Assessing inflammatory bowel disease-associated antibodies in Caucasian and First Nations cohorts

Charles N Bernstein MD[1,2], Hani El-Gabalawy MD[2], Michael Sargent[1,2], Carol J Landers PhD[4], Patricia Rawsthorne RN[1,2], Brenda Elias PhD[3], Stephan R Targan MD[4]

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BACKGROUND: First Nation populations in Canada have a very low incidence of inflammatory bowel disease (IBD). Based on typical infections in this population, it is plausible that the First Nations react differently to microbial antigens with a different antibody response pattern, which may shed some light as to why they experience a low rate of IBD.
OBJECTIVE: To compare the positivity rates of antibodies known to be associated with IBD in Canadian First Nations compared with a Canadian Caucasian population.
METHODS: Subjects with Crohn's disease, ulcerative colitis (UC), rheumatoid arthritis (RA) (as an immune disease control) and healthy controls without a personal or family history of chronic immune diseases, were enrolled in a cohort study aimed to determine differences between First Nations and Caucasians with IBD or RA. Serum from a random sample of these subjects (n=50 for each of First Nations with RA, First Nations controls, Caucasians with RA, Caucasians with Crohn's disease, Caucasians with UC and Caucasians controls, and as many First Nations with either Crohn's disease or UC as could be enrolled) was analyzed in the laboratory for the following antibodies: perinuclear antineutrophil cytoplasmic antibody (pANCA), and four Crohn's disease-associated antibodies including anti-*Saccharomyces cerevisiae*, the outer membrane porin C of *Escherichia coli*, I2 – a fragment of bacterial DNA associated with *Pseudomonas fluorescens*, and the bacterial flagellin CBir-1. The rates of positive antibody responses and mean titres among positive results were compared.
RESULTS: For pANCA, First Nations had a positivity rate of 55% in those with UC, 32% in healthy controls and 48% in those with RA. The pANCA positivity rate was 32% among Caucasians with RA. The rates of the Crohn's disease-associated antibodies for the First Nations and Caucasians were comparable. Among First Nations, up to one in four healthy controls were positive for any one of the Crohn's disease-associated antibodies. First Nations had significantly higher pANCA titres in both the UC and RA groups than Caucasians
DISCUSSION: Although First Nation populations experience a low rate of IBD, they are relatively responsive to this particular antibody panel.
CONCLUSIONS: The positivity rates of these antibodies in First Nations, despite the low incidence of IBD in this population, suggest that these antibodies are unlikely to be of pathogenetic significance.

Key Words: *Antibodies; Crohn's disease; Ethnicity; Inflammatory bowel disease; Rheumatoid arthritis; Ulcerative colitis*

L'évaluation des anticorps liés aux MII chez des cohortes de blancs et des Premières nations

HISTORIQUE : Les populations des Premières nations du Canada présentent une très faible incidence de maladies inflammatoires de l'intestin (MII). D'après les infections classiques au sein de cette population, il est possible que les Premières nations réagissent différemment aux antigènes microbiens ayant un mode de réponse anticorps différent, ce qui pourrait jeter la lumière sur les raisons de leur taux peu élevé de MII.
OBJECTIF : Comparer les taux de positivité des anticorps qu'on sait associés aux MII au sein de Premières nations du Canada à ceux d'une population blanche du Canada.
MÉTHODOLOGIE : Les sujets atteints de maladie de Crohn, de colite ulcéreuse (CU), de polyarthrite rhumatoïde (PR) (une maladie immunitaire témoin) et des sujets témoins en santé sans antécédents personnels ou familiaux de maladie immunitaire chronique ont participé à une étude de cohorte visant à déterminer les différences entre les Premières nations et les blancs atteints d'une MII ou de PA. Les chercheurs ont analysé en laboratoire le sérum d'un échantillon aléatoire de ces sujets (n=50 pour chacun des membres des Premières nations atteints de PR, des sujets témoins des Premières nations, des blancs atteints de PR, des blancs atteints de maladie de Crohn, des blancs atteints de CU et de sujets témoins blancs, et autant de membres des Premières nations atteints de maladie de Crohn ou de CU qu'on pouvait inscrire) afin d'y déceler les anticorps suivants : anticorps antineutrophiles cytoplasmatiques des polynucléaires (AANCp) et quatre anticorps liés à la maladie de Crohn, y compris l'anti-*Saccharomyces cerevisiae*, la membrane externe de la porine C de l'*Escherichia coli*, I2 – un fragment d'ADN bactérien associé au *Pseudomonas fluorescens* – et la flagelline bactérienne CBir-1. Les chercheurs ont comparé les taux de réponses anticorps positives et les titres moyens parmi les résultats positifs.
RÉSULTATS : Pour ce qui est des AANCp, les Premières nations avaient un taux de positivité de 55 % s'ils étaient atteints de CU, de 32 % s'ils étaient des sujets témoins et de 48 % s'ils étaient atteints de PR. Le taux de positivité aux AANCp s'élevait à 32 % chez les blancs atteints de PR. Les taux d'anticorps associés à la maladie de Crohn étaient comparables chez les membres des Premières nations et les blancs. Chez les membres des Premières nations, jusqu'à un sujet témoin en santé sur quatre était positif à l'un des anticorps associés à la maladie de Crohn. Les titres d'AANCp étaient considérablement plus élevés dans les groupes des Premières nations atteints de CU ou de PR que chez les blancs.
EXPOSÉ : Même si les populations des Premières nations présentent un faible taux de MII, ils sont relativement réactifs à ce groupe précis d'anticorps.
CONCLUSIONS : Les taux de positivité de ces anticorps au sein des Premières nations, malgré la faible incidence de MII dans cette population, indique que ces anticorps sont peu susceptibles d'avoir une signification pathogène.

Inflammatory bowel disease (IBD) affects approximately 0.5% (approximately 200,000) of Canadians (1). We previously reported on the epidemiology of IBD in Manitoba (2) and across five provinces (1) using population-based administrative data. While the rates in Canada are among the highest in the world (1,3,4), the rates in British Columbia, particularly for Crohn's disease, are much lower than elsewhere in Canada (1,3). One potential explanation for this difference in British Columbia is that nearly 25% of the population are visible minorities, of which many are Asian immigrants, thereby underscoring the importance of exploring these diseases in different ethnic/ancestral groups. In Manitoba, we previously reported the markedly lower rates of IBD among First Nations (FN) people compared with Caucasians (5,6) by a factor of 3 to 4 in ulcerative colitis and by a factor of 10 to 12 for Crohn's disease. The fourfold increased rates of ulcerative colitis versus Crohn's disease among the FN are consistent with the greater rates of ulcerative colitis in emerging nations compared with Crohn's disease, with the reverse being the case among most contemporary western nations including the Caucasian community of Canada (7). Approximately 10% of Manitobans are FN, and approximately one- half live in the city of Winnipeg while one-half live in rural communities.

[1]*IBD Clinical & Research Centre;* [2]*Department of Internal Medicine;* [3]*Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba;* [4]*Cedars Sinai Medical Center, Los Angeles, California, USA*
Correspondence: Dr Charles N Bernstein, IBD Clinical & Research Centre, University of Manitoba, 804F-715 McDermot Avenue, Winnipeg, Manitoba R3E 3P4. Telephone 204-789-3369, fax 204-789-3972, e-mail cbernst@cc.umanitoba.ca
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TABLE 1
Study population

	Females, %	Age, years (mean)
CD, Caucasian (n=50)	50	39.9
CD, First Nations (n=9)	67	35
UC, Caucasian (n=50)	50	39
UC, First Nations (n=11)	55	46
RA, Caucasian (n=50)	84	59.7
RA, First Nations (n=50)	78	46.7
Controls, Caucasian (n=50)	77	41.7
Controls, First Nations (n=50)	57	34.5

CD Crohn's disease; RA Rheumatoid arthritis; UC Ulcerative colitis

The lower rates of IBD are similarly evident among both urban and rural FN. The lower rate of IBD in the FN population does not reflect a general reduction in autoimmune disease in this ethnic/ancestral group – they have a significantly increased prevalence of rheumatoid arthritis (RA) compared with Caucasians (8-16). Using Manitoba Health administrative data, Manitoba registered FN were found to have an RA prevalence rate of 2% – twice that of the surrounding, predominantly Caucasian population (17).

Hence, it is important to study communities with discrepant rates of disease to investigate whether there are genetic or environmental etiological clues. The present study was one aspect of a larger cohort study in which Manitobans with either Crohn's disease, ulcerative colitis or RA, or were healthy controls without any autoimmune diseases and without first-degree relatives with autoimmune diseases were enrolled. We included persons who were either Caucasian or FN (Canadian Institutes of Health Research Team Grant in Arthritis and IBD). We took a random sample of these subjects and explored their responsiveness to a panel of antibodies associated with IBD. These antibodies are used diagnostically and may have prognostic roles; however, it is unclear whether they play any pathogenetic roles.

Increasingly, there is enthusiasm for the prospect that gut dysbiosis is central to the initiation of either form of IBD and that some bacterial species may be trigger(s) (18,19). Hence, antibodies to microbial antigens may represent an adaptive immune response that has more relevance than merely serving as a biomarker. An exploration of the antibody responses in FN populations – who generally have a low incidence of IBD – could help clarify the importance of these antibodies in pathogenesis. Furthermore, we determined the diagnostic utility of these antibodies in an FN population.

METHODS

Subjects

The Canadian Institutes of Health Research Team Grant in Arthritis and IBD specifically enrolled a cohort of Caucasian or FN Manitobans with Crohn's disease, ulcerative colitis or RA, and healthy controls (without any autoimmune disease or first-degree relatives with autoimmune disease). Persons of mixed race were excluded to minimize issues related to admixture. Consecutive subjects with IBD and RA meeting enrollment criteria presenting to the University of Manitoba Health Sciences Centre (Winnipeg, Manitoba) specialty clinics of two of the principal investigators (CNB and HEG), as well as RA patients living in FN communities where one principal investigator (HEG) holds clinics, were invited to enrol. Healthy controls of Caucasian and FN descent who answered advertisements posted at the University of Manitoba Health Science Centre specialty clinics and in the clinics of the participating FN communities were also invited to participate. Blood was drawn for serum and DNA extraction to determine differential expression of biomarkers and genes of Caucasians and FN (exploration of differences in genotype between the disease groups and among the different racial groups is underway). A random sample (n=50) of each category of enrollee was taken. The only exception was for FN with Crohn's disease (n=9) and FN with ulcerative colitis (n=11) due to a lack of subjects in those categories.

TABLE 2
Antibody results

		ASCA					
	pANCA	IgA	IgG	Panel*	I2	Omp-C	CBir-1
CD, Caucasian	10	50	62	66	14	24	38
CD, First Nations	33	33	22	33	0	22	22
UC, Caucasian	46	4	12	14	4	10	12
UC, First Nations	55	9	18	18	0	9	27
RA, Caucasian	32	2	4	8	2	18	2
RA, First Nations	48	2	10	10	8	18	4
Controls, Caucasian	16	6	16	18	2	4	2
Controls, First Nations	32	4	12	14	8	6	2

*Data presented as %. *Anti-*Saccharomyces cerevisiae *(ASCA) antibody panel: ASCA immunoglobulin (Ig) A or ASCA IgG (panel refers to either or both ASCA IgA and IgG positivity); CBir-1 Antibodies to a bacterial flagellin; CD Crohn's disease; I2 Antibodies to a fragment of bacterial DNA associated with* Pseudomonas fluorescens; *Omp-C Antibodies to the outer membrane porin C of* Escherichia coli; *pANCA Perinuclear antineutrophil cytoplasmic antibody; RA Rheumatoid arthritis; UC Ulcerative colitis*

Assays

Serum was aliquotted, frozen and shipped to the laboratory of one of the investigators (SRT). Assays were performed for perinuclear antineutrophil cytoplasmic antibody (pANCA), anti-*Saccharomyces cerevisiae* antibody (ASCA immunoglobulin [Ig]A + IgG and ASCA panel, which was the combined result), antibodies to the outer membrane porin C (OmpC) of *Escherichia coli*, antibodies to a fragment of bacterial DNA associated with *Pseudomonas fluorescens* (I2), which has been cloned from lamina propria mononuclear cells in active Crohn's disease, and to a bacterial flagellin (CBir-1). A T cell line specific for this flagellin induced colitis when transferred into naive severe combined immunodeficient mice. All serum assays were performed in a blinded fashion at Cedars-Sinai Medical Center (California, USA) as described previously (20-24). Antibody levels were determined, with results expressed as ELISA units (EU/mL), which are relative to Cedars-Sinai Laboratory standards and derived from a pool of patient sera with well-characterized disease found to have reactivity to the specific antigen. ELISA titres (mean ± SD) were compared among groups using nonparametric tests.

RESULTS

The average age of the study cohort was 43.4 years, and 66% were women (Table 1). FN demonstrated a fairly high level of pANCA positivity. While a positive rate of 55% for FN with ulcerative colitis (in a small sample size [n=11]) is within reported ranges for other ulcerative colitis populations, the rate in healthy controls was 32% and 48% in RA subjects (Table 2). Even among Caucasians with RA, the pANCA positive rate was 32%. There were comparable rates of Crohn's disease-associated antibodies in the FN and Caucasian populations; however, among FN, up to one in four controls have any one of these antibodies (Table 3). Therefore, although FN experience little IBD, they were relatively responsive to this particular antibody panel.

The ELISA titre values were comparable between groups for any one antibody studied (ie, between ethnic groups and between disease groups), except for pANCA titres, in which FN showed significantly higher pANCA titres in both the ulcerative colitis and RA groups than Caucasians (Table 4).

DISCUSSION

Because selective antibody expression could differentiate Crohn's disease from ulcerative colitis (ie, ASCA versus pANCA), there was some early enthusiasm that these antibodies might have some pathogenetic significance (25). Furthermore, because bacterial antigens are considered to have an important role in the pathogenesis of Crohn's disease and ulcerative colitis, the potential for an adaptive immune

response to microbial antigens in the pathogenesis is appealing. It has been shown that ASCA positivity predicted a greater likelihood of complications in pediatric Crohn's disease (26), and has been associated with a more aggressive Crohn's disease phenotype and the need for surgery (27-33). In patients undergoing ileoanal pouch surgery for ulcerative colitis, positivity for pANCA and anti-CBir-1 are associated with a risk for developing pouchitis (34).

While ASCA, anti-CBir-1, anti-OmpC and anti-I2 are positive in approximately 40% to 50% of patients with Crohn's disease, and pANCA is positive in approximately 50% to 75% of patients with ulcerative colitis (35,36), these antibodies have been reported to be uncommon in healthy controls (37-39). We corroborated these reports for antibodies to OmpC, I2 and Cbir-1 in Manitoba Caucasian and FN populations. However, the rates of ASCA positivity (for the panel) were 14% to 18% in controls, and similar for FN and Caucasian populations and, for pANCA, was quite high among FN (32% in healthy controls).

It was suggested that FN populations may be genetically programmed to respond differently to certain infections than Caucasians, as evidenced by their differential carriage of single nucleotide polymorphisms for vitamin D receptor, interferon-gamma, tumour necrosis factor-alpha, monocyte chemoattractant protein 1 and interleukin-6 (40). In fact, the rate of tuberculosis among Canadian FN is much higher than the rate in Canadian-born, non-FN (27.4 per 100,000 population versus two per 100,000 population in 2006) (41). It is, therefore, plausible that FN react differently to microbial antigens with a different antibody response pattern. In our study, this was shown not to be the case because they may respond at an even greater rate to these microbial antigens than Caucasians. In fact, for pANCA, FN had significantly higher mean titres among the ulcerative colitis and RA groups. Unfortunately, a limitation of our study was the small sample size of FN with either Crohn's disease or ulcerative colitis. In fact, the pANCA positivity rate is sufficiently high in non-IBD subjects, which means that this antibody is likely of little diagnostic utility in the FN population. Furthermore, our data suggest that these antibodies are less likely to be pathogenetic because they are equally evident among FN who do not have IBD as in Caucasians.

So why are FN individuals without IBD reacting to pANCA at this rate? pANCA cross-reacts with several commensal bacterial antigens (of which the target antigen resides within the neutrophil [42,43]). In FN populations, this perhaps reflects a generalized increased response to bacteria. FN generally reside in lower socioeconomic areas characterized by crowded living conditions, and *Helicobacter pylori* infection – as determined by seropositivity – is ubiquitous in some communities (44,45). Mitochondrial DNA, anthropological, archaeological, linguistic, taxonomic and genetic studies (46-51) suggest that Canadian FN are descendants from Central Asia (X2 haplotype) who crossed the Bering Strait 13,000 to 30,000 years previously. Canadian FN, therefore, differ significantly from both Asians and Caucasian subjects with respect to the allelic frequencies of several genes. pANCA and ASCA rates were only 6% and 4%, respectively, in a sample of normal Chinese subjects (52), and pANCA was only positive in 22% of a Korean ulcerative colitis population (53). The results of our study suggest that the FN of Manitoba represent an ethnic ancestral population distinct from other populations in which these IBD-associated antibodies have been studied.

TABLE 3
Percentage positive to any Crohn's disease (CD)-associated antibody or multiple antibody tests

	CD-associated antibodies, %		
	Any positive	3 of 4 positive	4 positive*
CD, Caucasian	70	6	10
CD, First Nations	33	0	0
UC, Caucasian	32	2	0
UC, First Nations	36	0	0
RA, Caucasian	26	2	0
RA, First Nations	30	2	0
Controls, Caucasian	16	2	0
Controls, First Nations	24	0	0

**All of Anti-*Saccharomyces cerevisiae *antibody panel, fragment of bacterial DNA associated with* Pseudomonas fluorescens *(I2), outer membrane porin C of* Escherichia coli *(Omp-C) and bacterial flagellin (CBir-1) are positive. RA Rheumatoid arthritis; UC Ulcerative colitis*

CONCLUSION

The high specificity of these antibodies for IBD and their prevalence in family members (38,54,55) suggest that they are specific to a predilection for IBD in Caucasians. The Crohn's disease-associated antibodies may also be predictive for Crohn's disease within an FN population, although we could not determine this because of the small sample size in our study (but could show that FN with Crohn's disease can be positive for these antibodies). However, the high rates of pANCA positivity in FN without ulcerative colitis and, possibly, the 18% of FN controls who were ASCA positive suggest that these antibodies are not likely to be of pathogenetic significance.

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TABLE 4
Comparison of ELISA titre results*

	pANCA	ASCA IgA	ASCA IgG	I2	Omp-C	CBir-1
Crohn's disease, Caucasian	71.2±20.5	66±29.7	57.9±37	51.3±29.6	50.4±29.9	46.4±19.8
Crohn's disease, First Nations	54.3±14.6	71.3±53.1	100±42.4	0	54.5±30.4	45.5±26.2
Ulcerative colitis, Caucasian	60±27	61±35.4	39.5±14.7	23.5±0.7	36.2±13.6	35±5.8
Ulcerative colitis, First Nations	111.2±42.5†	34	36±15.6	0	27	52.3±42.2
Rheumatoid arthritis, Caucasian	48.9±14.6	25	39.7± 29.1	88	39±12.1	30
Rheumatoid arthritis, First Nations	75.6± 43.7‡	27	48.6±17.5	67±60.9	46.3±25.1	28±4.2
Control, Caucasian	45.3±16.3	48±13.1	36.2±22.5	28	24±1.4	31
Control, First Nations	50.9±43.3	56±29.7	33.7±15.6	36.3±18.9	33±8.5	29

**Data presented as mean ± SD ELISA units/mL. †P=0.03 for ulcerative colitis First Nations versus ulcerative colitis Caucasian; ‡P=0.009 for rheumatoid arthritis First Nations versus rheumatoid arthritis Caucasian. ASCA Anti-*Saccharomyces cerevisiae *antibody; CBir-1 Antibodies to a bacterial flagellin; I2 Antibodies to a fragment of bacterial DNA associated with* Pseudomonas fluorescens*; Ig Immunoglobulin*; *Omp-C Antibodies to the outer membrane porin C of* Escherichia coli*; pANCA Perinuclear antineutrophil cytoplasmic antibody*

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