Title:

Expansion of interferon signaling-associated gene (ISAG)^{hi} T cells in early-onset Alzheimer's disease

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Key Points:

Question: Is early-onset Alzheimer's disease (EOAD) associated with a specific peripheral immune signature?

Findings: We performed single-cell RNA-sequencing of >182,000 peripheral blood mononuclear cells and identified striking expansion of a subset of CD4 T cells termed interferon (IFN) signaling-associated gene $(ISAG)^{hi}$ T cells. We confirmed this finding by isolating CD4 T cells from additional participants with EOAD and measuring increased expression of $ISAG^{hi}$ marker genes.

Meaning: $ISAG^{hi}$ cells, which are primed for antiviral response, are expanded in early- but not late-onset AD or primary familial tauopathy, suggesting they may provide unique insight into the immune response to EOAD.

Abstract

Importance: Altered immune signatures are emerging as a central theme in neurodegenerative disease, yet little is known about immune responses in early-onset Alzheimer's disease (EOAD).

Objective: To determine whether an EOAD-specific immune signal can be detected in blood.

Design: We examined single-cell RNA-sequencing (scRNA-seq) data from peripheral blood mononuclear cells (PBMCs) and droplet digital (dd)PCR data from CD4 T cells from participants with EOAD and clinically normal controls.

Setting: University of California, San Francisco Memory and Aging Center.

Participants: Samples were collected for the study from 2017 to 2021. Participants with EOAD were diagnosed with AD prior to age 65. Data were last analyzed September 2023.

Main Outcomes and Measures: The primary analysis (scRNA-seq) examined whether EOAD was associated with altered frequency of any PBMC subsets. Secondary analysis (ddPCR) measured marker gene expression in CD4 T cells.

Results: Over 182,000 individual PBMC transcriptomes were analyzed by scRNA-seq from 16 individuals (mean [SD] age at sample collection, 52.2 [9.6] years; 8 [50.0%] were female; 8 [50.0%] had clinical diagnosis of AD). The relative abundance of PBMC subsets was tested for association with EOAD while controlling for age and sex. Of 19 PBMC clusters, one cluster, representing interferon signaling-associated gene (ISAG)^{hi} T cells, was significantly expanded in EOAD (P = 0.005). A validation cohort consisting of 19 individuals (mean [SD] age at sample collection, 57.7 [4.1] years; 13 [68.4%] were female; 9 [47.4%] had clinical diagnosis of AD) was used for CD4 T cell isolation and ddPCR analysis. Expression of *MX1* and *IFI6*, marker genes for ISAG^{hi} T cells, was significantly elevated in participants with EOAD (P = 0.002 and P = 0.04, respectively). Secondary analyses of independent scRNA-seq datasets from later-onset AD and familial tauopathy indicated that ISAG^{hi} T cells are detected but not elevated in these related diseases.

Conclusions and Relevance: ISAG^{hi} T cells, which appear primed for antiviral activity, are significantly and specifically expanded in EOAD. Additional research into the role of this rare cell type in neurodegeneration is warranted.

Introduction

Approximately 5–10% of the ~7 million Americans living with Alzheimer's disease (AD)[1] experience symptom onset prior to age 65[2]. In this early-onset form of AD (EOAD), affected individuals are more likely to experience an aggressive clinical course, have an atypical clinical syndrome, encounter delays in diagnosis, and experience unique social disruptions due to their relatively young age[2]. The vast majority (≥90%) of EOAD cases are not inherited in an autosomal-dominant manner, and for these individuals, we understand relatively little about the genetic and other biological factors underpinning disease risk.

Recent reports using single-cell RNA-sequencing (scRNA-seq) have highlighted changes in peripheral blood and cerebrospinal fluid (CSF) leukocyte populations in AD[3], Lewy body dementia[4], familial tauopathy[5], and during aging[6]. To our knowledge, however, a global, unbiased scRNA-seq analysis of peripheral blood mononuclear cells (PBMCs) in EOAD has not been reported. Using scRNA-seq, we now find evidence for marked expansion of a small population of recently characterized CD4 T cells expressing very high levels of interferon (IFN) signaling-associated genes ($ISAG^{hi}$ T cells) in EOAD. We also observe global up-regulation of $ISAG^{hi}$ T-cell marker genes across additional lymphoid and myeloid PBMC types. Strikingly, although we also detect $ISAG^{hi}$ T cells in a well-known dataset of CSF leukocytes derived primarily from individuals with older-onset AD and mild cognitive impairment (MCI)[3], the cluster is not expanded in these cases, suggesting $ISAG^{hi}$ T cells may be specific to EOAD. In addition, a CD4 T-cell subtype that appears to be highly similar to $ISAG^{hi}$ T cells–with a similar antiviral gene expression signature–is markedly expanded in the CSF in the context of viral encephalitis[7], suggesting that EOAD-expanded $ISAG^{hi}$ T cells have antiviral properties.

Methods

After obtaining informed consent, PBMCs from study participants (Table 1) at the University of California, San Francisco Memory and Aging Center were analyzed by scRNA-seq essentially as described[5]. Details are described in eMethods. Raw sequencing reads were aligned to GRCh38-2020-A and feature-barcode matrices generated using Cell Ranger (v7.1.0) with intronic reads excluded. Cluster proportions were determined for individual samples by dividing the number of cells in a given cluster by the total number of cells in clusters representing all PBMCs, all T cells, or all CD4 T cells (after quality control) for each individual. Statistical differences in cell-type abundances by diagnosis were assessed via linear modeling, controlling for age and sex.

Results

After QC filtering, clustering of ~182,000 PBMCs generated 19 primary clusters consisting of all expected PBMC types. Comparison of relative cluster abundance in EOAD cases vs. controls revealed a single cluster (cluster 15) that was robustly expanded in EOAD (Figure 1, A). Expression of marker genes indicates that cluster 15 is a subtype of CD4 T cell (eFigure 1, A). Quantification of cluster 15 abundance relative to either all PBMCs, all T cells, or all CD4 T cells revealed significant expansion in EOAD that is driven primarily by females (Figure 1, B, C). To determine what type of CD4 T cell cluster 15 represents, we subsetted all T cells and reclustered them separately from all other cell types. Reclustering revealed this cell type in sub-cluster 11, which expresses uniquely high levels of IFN-signaling genes *MX1* and *IFI6* relative to all other T cells (Figure 1, D). As expected, sub-cluster 11 was also significantly expanded in EOAD relative to controls (FIgure 1, D).

What is the precise identity of this subset of CD4 T cells? Recent literature using scRNA-seq to analyze human leukocyte populations has revealed two poorly understood cell types:

ISAG^{hi} T cells, detected in peripheral blood[8], and antiviral CD4 T cells, detected in CSF[7]. Antiviral CD4 T cells were so named due to their marker gene expression and robust expansion in the CSF in the context of viral encephalitis[7]. Comparison of all marker genes for our sub-cluster 11 to the top 200 marker genes for CSF antiviral CD4 T cells revealed highly statistically significant overlap ($P = 6.5 \times 10^{-14}$; eTable 1)[9]. Moreover, all of the 12 most-significant marker genes originally reported for ISAG^{hi} T cells[8] are also top marker genes of our sub-cluster 11 and of antiviral CD4 T cells. Therefore, from here on we refer to the EOAD-expanded CD4 T cells as ISAG^{hi} T cells.

ISAG^{hi} T-cell abundance was consistent across scRNA-seq batches (eFigure 1, B) and was not driven by *APOE* ε4 status (eFigure 1, C). Moreover, although our control samples came from participants with a younger mean age (Table 1), there was no relationship between age and ISAG^{hi} abundance (eFigure 1, D). To increase the sample size of our scRNA-seq dataset, we included 7 additional control PBMC samples previously characterized by scRNA-seq[5]. We found that the expansion of ISAG^{hi} T cells relative to PBMCs and all T cells remained significant after addition of these independent controls, although one outlier control sample with very high levels of ISAG^{hi} T cells made the findings somewhat less robust (eFigure 2). We recently reported a reduction in peripheral non-classical monocytes in familial tauopathy[5]. Comparing the familial tauopathy and EOAD datasets, we found that non-classical monocytes are not reduced in EOAD, and ISAG^{hi} T cells are not expanded in familial tauopathy (eFigure 3). Moreover, secondary analysis of a well-known CSF leukocyte dataset[3] revealed that ISAG^{hi} T cells, although detected, are also not expanded in the CSF in an independent cohort consisting of mostly later-onset MCI and AD cases (eFigure 4). Collectively, these findings suggest that ISAG^{hi} T-cell expansion may be associated specifically with EOAD rather than LOAD or familial tauopathy.

Differential expression analysis revealed a high number of differentially expressed genes (DEGs) in classical and non-classical monocytes in EOAD, relative to cognitively normal

controls (eFigure 5, eTable 2). Remarkably, we found that, on average, ~18% of the significantly up-regulated genes across all clusters (excluding those with fewer than 10 up-regulated DEGs) were also ISAG^{hi} T-cell marker genes (eFigure 5). In EOAD, we thus observe both expansion of a CD4 T-cell subset expressing very high levels of genes associated with IFN signaling and up-regulation of many of the same genes across additional lymphoid and myeloid cell types.

To validate our primary scRNA-seq finding, we magnetically isolated CD4 T cells from an additional cohort of EOAD cases and control participants. A droplet digital (dd)PCR-based validation assay indicated highly efficient isolation of CD4 T cells (eFigure 6). Reasoning that increased expression of specific ISAG^{hi} marker genes from isolated CD4 T cells would be consistent with expansion of ISAG^{hi} T cells as well as ISAG up-regulation, we performed ddPCR for ISAG^{hi} T-cell marker genes *MX1* and *IFI6* (Figure 2, A). Cases and controls in the ddPCR cohort had similar average ages (Table 1; see also eMethods), excluding age as an explanatory factor for this finding. Strikingly, ddPCR confirmed increased *MX1* and *IFI6* signals in CD4 T cells from EOAD (Figure 2, B). Increased *MX1* was observed across two independent ddPCR batches and was driven by females (Figure 2, C).

Discussion

In this study, we found evidence for a unique peripheral immune signature in EOAD. Our findings complement and expand upon existing evidence of diverse T-cell signatures in other forms of AD[3,10], additional neurodegenerative diseases[4], and during aging[6]. Our study is limited by the relatively small sample sizes that are characteristic of scRNA-seq experiments. Future studies in diverse EOAD cohorts from additional recruitment sites will be needed to confirm the broad relevance of our findings to EOAD. In light of recent findings suggesting that (i) herpes zoster vaccination may be causally associated with reduced dementia risk in women[11]; (ii) viral encephalitis exposure markedly increases risk for AD[12]; and (iii) the ISAG^{hi} T cells increased in EOAD bear striking resemblance to antiviral CD4 T cells expanded in CSF in viral encephalitis[7],

our findings raise the intriguing possibility that this extreme form of AD is characterized by a potent antiviral T-cell response.

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Table 1. Demographic and experimental information for samples used in scRNA-seq and ddPCR studies

	scRNA-seq discovery study		ddPCR validation study	
	Controls	Cases	Controls	Cases
n	8	8	10	9
n per batch (Batch A, Batch B)	4, 4	4, 4	5, 5	4, 5
PBMCs analyzed, *n*	91,955	90,398	N/A	N/A
CD4 T-cell RIN, mean (SD)	N/A	N/A	9.5 (0.5)	9.5 (0.3)
Sex, *n* female (%)	4 (50)	4 (50)	7 (70)	6 (66.7)
Age, mean (SD)	44.6 (7.4)	59.9 (3.1)	56.9 (5.0)	58.6 (2.7)
APOE ε4 status *n* heterozygous, *n* homozygous	3, 0	5, 1	2, 0	5, 1
Clinical syndrome (*n*)	clinically normal (8)	AD (6), frontal AD (2)	clinically normal (10)	AD (9)
Global CDR, mean (SD)	0.0 (0.0)	1.1 (0.4)	0.1 (0.2)[#]	0.9 (0.2)

Abbreviations: AD, Alzheimer's disease; CDR, Clinical Dementia Rating scale; ddPCR, droplet digital PCR; PBMC, peripheral blood mononuclear cell; scRNA-seq, single-cell RNA sequencing; RIN, RNA integrity number; SD, standard deviation. [#]All clinically normal control participants except one in the ddPCR study had a Global CDR score of 0; the remaining control

had a global CDR score of 0.5 and was considered clinically normal by neurological and neuropsychological testing.

Figure 1. Expansion of ISAG^{hi} T cells in EOAD characterized by scRNA-seq.

A, Uniform manifold approximation and projection (UMAP) plot of ~182,000 PBMCs from EOAD cases and cognitively normal controls, colored by cluster identity. Major cell types are labeled within the plot. The inset (right) shows the primary T-cell grouping displayed in grey, with the ISAG^{hi} T-cell cluster shown in magenta. **B**, ISAG^{hi} T-cell abundance is quantified relative to all PBMCs (left; P = 0.005), all T cells (middle, P = 0.013), and all CD4 T cells (right; P = 0.016). **C**, Stratifying by sex, ISAG^{hi} T-cell relative abundance is significantly increased in EOAD only in females, expressed as a percentage of PBMCs (left, P = 0.006), T cells (middle, P = 0.01), and CD4 T cells (right, P = 0.008). **D**, Reclustering of all T cells (left) generates a T-cell subcluster (11) representing ISAG^{hi} T cells, which express high levels of marker genes *MX1* and *IFI6*, in addition to T-cell markers *CD4* and *CD3E* (middle). Quantification of the ISAG^{hi} subcluster relative to all T cells again indicates a significant increase in EOAD cases (right, P = 0.034).

Figure 2. ISAG^{hi} T-cell marker gene expression is increased in CD4 T cells in EOAD.

A, CD4 T cells were magnetically isolated from PBMCs and RNA was extracted; gene expression was determined by RT-ddPCR. **B**, Expression of *MX1* and *IFI6* was significantly increased in CD4 T cells from EOAD cases relative to cognitively normal controls (P = 0.002 and P = 0.04, respectively). **C**, *MX1* expression was significantly increased in two independent RT-ddPCR batches (P = 0.03, both batches). The increase in *MX1* expression observed in EOAD was driven by females (P = 0.0002). *CD4* was used as a reference gene.

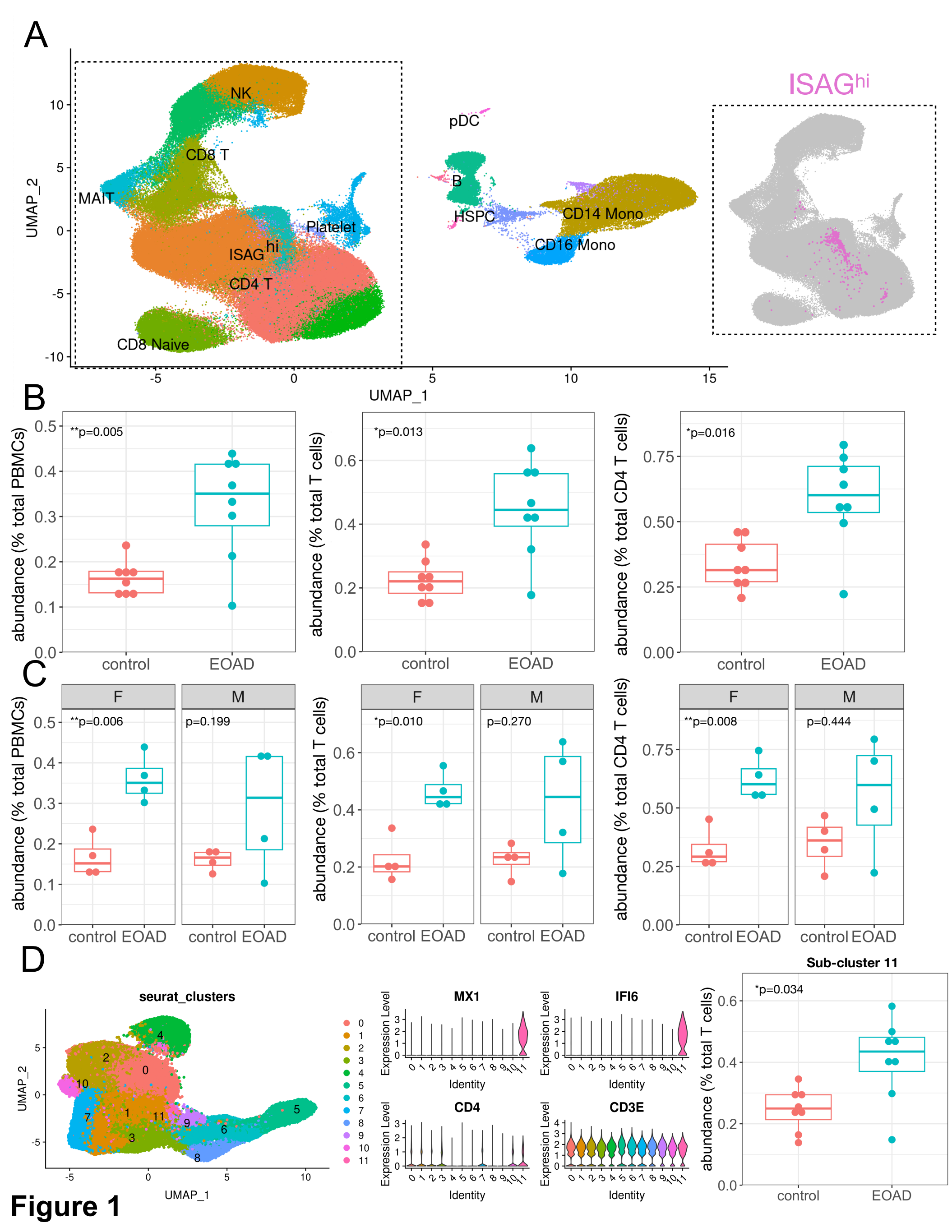
A
NK
CD8 T
MAIT
Platelet
ISAG hi
CD4 T
CD8 Naive
pDC
B
HSPC
CD14 Mono
CD16 Mono
ISAG hi
UMAP_1
UMAP_2
B
**p=0.005
*p=0.013
*p=0.016
abundance (% total PBMCs)
abundance (% total T cells)
abundance (% total CD4 T cells)
control
EOAD
C
F
M
**p=0.006
p=0.199
*p=0.010
p=0.270
**p=0.008
p=0.444
D
seurat_clusters
Expression Level
Identity
MX1
IFI6
CD4
CD3E
Sub-cluster 11
*p=0.034

Figure 1

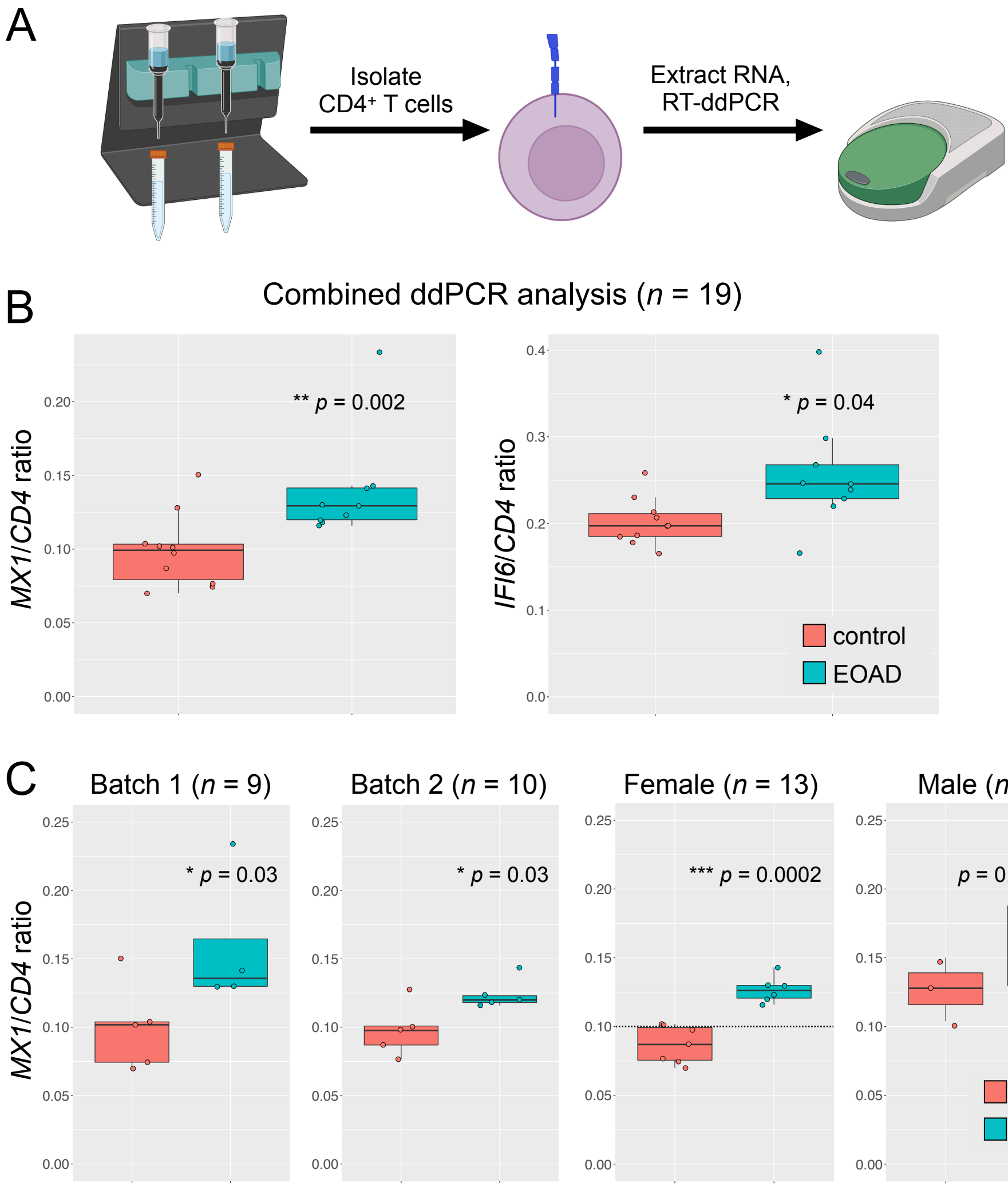
A
Isolate
CD4+ T cells
Extract RNA,
RT-ddPCR
B
Combined ddPCR analysis (n = 19)
MX1/CD4 ratio
** p = 0.002
IFI6/CD4 ratio
* p = 0.04
control
EOAD
C
Batch 1 (n = 9)
Batch 2 (n = 10)
Female (n = 13)
Male (n = 6)
MX1/CD4 ratio
* p = 0.03
* p = 0.03
*** p = 0.0002
p = 0.89
control
EOAD

Figure 2