vaccine-related side effects. Lastly, we will look at the immune response in children with

inflammatory bowel disease compared to their siblings, who we expect to mount a normal response.

WHAT WOULD MY CHILD HAVE TO DO?

This study will take place this fall and winter season of 2008. Your child will come to the pediatric gastroenterology clinic at the Alberta Children's Hospital for two visits.

1. Baseline visit:

At the first visit, we will collect demographic data (name, date of birth, address, and telephone number). Your child's medical history will be recorded. We will collect information on the number of stools (day, night), stool consistency, urgency, blood in the stool, activity level, and general well-being. A physical exam will be done. Your child will have a blood test. After the blood test, we will give the flu shot to your child. The duration of the first visit will be about 30 to 45 minutes.

You will be given a diary to record symptoms on a weekly basis for the next four weeks. You should also record the name and dose of any new drugs taken. [If your child is able to use a diary and has been using it for some time, he/she may complete it.]

2. Telephone follow-up:

We will telephone you 3 to 4 days after your child's flu shot to check for any adverse reactions. An adverse reaction to a vaccine is a response, which is more severe than usual, or is unusual / unexpected in character. This will take about 5 minutes.

3. Follow-up visit:

After 2 to 4 weeks, your child will return for a second visit ("Follow-up visit"). We will record any symptoms. A physical exam will be done. We will also review and collect the diary. A repeat blood test will be collected. The duration of the visit will be about 15 to 30 minutes.

4. Children with flu-like symptoms:

If your child develops a flu-like illness during the flu season (October to March), phone us at (403) 955-3198. These symptoms include fever AND cough AND one or more of the following: muscle aches, joint aches, sore throat, or fatigue. In children less than 5 years old, a fever is not required and symptoms of abdominal pain, nausea, vomiting, and diarrhea may be present. We will instruct your child to have a nose swab and blood test. This will check for influenza infection and check if your child's influenza strain matches any strains in the influenza vaccine.

5. Children < 9 years of age:

We will follow the recommendations by Health Canada for the flu shot in children < 9 years of age. If this is his/her first year of the flu shot, we will give two doses of the flu shot four weeks apart. For these children, the first visit will be identical to that described above in the "Baseline visit." A second visit will occur four weeks later when the second dose of the flu shot will be given. A third and final visit will occur two to four weeks later with identical events to the "Follow-up visit" described above.

WHAT ARE THE RISKS?

Taking part in this study may involve risks that are unforeseeable.

Venipuncture: This is a routine practice for getting blood samples. A needle will be put into a vein. Blood will be drawn into small tubes for lab tests. There may be a small amount of pain and/or bruising at the site where blood is drawn. A cream called EMLA may be put on before with a sticky patch to make it hurt less or not at all. Very rarely, there may be an allergic reaction to EMLA with hives, itchy skin, swelling, or difficulty breathing. Your child will be monitored by health care professionals while EMLA is being applied.

The flu shot: It is given via needle, usually into the arm. The expected side effects of the flu shot include: soreness, redness, or swelling at injection site; fever; headache; muscle ache; malaise; joint ache; shivering; sweating; fatigue; nausea; vomiting; and diarrhea. These side effects can be expected 6-12 hours after the flu shot and can last for 1-2 days. As mentioned above, the flu shot is recommended annually for all children with chronic diseases and children on drugs that suppress their immune system.

You will be advised at the start of the study to report freely and immediately to the medical staff any adverse events. You will also note in the diary any and all side effects (serious and non-serious) for the full length of the study.

WHAT ARE THE BENEFITS?

If you agree for your child to participate in this study, your child should benefit from receiving a flu shot to protect against the flu. This study will also provide information that may benefit future children with inflammatory bowel disease and help us to provide better vaccine protection in the future for children with inflammatory bowel disease.

DOES MY CHILD HAVE TO PARTICIPATE?

Taking part in this study is voluntary. If you choose not to have your child join or to stop participation, there will be no penalty or loss of benefits to which your child would otherwise be entitled. The relationship with your child's doctor and right to medical care will not be affected. You may withdraw consent at any time. Your child may be asked to stop the study by your doctor or the ethics board. If new information becomes available that might affect your willingness to participate in the study, we will inform you as soon as possible.

WILL WE BE PAID FOR PARTICIPATING OR DO WE HAVE TO PAY FOR ANYTHING?

There is no cost to participate in this study, or to receive blood tests or the influenza vaccine.

IF MY CHILD SUFFERS A RESEARCH-RELATED INJURY, WILL WE BE COMPENSATED?

In the event that your child suffers injury as a result of participating in this research, no compensation will be provided to you by the University of Calgary, the Calgary

Health Region, or the Researchers. You still have all your legal rights. Nothing said in this consent form alters your right to seek damages.

WILL MY CHILD'S RECORDS BE KEPT PRIVATE?

The information gathered on your child will be treated confidentially. Your child's name will not be mentioned in any verbal or written report. Your child's records may be reviewed to ensure accuracy. This will be done by authorized representatives of the University of Calgary Conjoint Health Research Ethics Board and regulatory agencies. The results of this study may be published in the medical literature. Your child's name will not appear on any data released. Only a patient number and initials will be shown.

All information will be kept private. The exception is when professional codes of ethics or legislation (or the law) require reporting. The information you give will be kept for at least seven years after the study is done. It will be kept in a safe area (i.e. locked filing cabinet). Your child's name or any other identifying information will not be attached to the information. The information gathered for this study may be looked at again in the future to help us answer other study questions. If so, the ethics board will first review the study. This will be done to ensure the information is used ethically.

CONTACTS FOR QUESTION / INFORMATION

For any concerns or problems, you should contact:

Dr. Iwona Wrobel or Dr. Jennifer deBruyn at (403) 955-7721

With any treatment or medical procedure, unforeseen effects are always possible.

If the case is urgent, contact the pediatric gastroenterologist on-call at the Alberta Children's Hospital at (403) 955-7211. You should go to the closest emergency department if this person cannot be reached.

If you have any concerns about any aspect of this study, you may contact the Ethics Resource Office, Internal Awards, Research Services, University of Calgary, at (403) 220-3782.

The doctor will provide you with any new information that may be learned in the study.

SIGNATURES

Your signature on this form indicates that you have understood to your satisfaction the information regarding your child's participation in the research project and agree to their participation as a subject. In no way does this waive your legal rights nor release the investigators, or involved institutions from their legal and professional responsibilities. You are free to withdraw your child from the study at any time without jeopardizing their health care.

Do you want the investigator(s) to inform your family doctor or pediatrician that your child is participating in this research study? Yes No

If so, please provide the doctor's name: _____

This study was explained to me by: _____

I agree for my child, _____, to take part in this study.

_____	_____	_____
Parent / Guardian's printed name	Signature	Date
_____	_____	_____
Child's printed name	Signature if applicable	Date
_____	_____	_____
Investigator/delegate's printed name	Signature	Date
_____	_____	_____
Witness' printed name	Signature	Date

The investigator or a member of the research team will, as appropriate, explain to your child the research and his or her involvement. They will seek your child's ongoing cooperation throughout the study.

The University of Calgary Conjoint Health Research Ethics Board has approved this research study.

A signed copy of this consent form has been given to you to keep for your records and reference.

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____	_____
Signature of Investigator or Designee	Date

Consent Form for Siblings of Children with Inflammatory Bowel Disease

Study title: IMMUNOGENICITY AND SAFETY OF INFLUENZA VACCINATION IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE

Principal Investigator: Dr. Iwona Wrobel, Alberta Children's Hospital, (403) 955-7721
Co-Investigators: Dr. Jennifer deBruyn, Dr. Otto Vanderkooi, Dr. Jennifer Athayde, Dr. Robert Hilsden, Alberta Children's Hospital, (403) 955-7721

Your child has been invited to participate in this research study because he / she is a sibling of a child with inflammatory bowel disease.

"You" may refer to you, your child, or your ward depending if you are a patient, parent, or legal guardian, respectively. You are being asked to give consent for yourself, your child, or your ward to take part in this research study. This consent form is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your child's participation will involve. Please inform us if you would like more detail about something mentioned here or information not included here. Take the time to read this carefully and to understand any accompanying information. You will receive a copy of this form.

BACKGROUND

Influenza (also known as the flu) is a common viral infection. It causes fever, headache, fatigue, and muscle aches. Also, sore throat, runny and stuffy nose, cough, red eyes, abdominal pain, nausea, and vomiting may occur. The Centers for Disease Control and Prevention recommends yearly influenza vaccination (also known as the flu shot) for children at increased risk for severe complications from the flu or flu-associated clinic, emergency department, or hospital visits. This includes children with chronic illnesses (including inflammatory bowel disease) and children on medications that suppress their immune system. These drugs are used to control inflammatory bowel disease symptoms. Catching the flu may lead to worsening of inflammatory bowel disease symptoms. Also, children on drugs that suppress the immune system may be at increased risk of infection and development of severe infection. Therefore, it is important to give the flu shot to all children with inflammatory bowel disease. However, we do not yet know if children on immune-suppressing drugs will have a good response to the vaccine and if the vaccine will trigger any inflammatory bowel disease symptoms. It is also recommended to give the flu shot to household contacts, such as siblings, of children with inflammatory bowel disease or children on immune-suppressing drugs. We are inviting 80 siblings to join our study.

WHAT IS THE PURPOSE OF THE STUDY?

This study will look at how well the flu shot works in children with inflammatory bowel disease. We will collect a blood test to check for an immune response. We will compare immune responses between children with inflammatory bowel disease and their healthy siblings, who are expected to mount a normal response.

WHAT WOULD MY CHILD HAVE TO DO?

The entire study will last over the fall and winter season of 2008. The study requires that your child come to the pediatric gastroenterology clinic at the Alberta Children's Hospital for two visits.

1. Baseline visit

At the first visit, we will collect demographic data (name, date of birth, address, and telephone number) and medical history. Your child will have a blood test drawn. After the blood test, we will give the flu shot to your child in the clinic. The duration of the first visit will be about 15 to 30 minutes.

2. Telephone follow-up

We will telephone you 3 to 4 days after your child's flu shot to check for any adverse reactions. An adverse reaction to a vaccine is a response, which is more severe than usual, or is unusual / unexpected in character. This will take about 5 minutes.

3. Follow-up visit

After 2 to 4 weeks, your child will return for a second visit to the clinic where a repeat blood test will be drawn. The duration of the second visit will be about 15 minutes.

4. Children <9 years of age

We will follow the recommendations by Health Canada for the flu shot in children < 9 years of age. If this is his/her first year of the flu shot, we will give two doses of the flu shot four weeks apart. For these children, the first visit will be identical to that described above in the "Baseline visit." A second visit will occur four weeks later when the second dose of the flu shot will be given. This will take about 10-15 minutes. A third and final visit will occur two to four weeks later with identical events to the "Follow-up visit" described above.

WHAT ARE THE RISKS?

Taking part in this study may involve risks that are unforeseeable.

Venipuncture: This is a routine practice for getting blood samples. A needle will be put into a vein. Blood will be drawn into small tubes for lab tests. There may be a small amount of pain and/or bruising at the site where blood is drawn. A cream called EMLA may be put on before with a sticky patch to make it hurt less or not at all. Rarely, there may be an allergic reaction to EMLA including hives, swelling, itchy skin, or difficulty breathing. Your child will be monitored by health care professionals while EMLA is being applied.

The flu shot: It is given via needle, usually into the arm. The expected side effects of the flu shot include: soreness, redness, or swelling at injection site; fever; headache; muscle ache; malaise; joint ache; shivering; sweating; fatigue; nausea; vomiting; and diarrhea. These side effects can be expected 6-12 hours after the flu shot and can last for 1-2 days. As mentioned above, the influenza vaccine is recommended annually for all children with chronic diseases and children on drugs that suppress their immune system.

You will be advised at the start of the study to report freely and immediately to the medical staff any adverse events.

WHAT ARE THE BENEFITS?

If you agree for your child to participate in this study, your child should benefit from receiving a flu shot to protect against the flu. This study will also provide information that may benefit future children with inflammatory bowel disease and help us to provide better vaccine protection in the future for children with inflammatory bowel disease.

DOES MY CHILD HAVE TO PARTICIPATE?

Taking part in this study is voluntary. If you choose not to have your child join or to stop participation, there will be no penalty or loss of benefits to which your child would otherwise be entitled. The relationship with your child's doctor and right to medical care will not be affected. You may withdraw consent at any time. Your child may be asked to stop the study by your doctor or the ethics board. If new information becomes available that might affect your willingness to participate in the study, we will inform you as soon as possible.

WILL WE BE PAID FOR PARTICIPATING OR DO WE HAVE TO PAY FOR ANYTHING?

There is no cost to participate in this study or to receive blood tests and the influenza vaccine.

IF MY CHILD SUFFERS A RESEARCH-RELATED INJURY, WILL WE BE COMPENSATED?

In the event that your child suffers injury as a result of participating in this research, no compensation will be provided to you by the University of Calgary, the Calgary Health Region, or the Researchers. You still have all your legal rights. Nothing said in this consent form alters your right to seek damages.

WILL MY CHILD'S RECORDS BE KEPT PRIVATE?

The information gathered on your child will be treated confidentially. Your child's name will not be mentioned in any verbal or written report. Your child's records may be reviewed to ensure accuracy. This will be done by authorized representatives of the University of Calgary Conjoint Health Research Ethics Board and regulatory agencies. The results of this study may be published in the medical literature. Your child's name will not appear on any data released. Only a patient number and initials will be shown.

All information will be kept private. The exception is when professional codes of ethics or legislation (or the law) require reporting. The information you give will be kept for at least seven years after the study is done. It will be kept in a safe area (i.e. locked filing cabinet). Your child's name or any other identifying information will not be attached to the information. The information gathered for this study may be looked at again in the future to help us answer other study questions. If so, the ethics board will first review the study. This will be done to ensure the information is used ethically.

CONTACTS FOR QUESTION / INFORMATION

For any concerns or problems, you should contact:
Dr. Iwona Wrobel or Dr. Jennifer deBruyn at (403) 955-7721.

With any treatment or medical procedure, unforeseen effects are always possible.

If the case is urgent, you can contact the pediatric gastroenterologist on-call at the Alberta Children's Hospital at (403) 955-7211. You should go to the closest emergency department if this person cannot be reached.

If you have any concerns about any aspect of this study, you may contact the Ethics Resource Offices, Internal Awards, Research Services, University of Calgary, at 220-3782. The doctor will provide you with any new information that may be learned in the study.

SIGNATURES

Your signature on this form indicates that you have understood to your satisfaction the information regarding your child's participation in the research project and agree to their participation as a subject. In no way does this waive your legal rights nor release the investigators, or involved institutions from their legal and professional responsibilities. You are free to withdraw your child from the study at any time without jeopardizing their health care.

Do you want the investigator(s) to inform your family doctor or pediatrician that your child is participating in this research study? Yes No

If so, please provide the doctor's name: _____

This study was explained to me by: _____

I agree for my child, _____, to take part in this study.

_____	_____	_____
Parent / Guardian's printed name	Signature	Date
_____	_____	_____
Child's printed name	Signature if applicable	Date
_____	_____	_____
Investigator/delegate's printed name	Signature	Date
_____	_____	_____
Witness' printed name	Signature	Date

The investigator or a member of the research team will, as appropriate, explain to your child the research and his or her involvement. They will seek your child's ongoing cooperation throughout the study.

The University of Calgary Conjoint Health Research Ethics Board has approved this research study.

A signed copy of this consent form has been given to you to keep for your records and reference.

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____ _____

Signature of Investigator or Designee Date