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Acod1 mediates anti-inflammatory Treg function in sepsis

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Sepsis is a serious, potentially fatal disease caused by the body's reaction to microbial invasions by bacteria, viruses, and fungi. Current research shows that the process of fighting sepsis passes through two phases. The first phase is a cytokine storm, and the second phase involves a cycle of pro-inflammatory and anti-inflammatory responses led by Regulatory CD4+ T-cells (CD4+ Tregs). Various immunomodulatory therapies have been proposed to break the cycle of pro- and anti-

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inflammatory reactions to sepsis. However, clinical trials are yet to show any promising results, indicating the need for further research into the mechanisms behind Treg dysfunction. We used next-generation sequencing (NGS) analysis of five datasets comprising of bulk RNA-seq and single-cell RNA-seq data to explore our research question. Our results identified Acod1 (Aconitate Decarboxylase 1) as a primary mediator of Treg suppression of immune cells as well as various metabolic pathways regulated by IL4 and IL10. scRNA-seq analysis showed that Acod1 and FoxP3 were localised in the same Treg-like cells. Further evidence from our study further suggests a mutual positive regulation loop between Acod1 and FoxP3 in sepsis. Additionally, CD36 was identified as a downstream target of Acod1. CD36 is a known metabolite regulator in Tregs, where it functions as a transporter of long fatty chains (LCFA) and is regulated by IL4 and IL10. Taken together, our results indicate that the metabolic CD36/Acod1 axis could be responsible for the continuous activation of Treg in sepsis. Thus, targeting this axis could prove valuable in improving the sepsis prognosis.

KEY WORDS: SEPSIS / Th17 / Treg / inflammation / long fatty chains / CD36

Growing evidence associates sepsis with a network of dysfunctional processes, including a poorly coordinated immune system response [Montero-Jodra *et al*. 2024]. Sepsis is a serious organ dysfunction that results in millions of fatal cases per year [Polat *et al*. 2017]. This disease can be caused by a variety of infections, including bacterial, viral, and fungal pathogens [Grondman *et al*. 2020]. Its main symptoms include fever, low blood pressure, an abnormal resting heartbeat tachycardia, and an uncharacteristically high white blood cell count [Ebrahim 2011]. The immune system during sepsis contributes to systemic inflammation, tissue damage, and organ failure affecting the kidney, respiratory system, heart, and brain [Delano *et al.* 2016]. Advances in understanding the pathophysiology of sepsis have led to the development of targeted therapies, such as immunomodulatory agents, that aim to restore immune system balance and improve patient outcomes [Boomer *et al.* 2014]. However, various trials utilising immune-modulatory agents to tackle sepsis have not produced promising results, highlighting the need for continued research to improve our understanding of the mechanisms behind this complex disease [Christaki *et al.* 2011].

Regulatory CD4+ T-cells (CD4+ Tregs) are essential in the pathophysiology of septic complications by suppressing the adaptive immune response. The sepsis course of diseases is thus defined by an initial cytokine storm caused by the innate immune system's response to the invading pathogen. This phase is usually followed by a cycle of hyper-hypoimmune reactions. This cycle results in cell exhaustion and cell death. Intriguingly, postmortem studies have discovered a significant reduction in the numbers of CD4 and CD8 T cells, pinpointing the role of dysfunctional Treg as a critical obstacle toward homeostasis during sepsis [Martin *et al.* 2020]. A higher frequency of Tregs has also been found in septic shock patients' blood. Additionally, a negative link between the frequency of Tregs and sepsis severity is evident in sequential organ failure, among other parameters [Hein *et al.* 2010, Cao *et al.* 2015]. These findings were further supported by animal studies that found that, in contrast to sham mice, CD4+CD25+ Tregs were considerably higher 24 hours after abdominal sepsis induction using cecal ligation and puncture (CLP) [Drechsler *et al.* 2021]

[Taylor *et al*. 2010].

The mechanisms behind Tregs dysfunction in sepsis remain unknown. Under normal conditions, Tregs are considered the guardians of the immune system due to their role in preventing excessive immune responses by suppressing other immune cells [Kondělková *et al*. 2010]. The suppression mechanisms can take two forms. The first, known as the indirect form, involves Tregs inhibition of dendritic cells through various pathways, including CTLA4, CD28, CD80, CD86, LFA1, A20, CD40-CDO4L, neuropilin 1, and LAG3 [Bhaumik et al., 2023]. CTLA-4 and FoxP3 were reported to be highly expressed in the blood of septic patients [Jiang *et al.* 2012]. On the other hand, the direct inhibition of CD4+ and CD8+ T cells includes the production of suppressive cytokines such as TGFβ, IL10, and IL35, consumption of cytokines such as IL2, and induction of apoptosis through the TRAIL, CD3, CD46, CD25, and BIM pathways. Interestingly, elevated IL-10 and TGF-β levels have been reported in the blood of septic patients [Bergmann *et al.* 2021, Monneret *et al*. 2008]. Additionally, current evidence has shown that Tregs can regulate ATP and ADP by interacting with CD73, CD39, APRT, A2A receptors, and p2ry11 receptors. Furthermore, Tregs can also regulate NFAT pathways through the IL4, ICER, PPARγ, GIT, and CBCLB pathways. It can also regulate calcium signalling in the responding cells by controlling the NFKB, PPP3CA, PPP3CB, PPP3CC, and IKAA pathways. Treg is known to be regulated by FoxP3 [Rizzo *et al.* 2018, Mickael *et al*. 2022]. However, FoxP3 levels in many transcriptomic studies of blood do not necessarily mirror real cases as the number of CD4+ and CD25+ FoxP3 is limited. The exact method by which the Treg function is destabilized remains unknown. scRNA-seq has the advantage of allowing inspection of Treg cells on molecular levels with higher accuracy.

In this report, we investigated the genetic networks controlled by FoxP3 that ultimately lead to excessive suppression of immune cells by Tregs. To do that, we analysed a host of microarray, bulk RNA seq, and scRNA-seq studies, as well as extensive evolutionary studies. Our results pinpointed the role of a dysfunctional metabolic network in Tregs, manifested by increased itaconate production through the upregulation of the gene Acod1 through putative interaction with other genes associated with metabolism and expressed particularly in the mitochondria, such as CD36.

Material and methods

Datasets

This study utilized five public datasets to investigate various aspects of sepsis pathogenesis and immune response. First, an analysis was conducted using the public microarray GSE65088 [Cakir *et al*. 2015]. The dataset included ex vivo blood samples divided into control and treated groups. The treated groups contained samples subjected to one of four types of infection caused by two bacterial microbes (*Staphylococcus aureus, Escherichia coli*) and two fungal pathogens (*Candida albicans, Aspergillus*

fumigatus). The control samples were treated with HBSS. All samples were treated for 4-8 hours, followed by standard processing using RNA isolation, and then bulk RNA-seq sequencing using Illumina [Cakir *et al*. 2015].

Subsequently, our study further explored the association of upregulated pathways in Tregs. This was done by comparing the transcriptome of FoxP3WT Tregs and FoxP3KO Tregs using the public RNA-seq dataset (GEO: GSE176236) [van der Veeken *et al*. 2022]. In this dataset, FoxP3WT Tregs were isolated from the lamina propria of FoxP3-DTR-GFP+ mice. Whereas in the case of knock-out mice, FoxP3DTR-GFP/loxp-Thy1.1-STOP-loxp-GFP markers were used to isolate FoxP3KO.

In the third data set we examined the presence of Acod1 in colon mononuclear cells using a public single-cell RNA-seq dataset (GEO: GSM4709925) Cells from the lamina propria layer were analyzed using 10x sequencing. Colons were extracted, and the epithelial layer was disrupted using EDTA. After that, the connective tissue was disgusted utilizing a mixture of collagenase D, DNAse, and Dispase. Cells were further enriched using a 40/80 percoll gradient before sequencing27.

The fourth dataset included five healthy controls and five patients with proven sepsis from the Emergency Department, Surgical Critical Care Division, Tongji Trauma Center, Tongji Hospital, and Tongji Medical College, China We examined scRNA-seq from the complete blood transcriptome of these patients (GEO: GSE224095).

In the fifth dataset, eight-week-old wild type (WT) or Irg1-/-C57bl/6 mice were infected with a combination of the 17 host-adapted Pseudomonas aeruginosa clinical isolates (106 total CFU per animal in 50 uL) or WT PAO1 The mice were sacrificed sixteen hours after infection, their lungs were removed, and single-cell suspensions of

Table 1. Summary of datasets investigated

Dataset	Type of study	Sample source
GSE65088	Microarray	Ex vivo blood samples
GSE176236	RNA-seq	$FoxP3^{WT}$ and $FoxP3^{KO}$ Tregs
GSM4709925	ScRNA-seq	Colon mononuclear cells
GSE224095	ScRNA-seq	Sepsis patients and healthy controls
GSE203352	ScRNA-seq	Infected WT and $Irg1^{-/-}$C57BL/6 mice lung suspensions

the lungs were prepared, followed by 10x scRNA-seq (GEO: GSE203352)

Microarray and RNA-seq analysis

RNA-seq analysis was done in R using limma [Kubick *et al.* 2020]. The limma RNA-seq differential gene expression approach was used to compute the non-parametric approximations of mean-variance relationships. This enabled the calculation of the weights and empirical Bayes shrinkage of variance parameters for a linear model analysis of log-transformed counts. Differential expression analysis was also performed to determine the differences in gene expression between infected and

non-infected samples by fitting a linear model to quantify the variability in the data using lmFit.

First, the statistically significant deferentially expressed genes (DEGs) were used for gene set enrichment analysis (GSEA) based on gene ontology biological process databases, GO (Biological Process, Cellular Component, and Molecular Function), KEGG (Pathways), Reactome (Pathways), and WikiPathways. The GeneTrail3 software was used for this. For this investigation, upregulated genes from each group were uploaded to the server. A gene enrichment analysis was conducted using overrepresentation. Over-representation analysis (ORA) evaluates if a group of variables is more common in a set of results than we would anticipate by chance. The ORA was run with its default settings to control the false discovery rate.

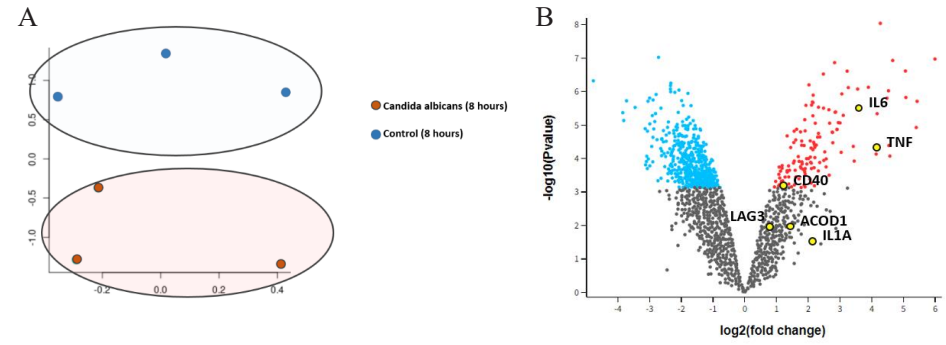
Second, using Cytoscape, we used BiNGO to find pathways that are overrepresented in infected septic samples. BiNGO is built as a plugin for Cytoscape, which is an open-source bioinformatics software platform [Mickael *et al*. 2023].

scRNA-seq analysis

Cell clustering was achieved, as previously noted, using the Seurat R tool[Satija *et al.* 2015]. Specifically, cell clustering was performed using the nonlinear dimensional reduction methodology in conjunction with the Uniform Manifold Approximation and Projection (UMAP) method. We analysed each cell cluster using paired differential expression analysis, using parameters recommended for data with batch effect, to find flag genes of the cell clusters with average fold change (FC) expression compared with other included clusters >2. Our study utilised Library Seurat and dplyr in R to analyse quality control metrics, filter cells, normalise data, cluster cells, and identify cluster biomarkers. Low-quality cells were removed using a threshold of 200 to 7,000 genes per cell, and cells with over 10% of the mitochondrial genome were excluded. The "sctransform" package was used for normalisation, and the "RunUMAP" function was used for clustering cells. The "FindAllMarkers" function identified specific markers for each cell cluster, and the "DoHeatmap" function displayed the top genes in each cluster. The "VlnPlot" function provided expression probability distributions across cell clusters.

Results and discussion

Several analyses of public data were conducted to pinpoint how Tregs contribute to sepsis. The first was the analysis of blood samples infected with different bacteria and fungi using GSE65088 [Cakir *et al*. 2015]. We identified that the genes transcribed significantly differed by the type of infection. However, some differentiated genes were common to all treated samples investigated (Fig. 1B). For example, LAG3 and CD40 were upregulated in C. albicans, indicating Treg could suppress dendritic cells in sepsis through indirect pathways (Figures 1A and 1B). Several proinflammatory markers were also upregulated, such as IL6, TNF, and IL1 (Fig. 1B). The Treg effect

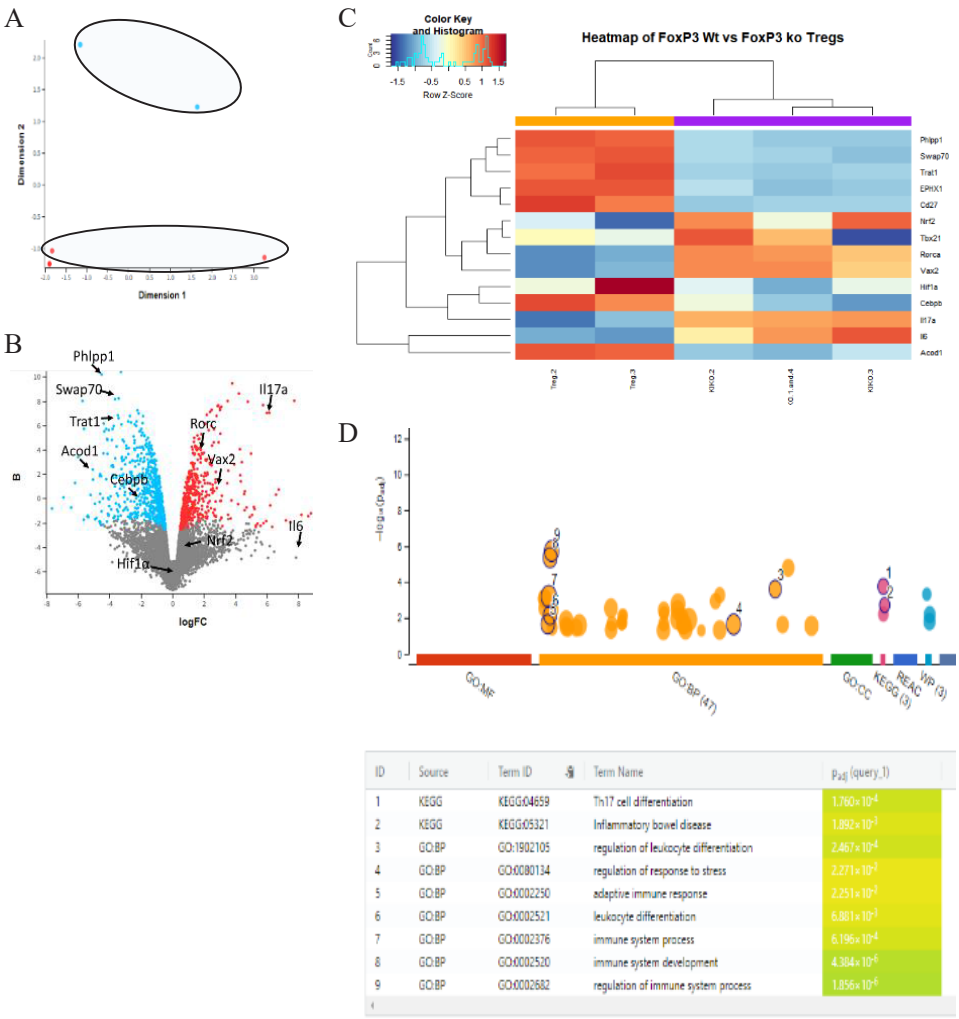


GO Description	P-value	Corrected P- value	Cluster frequency
response to stimulus	1.63E-28	2.86E-25	54/109 49.5%
response to stress	1.37E-23	1.20E-20	37/109 33.9%
immune response	1.81E-19	1.06E-16	21/109 19.2%
biological regulation	4.05E-19	1.48E-16	71/109 65.1%
cellular process	4.21E-19	1.48E-16	79/109 72.4%
immune system process	8.10E-19	2.37E-16	26/109 23.8%
positive regulation of cellular process	1.11E-17	2.79E-15	35/109 32.1%
negative regulation of biological process	6.71E-17	1.32E-14	34/109 31.1%
positive regulation of biological process	6.76E-17	1.32E-14	36/109 33.0%
regulation of cellular process	1.15E-16	2.02E-14	64/109 58.7%
negative regulation of cellular process	1.32E-16	2.10E-14	32/109 29.3%
regulation of biological process	3.06E-16	4.48E-14	65/109 59.6%
response to chemical stimulus	3.40E-15	4.59E-13	27/109 24.7%
regulation of metabolic process	3.82E-15	4.80E-13	42/109 38.5%
regulation of biological quality	5.69E-15	6.66E-13	29/109 26.6%
regulation of cellular metabolic process	1.34E-14	1.47E-12	40/109 36.6%
response to biotic stimulus	8.85E-14	9.16E-12	16/109 14.6%
regulation of developmental process	1.59E-13	1.56E-11	22/109 20.1%
defense response	8.20E-13	7.59E-11	17/109 15.5%
regulation of molecular function	1.32E-12	1.16E-10	22/109 20.1%
system development	1.43E-12	1.20E-10	33/109 30.2%
regulation of primary metabolic process	2.64E-12	2.11E-10	36/109 33.0%
regulation of apoptosis	3.37E-12	2.58E-10	19/109 17.4%
regulation of programmed cell death	4.30E-12	3.15E-10	19/109 17.4%
regulation of cell death	6.06E-12	4.26E-10	19/109 17.4%
metabolic process	8.42E-12	5.69E-10	57/109 52.2%
response to wounding	9.82E-12	6.39E-10	15/109 13.7%
regulation of multicellular organismal process	1.07E-11	6.69E-10	23/109 21.1%
homeostatic process	1.15E-11	6.96E-10	19/109 17.4%
cellular response to stimulus	1.40E-11	8.20E-10	19/109 17.4%
anatomical structure development	1.80E-11	9.84E-10	33/109 30.2%
cellular response to chemical stimulus	1.80E-11	9.84E-10	13/109 11.9%
primary metabolic process	1.85E-11	9.84E-10	52/109 47.7%
multicellular organismal development	1.90E-11	9.85E-10	35/109 32.1%
behavior	2.70E-11	1.36E-09	16/109 14.6%
inflammatory response	5.83E-11	2.85E-09	12/109 11.0%
anatomical structure morphogenesis	7.69E-11	3.65E-09	23/109 21.1%
regulation of transcription, DNA-dependent	8.64E-11	4.00E-09	23/109 21.1%
organ development	9.17E-11	4.13E-09	27/109 24.7%
regulation of RNA metabolic process	1.18E-10	5.18E-09	23/109 21.1%
developmental process	1.35E-10	5.81E-09	35/109 32.1%
regulation of immune system process	1.39E-10	5.83E-09	14/109 12.8%
regulation of phosphorylation	1.44E-10	5.90E-09	14/109 12.8%
regulation of biosynthetic process	2.18E-10	8.71E-09	31/109 28.4%
cellular metabolic process	2.27E-10	8.87E-09	49/109 44.9%
regulation of phosphorus metabolic process	2.41E-10	9.03E-09	14/109 12.8%
regulation of phosphate metabolic process	2.41E-10	9.03E-09	14/109 12.8%
response to other organism	2.83E-10	1.04E-08	12/109 11.0%
response to virus	3.04E-10	1.09E-08	8/109 7.3%
regulation of catalytic activity	3.81E-10	1.34E-08	18/109 16.5%

Fig. 1. Pro-inflammatory and metabolism pathways are both enriched after 8 hours of infection. (A) A significant number of genes were differentially expressed between treated and non-treated groups. (B) Several genes associated with Tregs show upregulation patterns. (C) 8 hours post-treatment of healthy human blood from participants shows Treg-associated pathways, namely IL4 and IL10. (D) BiNGO analysis process shows upregulation of the metabolic process.

was also found in the overrepresentation of various Treg pathways, such as IL10 and IL4, in the OVA (Fig. 1C). Interestingly, our results also showed that several metabolism-related genes were upregulated, such as Acod1 (Aconitate decarboxylase 1) (Fig. 1B and 1D).

Subsequent analysis was performed on the public GEO (GSE176236) to investigate the effect of FoxP3 on Treg. The results indicated that Acod1 is tightly controlled



ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	KEGG	KEGG:04659	Th17 cell differentiation	1.760×10^{-4}
2	KEGG	KEGG:05321	Inflammatory bowel disease	1.892×10^{-3}
3	GO:BP	GO:1902105	regulation of leukocyte differentiation	2.467×10^{-4}
4	GO:BP	GO:0080134	regulation of response to stress	2.271×10^{-3}
5	GO:BP	GO:0002250	adaptive immune response	2.251×10^{-3}
6	GO:BP	GO:0002521	leukocyte differentiation	6.881×10^{-3}
7	GO:BP	GO:0002376	immune system process	6.196×10^{-4}
8	GO:BP	GO:0002520	immune system development	4.384×10^{-6}
9	GO:BP	GO:0002682	regulation of immune system process	1.856×10^{-6}

Fig. 2. Acod1 mediates Treg function. (A) PCA shows clear clustering of samples based on FoxP3 expression. (B and C) Volcano plot and heat map showing upregulated genes. (D) Gene enrichment pathways showing Th17 regulation confirming selected genes role in Th17/Treg differentiation.

by FoxP3 in Tregs. To further investigate the role of Acod1 in Tregs, we compared the transcriptome of FoxP3WT Tregs and FoxP3KO Tregs using the public dataset (GEO: GSE176236). We performed RNA-seq analysis using edgeR and Glimma in R. We found that the samples separated according to their Principal component analysis (PCA) analysis, showing that FoxP3 played a major role in determining the differentiation pattern of colon-induced Tregs (Figure 2A). Acod1 and two of its

upstream regulators (e.g., Phlpp1 and Trat1 that control Acod1 expression through regulation of PKC) were upregulated in the WT Tregs. We found that Cebpb, which is directly upregulating Acod1, is also upregulated in WT Tregs (Figure 2B). Similarly, Swap70, which upregulates Nfkβ also positively regulates Acod1, is upregulated in the WT Tregs (Fig. 2B and 2C). Conversely, IL17A and IL6 were downregulated; however, the Th17 pathway was enriched, confirming selected genes' role in Treg function (Fig. 2D).

Next, our study analysed single cells from peripheral immune cells extracted from

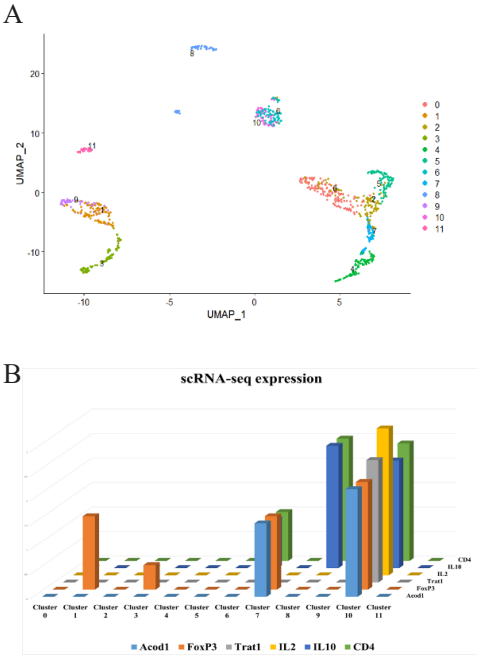


Fig. 3. Acod1 and FoxP3 share the same expression profile on a single cell level. (A) scRNA-seq of colon cells show that Acod1 and FoxP3, Trat1, IL10, IL2, CD27, CD25, and CD4 are co-expressed mainly in cluster number 10, which is highly likely to be Tregs.(B) Comparison of scRNA-seq expression between different clusters for Acod1 and genes associated with Tregs.

the colon. The results showed that Acod1 is particularly expressed in Tregs (Fig. 3A and 3B).

The next step was a thorough analysis of the difference in transcripts between control and sepsis patients on a single-cell level using GSE224095. Our results revealed that the cells could be clustered into 20 different types. Notably, most of the clusters showed mixtures of the two conditions which indicates that they are the same type of cells that have changed their expression levels based on sepsis. However several clusters were only upregulated in one of the conditions, such as clusters 12 and 17 which seem to be exclusively expressed in sepsis (Fig. 4A). To focus on FoxP3+ Tregs, we compared clusters based on their differential expression and identified that FoxP3 and CD4+ were differentially expressed in clusters 1,2,5,8,13,16 and 17. Shifting our focus to Acod1, our analysis showed that it was exclusively expressed in clusters 3,8 and 13 (Fig. 4B). Hence, we conducted a deferential expression analysis

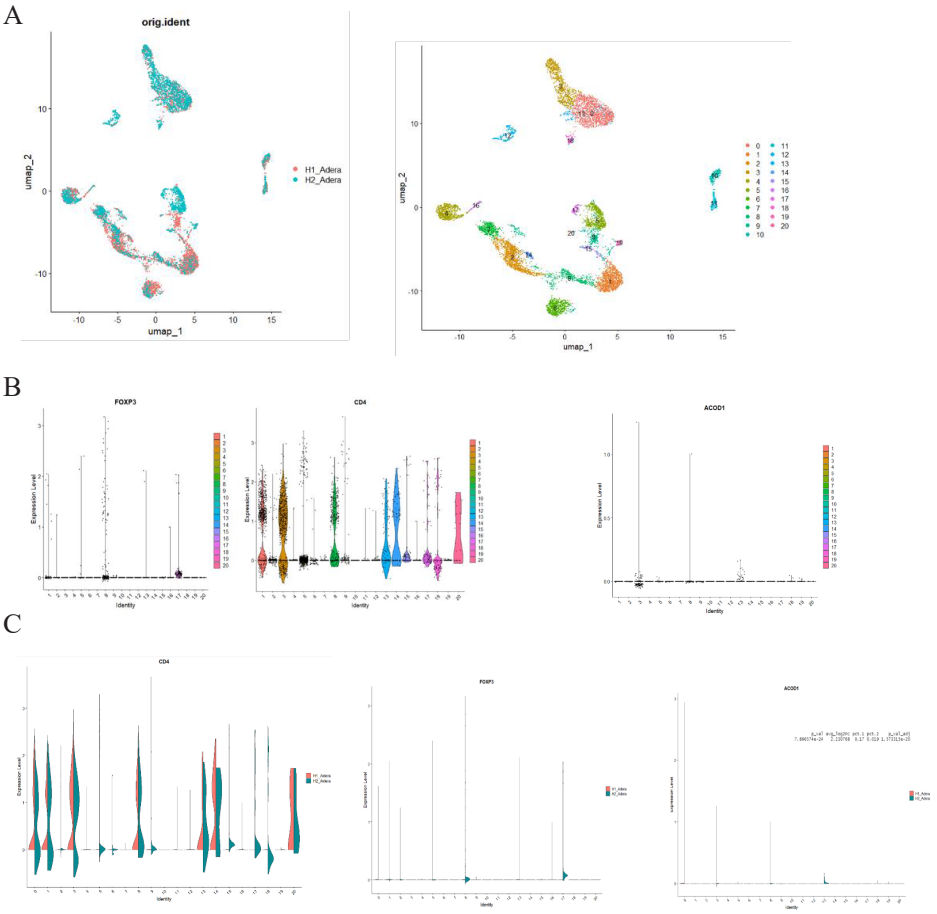


Fig. 4. Single-cell clustering using sepsis patients single-cell RNA seq. A) Our results identified 20 different cell clusters uniquely expressed in sepsis. B) FoxP3 and CD4+ are not ubiquitously expressed in all investigated cell types; rather, they are specifically expressed in certain cell clusters, indicating that they could be phenotyped as Treg-like cells. C) Acod1 is differentially expressed between the sepsis and control patients in a unique cluster marked by FOXP3+ CD4+.

based on clusters. Based on this analysis, we can infer that FoxP3 and CD4+ are solely deferentially expressed in cluster 8 between sepsis and control patients. Interestingly, Acod1 is differentially expressed between the sepsis and control patients in this particular cluster (cluster 8).

We examined the data more, by comparing other genes expressed between sepsis and non-sepsis patients in the cluster phenotyped as CD4+FOXP3+Acod1+ T cells. The genes differentially expressed included LYZ, S100A8, GPX1, FOS, MND1, CD36, NRG1, and CST3 among others (Figure 5A). To assess the location of Acod1 within the cell, we mined Human Atlas for its expression, our investigations indicate

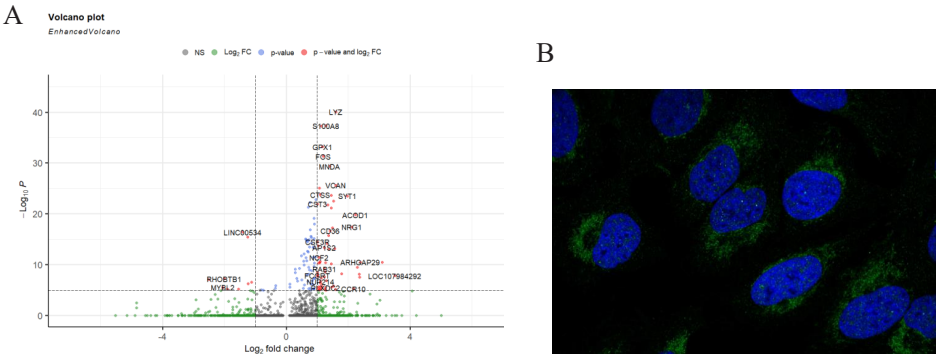


Fig. 5. Sepsis is associated with the upregulation of FoxP3 and various mitochondrial pathways including Acod1. (A) The volcano plot of cluster 13 shows that in addition to Acod1, several genes known to function in the mitochondrial metabolism axis are upregulated such as CD36. (B) Mining of expression of Acod1 in the cells in Human Atlas(c) shows that Acod1 is likely to be expressed in the mitochondria.

that Acod1 is likely to be expressed in the mitochondria (Fig. 5B).

Finally, we investigated the effect of Acod1 using scRNA-seq. In this interesting dataset, mice with Acod1 WT or Acod1-/- were infected to induce sepsis (GSE203352). Our results identified 23 different cell clusters (Figure 6A). Interestingly, we found that FoxP3 expression is affected by knocking-out Acod1 in sepsis (Figures 6B and 6C). This downregulation was also seen in CD4 expression and downstream metabolic interactors of Acod1, such as CD36 (Figure 6B and 6C).

Although the reason behind Tregs' continuous activation in sepsis is currently unknown [Cao *et al.* 2015], our results revealed that various metabolic pathways are enriched in sepsis. The results from our bulk RNA-seq analysis indicated that the IL4 and IL10 pathways are enriched in sepsis (Fig. 1). One of the main functions of IL10 is that it can resist switching metabolic pathways induced by inflammatory stimuli, where it reduces oxidative phosphorylation and glycolysis [Ip *et al.* 2017, Wang *et al*. 2022]. Also, by suppressing mTOR activity, IL-10 encourages the process of mitophagy, which removes damaged mitochondria with high reactive oxygen species and low membrane potential [Eddie *et al*. 2017, Stephen *et al*. 2023].

The metabolic regulator Acod1 was identified as a primary actor in Tregs during sepsis from our analysis. Acod1, through itaconate production, contributes to signal transduction and metabolic reprogramming as itaconate is a central source of acetyl-CoA [Thekla *et al.* 2021]. To perform its function, various signal transduction networks such as TLRs and IFNAR, adapter proteins (e.g., MYD88), ubiquitin ligases (e.g., A20), and transcription factors (e.g., NF-κB, IRFs, and STATs) control Acod1 expression, regulating itaconate production, oxidative stress, and antigen processing [Wu et al., 2020]. Our results showed that Acod1 and FoxP3 were expressed in Treg-like cells (Fig. 2 and 3). Additionally, our analysis suggests that FoxP3 and Acod1

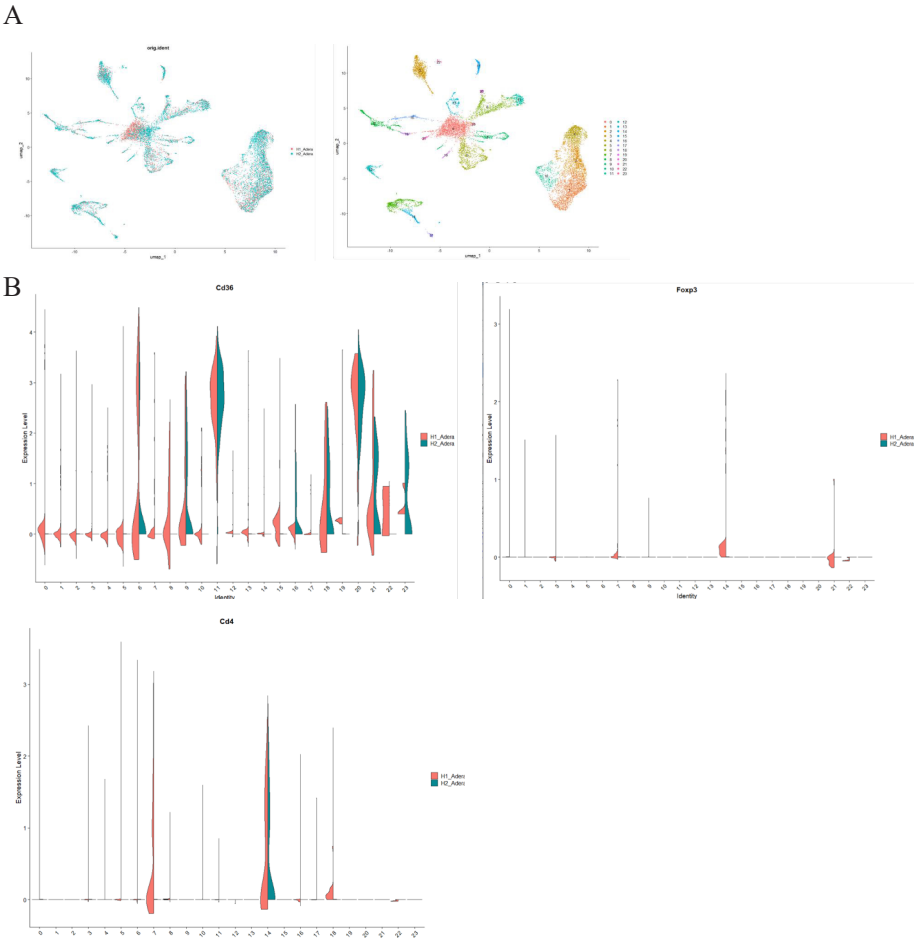


Fig. 6. Knocking out Acod1 had a significant effect on Treg function in sepsis. (A) Our single-cell RNA sequencing identified 23 unique cell clusters. (B) Looking closely into the change in each cluster's gene expression showed that FoxP3, CD4, and CD36 are downregulated in the Treg-like clusters.

mutually regulated each other's expression. Figures 2 and 6. During sepsis, our study showed that Acod1 and FoxP3 are upregulated in Treg-like cells (Fig. 4 and 5). Taken together, this suggests that Acod1 plays a critical role in sustaining Tregs during the hyper-hypoimmune reactions which characterise sepsis.

Our study also shows that Acod1 is associated with another metabolite regulator; CD36. CD36 is a class B scavenger receptor found on the surfaces of various immune cells, including macrophages, monocytes, and non-immune cells [Silverstein *et al.* 2009]. It binds to various extracellular ligands, including thrombospondin domain proteins, long-chain fatty acids (LCFAs), and molecules with molecular patterns [Chen

et al. 2022]. Recent studies have linked LCFA binding and transport to a potential binding pocket in the CD36 extracellular domain, although the atomic structure of CD36 remains unsolved [Hsieh *et al.* 2016]. CD36 is upregulated by cytokines such as CSF, IL4, and IL10 [Huh *et al*. 1996, Yang *et al*. 2022]. In Tregs, CD36 acts as a pattern recognition receptor that facilitates fatty acid (LCFA) transport. LCFA binding to CD36 triggers intracellular signalling events, adjusting lipid metabolism, including dissociation of SFK Fyn and enrichment of liver kinase B1, activating the AMPK pathway, and upregulating fatty acid oxidation (FAO) [Samovski *et al.* 2015, Horton *et al.* 2020, Wang *et al*. 2020]. In turn, FAO drives acetyl-CoA production from Itaconate [Harber *et al*. 2024]. Interestingly, our results show that knocking out Acod1 reduced the level of CD36 (Fig. 6). Thus, a hypothesis drawn from our study results surmises that during sepsis, the transport of LCFAs to the cell is mediated by CD36. The oxidization of LCFAs leads to the conversion of itaconate produced by Acod1 to acetyl-CoA. Itaconate further activates FoxP3 and mediates the production of IL10 and IL4. These anti-inflammatory cytokines maintain the activation of CD36, leading to a vicious cycle of anti-inflammatory responses in sepsis. In summary, our results indicate that targeting Acod1 can potentially ease the sepsis prognosis.

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Conflict of interest

The authors claim no conflict of interest exists.

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