IFITM1 is differentially expressed in the blood of patients with Crohn's Disease.

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The interferon pathway (1) is an innate immune signaling system that evolved to protect multicellular organisms from viral infections (2). The expression of multiple gene families is expressed following activation of interferon signaling (1); one of these is the antiviral interferon inducible transmembrane proteins, or IFITM gene family (3). Using publicly available datasets (4, 5), we identified the interferon induced transmembrane protein 1, IFITM1 (6-10), as a differentially expressed gene in the whole blood and CD4+ CD25+ regulatory T-cells of patients with Crohn's Disease. IFITM1 was expressed at significantly higher levels in the whole blood and T_{reg} of patients with Crohn's Disease than in non-affected control subjects. We previously reported that changes in gene expression of the double stranded ribonucleic acid sensor, 2'-5'-oligoadenylate synthetase, OAS2, were among the most significant quantitative differences in the gene expression of monocyte-derived macrophage and regulatory T-cells from patients with Crohn's Disease when compared to those not affected. Together, these data suggest that a primary defect in the coordination of the host response to viral infection in the hematopoietic compartment, through nucleic acid sensing and subsequent interferon signaling may be fundamentally linked with the pathogenesis of Crohn's Disease.

Keywords: IFITM, IFITM1, interferon induced transmembrane protein 1, Crohn's Disease, systems biology of Crohn's Disease.

1 The inflammatory bowel diseases, including Crohn's disease and ulcerative colitis,
2 affect nearly 1.8 million patients in the United States (11). Clinical observations and data from
3 genome-wide association studies (GWAS) have pointed to defects in pathogen defense and
4 pattern recognition as central to the pathogenesis of Crohn's disease (12-14). Specifically, one
5 clue as to the importance of pathogens and microbiota in the natural course of Crohn's disease
6 is the beneficial effect of antibiotics in that disease remission can in some cases be achieved
7 with the use of antibiotics alone (15, 16). This suggests that interactions between the host and
8 commensals or pathogenic bacteria are important for induction of inflammation. Studies of
9 mutant mice modeled to possess the ATG16L1 allele found by GWAS to confer risk of
10 development of Crohn's disease revealed that possession of the variant allele alone resulted in
11 no evident inflammatory phenotype, but in conjunction with murine norovirus infection resulted
12 in abnormal Paneth cell pathology of the small bowel, similar to that seen in patients with
13 Crohn's Disease to develop (17). Intriguingly, though virus infection triggered Paneth cell
14 pathology, antibiotic treatment resulted in resolution of this pathology (17). These data support
15 the notion that pathogen sensing, of both viruses and bacteria contribute to the disease
16 process in Crohn's disease. Unbiased, transcriptome-level analysis of gene expression in the
17 tissues of patients with Crohn's Disease can reveal the fundamental differences between the
18 transcriptional networks that govern cell biology in patients with Crohn's Disease versus that of
19 patients without an inflammatory bowel disease.
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22 In this study, we mined published transcriptome data (4) generated from the blood of
23 patients with Crohn's disease and found that the interferon induced transmembrane protein
24 IFITM1 was among the genes most differentially expressed in CD patient blood. The
25 expression of IFITM1 is induced following viral infections and triggering of the interferon
26 antiviral signaling pathway. Analysis of a separate dataset (5) revealed that the expression of
27 IFITM1 in the regulatory T-cells of patients with Crohn's disease was significantly increased as
28 compared to regulatory T-cells of control, non-affected subjects. IFITM1 expression is
abnormally elevated in the hematopoietic compartment of patients with Crohn's disease.

Methods

We used GSE94648 (4) and GSE23996 (5) for this analysis in conjunction with GEO2R. GSE94648 (4) was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with n=22 whole blood from controls and n=50 whole blood from patients with Crohn's Disease. GSE23996 (5) was generated using the HCI Human TReg Chip with n=6 CD patients (3 inactive CD patients and 3 active CD patients) and n=29 healthy controls (11 patients; 29 chips).

The Benjamin 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 IFITM1 in the blood of patients with Crohn's disease as compared to healthy controls, and between monocyte-derived macrophages from CD patients and from control subjects using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant.

Results

We assessed global gene expression behavior using two separate microarray datasets (4, 5) to determine which genes changed in expression most significantly in the blood and regulatory T-cells (T_{reg}) of patients with Crohn's disease compared to that of non-affected control subjects.

IFITM1 is differentially expressed in the whole blood and regulatory T-cells of patients with Crohn's Disease.

We identified IFITM1 as among the most differentially expressed genes in the blood of Crohn's disease. When ranking all of the genes in the transcriptome whose expression was

1 measured by microarray based on the difference in expression in the blood of patients with
2 Crohn's disease versus that of control, non-affected subjects, IFITM1 was ranked 1 out of
3 19674. Transcriptome-wide differential expression of IFITM1 in the blood of patients with
4 Crohn's Disease was statistically significant (Table 1; $p=6.5E-09$).

5 We analyzed a separate dataset that measured a more limited set of genes but
6 specifically in CD4+ CD25+ regulatory T-cells and found that IFITM1 was also a differentially
7 expressed gene in the T_{reg} of patients with Crohn's disease (Table 1). Differential expression of
8 IFITM1 in CD T_{reg} was ranked 85 out of 397; this was statistically significant (Table 1;
9 $p=4.01E-04$).

11 IFITM1 is expressed at significantly higher levels in the blood of patients with Crohn's Disease
12 than in the blood of non-affected control subjects.

13 Exact IFITM1 mRNA expression levels from the blood of patients with Crohn's disease
14 and from the blood of non-affected control subjects were extracted from this microarray
15 dataset, and a statistical test was used to determine whether this difference in expression was
16 statistically significant. IFITM1 was expressed at significantly higher levels in the blood of
17 patients with Crohn's disease than in non-affected control subjects and this difference was
18 statistically significant (Figure 1; $p<0.0001$).

20 IFITM1 is expressed at significantly higher levels in the regulatory T-cells of patients with
21 Crohn's Disease than in the blood of non-affected control subjects.

22 We also extracted IFITM1 mRNA expression levels from the T_{reg} of patients with Crohn's
23 disease and from the T_{reg} of non-affected control subjects, evaluating whether the difference in
24 mRNA expression was statistically significant. IFITM1 was expressed at significantly higher
25 levels in the T_{reg} of patients with Crohn's disease than in non-affected control subjects and this
26 difference was statistically significant (Figure 2; $p=0.0084$).

Discussion

We found here that IFITM1 was the single most differentially expressed gene in the blood of patients with Crohn's disease when compared to that of non-affected control subjects. IFITM1 was also differentially expressed in the T_{reg} of patients with Crohn's disease, and in both the whole blood and T_{reg}, IFITM1 was expressed at significantly higher levels in patients with Crohn's disease.

IFITM proteins block replication of enveloped viruses at a step in the viral life cycle before entry (3). IFITM1 has restriction activity against influenza A, Ebola virus and the severe acute respiratory syndrome (SARS) coronavirus, with its activity targeted towards the lysosome and viral dependencies on lysosomal cathepsins (18). As a family, IFITM proteins contain two transmembrane domains (3). IFITM1 possesses broad, ubiquitous expression (3). In B cells, IFITM1 has been demonstrated to interact with CD81 and indirectly with CD19 and CD21 but the functional significance of these interactions is unknown (19, 20). Mice deficient in the entire IFITM locus are developmentally normal despite reports that IFITM1 and IFITM3 may be important for germ cell development (21, 22)

Literature analysis reveals limited previously published information regarding the relevance of the IFITM gene family in inflammatory bowel diseases. In the related but distinct inflammatory bowel disease ulcerative colitis, polymorphisms in IFITM1 and IFITM3 were reported to associate with the risk of development of UC in a Korean population (23, 24).

Perturbed expression of the IFITM1 gene (25, 26) in patients with Crohn's disease and differential DNA methylation (27) of the IFITM1 locus in patients with ulcerative colitis has been reported in patients with Crohn's disease has been reported by multiple groups but all of these studies assessed intestinal tissues or colonic mucosa. There has been no report of IFITM1 differential expression in the blood of patients with Crohn's disease, thusfar.

In 1985, Kelly et al. reported that eight messenger RNAs were induced in HeLa cells following exposure to alpha and gamma interferons (8). These included a 2-5A synthetase and five unknown mRNAs that were induced 2-100 fold. One of these mRNAs encoded for what

1 would later become to be known as the interferon induced transmembrane protein 1, IFITM1.

2 We previously reported that the 2'-5' oligoadenylate synthetase OAS2 (28) was among
3 the most differentially expressed genes in the monocyte-derived macrophages and regulatory
4 T-cells of patients with Crohn's disease (29). Together these data reveal that two central targets
5 of the interferon-inducible gene expression program, OAS2 and IFITM1 are differentially
6 expressed in the blood cells of patients with Crohn's disease.

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8 The functional relevance of the differential and increased expression of IFITM1 in the
9 blood of patients with Crohn's disease may start to be assessed by *ex vivo* viral infection
10 across a panel of viruses, single- and double-stranded as well as RNA and DNA viruses using
11 patient blood and sorted white cell subsets from patient blood. Some of these effects may
12 only be recapitulated in the whole organism, and in these cases the use of DSS colitis murine
13 models of colitis may be required (30). IFITM1 deficient mice may also be assessed for the
14 propensity to develop inflammatory bowel disease-like pathologies. Differential expression of
15 IFITM1 must be validated in separate and larger cohorts of CD patients, and whether IFITM1
16 expression is dysregulated in other blood cell types must be assessed systematically. These
17 results demonstrate the value of unbiased, global differential gene expression analyses to
18 complement our understanding of the ways cell biology and gene expression are altered in
19 patients with Crohn's Disease. The sensing of viral infections in the hematopoietic tissues,
20 ensuing anti-viral response via interferon signaling and the effect of the interferon-inducible
21 gene expression program in the blood on the gut tissues may be a critical step in the
22 development of Crohn's Disease and/or in the induction of inflammatory states in patients with
23 Crohn's Disease.
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Rank	probe/ transcript ID	p-value	t	B	gene	gene name
GSE94648						
1	8519_at	6.5E-09	6.5696221	10.007017	IFITM1	interferon induced transmembrane protein 1
GSE23996						
85	6050785 NM_003641 NG_001336.2	4.01E-04	3.90249	-0.2467	IFITM1	interferon induced transmembrane protein 1

Table 1: IFITM1 is differentially expressed in the whole blood and regulatory T-cells of patients with Crohn's Disease.

The rank of differential expression relative to the entire transcriptome (GSE94648) or a selected set of genes expressed in the regulatory T-cell (GSE23996), the probe or transcript ID, the p-value, t, a moderated t-statistic, B, log-odds of differential expression between the groups compared (control and CD), the gene and gene name are represented in this chart.

Figure 1: IFITM1 is expressed at significantly higher levels in the blood of patients with Crohn's Disease than in the blood of non-affected control subjects.

The expression of IFITM1 in control blood (n=22) and in the blood of patients with Crohn's Disease (n=50) is graphically represented along with mean mRNA expression value and p-value.

Figure 2: IFITM1 is expressed at significantly higher levels in the regulatory T-cells of patients with Crohn's Disease than in the blood of non-affected control subjects.

The expression of IFITM1 in control T_{reg} (n=11 patients; n=29 chips) and in the T_{reg} of patients with Crohn's Disease (n=6 patients; n=6 chips: 3 patients [active CD] and 3 patients [inactive CD]) is graphically represented along with mean mRNA expression value and p-value.