Single-Cell RNA Sequencing of T Cells in Crohn's Disease Reply

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still know relatively little about early, accurate, and rapid diagnosis of CD and what critically determines the subsequent prognosis.[6] More high-quality studies about disease-specific single-cell RNA sequencing and pathways of immune cells are required for further insights into CD biology.

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Conflicts of interest
The authors disclose no conflicts.

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Reply. First of all, we thank Dr Du and colleagues for their interest in our study on single-cell RNA sequencing (scRNAseq) of T cells in Crohn's disease (CD), and we appreciate this opportunity to respond to their comments.

For this study, we recruited 3 CD patients with mild to moderate disease activity. Du et al suggest that studying patients with different degrees of intestinal inflammation, including severe inflammation, would have been preferable. We agree. However, as discussed in our article, the experimental and low-throughput character of our plate-based scRNAseq technique limited our study to a small sample size. Furthermore, Du et al mention, including patients with different degrees of inflammation would have added an extra layer of complexity to the data, which would have increased heterogeneity and decreased power to robustly define T-cell subtypes. In this experimental setup, we had to make a trade-off in terms of analytical capacity and power. Moreover, we would like to clarify that disease activity was assessed based on a combination of endoscopic and histologic features.

Du et al correctly point out that the Th1, Th2, and Th22 T-cell subtypes were not identified in our study, even though they (together with Th17, which could be reliably identified in our analysis) are known to play a key role in CD's characteristic pattern of enhanced inflammation, proinflammatory cytokine release, and impaired negative feedback.[1] However, identifying T-helper subpopulations with single-cell RNA expression techniques can be difficult because these subpopulations are mainly characterized by the production of cytokines, which poses 2 problems. First of all, many of these cytokines are not exclusive to a single subpopulation. IL-22, for example, can be produced by a range of cells, including Th17 and Th22 cells.[2] Second, even the most sensitive methods currently struggle to detect low-abundance, short-lived transcripts like cytokine transcripts.[3,4] However, novel scRNAseq platforms now hold the promise of improved sensitivity to capture cytokine transcripts.[5]

mRNA expression of well-known Th1, Th2, Th17, and Th22 cytokines was only detected in a small proportion of the 4070 T cells we examined (*IL1B* in 0.4%, *IL2* in 0.9%, *IL17A* in 1.0%, *IL17F* in 0.2%, *IL21* in 0.07%, and *IL22* in 2.2%). We characterized clusters based on the expression of many genes, rather than only the few well-known (cytokine) marker genes. Of note, the majority of cells expressing *IL17A,* as well as the majority of cells expressing *IL22*, clustered with cells that we collectively classified as Th17 cells. We further found that Th1, Th2, and Th22 cells clustered with other T-cell subtypes based on similar overall expression patterns. These subtypes are present in our dataset, however, they were not separately characterized. In scRNA studies, sequencing more cells allows for the identification of more cell subtypes through a higher resolution of the data. Therefore, future studies looking to examine specific T-cell subtypes, such as Th22 cells, should aim to increase resolution by either increasing the cell sample size, by specifically preselecting for T-cell subtypes using flow cytometry, or by simultaneously measuring protein and gene expression from single cells using techniques such as CITEseq.

Our study highlights the importance of disease- and location-specific studies in CD. It shows that scRNAseq studies have the potential to uncover biology that can identify targets for new therapeutic options or drug repurposing for CD. Follow-up studies with a higher resolution will therefore be valuable for further characterization of disease-specific and inflammation-specific (immune) cell subsets involved in CD pathogenesis.[6]

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Conflicts of interest
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Therapy of Hepatitis B: Is It Really Not Associated With Hepatitis B Surface Antigen Seroclearance ?

Dear Editors:

I read with interest the study on factors associated with rates of hepatitis B surface antigen (HBsAg) clearance in adults with chronic hepatitis B virus (HBV) infection.[1] This comprehensive review recognized some factors associated with the seroclearance of HBsAg. However, the therapy of HBV was not found to be an important factor for HBsAg loss. This point needs further clarification.

It is well-established that oral antiviral therapy cannot achieve a higher rate of HBsAg loss as compared with control.[2] In contrast, it is also well-known that the use of pegylated interferon, alone or in combination with oral antiviral drugs, could achieve a significantly higher incidence of HBsAg clearance as compared with patients receiving oral antiviral drugs alone.[3–5] The current meta-analysis excluded studies with <200 patients; thus, several valuable trials that evaluated pegylated interferon in the seroclearance of HBsAg, have been excluded in the analysis.[5] Owing to a higher incidence of adverse events with interferon, the interferon trials were generally fewer and smaller in size than the trials of oral antiviral therapy. Study selection in a meta-analysis may lead to inaccurate conclusions. Based on the available literature, the value of pegylated interferon in inducing higher seroclearance rate of HBsAg in a subset of chronic HBV patients should not be ignored. The conclusion that there is a similar low seroclearance rate between treated and untreated HBV patients seems to be inappropriate and misleading.

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Reply. We thank Dr Lo for his interest in our recent article[1] and his comments on the effect of treatments (antiviral agents and interferon-based therapy) on hepatitis B surface antigen (HBsAg) seroclearance.

We agreed with Dr Lo that previous trials and meta-analyses have shown that interferon-treated patients had a high HBsAg seroclearance rate,[2,3] but the generalizability of clinical trials is limited by their strict inclusion criteria and selection for patients who are more likely to respond and adhere to therapies. Therefore, to obtain a more realistic incidence rate of this key milestone in the natural history of hepatitis B infection, we excluded data from clinical trials. We found that the annual incidence rate of HBsAg seroclearance in untreated and treated patients was 1.31% (95% confidence interval, 0.98–1.67) and 0.82% (95% confidence interval, 0.34–1.50), respectively, compared with that of untreated patients. Furthermore, our systematic review only identified one article reporting the real-world annual incidence rate in interferon-α- or interferon-β-treated patients (HBsAg seroclerance rate; 1.80%; 95% confidence interval, 1.40–2.25),[4] and part of the reason for the lack of real-world data may have to do with the significant side effect profile of pegylated interferon.[5]

Therefore, although higher HBsAg seroclearance rates have been reported to be higher for pegylated interferon in prior clinical trials than the real-world data from our study,[2] and we do appreciate Dr Lo's comment about the value of pegylated interferon in inducing higher seroclearance rate of HBsAg in a subset of chronic HBV patients, the lack of optimism in our study conclusion regarding the incidence of HBsAg for the general real-world population of treated chronic hepatitis B patients was based on the available real-world data that we could evaluate.

However, the available data are limited, and we do believe that additional studies with individual patient level data from large real-world cohorts that also include pegylated interferon patients are necessary to better evaluate HBsAg seroclearance rates for the various subsets of patients in routine practice, both treated and untreated.