The interleukin 7 receptor is differentially expressed in the blood of patients with Crohn's Disease.

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The inflammatory bowel diseases include Crohn's Disease and Ulcerative Colitis (1). Crohn's Disease has been understood as a complex genetic disorder of multifactorial nature (2) but these factors remain incompletely understood. We mined multiple published and public datasets (3, 4, 5) to determine in an unbiased and systematic fashion which genes were expressed most differently in the blood cells of patients with Crohn's Disease as compared to healthy controls. We found that the gene encoding the interleukin 7 receptor, IL7R, was among the genes whose expression changed most significantly between patients with Crohn's Disease as compared to healthy controls, in the whole blood, in monocyte-derived macrophages, and in regulatory T-cells (T_{reg}). While patients with Crohn's Disease expressed significantly lower levels of IL-7R in the whole blood and in monocyte-derived macrophage than healthy controls, patients with Crohn's Disease expressed significantly higher levels of IL-7R in T_{reg}. IL-7R expression has been studied in the intestinal tissues of patients with Crohn's Disease but this is the first study to document differential expression of the IL-7 receptor in the blood cells of patients with Crohn's Disease.

Keywords: Crohn's Disease, IBD, CD, IL7R, IL-7, interleukin 7, genetics of CD

1 Almost 20 years after the identification of the IBD1 susceptibility locus as NOD2 (6), the
2 genes that conspire to produce the Crohn's Disease phenotype have still not been defined. The
3 number of patients diagnosed with Crohn's Disease in the developed world has rapidly
4 increased over the past several decades (7) and the cause of this increase remains unknown
5 but has been attributed to lifestyle differences including stress, diet, and increased hygiene (8)
6 leading to a shift from Th2 to Th1-mediated responses due to lack of helminth infection (9).
7 However, study of Ashkenazi Jewish populations and families with inborn errors of immunity
8 have demonstrated that Crohn's Disease, more so than Ulcerative Colitis, is a disease caused
9 by genetic susceptibility (2). Complicating the understanding of CD susceptibility genetics is the
10 fact that in a single person, multiple genes can contribute to a complex genetic disorder, and
11 within an affected population, different genes can collaborate to cause the same complex
12 genetic disorder in different patients.
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14 In this study, we used an unbiased and systems-level method to compare the blood cell
15 transcriptomes patients with Crohn's Disease with that of healthy patients, in the whole blood, in
16 monocyte-derived macrophage, and in regulatory T-cells (T_{reg}) using published and public
17 datasets (3, 4, 5). We found that the gene encoding the interleukin-7 receptor was among the
18 genes whose expression was most different in patients with Crohn's Disease across the whole
19 transcriptome - in the whole blood, in monocyte-derived macrophages, and in T_{reg} . These data
20 suggest that IL-7R biology, not just in the intestinal tissues, but in the hematopoietic system,
21 may be important to the pathogenesis of Crohn's Disease.
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24 **Methods**

25 We utilized GSE126124 (3), GSE60083 (4), and GSE23996 (5) for this study in
26 conjunction with GEO2R. GSE126124 was generated using Affymetrix Human Gene 1.0 ST
27 Array technology with n=39 CD patients and n=32 healthy controls. GSE60083 was generated
28 using Illumina HumanWG-6 v3.0 expression beadchip technology with n=58 CD patients and

n=42 healthy controls. GSE23996 was generated using the HCI Human TReg Chip with n=6 CD patients and n=11 healthy controls.

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 IL-7R in 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 probed three separate datasets, public and published, to identify genes that are transcriptionally perturbed in the blood of patients with Crohn's Disease. We identified IL7R as one of the most differentially expressed genes transcriptome-wide, across all three datasets.

The gene encoding the interleukin 7 receptor is differentially expressed in the blood cells of patients with Crohn's Disease.

IL7R was among the genes whose expression changed most significantly when comparing the whole blood transcriptomes of patients with Crohn's Disease versus that of healthy controls (Table 1). IL7R was ranked 22 out of 33297 total transcripts; this differential expression was statistically significant (Table 1; $p=6.63E-11$).

When comparing the transcriptomes of monocyte-derived macrophages, from patients with Crohn's Disease versus that of healthy controls, IL7R was again identified as among the genes whose expressed changed most significantly transcriptome-wide. IL7R was ranked 85 out of 10468 total transcripts, and this was also statistically significant (Table 1; $p=0.0031219$).

A third comparative transcription analysis, between the regulatory T-cells of patients with Crohn's Disease versus that of healthy controls, revealed that IL7R was also among the genes

whose expression changed most significantly in CD25⁺ CD4⁺ Treg. IL7R was ranked 85 out of 397 total transcripts, and this was also statistically significant (Table 1; $p=4.3E-03$).

Thus, in the whole blood, the monocyte-derived macrophages, and in the regulatory T-cells of patients with Crohn's Disease, the IL-7 receptor is among the genes whose expression changes most significantly. In the whole blood and in macrophages, this was transcriptome-wide.

IL-7R is expressed at lower levels in the whole blood and monocyte-derived macrophages of patients with Crohn's Disease as compared to healthy controls.

We then compared the expression levels of IL-7R between patients with Crohn's Disease and healthy controls. Patients with Crohn's Disease expressed significantly lower levels of IL-7R in the whole blood (Figure 1A, $p<0.0001$) and in the monocyte-derived macrophages than healthy controls did (Figure 1B, $p=0.0053$).

IL-7R is expressed at higher levels in the regulatory T-cells of patients with Crohn's Disease as compared to healthy controls.

When comparing expression of IL-7R between patients with Crohn's Disease and healthy controls in regulatory T-cells, we found that IL-7R was expressed at higher, rather than lower levels in T_{reg} when compared to healthy controls. This was also statistically significant (Figure 2; $p=0.0444$).

Discussion

We studied multiple published and public datasets (3, 4, 5) to describe the transcriptional landscape of the blood cells of patients with Crohn's Disease in terms of the most significant gene expression changes compared to blood from healthy patients. We found that the receptor for IL-7 was among the genes whose expression was most different when comparing the whole blood, the monocyte-derived macrophages, and the regulatory T-cells from patients with Crohn's Disease versus that of healthy controls.

IL-7 is required for both B- (10, 11) and T-cell (12, 13) development in mice but IL-7 is not absolutely required for B-cell development in humans (14). Studies of mice deficient in the IL-7 receptor demonstrated that IL-7 signaling is required for steps in lymphocyte maturation early in development and before T-cell receptor rearrangement events (15). Mice deficient in the IL-2 receptor maintain a population of immature CD4⁺ CD25⁺ Foxp3^{high}, but double deficiency in IL-2R β and the alpha chain of the IL-7R (IL-7R α) leads to complete deficiency in CD4⁺ CD25⁺ Foxp3⁺ T_{reg} (16). This demonstrates that IL-7 signaling downstream of the IL-7 receptor in collaboration with IL-2 is important for the development of regulatory T-cells. Gamma delta T-cells of the blood are also affected by absence of IL-7 as IL7 receptor deficient mice lack both splenic and intestinal epithelial gamma delta T-cells (17). IL-7 is produced in both the thymus (18), likely by cortical epithelial cells (19) and in the bone marrow (20), as well as by multiple other cell types including traditional (21) and follicular dendritic cells (22), the intestinal epithelium (23), fetal and adult liver (24, 25) as well as keratinocytes (26) (27). IL-7 controls the size of the peripheral T-cell pool by providing proliferation cues to expand the size of the pool if it is insufficient (28). If the size of the pool is too large, IL-7 becomes limiting, is then unable to support survival and proliferation of all cells, and the size of the pool shrinks. At the molecular level, IL-7 exerts this function by controlling expression of apoptotic regulators like Bcl-2 (29).

A recent study of showed an accumulation of mRNA message for the IL-7R in the intestinal tissues (specifically, the colon) of patients with Crohn's Disease that were refractory to treatment, as compared to patients who had responded to treatment and as compared to healthy controls (30). Inflammation in the intestines lead to production of IL-7, which in periphery, in turn, activated effector T-cells (T_{eff}) that expressed the IL-7R, induced expression of the $\alpha 4\beta 7$ integrin, supporting infiltration of the gut with activated IL7R α ⁺ $\alpha 4\beta 7$ ⁺ T_{eff}. In this way, local IL-7 production by primary inflammatory events promoted an inflammatory circuit constituted by activated T-cells from the periphery that maintain an inflammatory state. Consistent with these findings, blockade of the IL-7 receptor ameliorated inflammation in this

1 study and in another study that examined T-bet deficient mice and found that T-bet controlled
2 expression of IL-7R to promote gut homeostasis (31).

3 IL-7R is a key molecule that can control intestinal homeostasis by acting as the receptor
4 for a molecule that has been termed a “fuel” for autoimmune and inflammatory disease (32).
5 While other groups have previously reported accumulation of mRNA message for IL-7R in the
6 colonic tissues of patients with Crohn’s Disease that were refractory to treatment, we found that
7 the IL-7R receptor was among the genes most differentially expressed in the whole
8 transcriptome of patients with Crohn’s Disease, in the whole blood and in monocyte-derived
9 macrophages, at significantly lower levels than in controls. We also found that IL-7R mRNA
10 was expressed significantly higher levels in regulatory T-cells compared to healthy controls.
11 Total resolution of the damaging inflammatory process in Crohn’s Disease in the individual
12 patient and at the population level will not occur without the understanding that the
13 hematopoietic system is critical to the pathogenesis of inflammatory bowel disease (33-36).
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Rank	ID	p-value	t	B	logFC	Gene	Gene name
GSE126124							
22	8104901	6.63E-11	-7.6405203	14.5677717	-0.82399623	IL7R	interleukin 7 receptor
GSE60083							
85	ILMN_1691341	0.0031219	-3.028157	-1.777	-0.45135156	IL7R	interleukin 7 receptor
GSE23996							
136	6050482	4.3E-03	3.04791	-2.4749	0.67301	IL7R	interleukin 7 receptor

Table 1: The gene encoding the interleukin 7 receptor 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.

Figure 1: IL-7R is expressed at lower levels in the whole blood and monocyte-derived macrophages of patients with Crohn's Disease as compared to healthy controls.

Figure 1A: IL-7R expression in the whole blood of healthy patients (green), and in the whole blood of patients with Crohn's Disease (red) in each patient or control is displayed with the mean mRNA expression value marked and p-value listed.

Figure 1B: IL-7R expression in the monocyte-derived macrophages of healthy patients (green), and in the monocyte-derived macrophages of patients with Crohn's Disease (red) in each patient or control is displayed with the mean mRNA expression value marked and p-value listed.

Figure 2: IL-7R is expressed at higher levels in the regulatory T-cells of patients with Crohn's Disease as compared to healthy controls.

IL-7R expression in the regulatory T-cells (T_{reg}) of healthy patients (green), and in the T_{reg} of patients with Crohn's Disease (red) in each patient or control is displayed with the mean mRNA expression value marked and p-value listed.