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Granulocyte-Macrophage Colony-Stimulating Factor Auto-Antibodies: A Marker of Aggressive Crohn's Disease

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Abstract

Background & Aims—Neutralizing auto-antibodies (Ab) against granulocyte-macrophage colony-stimulating factor (GM-CSF Ab) have been associated with stricturing ileal Crohn's disease (CD) in a largely pediatric patient cohort (total 394, adult CD 57). The aim of this study was to examine this association in two independent predominantly adult inflammatory bowel disease patient cohorts.

Methods—Serum samples from 745 subjects from the NIDDK IBD Genetics Consortium and 737 patients from Australia were analyzed for GM-CSF Ab and genetic markers. We conducted multiple regression analysis with backwards elimination to assess the contribution of GM-CSF Ab levels, established CD risk alleles and smoking on ileal disease location in the 477 combined CD subjects from both cohorts. We also determined associations of GM-CSF Ab levels with complications requiring surgical intervention in combined CD subjects in both cohorts.

Results—Serum samples from CD patients expressed significantly higher concentrations of GM-CSF Ab when compared to Ulcerative Colitis or controls in each cohort. Non-smokers with ileal CD expressed significantly higher GM-CSF Ab concentrations in the Australian cohort ($p=0.002$). Elevated GM-CSF Ab, ileal disease location and disease duration greater than 3 years were independently associated with stricturing/penetrating behavior and intestinal resection for CD.

Conclusions—The expression of high GM-CSF Ab is a risk marker for aggressive CD behavior and complications including surgery. Modifying factors include environmental exposure to smoking and genetic risk markers.

Keywords

Inflammatory bowel disease; granulocyte-macrophage colony-stimulating factor antibody; Crohn's Disease, smoking

Background and Significance

The onset of Inflammatory Bowel Disease (IBD) requires multiple factors. Key among them is the genetic composition of the host, environmental interactions, and a dysregulated innate and adaptive mucosal immune response^{1,2}. Several genome wide association studies have identified polymorphisms within genomic regions that correspond to immune pathways. These pathways are involved in activating inflammation in response to antigen exposure³⁻⁵.

Bacterial recognition and autophagy are two important processes that drive the appropriate response to antigens at the intestinal level. Functional mutations in *NOD2*, an intracellular pattern recognition receptor in Paneth cells, are a common and well replicated CD risk factor^{6,7}. In addition, defects in *ATG16L1* and *IRGM*, two well described autophagy genes, also increase risk for IBD susceptibility^{8,9}.

Endogenous auto-antibodies to cytokines which may have an activating or neutralizing effect are proposed as additional modulators of mucosal inflammation. Cytokine auto-antibodies may create a relative immunodeficient state in IBD predisposing patients to chronic inflammation. In one study neutralizing antibodies to transforming growth factor (TGF)- β were measured in sera from patients with Ulcerative Colitis (UC) (n=136) and were found to be elevated ($P < 0.01$) when compared with levels in unaffected individuals. In addition neutralizing antibodies to IL-10 ($P < 0.05$) were elevated in a subset of patients with Crohn's disease (CD)¹⁰. In a subsequent study, neutralizing autoantibodies (Ab) against granulocyte macrophage colony-stimulating factor (GM-CSF Ab) were elevated in adult and pediatric CD patients and particularly in those patients with ileal disease involvement and stricturing behavior ($P < .001$). In addition CD patients with increased GMCSF Ab had reduced neutrophil phagocytic capacity¹¹. An important parallel finding in this study was that *NOD2* deficient mice treated with neutralizing antibodies to GM-CSF and subsequently exposed to NSAIDS, developed a transmural ileitis. This study and others demonstrate that deficiency of the important hematopoietic growth factor GM-CSF can contribute to a relative immunodeficiency¹². Idiopathic pulmonary alveolar proteinosis (I-PAP) for example, is a rare lung disorder of impaired macrophage function and due to elevated GM-CSF autoantibodies that neutralize the bioactivity of the growth factor GM-CSF^{13,14}.

We therefore asked if elevated GMCSF Ab would be associated with a stricturing ileal phenotype in an adult CD population.

Materials and Methods

Study Subjects

Our study recruited subjects from two study cohorts, the NIDDK IBD Genetics consortium and the Brisbane node of the ANZ IBD Consortium, Australia. The NIDDK IBD cohort included 350 unaffected controls, 139 UC and 253 CD subjects. The Brisbane cohort included 257 unaffected controls, 255 UC and 224 CD subjects. All research protocols for

human subjects' research were reviewed and approved by individual Institutional Review Boards (IRB) at five institutions involved in the NIDDK IBD genetics consortium and Brisbane, Australia. All IBD cases were previously diagnosed using standard criteria. IBD phenotypes including CD and UC sub-phenotypes were classified according to the Montreal Classification¹⁵. A standardized clinical questionnaire was completed for each patient at the time of enrollment and entered into a database program. The questionnaire included date of birth, sex, age at diagnosis, ethnicity, disease location, disease behavior, family history, extraintestinal manifestations, surgeries, smoking status at diagnosis, and therapeutic management at the time of study enrollment. This information was stripped of identifying information and entered in the databases linked to the serum samples.

Genotyping Methods

A blood sample was obtained at the time of study enrollment for DNA and serum isolation. DNA was isolated using standard protocols at each center. Seven single nucleotide polymorphisms (SNPs) were genotyped. SNP Genotyping was completed by various methods. These included the Illumina GoldenGate Genotyping Assay which is a flexible, pre-optimized assay that uses a discriminatory DNA polymerase and ligase to interrogate multiple loci simultaneously¹⁶. Some samples were genotyped using Sequenom, a technology that uses primer extension chemistry and mass spectrometric analysis¹⁷. Some SNPs were determined using TaqMan MGB technology from Applied Biosystems and following the manufacturer's recommendations.

GM-CSF Ab Enzyme-Linked Immunosorbent Assay

Serum samples were stored at -80 degrees Celsius within 4 hours of isolation. Samples were sent for batch analysis of GM-CSF Ab to the Trapnell laboratory in Cincinnati. GM-CSF Ab to glycosylated GM-CSF were quantified in human serum by enzyme-linked immunosorbent assay (ELISA)¹⁸. In our prior report¹¹ we found that antibodies to both glycosylated and non-glycosylated GM-CSF were elevated in adult and pediatric CD compared to UC and controls. However, we identified a strong association with stricturing/penetrating behavior only for antibodies to glycosylated GM-CSF, and so the current study sought to replicate this association.

Statistical analysis

Statistical analyses were performed using SPSS version 19.0 (SPSS Inc., IBM Corporation, Somers NY, USA), Minitab Statistical Software, Release 16 (State College, Pennsylvania), GraphPad Prism version 5.00 (San Diego California, USA), and SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Demographic and baseline disease variables were summarized by cohort. Simple analyses were conducted by the Pearson chi-square test to compare discrete variables, and by 2-sample t test, 1-way ANOVA or the nonparametric alternative, the Kruskal-Wallis test, to compare continuous variables. All statistical tests were two-sided, and results were interpreted at the significance level of 0.05; variables with P-values < 0.05 were reported as statistically significant. Odds ratio estimates were computed with 95% confidence intervals. Multiple linear regression and logistic regression analyses were conducted to determine relationships of serum GM-CSF Ab levels or its elevation (5 mcg/mL) respectively with disease location, behavior, and surgery, controlling for the effects of other factors. Factors controlled for included age of diagnosis (by Montreal classification), gender, disease duration, and *NOD2* SNP carriage. We evaluated the sensitivity of the regression results against the definition of GM-CSF Ab elevation by using different cutoff values (2, 3 or 4 mcg/mL). Consistent results were obtained (see Tables, Supplemental Digital Content 1-4, <http://links.lww.com/IBD/A148>, <http://links.lww.com/IBD/A149>, <http://links.lww.com/IBD/A150>, and <http://links.lww.com/IBD/A151>) and validated our main results. The multiple regression analyses were conducted in the

combined cohorts (NIDDK and Brisbane). We included cohort effect in the regression analyses in order to account for any non-random discrepancies between the two cohorts unaccounted for by the aforementioned factors. We also included interaction effects by the cohort and smoking with the other factors included in the analyses to entertain the possibility that the cohort and smoking factor may modify the effects of the other factors such that the elevation of serum GM-CSF Ab level may only be significantly associated with ileal disease location among current smokers or ex-smokers. Non-significant interactions indicate no significant modifications by the cohort or smoking factor and allow interpreting the effects of the factors (main effects) as they appear. Conversely significant interactions indicate significant modifications and require the effects of the factors to be interpreted separately by the levels of the cohort or smoking factor. We simplified statistical models by removing non-significant interaction effects until all remaining interaction effects were significant via backward variable selection procedure. We reported the resulting parsimonious model results here, interpreting only significant interaction and main effects in the forms of odds ratio estimates. We therefore reported OR estimates in the tables for the regression models after accounting for significant interactions between the individual factors considered. In such a case in which there were significant interactions between the individual factors, the OR estimates for the individual effects were not provided because the significant interactions mean that the individual effects are modified by these interactions. In cases where significant interactions were not identified, then the OR estimates for the individual factors were reported.

Results

Clinical and Demographic Characteristics

Descriptive characteristics of the two IBD cohorts are listed in Table 1. Clinical and demographics features of the two cohorts were similar with the following exceptions. The proportion of unaffected males in the Brisbane cohort was significantly higher ($p=0.002$). Unaffected and UC cases from NIDDK were younger ($p<0.001$, and $p=0.006$, respectively). The proportions of unaffected and CD cases of European ancestry from Brisbane were higher ($p<0.001$ and $p<0.001$, respectively). Smokers and ex-smokers from Brisbane were significantly higher ($p=0.001$, $p=0.01$, and $p=0.001$ for unaffected, UC and CD subsets, respectively). The proportion of unaffected controls with a history of appendectomy were greater in the Brisbane cohort ($p<0.001$).

The phenotypic characteristics of the two IBD cohorts are listed in Table 2. The NIDDK cohort had a higher proportion of younger patients ($p=0.02$). Brisbane UC patients had a higher proportion of cases with disease duration longer than 3 years ($p=0.01$). Isolated Ileal disease was higher among Brisbane CD cases ($p<0.001$). The proportions of patients who had intestinal resection for surgery was higher in Brisbane ($p=0.008$ and $p=0.01$ for UC and CD subsets, respectively). Isolated proctitis in UC was greater in the NIDDK cohort ($p=0.001$). Exposure to biologic medication was significantly higher in the NIDDK UC cohort ($p=0.026$).

Initial studies were undertaken to define levels of GM-CSF Ab in serum from large cohorts of adult IBD and control patients. Using an assay designed to detect binding of GM-CSF Ab to glycosylated GM-CSF we found that patients with CD expressed significantly higher concentrations when compared to Ulcerative colitis or controls in each cohort, and for the combined cohorts (Figure 1). However, median GM-CSF Ab concentrations were significantly different between the two cohorts ($p=0.04$, $p<0.001$, and $p=0.007$ for unaffected, UC and CD, respectively). Interestingly, median GM-CSF Ab concentrations were significantly higher in non-smokers compared to smokers with CD in the Brisbane cohort ($p=0.002$) and a similar trend was observed for the NIDDK cohort although not

statistically significant (Figure, Supplemental Digital Content 5, <http://links.lww.com/IBD/A152>). A similar result was observed for the combined cohort, demonstrating that smoking was associated with reduced GM-CSF Ab concentration.

We then asked whether GM-CSF Ab level would vary with IBD risk allele carriage. Serum GM-CSF Ab expression level was elevated in CD patients independent of SNP carriage for the genetic markers *NOD2*, *ATG16L1*, *IRGM*, *JAK2* or *STAT3* (Tables, Supplemental Digital Content 6, <http://links.lww.com/IBD/A153>). This was true whether the cohorts were analyzed individually, or combined. Associations between smoking and elevated GM-CSF Ab, and between elevated GM-CSF Ab and ileal CD location, stricturing/penetrating disease behavior, and surgery were examined using multiple linear and logistic regression as described in the statistical analysis section after accounting for the effects of age of diagnosis, gender, disease duration, *NOD2* risk allele carriage, smoking status, and perianal disease location.

First, we examined the effect of smoking upon serum GM-CSF Ab concentration (Table 3). Significant two way and three way interactions of smoking status with ileal location and cohort indicate a modified effect of smoking status, different by ileal location and between the cohorts. Current or past smoking was significantly associated with the lower odds of GM-CSF Ab elevation in the NIDDK cohort with ileal location (OR=0.32 (0.17–0.61)). A similar trend was observed within the Brisbane cohort with ileal location but did not reach significance. No significant effect was observed within the smaller subset of CD patients with colon-only location. These results conversely demonstrated that non-smoking status at diagnosis was associated with elevated serum GM-CSF Ab amongst CD patients with ileal location, with a significant effect observed within the NIDDK cohort.

Second, we tested the relationship between elevated serum GM-CSF Ab concentration and ileal disease location (Table 4). Significant two way and three way interactions with smoking status at diagnosis and cohort suggest a modified effect of elevated GM-CSF Ab level, different by smoking status at diagnosis and between the cohorts. Elevated GM-CSF Ab level was associated with the higher odds of ileal location, and was only significant for non-smokers. Among current or ex-smokers at the time of diagnosis, no significant association was found and the direction of the association was not consistent between the cohorts. This analysis held true for all the GM-CSF Ab cutoff values tested (Table, Supplemental Digital Content 2, <http://links.lww.com/IBD/A149>).

Third, we tested the relationship between elevated serum GM-CSF Ab concentration and stricturing (B2) or penetrating (B3) disease behavior (Table 5). Absence of significant interaction suggests a consistent effect of GM-CSF Ab elevation between the cohorts and by smoking status. Elevated GM-CSF Ab level was significantly associated with higher odds of stricturing or penetrating disease behavior uniformly in both cohorts regardless of their smoking status. A significant interaction of ileal location with the cohort indicates that ileal location was associated differently between the cohorts. Ileal location was significantly associated with higher odds of the more aggressive CD phenotypes in both the cohorts with the odds much higher in the NIDDK cohort. As expected, disease duration greater than 3 years was independently significantly associated with higher odds ($p=0.015$) for stricturing/penetrating disease behavior.

Finally, we tested the relationship between elevated serum GM-CSF Ab concentration and a surgical event (intestinal resection) (Table 6). Absence of significant interaction suggests a consistent effect of GM-CSF Ab concentration; elevated GM-CSF Ab level was significantly associated with the higher odd of surgery uniformly in both the cohorts regardless of their smoking status (OR=1.80, (1.17–2.76)). Ileal location was also uniformly

significantly associated with the higher odds for surgery (OR=2.30, (1.35, 3.91)). Disease duration greater than 3 years was also independently significantly associated with surgery ($p=0.0005$). We observed an interaction between *NOD2* risk allele carriage, smoking status at diagnosis, and cohort. In this regard, smokers within the NIDDK cohort who carried a *NOD2* risk allele exhibited a greater risk for surgery. A similar trend was observed within the Brisbane cohort which did not reach significance. Collectively, these results demonstrated that elevated GM-CSF Ab was associated with increased risk for surgery in CD, after controlling for *NOD2* risk allele carriage, ileal disease location, smoking status, and duration of disease.

Discussion

There has been tremendous progress in the discovery of genetic markers that predict the risk of developing Inflammatory Bowel Disease. The 71 confirmed Crohn's susceptibility loci explain just 23.1% of the heritability¹⁹. Many risk polymorphisms implicate defects in innate immunity in CD^{19,20}. Other factors that may contribute to CD expression could result from the abnormal immune response in genetically predisposed individuals. GM-CSF is produced by several lamina propria immune cells²¹ and has a role in promoting intestinal epithelial barrier integrity, stimulating crypt cell proliferation in acute injury, and decreasing inflammation²²⁻²⁴. Recombinant GM-CSF has also been examined in clinical trials with both favorable and negative efficacy results suggesting an effect in a subset of patients²⁵⁻³⁰. Elevated GM-CSF antibodies have been shown to impair neutrophil cell function in patients with CD and to exacerbate NSAID-induced transmural ileitis in mouse models¹¹.

In this cross-sectional study we examined the correlation of GM-CSF neutralizing Ab with CD location and behavior. Similar to the study by Han et al¹¹, we confirmed that GMCSF Ab expression is elevated in CD when compared to UC or unaffected individuals. Serum GM-CSF Ab expression level was elevated in CD patients independent of SNP carriage for the genetic markers *NOD2*, *ATG16L1*, *IRGM*, *JAK2* or *STAT3*. Studies regarding a potential association between *NOD2* genotype and antimicrobial serologies have yielded conflicting results, although several have suggested that CD patients with *NOD2* risk allele carriage are more likely to be positive for ASCA. The lack of association between the *NOD2* genotype and serum GM-CSF antibody concentration in the current study is consistent with our prior report in 354 primarily pediatric-onset IBD patients, and so is unlikely to be due to a methodological issue¹¹. Future studies will utilize genome-wide approaches and larger cohorts to test for genetic variants associated with GM-CSF Ab concentration.

We also examined for the first time, the effect of smoking on GM-CSF Ab expression. Our primary finding was that smokers with ileal CD in both the Brisbane and NIDDK CD cohorts had significantly lower GM-CSF Ab concentrations. This result was surprising, as smoking is associated with a higher prevalence of complicated ileal CD³¹ which is less responsive to anti-TNF therapy. In our prior report¹¹ we showed that patients with elevated GM-CSF antibodies are more likely to have elevated titers of ASCA. Therefore, similar immune pathways may drive production of GM-CSF antibodies and ASCA. A prior study³² reported that the frequency of ASCA seropositivity was significantly lower in smokers with adult-onset CD compared to non-smokers with adult-onset CD. The authors concluded that smoking may regulate immune responses to intestinal antigens. A more recent study confirmed a trend towards a lower rate of ASCA seropositivity in adult-onset CD smokers compared to non-smokers, although this did not reach significance³³. Smoking has immune-suppressive effects which could result in reduced production of ASCA and GM-CSF auto-antibodies. These include suppression of dendritic cell maturation and antigen presentation, inhibition of T cell antibody-forming responses, and reduction in circulating levels of

immune-globulins. Nicotine and more recently carbon monoxide have been implicated in mediating these effects³⁴. However, a large cohort study of adults with pulmonary alveolar proteinosis did not identify an association between smoking status and serum GM-CSF Ab concentration³⁵. Conversely, smoking may augment auto-antibody production in rheumatoid arthritis and lupus³⁴. Collectively, these data suggest that smoking may exert a fundamentally different effect with regard to development of sero-reactivity to intestinal versus extra-intestinal antigens. This mechanism will be addressed in future studies

Smoking, elevated GM-CSF Ab, and perianal disease were significantly associated with ileal disease location in the combined cohort analysis. Smokers had significantly higher odds (by 6.76 times) of having ileal disease location compared to non-smokers in the Brisbane cohort when GM-CSF Ab was less than 5mcg/mL. Consistent with prior studies, perianal location was associated with significantly lower odds (by 0.461 times) of having ileal disease location. After controlling for these factors, elevated GM-CSF Ab (> 5mcg/mL) was associated with significantly higher odds (by 2.27 times) of having ileal disease location among non-smokers both in the Brisbane and the NIDDK cohort.

Ileal CD, elevated GM-CSF Ab, and disease duration longer than 3 years were risk factors for stricturing or penetrating disease behavior and for surgery. Interestingly, smoking and *NOD2* risk genotypes, factors that are associated with ileal disease location were not independent risk factors for stricturing or penetrating behavior in our combined cohort analysis except as effect modifiers for surgery. Prior studies looking at the effect of smoking in CD report a higher prevalence of ileal disease and a lower prevalence of colonic involvement^{36, 37}. Smoking is also linked to a greater likelihood of more complicated CD behavior including more frequency of stricturing or penetrating disease behavior, more frequent disease flares, and a higher risk of surgery³⁸⁻⁴². In our study, non-smokers with ileal CD and high GM-CSF Ab concentration had the greatest frequency of stricturing/penetrating CD behavior. Thus, smoking appeared to offer some protection against high GM-CSF Ab production and a resultant lower frequency of stricturing or penetrating disease behavior among patients with ileal disease.

The processes by which deficiency of GM-CSF culminates in a more severe CD phenotype are currently being explored. In a recent study, *NOD2* deficient and WT mice demonstrated an increase in CCL25, a chemokine involved in immune cell homing to the small intestine, and CCR9 its cognate receptor after neutralization of GM-CSF. This correlated with an increased fraction of CCR9⁺ lymphocytes and a transmural ulcerating ileal disease^{11, 43}. Samson et al⁴³ also observed an increase in ileal CCR9⁺ lamina propria mononuclear cells (LPMC) obtained from ileal biopsies and in CCR9⁺ lymphocytes from peripheral blood in pediatric CD patients expressing high neutralizing antibodies to GM-CSF. Therapeutic blockade of CCR9 can ameliorate early murine ileitis⁴⁴ and a phase II study of a CCR9 antagonist has demonstrated efficacy in patients with moderate to severe CD^{43, 45}.

In our study elevated GM-CSF Ab was associated with higher incidence of surgery for IBD. One limitation was that since this was a cross-sectional study, we could not determine if elevated GM-CSF Ab expression results in an earlier time to surgery. Another limitation of our study was that serum samples and phenotype data from patients were obtained only at the time of enrollment. As such, we could not assess the direct impact of GM-CSF antibody titers on disease activity. Longitudinal assessment and disease activity measurements using standardized instruments such as the CDAI, may be more useful in correlating with mucosal inflammation.

Our study confirms that the expression of elevated GM-CSF Ab in Crohn's disease is correlated with aggressive disease behavior. Smoking appeared to be a negative modifier of

GM-CSF Ab concentration in this study. Longitudinal studies including dosage of nicotine exposure, along with other factors such as exercise and dietary factors are indicated to better define the role of smoking in GM-CSF Ab production. The studies primary finding was that CD patients with elevated GM-CSF auto-antibodies were more likely to experience stricturing/penetrating behavior. Longitudinal studies are needed to examine the natural history of GM-CSF Ab expression and its effect on the bioavailability of the GM-CSF cytokine. It would also be beneficial to identify the antigenic trigger for the expression of autoantibodies. This will require an examination of the composition of the intestinal microbiota and other mediators of the intestinal barrier integrity. The results of this kind of study will help to identify the subset of patients expressing high GMCSF Ab who may benefit from more aggressive therapy to induce disease remission and mucosal healing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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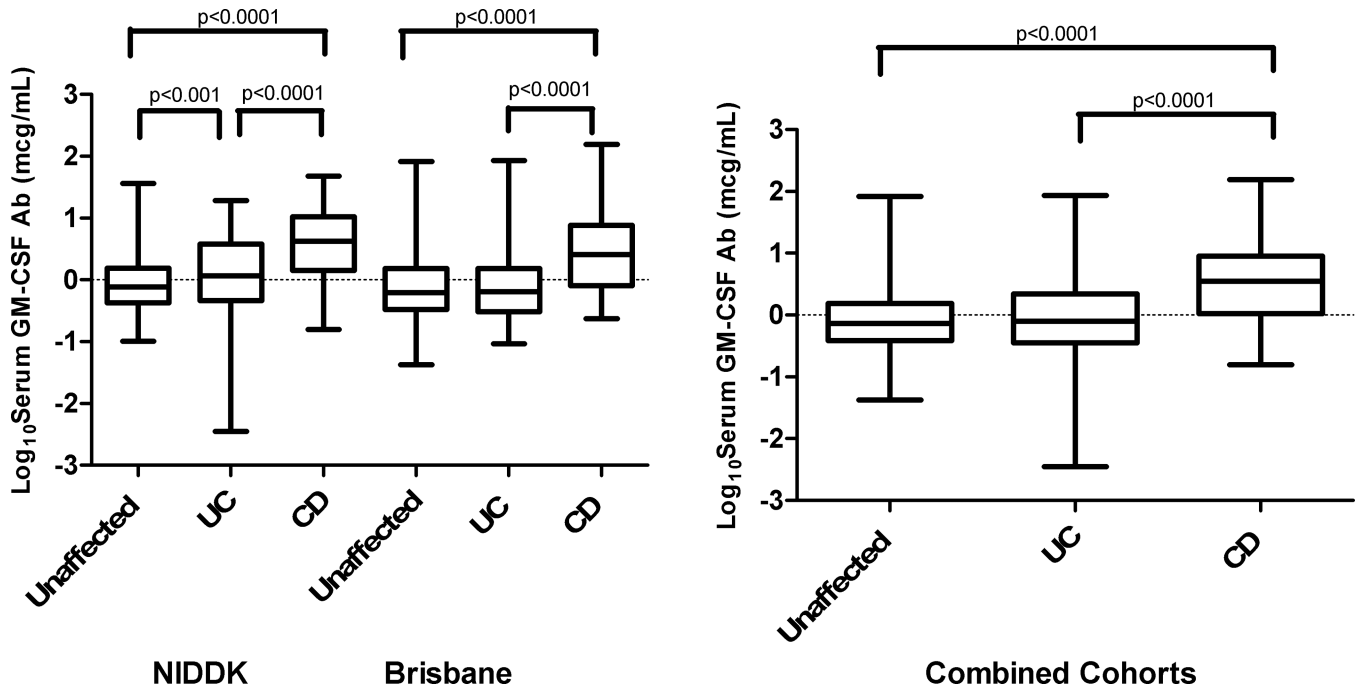
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**Figure 1.**

Serum GM-CSF Ab Levels in Unaffected, UC, and CD Patients. The Log₁₀ transformation of serum GM-CSF Ab ($\mu\text{g}/\text{ml}$) are shown for the NIDDK cohort, the Brisbane cohort, and the combined cohort. The middle line represents the median, and the lower edge and the upper edge of the box represent the 25% and 75% quartiles. The bottom and top lines represent the minimum and maximum values, respectively. For the Brisbane cohort there were 257 Unaffected, 255 UC and 224 CD subjects. For the NIDDK cohort there were 350 Unaffected, 139 UC and 253 CD subjects.

Kruskal-Wallis with Dunn's post-test revealed significant differences between Unaffected and CD subjects, and UC and CD subjects in both cohorts analyzed separately and when combined. Pairwise comparison of each disease phenotype between the two cohorts revealed that the median log transformed serum GM-CSF Ab level in the NIDDK UC group was higher than the Brisbane UC group.

Table 1
Demographic Characteristics and Serum GM-CSF Ab Concentrations of the Cohorts.

Diagnosis	Unaffected	Brisbane	UC	Brisbane	CD	Brisbane
Cohort	NIDDK		NIDDK		NIDDK	
Count	350	257	139	255	253	224
Male/female (% male)	116/233 (33.2)	118/139 (45.9)**/	68/71 (48.9)	116/139 (45.5)	121/132 (47.8)	103/121 (46.0)
Age, mean ± S.d. (range)	33.1±12.9 (16–76)	58.3 ± 13.5** (22–83)	43.6 ± 16.6 (1–85)	48.6 ± 16.1** (18–86)	41.2 ± 15.1 (3–86)	42.9 ± 13.8 (16–81)
Race						
European Ancestry (%)	284 (81.1)	248 (96.5)**	126 (90.6)	218 (85.5)	218 (86.2)	222 (99.1)**
Other	65	9	13	16	35	2
Smokers (at diagnosis)						
Yes (%)	30(8.7)	12(6.6)**	14(10.1)	36(15.8)*	46(18.3)	108(48.2)**
Ex-smoker(%)	27(7.8)	103(56.6)	23(16.5)	59(25.9)	24(9.6)	19(8.5)
No (%)	289(83.5)	67(36.8)	102(73.4)	133(58.3)	181(72.1)	97(43.3)
Appendectomy						
Yes (%)	18(5.2)	67(26.5)**	6(4.6)	20(9.14)	25(10.6)	33(15.5)
No (%)	330(94.8)	186(73.5)	124(95.4)	199(90.9)	210(89.4)	180(84.5)
GM-CSF Ab (mcg/mL)						
Median (IQR)	0.77 (41, 1.5)	0.62 (0.33, 1.5)*	1.16 (0.46, 3.8)	0.65 (0.31, 1.5)**	4.24 (1.4, 10.6)	2.59 (0.81, 7.6)**

/ p-value <0.05 denotes significantly different proportions of affection subsets between NIDDK and Brisbane cohorts; specific values are cited in Results section

Table 2

Phenotypic Characteristics of the IBD Patients.

Diagnosis	UC		CD	
	NIDDK	Brisbane	NIDDK	Brisbane
Cohort				
Age at diagnosis, mean \pm s.d. (range)	32.7 \pm 16 (3–82)	32.8 \pm 14.6 (3–75)	26.9 \pm 13.1 (0–80)	27.1 \pm 11.1 (2–66)
Age at diagnosis of CD (Montreal A)				
A1 (\leq 16 years)	20 (14.4)	26 (10.2)	48 (19)	23 (10.3)* ²
A2 (17 – 40 years)	83 (59.7)	155 (60.4)	170 (67.2)	173 (77.2)
A3 (> 40 years)	36 (25.9)	73 (28.5)	35 (13.8)	28 (12.5)
Disease duration > 3 years (%)	110 (79.1)	226(88.3)*	225 (88.9)	204 (91.1)
Disease location, CD (Montreal L)				
L1 ileal			56 (22.1)	109 (48.7)**
L2 colonic			64 (25.3)	26 (11.6)
L3 ileocolonic			128 (50.6)	88 (39.3)
Perianal \pm other involvement			78 (30.8)	63 (28.1)
Disease behavior, CD (Montreal B)				
B1 non-stricturing, non enetrating			113 (44.7)	83 (37.1)
B2 stricturing			63 (24.9)	64 (28.6)
B3 penetrating – excludes perianal			71 (28.1)	77 (34.4)
Surgery for IBD – Yes (%)	22 (15.8)	66(25.8)**	136 (53.8)	147 (65.6)*
Disease location, UC (Montreal E)				
E1 ulcerative proctitis	21 (15.1)	12 (4.7)**		
E2 left-sided UC (distal UC)	37 (26.6)	91 (35.5)		
E3 extensive UC (pancolitis)	79 (56.8)	136 (53.1)		
Exposure to IBD Medication – Yes (%)				
5-Amino Salicylic Acids³	127 (91.4)	212 (82.8)	189 (74.7)	62 (27.7)
Immunomodulators⁴	64 (46.0)	108 (42.2)	167 (66.0)	167 (74.6)
Biologics⁵	23 (16.5)	23 (9.0)	100 (39.5)	74 (33.0)*

²P-value <0.05 denotes significantly different proportions of affection subsets between NIDDK and Brisbane; specific values are cited in results section

³5-ASAs: Balsalazide, Mesalamine, Olsalazine, Sulfasalazine

⁴Immunomodulators: Imuran, Methotrexate, Purinethol, Neoral

⁵Biologics: Adalimumab, Certolizumab, Infliximab

Table 3Multiple Logistic Regression Results for Elevated Serum GM-CSF Ab Concentration (≥ 5 mcg/mL)

Factors	P-values		
Age at diagnosis (>16yrs)	0.6758		
Gender	0.8317		
NOD2 risk allele carriage	0.7367		
Cohort	0.7530		
Smoking	0.0686		
Ileal Location	0.0086		
Perianal	0.3149		
Disease duration (>3yrs)	0.5496		
Cohort*smoking	0.0322		
smoking*Ileal Location	0.0015		
Cohort*smoking*Ileal Location	0.0038		
Factors	By Ileal location	By Cohort	OR (95%CI)
Smoking Yes vs. No	Yes	Brisbane	0.70 (0.35–1.41)
		NIDDK	0.32 (0.17–0.61)
	No	Brisbane	0.63 (0.21–1.89)
		NIDDK	4.35 (0.83–20.4)

A multiple logistic regression model was fitted with elevated GM-CSF-Ab as the response variable. Main effects for the variables age at diagnosis, gender, NOD2 allele, Cohort, Smoking, Ileal location, Perianal location and disease duration were included in the model automatically, while interaction terms between these main effects were included only if significant after backward elimination. Table 3 displays the p-values for all fitted terms in the final model, as well as the estimated odds ratios (OR) for significant association of Elevated GM-CSF-Ab with smoking. The odds ratio estimates were broken down by Ileal location and cohort as the significant association was defined by interactions of smoking with Ileal location and cohort factors.

Table 4

Multiple Logistic Regression Results for Ileal Disease Location.

Fctors	P-values		
Age at diagnosis (>16yrs)	0.6809		
Gender	0.3071		
NOD2 risk allele carriage	0.1408		
Cohort	0.2269		
Smoking	0.0044		
Elevated GM-CSF Ab	0.0108		
Perianal location	0.0029		
Smoking* Elevated GM-CSF Ab	0.0023		
Cohort*Smoking	0.0010		
Cohort*Smoking* Elevated GM-CSF Ab	0.0048		
Factors	By Smoking	By Cohort	OR (95%CI)
GM-CSF Ab Elevation Yes vs. No	Yes	Brisbane	0.19 (0.04–0.81)
		NIDDK	2.67 (0.88–8.06)
	No	Brisbane	2.27 (1.21–4.26)
		NIDDK	2.25 (1.21–4.25)

A multiple logistic regression model was fitted with Ileal CD location as the response variable. Main effects for the variables age at diagnosis, gender, NOD2 allele, Cohort, Smoking, elevated GM-CSF Ab ($> 5\text{mcg/mL}$), and Perianal location were included in the model automatically, while interaction terms between these main effects were included only if significant after backward elimination. Table 4 displays the p-values for all fitted terms in the final model, as well as the estimated odds ratios for association of Ileal CD location with Elevated GM-CSF-Ab. The odds ratio estimates were broken down by smoking and cohort as the significant association was defined by interactions of elevated GM-CSF-Ab with smoking and cohort.

Table 5

Multiple Logistic Regression Results for Stricturing/Penetrating Behavior.

Factors	P-values	
Age at diagnosis (>16yrs)	0.6526	
Gender	0.1788	
NOD2 risk allele carriage	0.7334	
Cohort	0.0205	
Smoking	0.8690	
Elevated GM-CSF Ab	0.0001	
Ileal Location	0.0481	
Perianal location	0.2250	
Disease Duration (>3yrs)	0.0146	
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Cohort* Ileal Location	0.0722	
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Factors	By Cohort	OR (95%CI)
Ileal Location Yes vs. No	Brisbane	2.48(1.01–6.10)
	NIDDK	7.09(3.47–14.49)
Disease Duration >3yrs vs. 3		2.46 (1.20–5.07)
Elevated GM-CSF Ab Yes vs. No		2.37 (1.53–3.67)

A multiple logistic regression model was fitted with Stricturing/Penetrating behavior as the response variable. Main effects for the variables age at diagnosis, gender, NOD2 allele, Cohort, Smoking, elevated GM-CSF Ab ($> 5\text{mcg/mL}$), Perianal location and disease duration were included in the model automatically, while interaction terms between these main effects were included only if significant after backward elimination. Table 5 displays the p-values for all fitted terms in the final model, as well as the estimated odds ratios for association of Stricturing/Penetrating CD behavior with Ileal CD location, disease duration longer than 3 years, and Elevated GM-CSF-Ab. The Ileal location odds ratio estimates were broken down by cohort as the significant association was defined by interaction of Ileal location with cohort. OR estimates are reported for the individual factors disease duration and elevated GM-CSF Ab as significant interactions for these factors were not identified.

Table 6

Multiple Logistic Regression Results for Surgery.

Factors	P-values
Age at diagnosis (>16yrs)	0.5205
Gender	0.8879
NOD2 risk allele carriage	0.2884
Cohort	<.0001
Smoking	0.2016
Elevated GM-CSF Ab	0.0078
Ileal Location	0.0022
Perianal location	0.2866
Disease Duration (>3yrs)	0.0005
Cohort*NOD2	0.0346
NOD2*smoking	0.0356
Cohort*smoking	0.0330
Cohort*Perianal	0.0020

Factors	By Smoking	By Cohort	OR (95%CI)
NOD2 Mutation Yes vs. No	Yes	Brisbane	1.98(0.86–4.57)
		NIDDK	5.92(1.90–18.18)
	No	Brisbane	0.62(0.26–1.49)
		NIDDK	1.86(0.96–3.58)
Ileal Location Yes vs. No			2.30 (1.35–3.91)
Disease Duration >3yrs vs. 3			3.75 (1.79–7.88)
Elevated GM-CSF Ab Yes vs No			1.80 (1.17–2.76)

A multiple logistic regression model was fitted with Surgery as the response variable. Main effects for the variables age at diagnosis, gender, NOD2 allele, Cohort, Smoking, elevated GM-CSF Ab ($< 5\text{mcg/mL}$), Ileal CD location, Perianal location and disease duration were included in the model automatically, while interaction terms between these main effects were included only if significant after backward elimination. Table 6 displays the p-values for all fitted terms in the final model, as well as the estimated odds ratios for association of surgery with NOD2 risk allele carriage, Ileal CD location, disease duration longer than 3 years, and Elevated GM-CSF-Ab. The NOD2 risk allele odds ratio estimates were broken down by smoking and cohort as the significant association was defined by interactions of NOD2 risk allele carriage with smoking and cohort. OR estimates are reported for the individual factors ileal location, disease duration, and elevated GM-CSF Ab as significant interactions for these factors were not identified.