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Single-Cell RNA Sequencing of Blood and Ileal T Cells From Patients With Crohn's Disease Reveals Tissue-Specific Characteristics and Drug Targets

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Inflammatory Bowel Disease; Genome-wide Association Study; Immune Response; Genetics

Crohn's disease (CD) is a chronic inflammatory disease predominantly affecting the terminal ileum. The ~200 CD-risk loci identified by genome-wide association studies are enriched for genes involved in T-cell signaling, highlighting the importance of T cells in CD pathology.[1,2] It is crucial to study T cells in their disease-relevant context, namely the

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Author contributions

Werna T. Uniken Venema and Michiel D. Voskuil contributed equally to this work and are shared first authors. Werna T. Uniken Venema, Michiel D. Voskuil, and Eleonora A. Festen participated in the conception, design and coordination of the study. Gerard Dijkstra, Rinse K. Weersma, and Eleonora A. Festen recruited patients. Werna T. Uniken Venema, Michiel D. Voskuil, and Bernadien H. Jansen performed the laboratory experiments. Werna T. Uniken Venema, Michiel D. Voskuil, Gerben van der Vries, Arnau Vich Vila, and Eleonora A. Festen performed the data analyses and data interpretation. Bana Jabri, Klaas Nico Faber, Gerard Dijkstra, Ramnik J. Xavier, Cisca Wijmenga, and Daniel B. Graham provided support with data interpretation. All authors assisted in the writing and reviewing of the manuscript and approved of sending it out for publication.

Conflicts of interest

There are no (potential) conflicts of interest to declare.

Writing assistance

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Supplementary Material

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intestinal mucosa. Although human single-cell atlases are in development,[3] location-and disease-specific single-cell RNA sequencing (scRNAseq) datasets are still scarce. In this study, we used scRNAseq of disease-relevant cells to examine pathomechanisms and identify potential drug targets on a cellular level in CD.

Methods

We performed flow cytometry and scRNAseq of 5292 CD3-positive T lymphocytes isolated from peripheral blood (PBLs) and ileal biopsy specimens from 3 patients with CD with mild to moderate disease activity (Figure 1, step 1). Biopsy specimens were dissociated and separated into intraepithelial T lymphocytes (IELs) and lamina propria T lymphocytes (LPLs).[4] Then, we integrated T-cell transcriptomes with genome-wide association study CD-risk loci and drug-target identification resources. Through a literature search and online database analysis, we identified 179 CD-risk genes and 2712 drug-target genes that we aligned to differentially expressed genes. Then, we selected genes encoding proteins targeted by drugs currently available for (clinical trials in) humans (Supplementary Methods).

Results

After quality control, 4070 T cells remained for analysis. These cells expressed 966 genes per cell, on average, and 41,134 distinct genes in total (Figure 1, step 2). IELs, LPLs, and PBLs showed markedly different expression profiles (Supplementary Figure 1 and Supplementary Table 1).

Unsupervised clustering of scRNAseq data identified 6 distinct T-cell types that have different distributions in IELs, LPLs, and PBLs: cytotoxic T lymphocytes (CTLs) dominate IELs, quiescent T cells dominate the PBL reservoir, and T-helper 17 (Th17) cells dominate LPLs (Figure 1, step 3). In peripheral blood, T-regulatory (Treg) cells intermixed with effector T cells (effector/Treg cells) and quiescent T cells (Treg/quiescent cells). Treg/quiescent cells also were present among IELs and LPLs. Among IELs and LPLs, we identified a cluster of cells of a previously undefined cell type characterized by expression of *CD3* and *REG1A/B* and confirmed this with immunofluorescence staining. All T-cell subtypes were present in all patients.

Strikingly, all cell-subtype clusters consisted of epitopic CD8$\alpha\beta$-positive and -negative cells. Moreover, *CD8A* and *CD8B* transcripts were expressed in epitopic CD8$\alpha\beta$-positive and -negative cells, which was confirmed in a publicly available naïve $CD4^+$ T-cell scRNAseq dataset.[5]

Using permutation analysis, we found that IELs and LPLs expressed significantly more CD-risk genes than expected by chance ($P = .00389$ and $P < .00001$, respectively), suggesting that cells within these compartments play a role in CD inflammation.[2] PBLs were not enriched for CD-risk gene expression ($P = .45954$). Th17 cells showed the largest number of over-expressed CD-risk genes and were most specifically enriched for CD-risk gene expression ($P < .00001$). Mucosal CTL and Treg/quiescent and peripheral blood effector/Treg cells also were significantly enriched for CD-risk gene expression ($P = .0278$, $P = .02477$, and $P = .00081$, respectively).

We investigated which drug targets are expressed by Th17 cells and CTLs, cell types with well-characterized expression signatures that play a central role in CD pathogenesis (Table 1). Th17 cells showed up-regulation of *IL17A,* whose gene product is targeted by secukinumab. *ITGAE,* whose gene product is targeted by etrolizumab, was upregulated in mucosal CTLs. *S1PR5,* up-regulated in peripheral blood CTLs, is a known drug target for ozanimod. Potential targets for drug repositioning include *PDE4D* in mucosal Th17 cells, a target for apremilast, under investigation for treatment of ulcerative colitis; *ITGB2* in peripheral blood CTLs, a target for lifitegrast, approved for keratoconjunctivitis sicca; and *ALOX5AP* in mucosal CTLs, a target for fiboflapon, under investigation for asthma.

Discussion

We have demonstrated that multiple ileal mucosal T-cell subtypes and 1 peripheral blood T-cell subtype from patients with CD are enriched for CD-risk gene expression. T-cell subtypes known to be involved in CD pathogenesis provide promising targets for future cell type-specific therapies in patients with CD. A limitation of our study is the small sample, which might decrease the amount of variation covered. However, most cell type-specific gene expression signatures remained after correcting for inter-individual differences. Because location-and disease-specific scRNAseq data are still limited, detailed datasets like ours are an important reference for furthering our understanding of the molecular processes leading to health and disease and identifying potential targets for drug development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper:

CD	Crohn’s disease
CTL	cytotoxic T lymphocyte

IEL	intraepithelial T lymphocyte
LPL	lamina propria T lymphocyte
PBL	peripheral blood T lymphocyte
scRNAseq	single-cell RNA sequencing
Th17	T-helper 17 cell
Treg	T-regulatory cell

References

1. Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet 2015;47:979–986. [PubMed: 26192919]
2. Farh KK-H, Marson A, Zhu J, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature 2015;518:337–343. [PubMed: 25363779]
3. Regev A, Teichmann SA, Lander ES, et al. The human cell atlas. Elife 2017;6:e27041.
4. Raine T, Liu JZ, Anderson CA, et al. Generation of primary human intestinal T cell transcriptomes reveals differential expression at genetic risk loci for immune-mediated disease. Gut 2015;64:250–259. [PubMed: 24799394]
5. Zheng GXY, Terry JM, Belgrader P, et al. Massively parallel digital transcriptional profiling of single cells. Nat Commun 2017;8:14049. [PubMed: 28091601]

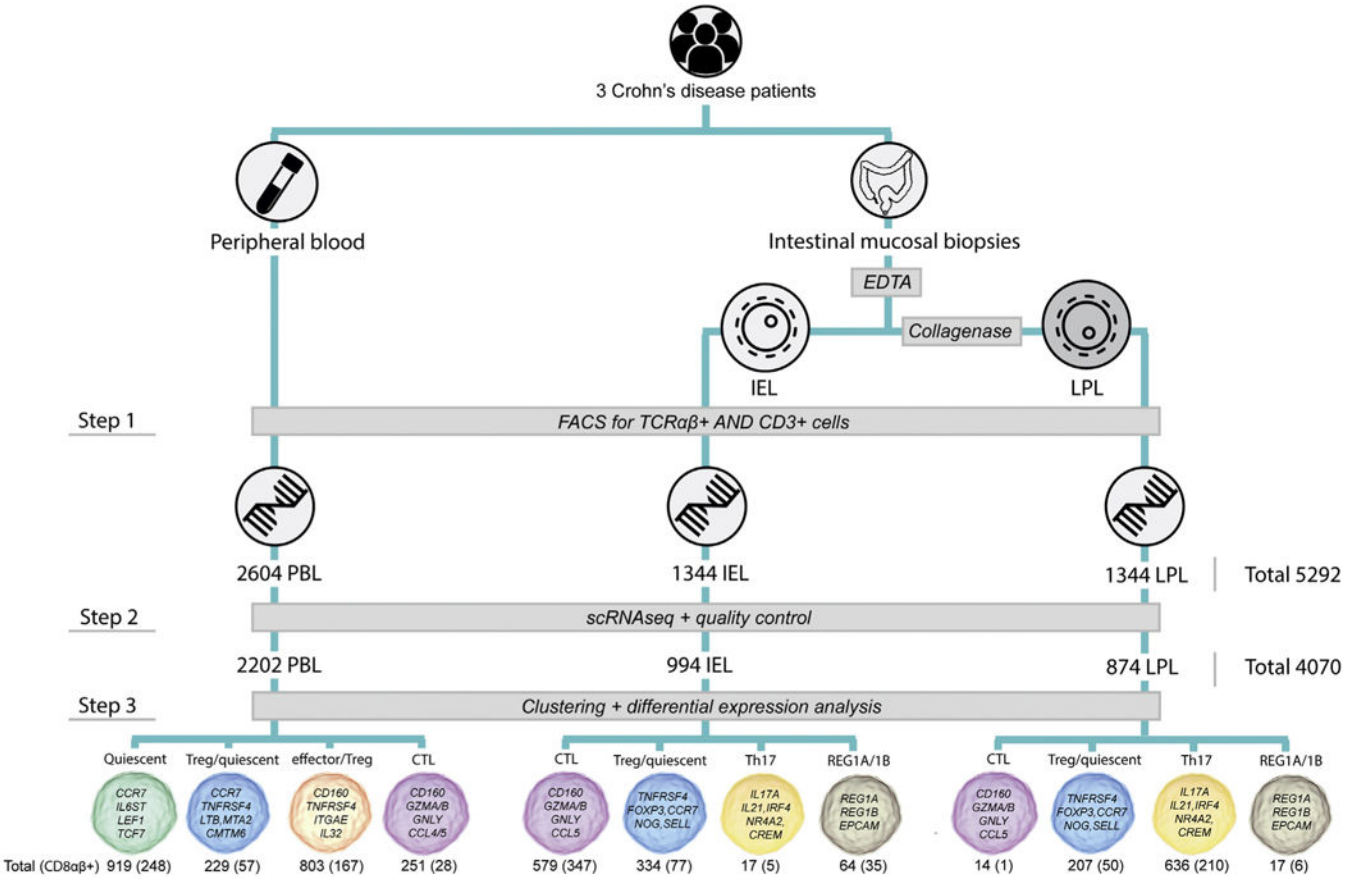

Figure 1.

Experimental flowchart. EDTA and collagenase are treatments used to isolate IELs and LPLs, respectively. T-cell subtype characterization was performed as described in the Supplementary Methods. The number of epitopic CD8$\alpha\beta$-positive T cells measured by flow cytometry is presented within brackets. FACS, fluorescence-activated cell sorting; TCR, T-cell receptor.

Table 1.

Overexpression of CD-Risk Genes and Genes Encoding Potential Drug Targets in Ileal Mucosal Th17 Cells and CTLs

	Mucosal Th17 cells		Peripheral blood CTL		Mucosal CTL	
	Gene	**Drug or compound**	**Gene**	**Drug or compound**	**Gene**	**Drug or compound**
CD-risk gene	*CCL20*	Chemoattractant for various immune cells	*CTSW*	Regulation of T-cell cytolytic activity	*PLCG2*	Transmembrane signaling of immune system receptors
	DNAJB4	Heat shock protein, involved in protein folding	*LSP1*	Adhesion and transendothelial migration	*PTPN22*	Negative regulator of TCR signaling, positive regulator of TLR signaling
	IFNG	Cytokine, involved in adaptive–innate immunity	*PRKCB*	Apoptosis regulation	*SOCS1*	Cytokine-inducible negative regulation of cytokine signaling
	IRF4	Regulation of mucosal Th17 cell differentiation	*PTPRC*	T-cell antigen receptor signaling regulation		
	MAP3K8	T-helper cell differentiation and IFN gamma expression				
Known CD-drug target	*IL17A*	Secukinumab	*S1PR5*	Ozanimod	*CD3D/E/G*	NI-0401
			FCGR3A	IgG class mAbs[a]	*ITGAE CCR9 TGFBR1*	Etrolizumab Vercirnon Mongersen[c]
Candidate for drug repositioning	*DNAJB1*	Apatorsen	*CCL5*	Heparin compounds	*CCL5*	Heparin compounds
	SIK1	Dabrafanib	*FGR*	Dasatinib	*ADRB1*	STD-101-D1
	HSP90AA1	Nedocromil	*FCGR3A*	IgG class mAbs[b]	*ALOX5AP*	Fiboflapon
	PTGER4	Rivenprost; limaprost; dinoproston	*ITGB2*	Lifitegrast	*SLAMF7*	Elotuzumab
	PDE4D	Apremilast	*ADRB2*	β_2-adrenergic receptor blockers	*MDM4*	ALRN-6924

NOTE. Ileal mucosal Th17 cells, peripheral blood CTLs, and ileal mucosal CTLs show the largest number of significantly overexpressed CD-risk genes. The top 5 most significantly overexpressed CD-risk genes are listed in the first section of this table. In the second section, the top 5 most significantly overexpressed known CD drug targets and candidates for drug repositioning, per cell type, are listed. Selection of CD-risk genes is explained in the CD-Risk Genes section in the supplement. “Known CD-drug target” refers to genes encoding targets for drugs currently approved or under investigation for treatment of CD in humans. “Candidate for drug repositioning” refers to genes encoding targets for drugs currently approved for or under investigation in humans for other diseases than CD (Drug-Target Genes section in supplement).

IFN, interferon; IgG, immunoglobulin G; mAbs, monoclonal antibodies; TCR, T-cell receptor; TLR, toll-like receptor.

[a]For example, adalimumab, etanercept, and natalizumab.

[b]For example, alefacept and alemtuzumab.

[c]Indirect target.