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A single-cell view of biliary atresia

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Abstract

A new study elevates our understanding of how the immune system regulates the pathogenesis of biliary atresia through a powerful single-cell approach. Cell-specific transcriptome analyses indicate key roles for macrophages, T cells and B cells in hepatobiliary injury of affected infants.

Biliary atresia is a severe inflammatory and fibrosing cholangiopathy of early infancy and the primary cause of end-stage cirrhosis in children. Studies in the past two decades have substantially advanced our understanding of the pathogenesis of bile duct injury and the resultant clinical phenotype¹. For example, environmental triggers of biliary atresia, historically linked to the detection of viruses in tissues and to the seasonal clusters of cases, now include the toxin biliatresone, which causes injury to bile duct epithelial cells¹. Based on the onset of disease in the early postnatal period, genetic determinants have been investigated, but sequence variants in *ADD3*, *FOXA2*, *PKD1L1* and other genes are only found in small subsets of affected infants¹. With a broader scope, studies in biliary atresia focusing on the immune system have given unique insight into how myeloid and lymphoid cells regulate pathogenic mechanisms of the disease.

Early gene expression studies of diseased livers in children with biliary atresia reported a differential expression of pro-inflammatory genes, suggesting a dominant T helper 1 (T_H1) cell response as evidenced by a high expression of IFN γ ². Testing the relevance of this finding in neonatal mice, a lack of functional interferon rendered mice resistant to bile duct inflammation and obstruction induced by rotavirus infection³. Subsequent studies followed this paradigm and used genetically engineered mice and antibody-mediated cell depletion to show the relevance of regulatory T cells in controlling disease susceptibility during the early postnatal period. These studies also demonstrated a role for a T_H1 cell-rich environment created by the activation of macrophages, plasmacytoid dendritic cells, natural killer cells and CD8⁺ T cells, and the expression of integrins, cytokines and chemokines, such as IL-8, IL-15, and TNF⁴. In addition, B cells from experimental mouse models of biliary atresia produce a variety of soluble mediators that activate T cells⁵, with effector lymphocytes

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Competing interests

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releasing cytokines and cytotoxic granules to attack cholangiocytes. In parallel to this pro-inflammatory environment in the biliary system, studies in mice and in human tissues uncovered a type 2 response with the recruitment of type 2 innate lymphoid cells that release IL-13 and IL-33 in an attempt to repair the duct epithelium but secondarily promote fibrosis^{4,6}. Despite the many advance ments with conventional reductive approaches in gene-targeted mice and cell culture systems, the field of biliary atresia was lacking an all-inclusive human-based strategy to evaluate the biological behaviours of all hepatic inflammatory cells simultaneously.

A new study by Wang et al.⁷ begins to fill this experimental void by extensively profiling hepatic and circulating immune cells at the single-cell level using liver biopsy samples collected at the time of diagnosis of biliary atresia (BOX 1). First, the investi gators phenotyped 927 patients with biliary atresia, 248 patients with choledochal cyst and more than 11,000 healthy controls, and found that elevated serum levels of γ -glutamyl transpeptidase, aminotransferases and bilirubin are characteristic of biliary atresia. For single-cell experiments, they generated hepatic mononuclear single-cell transcriptomes and performed lymphocyte single-cell V(D)J profiling to assess T cell clonotypes and gene expression levels in six patients with biliary atresia and control children with normal liver function who required surgery for either choledochal cysts or other non-cholestatic diseases⁷. In support of previous studies, the numbers of cytotoxic T_H1 cells and transitional cytotoxic T helper 17 (T_H17) cells were increased in biliary atresia relative to controls. However, this finding was accompanied by a new finding of decreased CX3CR1⁺CD8⁺ T effector (T_{eff}) cells that were posited as contributing to a pro-fibrogenic environment through the inability to clear CX3CL1-expressing hepatic stellate cells. Bulk RNA sequencing of CX3CR1⁺CD8⁺ T_{eff} cells revealed enrichment of migration genes and suppressed expression of the fibrogenic genes *COL1A1* and *COL1A2* in hepatic stellate cells from co-culture experiments. Furthermore, there was reduced co-localization of cytotoxic lymphocytes with hepatic fibroblasts in biliary atresia⁷. These findings suggest that a dysregulated cytotoxic response dependent on CX3CR1⁺CD8⁺ T_{eff} cells is responsible for an unopposed activation of hepatic fibroblasts leading to early fibrosis.

The second major key point in this study is specific to B cells. Unexpectedly, the researchers observed B cell lymphopoiesis in the liver weeks after birth in biliary atresia and controls⁷. This finding is contrary to the paradigm that B cell lymphopoiesis switches to the bone marrow shortly after birth⁸. In patients with biliary atresia, somatic hypermutation within the variable regions and class switch recombination are both decreased⁷. The researchers postulate this effect is due to decreased central tolerance and resultant activation of auto reactive hepatic B cells in the face of disease. In support of this finding, they find that patients with biliary atresia have increased IgG-specific autoantibodies to multiple cytosolic and nuclear antigens.

Given that B cells not only produce antibodies but also orchestrate T cell, natural killer cell and myeloid cell function via antigen processing, Wang et al.⁷ investigated B cell-modifying therapies in both mice and humans. In mice, B cell depletion with a CD20-targeted antibody significantly decreased body weight loss and decreased jaundice compared with infected control mice ($P < 0.001$). In human studies, they treated four patients with biliary atresia

with a single dose of rituximab (a B cell-depleting antibody). Comparison of single-cell transcriptomes from patients with biliary atresia with and without rituximab treatment revealed decreased clonal transition from CX3CR1⁺CD8⁺ T_{eff} cells to CD8 tissue resident memory cells, increased expression of *CX3CR1* and cytotoxic gene expression, and increased T_H17 gene signatures in treated patients. However, the clinical significance of these findings is currently unknown as no clinical outcomes were reported⁷.

A strength of the experimental approach by Wang et al.⁷ is their use of in vitro and mouse model systems to validate the biological implications of single-cell expression data. Although comprehensive, one assumes the reported data represent a fraction of the vast amount of information that can be generated with this technology; therefore, we expect future findings from ongoing analyses of this rich dataset. The finding of suppressed inflammatory and phagocytic function by macrophages is particularly intriguing given previous reports of macrophage activation and high expression of TNF in experimental biliary atresia and in human liver biopsy samples⁹. In this context, the inclusion of choledochal cyst and non-cholestatic individuals as control groups raises the question of how much the cholestatic environment typical of biliary atresia might have influenced the gene expression profiles of single cells. Finally, the inclusion of data from four infants treated with B cell-depleting antibodies (rituximab) generates excitement in the field, but its value is limited by a small cohort, paucity of clinical data, safety concerns, and co-administration of other therapeutic agents such as ursodeoxycholic acid and corticosteroids, which might be used clinically in some centers despite no clear evidence of efficacy.

The advanced stage of biliary injury already present at the time of diagnosis of biliary atresia and the limited access to tissues have made it particularly difficult to study this disease in patients. The report by Wang et al.⁷ overcomes these barriers and opens wide experimental windows for additional analyses to further map the functional profiles of individual cell types, generate new hypotheses and refine existing pathogenic models of disease. It also generates enthusiasm for the application of the powerful single-cell technologies to a much larger cohort of biliary atresia. This approach addresses a critical need because the stage of disease is notably variable, even when patients are age-matched. Studies in a broader population will enable solid insight into disease processes and inform which pathways (for example, CX3CR1 or CX3CL1) or cell populations (for example, B cells) can be targeted therapeutically to block progression to end-stage liver disease and prolong survival with the native liver.

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Box 1 |

Key Findings by Wang et al.

Design

- Powerful approach studying mononuclear single-cell transcriptomes and lymphocyte single-cell V(D)J profiling in the livers of patients with biliary atresia at the time of diagnosis and in controls.

Key findings

- Decreased CX3CR1⁺CD8⁺ T effector cells in patients with biliary atresia. This cell population is instrumental in mitigating fibrosis through decreased collagen expression by hepatic stellate cells and cytotoxic effects on fibroblasts.
- Autoantibodies are expanded in the livers of patients with biliary atresia. B cell-depletion therapy with rituximab in four patients with biliary atresia increased CX3CR1 expression and decreased autoantibody production.
- Inflammatory and chemotactic genes are suppressed in hepatic myeloid cells from affected patients.

Scientific advancement

- This powerful single-cell approach validated with in vitro assays, mouse models and human data provides new insights into how immune cell types might work in concert to drive the pathogenesis of biliary atresia in humans.