The gene encoding the B-cell activating factor and B-lymphocyte stimulator (BAFF/BLyS), TNFSF13B, is differentially expressed in the blood of patients with Crohn's Disease.

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Inflammatory bowel diseases (IBD) include Crohn's Disease and Ulcerative Colitis (1). We mined published microarray data to understand how gene expression in the hematopoietic compartment of patients with Crohn's Disease is most different from that of healthy controls (2-4). Across two datasets (2, 3), we found that BAFF, also known as the B-lymphocyte stimulator (BLyS), encoded by the gene TNFSF13B (5), was differentially expressed in the blood of patients with Crohn's Disease. Analysis of a third dataset (4) revealed that BAFF was among the genes most differentially expressed in monocyte-derived macrophages from patients with Crohn's Disease. Serum BAFF, fecal BAFF, and BAFF expression in the intestinal mucosa has been demonstrated to be increased in patients with IBD (6, 7). We show here that expression of BAFF in the peripheral blood of patients with Crohn's Disease is also increased.

Keywords: inflammatory bowel disease, Crohn's Disease, TNFSF13B, BAFF, BLyS, CD257, TALL-1, systems biology of Crohn's Disease.

1 Inflammatory bowel diseases, including Crohn's Disease, affect the gastrointestinal tract
2 but have their origins in the immune system (1, 8, 9). Bone marrow transplantation of cancer
3 patients with Crohn's Disease has shown that changing only the blood-forming tissues of a
4 patient can be efficacious in maintaining durable remission of CD (10). This, in conjunction
5 with information learned from genetic linkage studies (11), demonstrates that the
6 hematopoietic compartment is essential in the pathogenesis of Crohn's Disease, a disorder
7 whose primary manifestation is in the gut.
8

9 The study of gene expression is critical for the understanding of what genes and
10 pathways are altered in disease (12). To understand how gene expression is most different in
11 the blood of patients with Crohn's Disease, we used three independent published datasets to
12 compare the global gene expression profiles of blood from patients with CD and from healthy
13 controls. We identified the gene encoding CD257, also known as BAFF/BLyS, TNFSF13B, as
14 among the genes most differentially expressed in the blood of patients with Crohn's Disease.
15 TNF superfamily members are essential molecules in the immune system and are implicated in
16 a variety of immune deficiencies, autoimmune disorders, in cancer, and in the inflammatory
17 bowel diseases (13, 14).
18

19 **Methods**

20 We utilized GSE126124 (2), and GSE94648 (3) and GSE60083 (4), for this study in
21 conjunction with GEO2R. GSE126124 was generated using Affymetrix Human Gene 1.0 ST
22 Array technology with n=39 CD patients and n=32 healthy controls. GSE94648 was generated
23 using Affymetrix Human Genome U133 Plus 2.0 Array with n=41 CD patients with active
24 disease, n=9 CD patients with inactive disease, and n=22 healthy controls. GSE60083 was
25 generated using Illumina HumanWG-6 v3.0 expression beadchip technology with n=58 CD
26 patients and n=42 healthy controls.
27
28

The Benjamini and Hochberg method of p-value adjustment was used for ranking of
differential expression but raw p-values were used for assessment of statistical significance of

global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used.

A statistical test was performed to evaluate the significance of difference between mRNA expression levels of TNFSF13B in the peripheral blood of patients with Crohn's Disease versus in healthy controls using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant.

Results

We performed comparative transcriptome profiling of the peripheral blood from patients with Crohn's Disease and from control subjects to understand how gene expression was most different in the hematopoietic compartment of patients with Crohn's Disease.

TNFSF13B is differentially expressed in the blood of patients with Crohn's Disease.

We identified TNFSF13B as among the genes whose expression was most quantitatively different in the blood of patients with Crohn's Disease when compared to the blood of control subjects, using published microarray data (2). When sorting all of the genes by the degree of change in expression between diseased and control subjects, TNFSF13B ranked 2953 out of 33297 total transcripts (Table 1). Differential expression of TNFSF13B in the blood of patients with Crohn's Disease was statistically significant (Table 1; $p=4.59E-04$).

In second dataset, we again identified TNFSF13B as differentially expressed in the blood of patients with Crohn's Disease. When sorting all of the genes by the degree of change in expression between diseased and control subjects, TNFSF13B ranked 200 out of 19674 total transcripts (Table 2). Differential expression of TNFSF13B in the blood of patients with Crohn's Disease was statistically significant (Table 2; p-value: $5.8E-05$).

TNFSF13B is differentially expressed in monocyte-derived macrophage from patients with Crohn's Disease.

1 In a third dataset, we identified TNFSF13B as a differentially expressed gene in
2 monocyte-derived macrophage (MDM) from patients with Crohn's Disease when compared to
3 the MDM from control subjects. When sorting all of the genes by the degree of change in
4 expression between diseased and control subjects, TNFSF13B ranked 367 out of 10468 total
5 transcripts (Table 3). Differential expression of TNFSF13B in MDM from patients with Crohn's
6 Disease was statistically significant (Table 3; $p=0.0133479$). A second transcript encoded by
7 the TNFSF13B was also differentially expressed in MDM from patients with Crohn's Disease.
8 When sorting all of the genes by the degree of change in expression between diseased and
9 control subjects, here, TNFSF13B ranked 1178 out of 10468 total transcripts (Table 3).
10 Differential expression of this second TNFSF13B transcript in monocyte-derived macrophage
11 from patients with Crohn's Disease was also statistically significant (Table 3; $p=0.047553$).
12

13 TNFSF13B is expressed at significantly higher levels in the blood of patients with active
14 Crohn's Disease when compared to the blood of healthy controls.

15 We extracted exact mRNA expression levels for TNFSF13B in $n=22$ healthy controls
16 and $n=41$ patients with active Crohn's Disease to directly compare TNFSF13B expression in
17 the peripheral blood between diseased and control subjects. TNFSF13B was expressed at
18 significantly higher levels in the blood of patients with Crohn's Disease as compared to healthy
19 controls. While the median TNFSF13B level in healthy controls was 11.84 AU (arbitrary units),
20 the median TNFSF13B mRNA level in patients with active CD was 12.22 AU. The mean
21 TNFSF13B mRNA expression level in healthy controls was 11.31 ± 0.2393 AU, while the mean
22 TNFSF13B expression level in patients with active CD was 11.58 ± 0.2479 AU. Increased
23 expression of TNFSF13B in the peripheral blood of patients with CD was statistically significant
24 (Figure 1; $p<0.0001$).
25
26

27 Thus, we found that the gene encoding the B-cell activating factor / B-lymphocyte
28 stimulator was among the genes whose expression was most different in the blood of patients

1 with Crohn's Disease when compared to that of healthy control subjects. TNFSF13B was
2 expressed at significantly higher levels in the blood of CD patients when compared to controls.

4 **Discussion**

5 We identified the TNF receptor superfamily member TNFSF13B, also known as CD257,
6 BAFF, BLyS, and TALL-1, as differentially expressed in the blood of patients with Crohn's
7 Disease and in CD patient monocyte-derived macrophage using published microarray datasets
8 (2-4) BAFF was expressed at significantly higher levels in the peripheral blood of patients with
9 Crohn's Disease as compared to healthy controls.

10 BAFF is a Type II membrane protein and ligand of the TNF receptor superfamily (5).
11 BAFF/BLyS interacts with the BAFF receptor (BAFF-R), also known as BR3, encoded by
12 TNFRSF17; with TACI, also known as CD267, encoded by TNFRSF13B, and with BCMA also
13 known as CD269, encoded by TNFRSF13C (15, 16). Upon cleavage by furin at a furin
14 consensus site, BAFF can be released as a soluble factor in trimeric form. Quaternary
15 structure of BAFF trimers is dependent on pH. At neutral or basic pH, 20 molecules of the
16 BAFF trimeric ligand associate to form a 60-mer assembly that resembles a viral-like structure.
17 At acidic pH, this 60-mer disassembles into its monomeric trimer form (15).

18 Functionally, BAFF provides survival signals for the development of B-cells (17, 18).
19 The secondary lymphoid organs of BAFF deficient mice are almost completely devoid of
20 follicular and marginal B-lymphocytes (17). BCMA-deficient mice had intact B-lymphocyte
21 compositions in the secondary lymphoid organs, demonstrating that BAFF provides essential
22 survival signals for B-lymphocyte development independent of signaling through BCMA (17).
23 While mature B-1 lymphocytes were present in BAFF/BLyS-deficient mice, there was an
24 absence of nearly all other mature B-lymphocytes with a block at the T1 transitional
25 developmental stage. Consistent with a role for BAFF/BLyS in providing developmental
26 survival signals to B-lymphocytes through interactions with its cognate receptors including
27 TACI, treating mice with soluble TACI receptor lead to a specific decrease in mature B-
28

lymphocyte populations in the periphery (18). The exact nature of the signaling cascades produced following engagement of the BAFF ligand with the BAFF receptor is incompletely understood but may involve classical NF- κ B signals as B-cell development in NEMO deficient mice is arrested at the same stage as in BAFF-R deficient mice, and constitutive activation of IKK2 permits B-cell survival independent of BAFF (19).

In the intestinal mucosa of patients with Crohn's Disease, levels of BAFF were found to be significantly increased (6). Fecal BAFF was also found to be increased in patients with Crohn's Disease and suggested to be a biomarker to help distinguish between irritable bowel syndrome (IBS) from inflammatory bowel disease (IBD) (7). Serum BAFF was also found to be elevated in patients with Crohn's Disease (6). BAFF/BLyS is important for the survival of B cells and when over-expressed in transgenic mice can lead to autoimmunity that resembles systemic lupus erythematosus and Sjögren's syndrome. This autoimmunity arises at least in part through T-cell independent activation of low-affinity B-cells reactive to self (20, 21).

There is literature regarding a potential role for B-cell abnormalities in patients with Crohn's Disease. One study revealed a decrease in memory IgM⁺ B-cells in patients with IBD (22). A second study investigating B-cell dynamics in the blood of patients with Crohn's Disease demonstrated that the number of CD38^{high} CD24^{high} transitional B-cells was significantly higher in the blood of patients with Crohn's Disease. CD21^{low} B-cells (23). CD27⁺ IgM⁺ IgD⁺ natural effector B-cells were also significantly decreased in CD patient blood, consistent with earlier findings (23). Remarkably, Crohn's Disease patients treated with infliximab, a tumor necrosis factor inhibitor, displayed transitional B-cell counts similar to control (significantly less than patients with Crohn's Disease not receiving infliximab) (23). Somatic hypermutation is a mechanism by which B-cells can introduce genetic variation into immunoglobulin gene fragments in B-cells through a mutagenic process involving the activation-induced cytidine deaminase (AID) (24) as they encounter diverse microbial stimuli in an attempt to mount a humoral response. B-cells from patients with Crohn's Disease displayed defects in somatic hypermutation (SHM) as assessed by IGHV gene mutation levels.

SHM mutation level in the IGHV gene of natural effector B-cells from patients with Crohn's Disease was low in number compared to B-cells from healthy controls, and the amino acids targeted for mutation were poorly selected for, relative to mutations found in the complementary-determining region (CDR) of the IGHV gene in B-cells from healthy patients (23). Shorter length of CDR3 is indicative of IGH gene rearrangements, found in antigen-experienced B-cells. The CDR3 length of the IGHV gene in B-cells from patients with Crohn's Disease was significantly longer compared to CDR3 length in B-cells from control subjects. Moreover, while control effector B-cells had divided on average 9 times, effector B-cells from patients with Crohn's Disease divided only twice, similar to naïve mature B-cells. Together, these data indicated that IgM+ IgD+ memory B-cells from patients with Crohn's Disease, in peripheral circulation, displayed abnormalities in somatic hypermutation and in their replication history. In contrast, memory B-cells expressing IgA or IgG immunoglobulin transcripts displayed higher than average SHM frequencies as assessed by IGHV3 and IGHV4 mutation level. The CDR3 length of these genes was similar to control. Assessing SHM frequency in IGHV genes for IgA and IgG transcripts revealed that B-cells from patients with Crohn's Disease displayed a preference for IgA1 and IgG2 usage. Together, these data reveal changes in the composition of the B-lymphocyte population in CD patient blood, and demonstrate that B-cells from patients with Crohn's Disease manifest defects in somatic hypermutation.

We found that BAFF/BLyS is expressed at significantly higher levels in the peripheral blood of patients with active Crohn's Disease than in the blood of control subjects. We also found that BAFF/BLyS was among the genes whose expression is most different when comparing the blood and monocyte-derived macrophage of patients with CD with that of control subjects. If validated in larger and separate cohorts, the functional significance of BAFF/BLyS differential expression in B-lymphocytes from patients with Crohn's Disease should be evaluated.

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Rank	ID	p-value	t	B	Gene	Gene name
2953	7969986	4.59E-04	3.6697399	-0.3367351	TNFSF13B	tumor necrosis factor superfamily member 13b

Table 1: The gene encoding the B-cell activating factor / B-lymphocyte stimulator is differentially expressed in the blood cells of patients with Crohn's Disease.

The rank of global differential expression in each dataset, the transcript or probe ID, the p-value with respect to differential expression transcriptome-wide, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared (Crohn's Disease and healthy controls), the log fold-change in mRNA expression between the two groups, the gene and gene name are represented in this chart.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
200	10673_at	5.8E-05	4.2703424	1.698022		TNFSF13B	tumor necrosis factor superfamily member 13b

Table 2: The gene encoding the B-cell activating factor / B-lymphocyte stimulator is differentially expressed in the blood cells of patients with Crohn's Disease.

The rank of global differential expression in each dataset, the transcript or probe ID, the p-value with respect to differential expression transcriptome-wide, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared (Crohn's Disease and healthy controls), the log fold-change in mRNA expression between the two groups, the gene and gene name are represented in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
367	ILMN_1758418	0.0133479	-2.518627	-2.965	TNFSF13B	tumor necrosis factor superfamily member 13b
1178	ILMN_2066858	0.047553	-2.005744	-3.967	TNFSF13B	tumor necrosis factor superfamily member 13b

Table 3: The gene encoding the B-cell activating factor / B-lymphocyte stimulator is differentially expressed in monocyte-derived macrophage from patients with Crohn's Disease.

The rank of global differential expression in each dataset, the transcript or probe ID, the p-value with respect to differential expression transcriptome-wide, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared (Crohn's Disease and healthy controls), the log fold-change in mRNA expression between the two groups, the gene and gene name are represented in this chart.

Figure 1: TNFSF13B is expressed at significantly higher levels in the peripheral blood of patients with Crohn's Disease as compared to healthy controls.

TNFSF13B expression in the whole blood of healthy control subjects (left), and in the whole blood of patients with Crohn's Disease (right) is displayed with the mean mRNA expression value marked and p-value listed.