



A novel subpopulation of monocytes with a strong interferon signature indicated by SIGLEC-1 is present in patients with in recent-onset type 1 diabetes

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Abstract

Aims/hypothesis Type 1 diabetes is a T cell-mediated autoimmune disease characterised by pancreatic beta cell destruction. In this study, we explored the pathogenic immune responses in initiation of type 1 diabetes and new immunological targets for type 1 diabetes prevention and treatment.

Methods We obtained peripheral blood samples from four individuals with newly diagnosed latent autoimmune diabetes in adults (LADA) and from four healthy control participants. Single-cell RNA-sequencing (scRNA-seq) was performed on peripheral blood mononuclear cells to uncover transcriptomic profiles of early LADA. Validation was performed through flow cytometry in a cohort comprising 54 LADA, 17 adult-onset type 2 diabetes, and 26 healthy adults, matched using propensity score matching (PSM) based on age and sex. A similar PSM method matched 15 paediatric type 1 diabetes patients with 15 healthy children. Further flow cytometry analysis was performed in both peripheral blood and pancreatic tissues of non-obese diabetic (NOD) mice. Additionally, cell adoptive transfer and clearance assays were performed in NOD mice to explore the role of this monocyte subset in islet inflammation and onset of type 1 diabetes.

Results The scRNA-seq data showed that upregulated genes in peripheral T cells and monocytes from early-onset LADA patients were primarily enriched in the IFN signalling pathway. A new cluster of classical monocytes (cluster 4) was identified, and the proportion of this cluster was significantly increased in individuals with LADA compared with healthy control individuals (11.93% vs 5.93%, $p=0.017$) and that exhibited a strong IFN signature marked by *SIGLEC-1* (encoding sialoadhesin). These SIGLEC-1⁺ monocytes expressed high levels of genes encoding C-C chemokine receptors 1 or 2, as well as genes for chemoattractants for T cells and natural killer cells. They also showed relatively low levels of genes for co-stimulatory and HLA molecules. Flow cytometry analysis verified the elevated levels of SIGLEC-1⁺ monocytes in the peripheral blood of participants with LADA and paediatric type 1 diabetes compared with healthy control participants and those with type 2 diabetes. Interestingly, the proportion of SIGLEC-1⁺ monocytes positively correlated with disease activity and negatively with disease duration in the LADA patients. In NOD mice, the proportion of SIGLEC-1⁺ monocytes in the peripheral blood was highest at the age of 6 weeks (16.88%), while the peak occurred at 12 weeks in pancreatic tissues (23.65%). Adoptive transfer experiments revealed a significant acceleration in diabetes onset in the SIGLEC-1⁺ group compared with the SIGLEC-1⁻ or saline control group.

Conclusions/interpretation Our study identified a novel group of SIGLEC-1⁺ monocytes that may serve as an important indicator for early diagnosis, activity assessment and monitoring of therapeutic efficacy in type 1 diabetes, and may also be a novel target for preventing and treating type 1 diabetes.

Data availability RNA-seq data have been deposited in the GSA human database (<https://ngdc.cncb.ac.cn/gsa-human/>) under accession number HRA003649.

Keywords Interferon response · Latent autoimmune diabetes in adults · SIGLEC-1⁺ monocytes · Single-cell transcriptomics · Type 1 diabetes

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Extended author information available on the last page of the article

Abbreviations

CD11b	αM-integrin
cDC2	Conventional type 2 dendritic cell
DEG	Differentially expressed gene

Research in context

What is already known about this subject?

- Type 1 diabetes is a T cell-mediated autoimmune disease characterised by pancreatic beta cell destruction, and LADA is a form of type 1 diabetes with late destruction of beta cells
- Prior genetic investigations have linked IFN signalling to initiation of type 1 diabetes

What is the key question?

- Which cell population plays the crucial role in the initial immune responses that induce type 1 diabetes onset?

What are the new findings?

- The population of SIGLEC-1-expressing classical monocytes is significantly increased in individuals with recent-onset type 1 diabetes, exhibiting a strong IFN signature
- This SIGLEC-1⁺ subset of monocytes expresses high levels of genes encoding chemoattractants for T cells and NKs, and relatively low levels of genes for co-stimulatory and HLA molecules
- The findings suggest that SIGLEC-1⁺ monocytes have the potential to be recruited to pancreatic islets and play a crucial role in induction of destructive insulinitis, warranting further investigation into their involvement in the onset of type 1 diabetes

How might this impact on clinical practice in the foreseeable future?

- Our study identifies a novel group of SIGLEC-1⁺ monocytes that may serve as an important indicator for early diagnosis, activity assessment and monitoring of therapeutic efficacy in type 1 diabetes, and may also be a new target for prevention and treatment of type 1 diabetes

FCM	Flow cytometry
GADA	GAD65 antibody
GDT	$\gamma\delta$ T cell
GO	Gene Ontology
ICR	Institute of Cancer Research
LADA	Latent autoimmune diabetes in adults
Lin	Lineage
Ly6c	Lymphocyte antigen 6 complex locus C
MAIT	Mucosal-associated invariant T cell
MX1	Myxovirus Resistance 1
NK	Natural killer cell
NKT	Natural killer T cell
NOD	Non-obese diabetic
PBMC	Peripheral blood mononuclear cell
pDC	Plasmacytoid dendritic cell
PLXNB2	Plexin B2
PSM	Propensity score matching
scRNA-seq	Single-cell RNA-sequencing
Sema4D	Semaphorin 4D
SIGLEC-1	Sialoadhesin
STAT1	Signal transducer and activator of transcription 1
Teff	Effector T cell

Tem	Effector memory T cell
Treg	Regulatory T cell
UMAP	Uniform Manifold Approximation and Projection

Introduction

Type 1 diabetes, an autoimmune condition that destroys pancreatic beta cells, affects over 9 million people worldwide (2% of people with diabetes overall) [1]. Its symptoms vary from polyuria, polydipsia and weight loss to severe diabetic ketoacidosis [2]. Currently available treatments, mainly relying on exogenous insulin supplementation, fail to address the immune aspect, highlighting the need for studies to identify safe and effective immunotherapy.

Type 1 diabetes is typically categorised into two forms: paediatric-onset and adult-onset, the latter also being known as latent autoimmune diabetes in adults (LADA) [3]. LADA exhibits clinical similarities to type 2 diabetes but involves the gradual destruction of pancreatic beta cells by T cells [4]. Consequently, capturing the

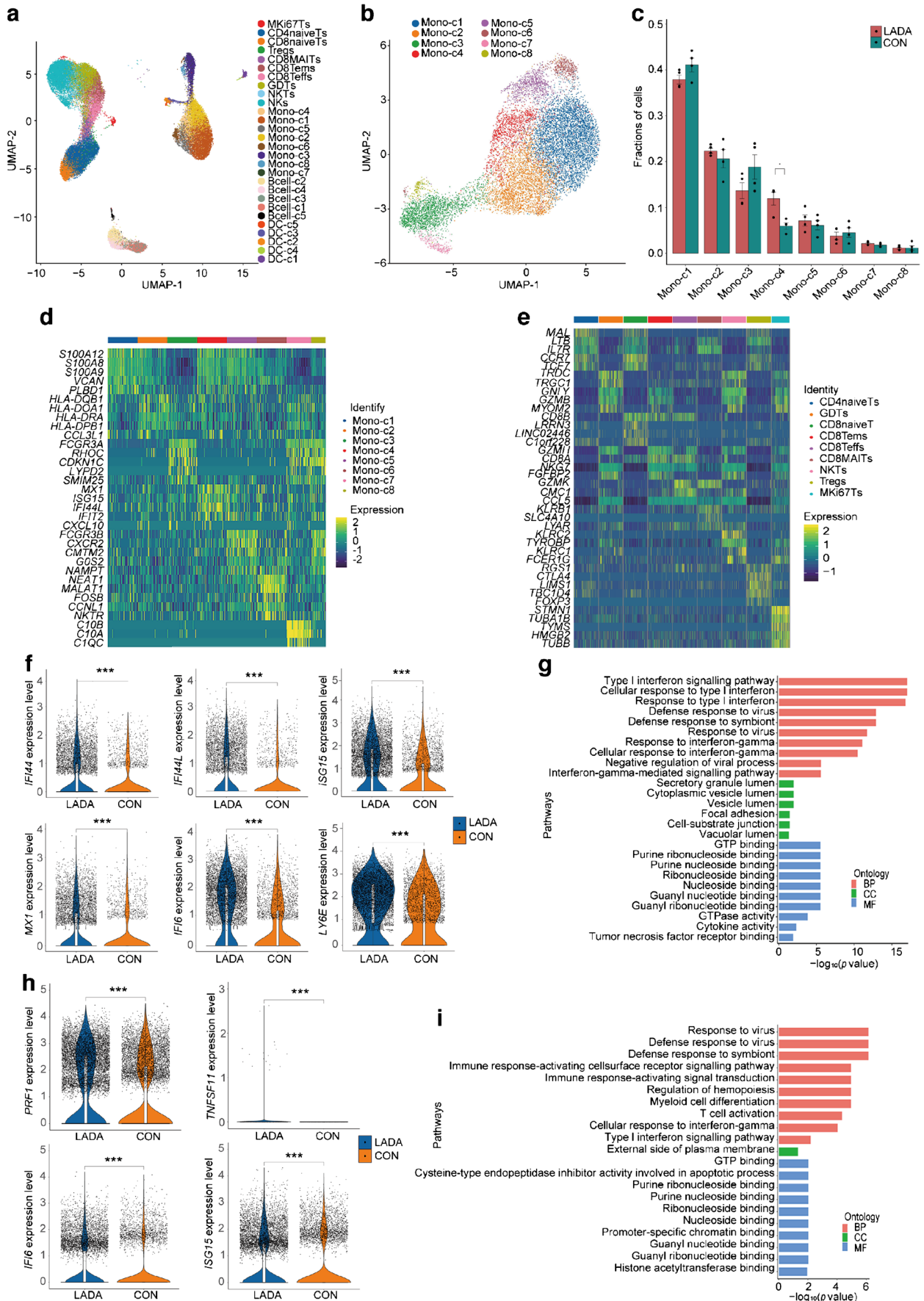


Fig. 1 Variations in immune cell composition and function between participants with LADA and healthy control participants. **(a)** Relative abundance of all cell subtypes, including T cells, B cells, monocytes, conventional type 2 dendritic cells, plasmacytoid dendritic cells, NKs and plasma cells, in both the LADA and control groups. **(b)** Two-dimensional UMAP dimensionality reduction plot showing the clusters of monocytes (c1–c8), with each cluster colour-coded as indicated. **(c)** Proportion of each monocyte cluster in the LADA and control groups. **(d, e)** Heatmaps displaying the top five DEGs in the identified monocyte subsets **(d)** and T cell subsets **(e)**; expression level, indicated by colour, is based on *z* score. **(f)** Differential expression analysis of IFN-related genes in monocytes between LADA and control groups. **(g)** GO enrichment analysis of upregulated genes expressed by monocytes in the LADA group compared with the control group. **(h)** Differential expression analysis of genes associated with cytotoxic activity and IFN response in T cells between LADA and control groups. **(i)** GO enrichment analysis of upregulated genes expressed by T cells in the LADA group compared with the control group. Student's *t* tests were used for normal variances, Welch's *t* tests for unequal variances, and Wilcoxon rank-sum tests for non-normal distributions. **p*<0.05, ****p*<0.001. Mono-c1 to -c8, clusters 1 to 8 of monocytes; Bcell-c1 to -c5, clusters 1 to 5 of B cells; DC-c1 to -c5, clusters 1 to 5 of dendritic cells (DCs); CON, control; BP, biological process; CC, cellular component; MF, molecular function

immunological changes in early LADA stages is easier than in paediatric-onset type 1 diabetes. However, to date, autoantibodies against islets remain the only established disease-specific markers for type 1 diabetes [3].

Type 1 diabetes begins with islet autoantigen presentation to CD4⁺ T cells, triggering pancreatic inflammation and beta cell destruction via various CD4⁺ T cell subsets. Individuals with type 1 diabetes display elevated levels of proinflammatory monocytes, notably in the subpopulations characterised by CD14⁺ and CD16⁺, as well as CD14⁺ CD16⁻ expression [5, 6]. Research by Ren et al [7] in newly diagnosed type 1 diabetes children highlighted significantly elevated CD14⁺ CD16⁺ monocyte levels, known for their enhanced antigen presentation and proinflammatory activity. In summary, specific monocyte subpopulations probably contribute to T cell activation and the destruction of islet cells during the onset of type 1 diabetes.

Previous genetic investigations have connected IFN signalling to the development of type 1 diabetes. In the non-obese diabetic (NOD) mouse model, early IFN α production in the pancreas triggers IFN signalling, attracting diabetogenic T cells [8, 9]. Blocking IFN signalling has been shown to delay or prevent diabetes [8, 10]. Two independent studies within the BABYDIET [11] and DIPP [12] cohorts have identified a robust IFN-inducible transcriptional signature in the blood of at-risk children. Furthermore, laser-captured insulitic islets from individuals with newly diagnosed type 1 diabetes demonstrated significantly increased expression of IFN-stimulated genes [13]. Notably, treatment of hepatitis or multiple sclerosis with IFN α/β has been shown to be associated with an elevated risk of developing diabetes [14–16]. Conversely, in individuals with defects in the *AIRE* gene (encoding autoimmune regulator), which

affects central T cell tolerance, there is a notable inverse relationship between the presence of IFN α -neutralising antibodies and the onset of type 1 diabetes. This indicates that antibodies targeting IFN α might play a protective role in preventing the development of type 1 diabetes in this specific group [17]. However, the link between circulating immune cells, IFN responses and the initiation of diabetes remains underexplored.

This study aims to identify and assess the cell populations responsible for the initial immune responses triggering the onset of type 1 diabetes.

Methods

Study participants and matching process

Single-cell RNA-sequencing (scRNA-seq) group In 2021, four participants with LADA within 1 month of diagnosis were enrolled and matched with four healthy controls. Matching was based on sex/gender, BMI and ethnicity, with all participants being Asian, split evenly by sex, and having a BMI difference of less than ± 2 kg/m² (see electronic supplementary material [ESM] Methods).

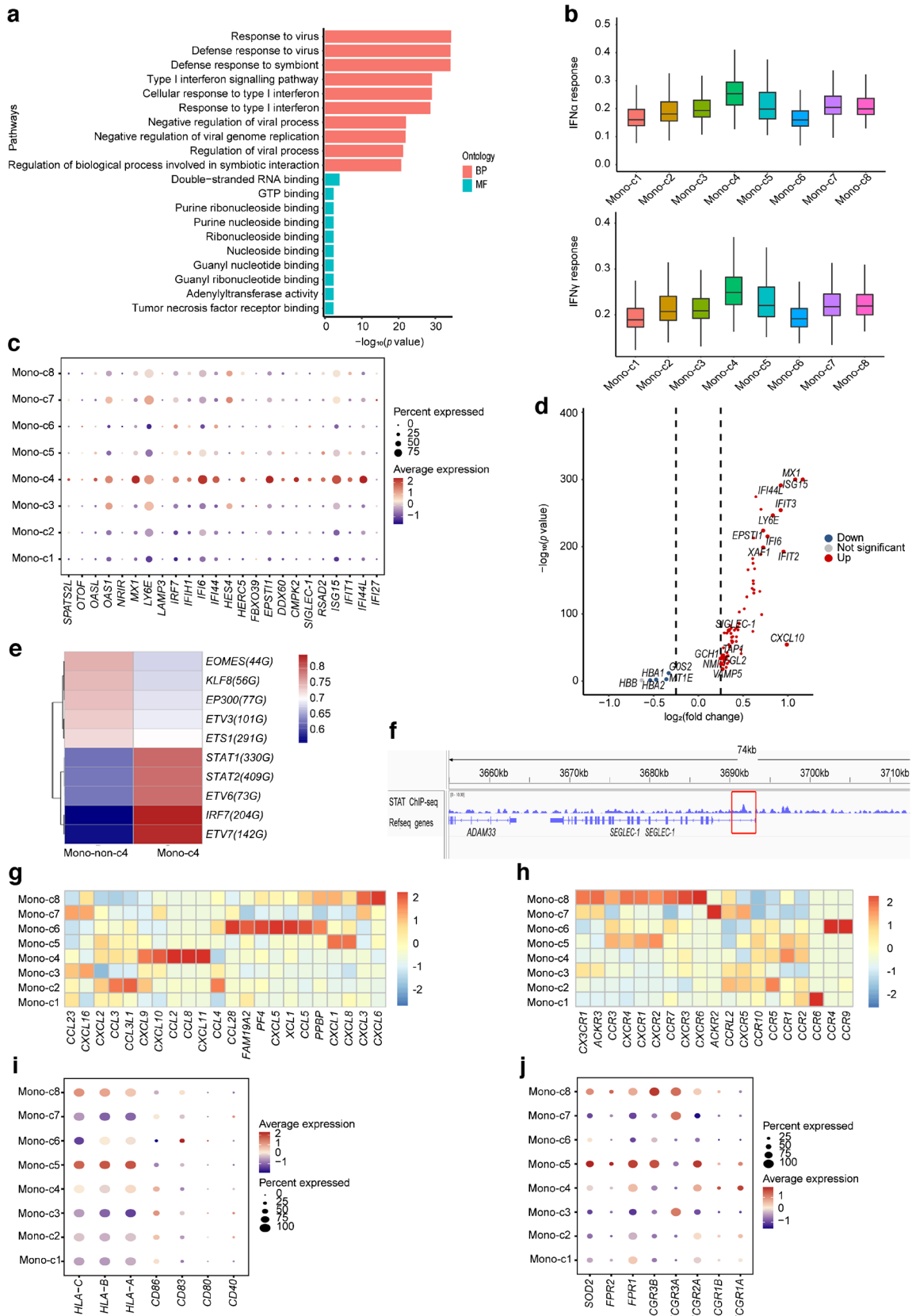
Flow cytometry (FCM) analysis groups In 2022, a diverse group of adult participants was enrolled: 54 with LADA, 17 with type 2 diabetes and 26 healthy individuals. They were matched using propensity score matching (PSM) based on age and sex, pairing 26 LADA patients with healthy individuals and 17 LADA patients with type 2 diabetes patients. Additionally, 15 children with type 1 diabetes and 15 healthy children formed a paediatric cohort, matched using PSM focusing on age and sex. All participants were of Asian ethnicity.

More details on healthy control selection, and inclusion and exclusion criteria [3, 18–20] are available in the ESM Methods.

Ethical considerations The independent ethics council of the Affiliated Hospital of Qingdao University, China, authorised this research, and every participant, including children, signed written informed consent. In the case of children, their guardians also provided consent by signing the required forms. All tests and blood sampling complied with the committee recommendations of ethical and biosafety regulations.

Mouse handling and experimental protocols

Six-week-old male NOD mice from Changzhou Cavens Experimental Animal Company (China) and Institute of Cancer Research (ICR) mice from Beijing Huafukang



◀ **Fig. 2** Differential gene expression and enrichment analysis of cluster 4 monocytes. **(a)** GO enrichment analysis of upregulated genes expressed by cluster 4 monocytes compared with other monocyte clusters. **(b)** Ucell analysis of genes related to IFN α and IFN γ signalling pathways in each monocyte cluster. **(c)** Dot plot showing the expression of IFN-stimulated genes in identified monocyte subsets; the size of the dot indicates the proportion of expression, and the shade of the colours indicates the average expression amount (z score). **(d)** Volcano plot showing DEGs between cluster 4 and non-cluster 4 monocytes. **(e)** Heatmap comparing regulon activity between cluster 4 and non-cluster 4 monocytes; activity levels are indicated by colour based on z score, as shown in the key; the numerals adjacent to each transcription factor indicate the count of target genes regulated, where 'G' stands for 'genes'. **(f)** GM12878 ChIP-seq data showing that STAT1 binds significantly to the promoter region of the *SIGLEC-1* gene. **(g, h)** Heatmaps showing the expression of chemokine genes **(g)** and chemokine receptor genes **(h)** in identified monocyte subsets; expression levels are indicated by colour based on z score, as shown in the key. **(i, j)** Dot plots showing the expression of genes relating to antigen presentation and co-stimulation **(i)** and phagocytosis and metabolic activity **(j)** in identified monocyte subsets; the size of the dot indicates the proportion of expression, and the shade of the colours indicates the average expression amount. BP, biological process; MF, molecular function; Mono-c1 to -c8, clusters 1 to 8 of monocytes, Mono-non-c4, all monocyte clusters except cluster 4

Biotechnology Company (China) were used in this study. All mice were kept in pathogen-free facilities. The mice were randomly divided into three time-point groups, and killed at 6, 12 and 24 weeks of age for collection of peripheral blood samples and pancreatic tissues. We also performed monocyte adoptive transfer and clearance assays on 6-week-old NOD and ICR mice. This was followed by an 8-week observation period, during which we assessed islet inflammation and the incidence of diabetes. All experiments followed strict randomisation protocols (see ESM Methods).

Single-cell preparation

We collected blood samples from human participants and mice, then isolated peripheral blood mononuclear cells (PBMCs). For pancreatic single-cell suspensions, we treated mouse pancreatic fragments with trypsin and DNase, followed by ending the digestion and releasing single cells (see ESM Methods).

scRNA-seq library construction and analysis

Library construction The library was developed using GEX-SCOPE kit [21].

Primary analysis of raw read data Gene expression matrices were developed with the CeleScope workflow.

Quality control, dimension reduction and clustering Cells were filtered for gene and unique molecular identifier (UMI) counts; clustering was done with Seurat [22] and visualised using Uniform Manifold Approximation and Projection (UMAP).

Analysis of differentially expressed genes and cell type annotation Differentially expressed genes (DEGs) were identified with the Seurat FindMarkers function [23]; cell types were annotated from canonical markers and literature [24, 25].

Pathway enrichment analysis Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted with the 'clusterProfiler' package in R [26].

Ucell gene set scoring Gene set scoring was performed using the R package Ucell [27].

Cell–cell interaction analysis Cell–cell interaction was analysed using CellPhoneDB [28].

Further details are available in the ESM Methods.

FCM analysis and cell sorting

Human PBMCs were stained for surface markers and intracellular cytokines, and mouse monocytes/macrophages were stained and sorted using specific antibodies. All data were analysed using FlowJo software (version 10.8.1, <https://www.flowjo.com/>), with operators blinded to sample details (see ESM Methods).

Morphometric analysis of pancreatic insulinitis

Islet inflammation in mice was assessed using histological scoring [29, 30]. Operators were blinded to sample sources (see ESM Methods).

Statistics and repeatability

For statistical analysis, the study used unpaired Student's t tests, Welch t tests for unequal variances, or Wilcoxon rank-sum tests for non-normally distributed data, depending on data distribution and variance. For comparisons involving three or more groups, ANOVA was employed. A p value < 0.05 was considered statistically significant (see ESM Methods).

Results

Single-cell transcription for characterisation of immunological features in new-onset LADA

We evaluated the transcriptome profiles of PBMCs from four individuals with LADA and four healthy control individuals using scRNA-seq in order to define the immunological characteristics of patients with LADA (ESM Fig. 1a). The clinical features of the study participants are presented in ESM Table 1.

Our scRNA-seq analysis profiled an estimated 24,831 cells from participants with LADA and 16,848 cells from healthy control participants. Quality control charts for all eight samples

are shown in ESM Fig. 1b. We applied UMAP clustering, and identified seven major cell populations based on typical gene markers for each cell cluster, comprising 17,700 T cells, 3227 B cells, 12,358 monocytes, 548 conventional type 2 dendritic cells (cDC2s), 400 plasmacytoid dendritic cells (pDCs), 7298 natural killer cells (NKs) and 148 plasma cells (ESM Fig. 2a,b). In addition to this classification, we identified the top five DEGs and characteristic marker genes for each of these seven cell types (ESM Fig. 2c,d). ESM Fig. 2e,f and ESM Table 1 show the quantities of each cell type and their relative proportions within PBMCs for each donor.

Further analysis involved subclustering of transcriptionally distinct subsets within the aforementioned cell types (Fig. 1a). Among the monocytes, we identified eight

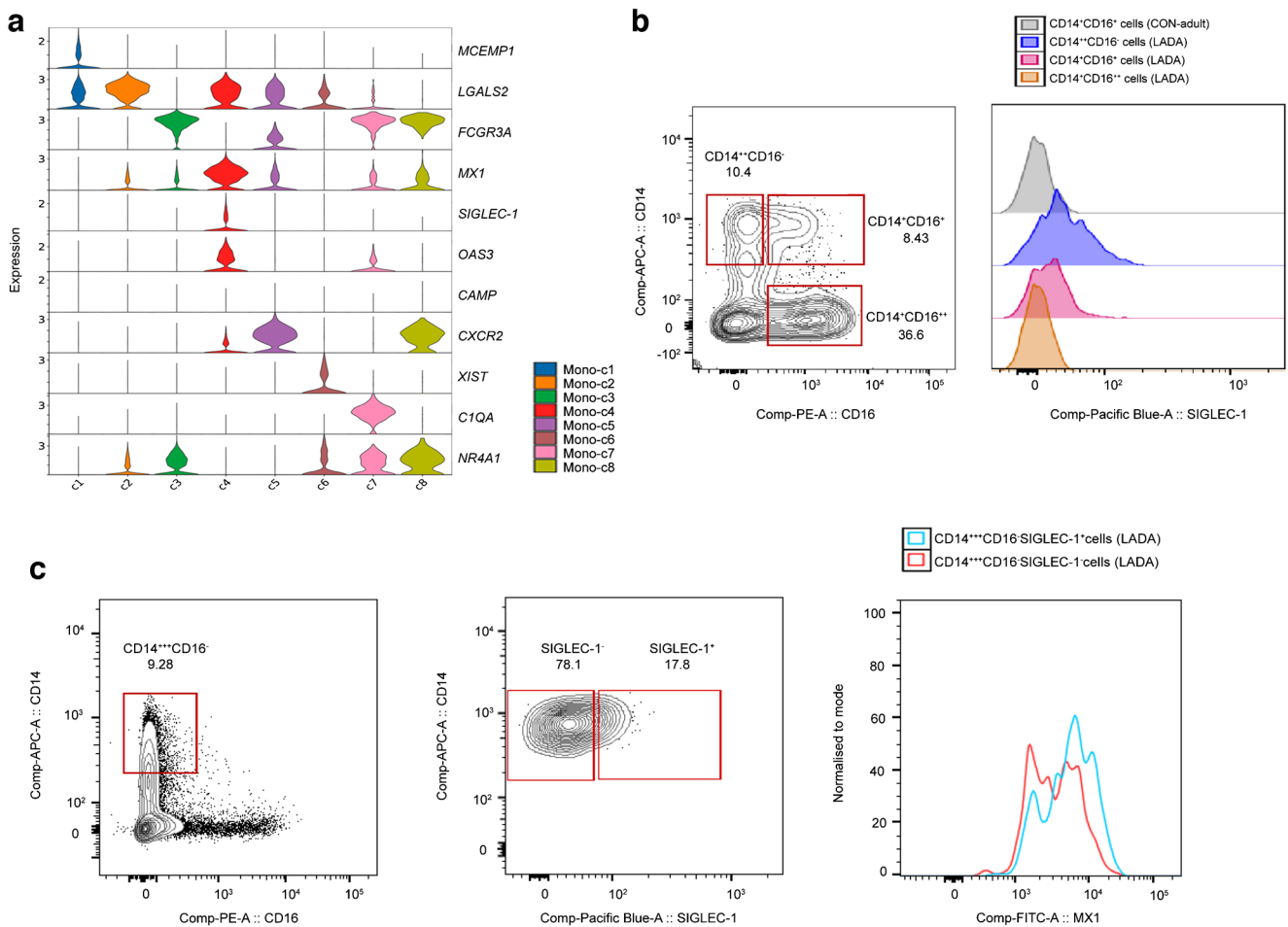


Fig. 3 Verification of SIGLEC-1 as a specific surface marker of cluster 4 monocytes. **(a)** Violin plot of the characteristic marker genes of eight monocyte subsets. **(b)** FCM analysis to examine SIGLEC-1 expression on classical (CD14⁺⁺ CD16⁺), intermediate (CD14⁺ CD16⁺) and non-classical (CD14⁻ CD16⁺) monocytes in CD45⁺ Lin⁻ in peripheral blood of participants with LADA. **(c)** FCM analysis to examine the expression of intracellular MX1 proteins related to IFN signalling in SIGLEC-1⁺ and SIGLEC-1⁻ monocytes. Mono1 to

Mono8, clusters 1 to 8 of monocytes; Comp-APC-A::CD14, the compensated area signal of allophycocyanin (APC) fluorescence, specifically detecting the CD14 marker; Comp-PE-A::CD16, the compensated area signal of phycoerythrin (PE) fluorescence for identifying the CD16 marker; Comp-Pacific Blue-A::SIGLEC-1, the compensated area signal of Pacific Blue dye for detecting SIGLEC-1; Comp-FITC::MX1, the compensated FITC signal for MX1 marker detection

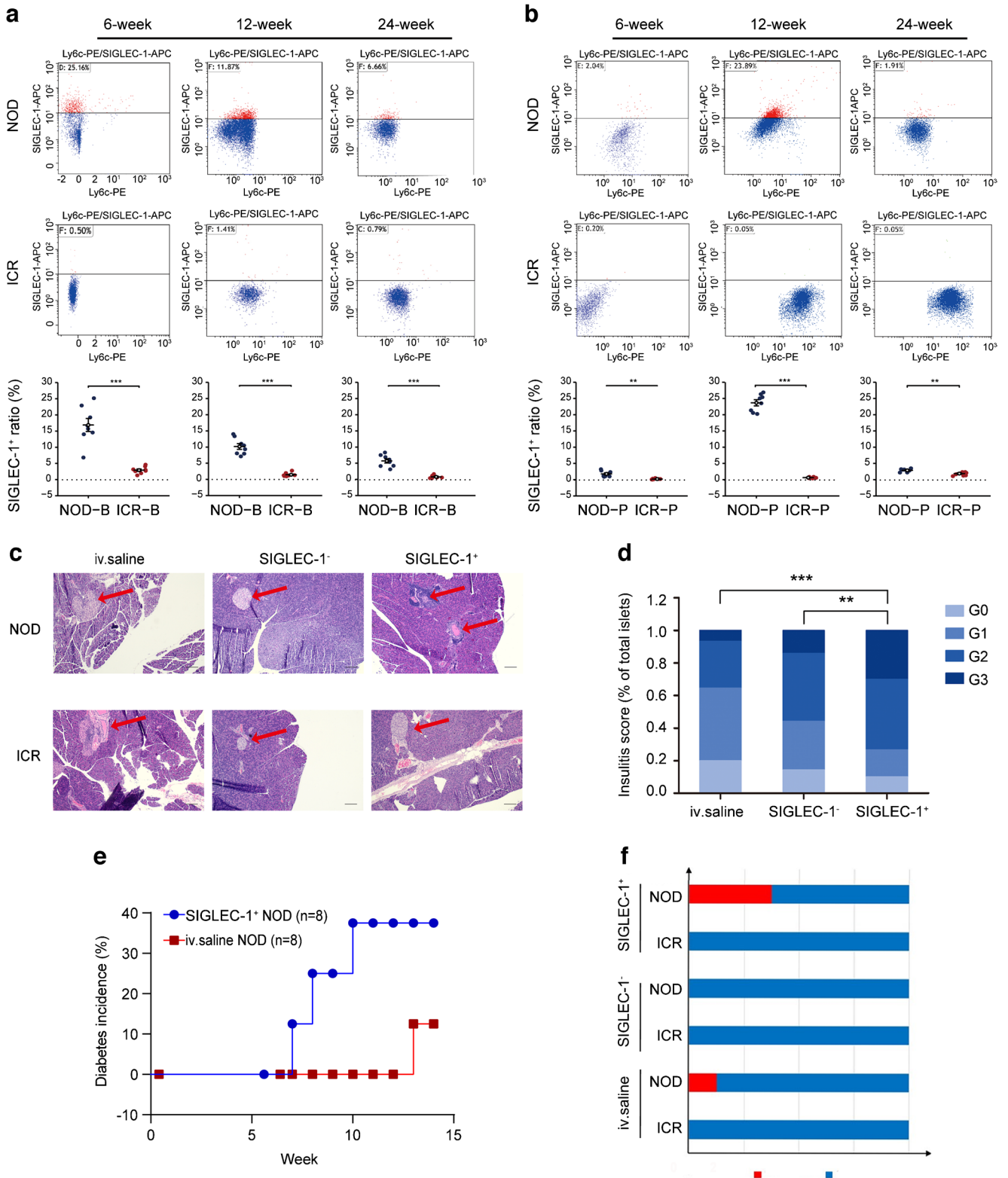


Fig. 4 FCM analysis of SIGLEC-1⁺ monocytes/macrophages in NOD and ICR mice. **(a)** FCM analysis of the CD11b⁺ Ly6c⁺ SIGLEC-1⁺ monocytes in the peripheral blood of NOD and ICR mice at 6, 12 and 24 weeks ($n=6-8$). The panels are labelled as 'Ly6c-PE/SIGLEC-1-APC', where 'Ly6c-PE' refers to the detection of the Ly6c marker using phycoerythrin (PE) fluorescence and 'SIGLEC-1-APC' to the detection of the SIGLEC-1 marker using allophycocyanin (APC) fluorescence. These markers are used to identify and analyse the population of monocytes in the blood samples. NOD-B, peripheral blood of NOD mice; ICR-B, peripheral blood of ICR mice. **(b)** FCM analysis of F4/80⁺ CD11b⁺ Ly6c⁺ SIGLEC-1⁺ macrophages in the pancreatic tissues of NOD and ICR mice at 6, 12 and 24 weeks ($n=7-8$). Similar to **(a)**, the panels in **(b)** are also labelled to indicate the use of PE and APC fluorescent dyes to detect Ly6c and SIGLEC-1 markers, respectively. NOD-P, pancreatic tissues of NOD mice; ICR-P, pancreatic tissues of ICR mice. **(c)** H&E staining in NOD and ICR mice after i.v. administration of saline, infusion of SIGLEC-1⁺ monocytes and infusion of SIGLEC-1⁻ monocytes (scale bar, 250 μ m). Arrows highlight pancreatic islets infiltrated by inflammatory cells, illustrating the extent of inflammation in different treatment groups. **(d)** Comparative analysis of islet inflammatory scores in NOD mice among the SIGLEC-1⁺, SIGLEC-1⁻ and i.v. saline groups. A score of G1 indicates peri-insulinitis, characterised by infiltration limited to the periphery of the islet. A score of G2 indicates infiltration covering less than 50% of the islet area. A score of G3 indicates severe insulinitis, with 50% or more of the islet area infiltrated, and disruption of islet structure. ** $p<0.01$ and *** $p<0.001$ represent significant increases in severe insulinitis (G3 scores) in SIGLEC-1⁺ mice compared with the SIGLEC-1⁻ and saline-treated groups, respectively. **(e)** Natural progression of type 1 diabetes development in the SIGLEC-1⁺ and i.v. saline groups. Type 1 diabetes development was monitored by weekly glycosuria testing and confirmed by a blood glucose level exceeding 13.3 mmol/l. **(f)** Incidence rates of type 1 diabetes in each group after monocyte transfusion or i.v. saline injection ($n=8$). Student's t tests for normal variances, Welch's t tests for unequal variances, and Wilcoxon rank-sum tests for non-normal distributions. ** $p<0.01$, *** $p<0.001$

transcriptionally distinct clusters of monocytes, further categorised as classical monocytes, defined by the expression of *CD14* and *VCAN* (clusters 1, 2, 4, 5 and 6), and intermediate/non-classical monocytes expressing *FCGR3A* (encoding CD16) (clusters 3, 7 and 8) (Fig. 1b–d and ESM Fig. 3). Within the T cell population, we identified nine distinct subsets: CD4⁺ naive T cells (CD4naiveTs), CD8⁺ naive T cells (CD8naiveTs), regulatory T cells (Tregs), CD8⁺ effector T cells (CD8Teffs), CD8⁺ effector memory T cells (CD8Tems), CD8⁺ mucosal-associated invariant T cells (CD8MAITs), $\gamma\delta$ T cells (GDTs), proliferating T cells marked by MKi67 (MKi67⁺ T cells, MKi67Ts) and natural killer T cells (NKTs) (Fig. 1e and ESM Fig. 4). Similarly, B cells were classified as naive B cells (clusters 1 and 3), memory B cells (clusters 2 and 4) or plasma cells (cluster 5). Dendritic cells were classified as plasmacytoid (clusters 1 and 4), conventional type 2 (clusters 2 and 4) and conventional type 1 (cluster 5). Subclassifications and associated DEGs for each subset of B cells and dendritic cells are shown in ESM Figs 5 and 6. Importantly, we observed a significant increase in the proportion of cluster 4 among monocytes in donors with LADA compared with healthy donors (11.93% vs 5.93%;

$p=0.017$), while no significant differences in other subsets were observed between the two groups.

GO enrichment analysis was then performed to annotate the functions of DEGs in individuals with LADA and healthy control individuals. The DEGs that were upregulated in monocytes of participants with LADA were primarily enriched in IFN-related signalling pathways [31, 32], with the most significant differences being observed in expression of *IFI44*, *IFI44L*, *MX1*, *ISG15*, *IFI6* and *LY6E* (Fig. 1f,g and ESM Table 2). In T cells, the DEGs upregulated in participants with LADA were enriched in the biological processes of T cell activation and IFN responses, and included *PRF1*, *TNFSF11*, *ISG15* and *IFI6* (Fig. 1h,i and ESM Table 3). We also found that IFN-related pathways were involved in the B cell activation that occurs in LADA patients. Additionally, the DEGs upregulated in individuals with LADA were enriched in T cell regulation and B cell activation in NKs, antigen processing and presentation in dendritic cells, and mRNA processing in plasma cells. Detailed GO analysis information for each cell type in LADA participants, compared with the healthy control participants, is presented in ESM Fig. 7.

Immunological features of cluster 4 monocytes

We further analysed GO enrichment for each monocyte subset (ESM Fig. 8). The results indicated that the genes upregulated in cluster 4 monocytes were mainly associated with IFN-related signalling pathways, such as defence response to the virus and type I IFN signalling pathway, compared with other monocyte clusters (Fig. 2a). Ucell analysis verified that genes upregulated in cluster 4 monocytes were especially enriched in IFN α and IFN γ responses (Fig. 2b). The IFN signature of cluster 4 included genes such as *IFI44*, *IFI44L*, *EPSTI1*, *MX1*, *HERC5*, *IFIT1*, *RSAD2*, *SIGLEC-1*, *ISG15*, *IFI6*, *CMPK2*, *LY6E*, *OAS1* and *OASL* (Fig. 2c).

Analysis was then performed for DEGs between cluster 4 and non-cluster 4 monocytes (ESM Table 4). Cluster 4 cells were more enriched in IFN-related signalling genes than those of other subsets [31, 33]; these genes included *IFI44L*, *EPSTI1*, *ISG15*, *IFIT2*, *IFIT3*, *IFI6*, *LY6E*, *XAF1* and *SIGLEC-1* (Fig. 2d). Additionally, several transcription factors were upregulated in cluster 4 monocytes, such as *ETV6*, *ETV7*, *IRF7*, *STAT1* and *STAT2* (Fig. 2e). GM12878 ChIP-seq data from ENCODE (<https://www.encodeproject.org/>) revealed that signal transducer and activator of transcription 1 (STAT1) binds to 35 of the 76 upregulated DEGs in cluster 4 monocytes, whereas ETV6 bound to one upregulated DEG. No data are available for ETV7, IRF7 and STAT2. A notable binding affinity of STAT1 to the promoter region of the *SIGLEC-1* gene was observed in GM12878 (Fig. 2f).

Additionally, the expression levels of genes encoding IFN-inducible chemokines were analysed. Cluster 4 cells had

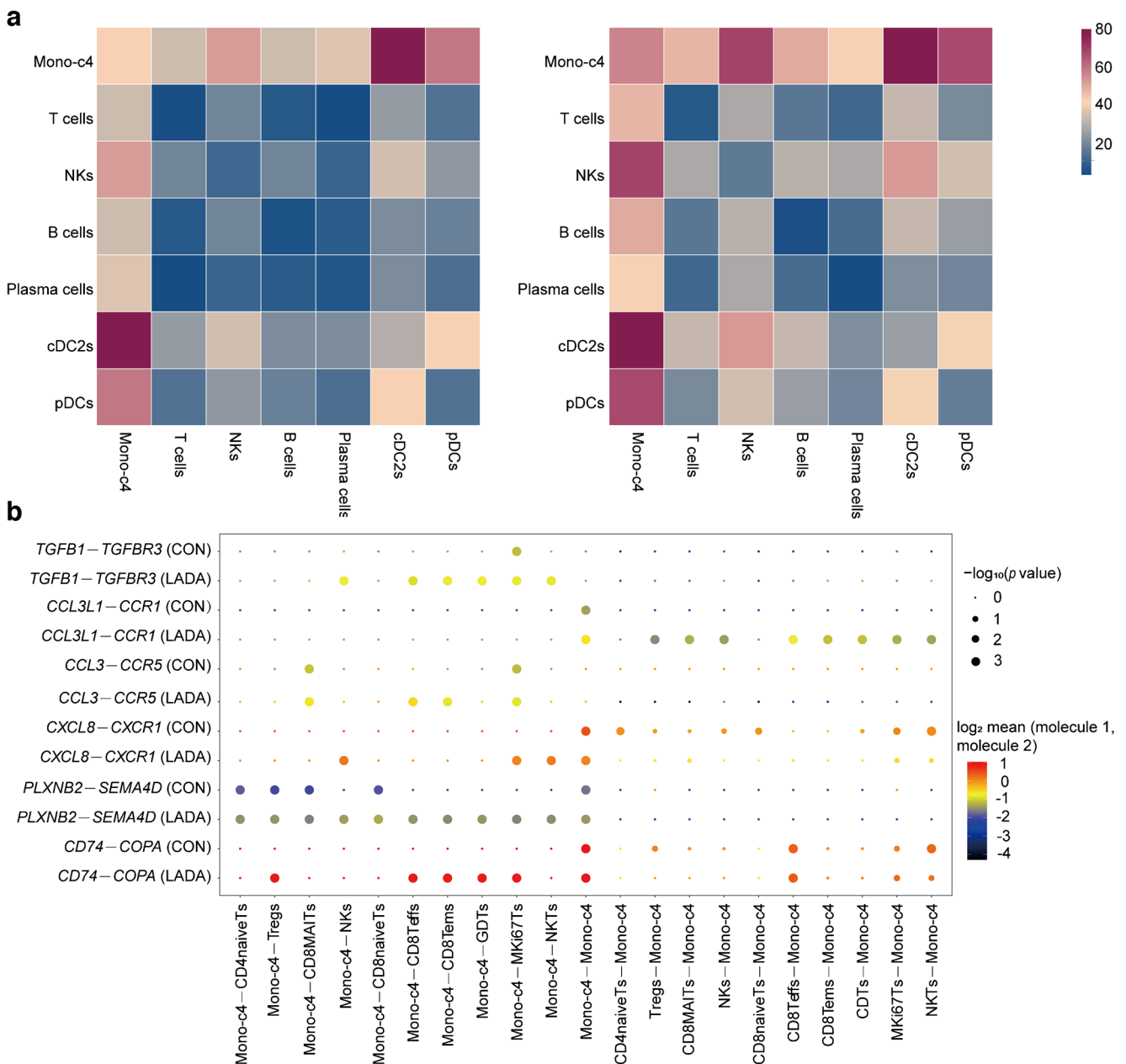


Fig. 5 Cell-to-cell communication between cell subsets. **(a)** Heatmap of the number of interacting ligands and receptors across various cell subsets; colour intensity of each cell corresponds to the count of

receptor–ligand interactions. **(b)** Significant ligand and receptor interactions between cluster 4 monocytes and T cells/NKs. Mono-c4, cluster 4 of monocytes

higher expression levels of *CXCL9*, *CXCL10*, *CXCL11*, *CCL2/MCP-1* and *CCL8/MCP-2* (Fig. 2g). High expression levels were also observed in cluster 4 cells for *CCR1* and *CCR2*, which encode proteins that play a role in myeloid infiltration into the pancreas (Fig. 2h) [34]. Cluster 4 monocytes showed substantially lower expression of genes associated with antigen presentation (*HLA-ABC* and *HLA-DR*), co-stimulation (*CD40*, *CD80*, *CD83* and *CD86*) and phagocytosis (*FCGR1A*, *FCGR1B*, *FCGR2A*, *FCGR3A*, *FCGR3B*, *FPR1*, *FPR2* and *SOD2*) than monocytes in clusters 5 and 8 (Fig. 2i,j).

Verifying SIGLEC-1 as a specific surface marker of cluster 4 monocytes

SIGLEC-1 (sialoadhesin, also known as CD169), a transmembrane receptor and an IFN signature [35, 36] is highly and exclusively expressed on cluster 4 monocytes (Fig. 3a). We therefore performed an FCM analysis to verify whether SIGLEC-1 can act as a specific surface marker to identify cluster 4 monocytes. Within the $CD45^+$ lineage-negative (Lin^-) cell population, we identified three

monocyte subsets: classical (CD14⁺⁺ CD16⁻), intermediate (CD14⁺ CD16⁺) and non-classical (CD14⁺ CD16⁺⁺) monocytes. SIGLEC-1 expression on classical monocytes was much higher than that on intermediate or non-classical monocytes (Fig. 3b). Further FCM analysis was performed to examine the expression of other IFN signalling-related proteins in SIGLEC-1⁺ and SIGLEC-1⁻ monocytes. The results showed co-expression of SIGLEC-1 with the intracellular protein myxovirus resistance 1 (MX1) (Fig. 3c), suggesting that SIGLEC-1 may be a convenient surface marker to represent cluster 4 monocytes.

Role of SIGLEC-1⁺ monocytes in islet inflammation and type 1 diabetes onset

We then explored the existence and proportions of SIGLEC-1⁺ monocytes/macrophages in the peripheral blood and pancreatic tissues of NOD and ICR mice. Monocytes were identified through their expression of α M-integrin (CD11b) and lymphocyte antigen 6 complex locus C (Ly6c; CD11b⁺ Ly6c⁺), while macrophages were identified by their expression of EGF-like module-containing mucin-like hormone receptor-like sequence 1 (F4/80), in addition to CD11b and Ly6c (F4/80⁺ CD11b⁺ Ly6c⁺). The mice were euthanised at 6, 12 and 24 weeks of age, their peripheral blood samples were collected, and the pancreatic tissues were harvested. Compared with 6-week-old ICR mice, a significantly larger proportion of SIGLEC-1⁺ cells (16.88%) was observed in the peripheral blood of 6-week-old NOD mice, but only a small proportion (1.78%) was detected in their pancreatic tissues. As NOD mice aged to 12 weeks, there was a significant decrease in the levels of circulating SIGLEC-1⁺ cells (from 16.88% to 10.17%, $p=0.0087$), coupled with a marked increase in SIGLEC-1⁺ cells infiltrating the pancreatic tissues (from 1.78% to 23.65%, $p=6.78 \times 10^{-9}$). By the age of 24 weeks, NOD mice exhibited substantially lower proportions of SIGLEC-1⁺ cells in both peripheral blood (reducing from 10.17% to 5.71%, $p=0.0012$) and pancreatic tissues (decreasing from 23.65% to 2.87%, $p=4.18 \times 10^{-8}$). However, this cell subpopulation was present at a negligible level in the peripheral blood and pancreatic tissues of the ICR mice at all ages (Fig. 4a,b).

To explore the role of SIGLEC-1⁺ monocytes in diabetes onset, we performed adoptive transfer experiments. SIGLEC-1⁺ and SIGLEC-1⁻ monocytes from 6-week-old NOD mice were transferred to NOD and ICR mice of the same age ($n=8$ for each group). After 8 weeks, NOD mice receiving SIGLEC-1⁺ monocytes had significantly more islets with severe insulinitis, characterised by an insulinitis score of G3 [29, 30], compared with NOD mice that received SIGLEC-1⁻ monocytes or physiological saline (0.9% NaCl) (Fig. 4c,d). The SIGLEC-1⁺ group also showed accelerated diabetes onset over the 8-week

observation period. Specifically, within the SIGLEC-1⁺ group, three of eight mice developed diabetes, but none in the SIGLEC-1⁻ group experienced diabetes during this observation period. In contrast, ICR mice remained free of insulinitis or diabetes (Fig. 4e,f). We also used a C chemokine receptor 2 (CCR2) antagonist [37] to inhibit classical monocyte recruitment in 6-week-old NOD mice. While this reduced the number of islets with insulinitis scores of G2 and G3 [29, 30], no distinct differences in diabetes incidence emerged within the 8-week observation period (ESM Fig. 9). We meticulously monitored each experimental group for adverse events throughout the study. Importantly, no mice in the intervention group died, underscoring the safety of the experimental protocol.

Interaction between cluster 4 monocytes and other cells in LADA patients

To determine the mechanisms of initiation of islet inflammation by cluster 4 monocytes in LADA patients, we examined their potential interactions with various cell types. Cluster 4 monocytes exhibited stronger interactions with T cells and NKs in participants with LADA than in control participants (Fig. 5a). Further ligand–receptor analysis revealed potential interactions involving CD8⁺ T cells, NKTs and NKs in participants with LADA through various axes, including *TGFB1–TGFB3*, *CD74–COPA*, *CCL3–CCR5* and *CXCL8–CXCR1*. Cluster 4 monocytes engaged with all T/NK cell subsets, including Tregs and CD4⁺ naive T cells, through the plexin B2 (PLXNB2)–semaphorin 4D (Sema4D) interaction (Fig. 5b). In addition, the majority of T cell subsets and NKs communicated with cluster 4 monocytes via the *CCL3L1–CCR1* signalling axis in participants with LADA, but not in control participants. These results demonstrate extensive interactions of cluster 4 monocytes with T cells and NKs, which are known to be crucial for the destruction of islet beta cells in individuals with LADA.

Elevated SIGLEC-1⁺ monocyte levels as a potential biomarker of type 1 diabetes onset

We performed FCM analysis of CD45⁺ Lin⁻ CD14⁺⁺ SIGLEC-1⁺ monocytes in participants with various types of diabetes. The clinical variables of the recruited participants and the matched individuals are shown in ESM Table 5. Our analysis revealed a median fraction of SIGLEC-1⁺ monocytes of 5.79% in the peripheral blood of individuals with LADA, which was significantly different from the mean fractions of 1.76% observed in those with type 2 diabetes and 2.04% in healthy control individuals. In order to ensure group homogeneity, we paired 26 participants with LADA with healthy control participants and matched 17

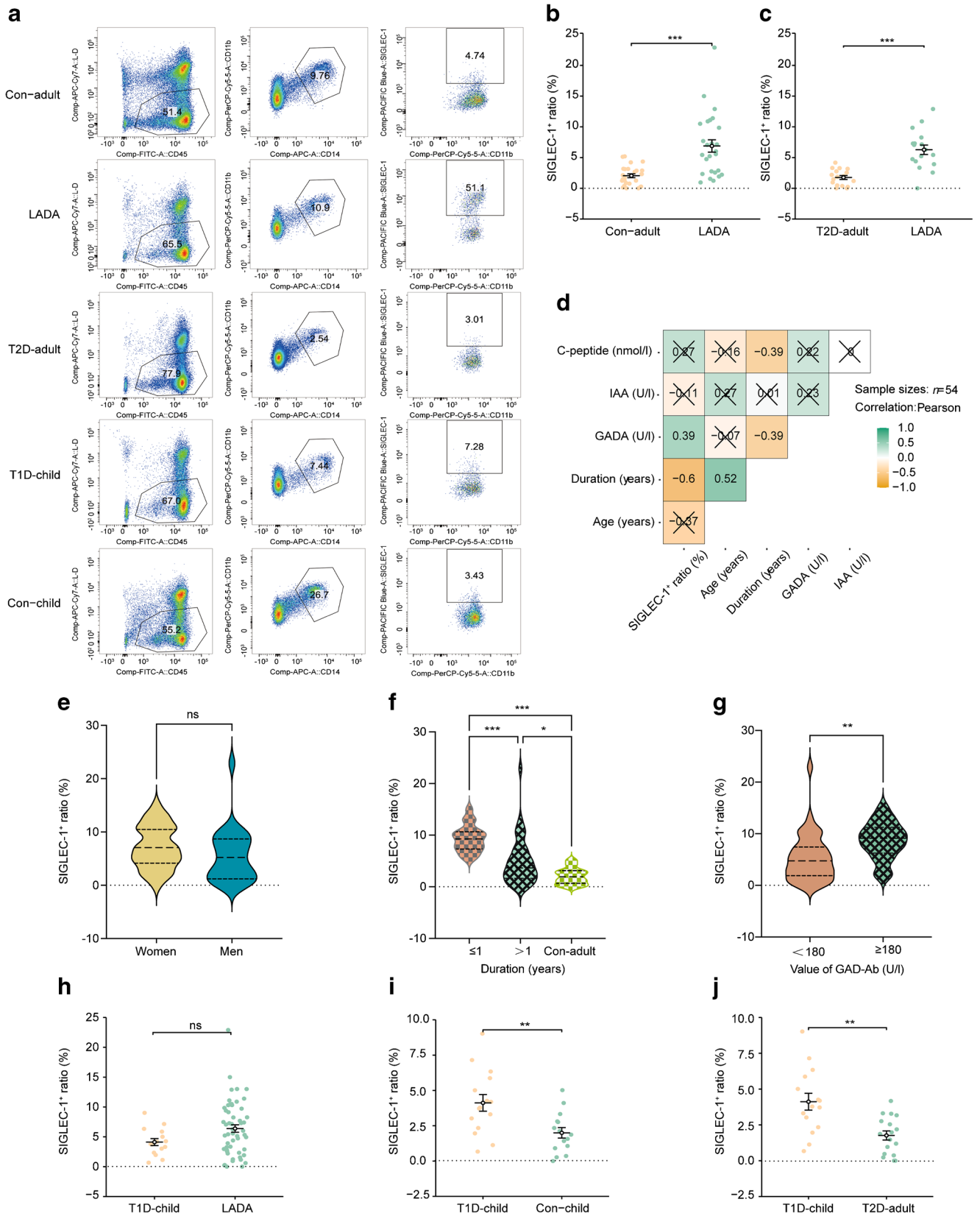


Fig. 6 FCM analysis of SIGLEC-1⁺ monocytes in individuals with various types of diabetes. **(a)** FCM analysis of the SIGLEC-1⁺ cell population in CD14⁺ CD11b⁺ monocytes among various groups, including LADA patients, type 2 diabetes (T2D-adult) patients and healthy adults (Con-adult), as well as paediatric type 1 diabetes (T1D-child) patients and healthy children (Con-child). Comp-APC-Cy7-A::L-D, the compensated area signal of APC-Cy7 fluorescence, is specifically used for detecting the Live/Dead (L-D) dye, a marker for assessing cell viability; Comp-FITC-A::CD45, the compensated FITC area signal for CD45 marker detection; Comp-PerCP-Cy5.5-A::CD11b, the compensated area signal of the peridinin–chlorophyll–protein complex–cyanine5.5 (PerCP-Cy5.5) fluorescent dye, specifically for detecting the CD11b marker; Comp-APC-A::CD14, the compensated area signal of APC fluorescence, specifically detecting the CD14 marker; Comp-Pacific Blue-A::SIGLEC-1, the compensated area signal of Pacific Blue dye for detecting SIGLEC-1. **(b, c)** Comparison of the SIGLEC-1⁺ ratio between healthy adults (Con-adult) and matched individuals with LADA ($n=26$ for each group), as well as type 2 diabetes (T2D-adult) patients and matched LADA patients ($n=17$ for each group). **(d)** Spearman correlation analysis showing the relationship between the SIGLEC-1⁺ ratio and variables such as age, disease duration, GADA, IAA and C-peptide levels within participants with LADA ($n=54$). A cross indicates non-significant at $p<0.05$. **(e)** Comparison of the SIGLEC-1⁺ ratio among men and women within the 54 participants with LADA. **(f)** Comparison of the SIGLEC-1⁺ ratio between the 54 participants with LADA with different disease durations (≤ 1 year and > 1 year) and healthy adults (Con-adult). **(g)** Comparison of the SIGLEC-1⁺ ratio among the 54 LADA participants with different GAD-Ab levels (< 180 IU/ml and > 180 IU/ml). **(h–j)** Comparison of the SIGLEC-1⁺ ratio between paediatric type 1 diabetes (T1D-child) patients ($n=15$), healthy children (Con-child) ($n=15$), LADA patients ($n=54$) and adults with type 2 diabetes (T2D-adult) ($n=17$). Student's t tests for normal variances, Welch's t tests for unequal variances, and Wilcoxon rank-sum tests for non-normal distributions. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

participants with LADA participants with 17 with type 2 diabetes. The results unequivocally indicated that the SIGLEC-1⁺ monocyte ratio in the LADA group was notably higher in comparison with both the healthy control group ($p=4.13 \times 10^{-5}$) and the type 2 diabetes group ($p=2.11 \times 10^{-5}$) (Fig. 6a–c).

Among the 54 individuals with LADA, we found no statistically significant correlation between SIGLEC-1⁺ ratio and age (Fig. 6d). However, there was a positive correlation between SIGLEC-1⁺ ratio and disease activity, as indicated by GAD65 antibody (GADA) levels (Pearson's $r=-0.6$). SIGLEC-1⁺ ratio and disease duration showed a less substantial correlation ($r=0.39$). There was no significant difference in SIGLEC-1⁺ ratio between male and female participants with LADA (Fig. 6e).

Notably, LADA patients with a disease duration of 1 year or less exhibited a significantly higher SIGLEC-1⁺ ratio than those with a longer disease duration (Fig. 6f). Additionally, LADA patients with elevated GADA levels (≥ 180 IU/ml) had a significantly higher SIGLEC-1⁺ ratio (Fig. 6g).

In our parallel paediatric cohort of 15 individuals with type 1 diabetes and 15 healthy control individuals, we found that the SIGLEC-1⁺ ratio in the paediatric type 1 diabetes group was notably higher, with a mean fraction of 4.12%, compared with 2.0% in the paediatric control group and 1.76% in the adult-onset type 2 diabetes group ($p=0.005$ and 0.001 , respectively). Furthermore, the SIGLEC-1⁺ ratio observed in the paediatric type 1 diabetes group (4.12%) was not significantly different from that in the adult LADA group (5.79%, $p=0.0874$), indicating a consistent pattern of SIGLEC-1⁺ expression across type 1 diabetes patient populations (Fig. 6h–j).

Discussion

This study revealed several novel findings concerning immune cells and type 1 diabetes onset, as depicted in Fig. 7. First, the proportion of SIGLEC-1-expressing classical monocytes (CD45⁺ Lin⁻ CD14⁺ SIGLEC-1⁺) increased significantly in individuals with early-stage type 1 diabetes, with a strong IFN signature; second, the SIGLEC-1⁺ monocytes expressed high levels of genes encoding chemoattractants for T cells and NKs, and relatively low levels of genes for co-stimulatory and HLA molecules; third, the SIGLEC-1⁺ ratio positively and negatively correlates with disease activity and duration, respectively; and finally, SIGLEC-1⁺ monocytes may be recruited to the islets, which is crucial in inducing destructive insulinitis and onset of type 1 diabetes.

There are some limitations to this study. First, the cell adoptive transfer assay results are based on a small sample size (eight animals per group), indicating the need for more extensive studies to confirm the role of these cells in autoimmune diabetes. Second, the research did not specifically eliminate SIGLEC-1⁺ monocytes in NOD mice, limiting direct assessment of their role in insulinitis and type 1 diabetes development. Third, the study also excluded adults with paediatric-onset type 1 diabetes, a critical group for understanding the disease's early stages in adulthood. Given the rapid progression of type 1 diabetes after onset, capturing the initial stage of such diabetes in adulthood poses challenges. Future research should explore this population for a more comprehensive understanding of the role of SIGLEC-1 in the development of type 1 diabetes. Additionally, while the study found no significant gender differences in SIGLEC-1⁺ ratios in LADA patients, it did not thoroughly investigate gender-specific factors, which should be a focus in future research to understand sex/gender-related variations in type 1 diabetes.

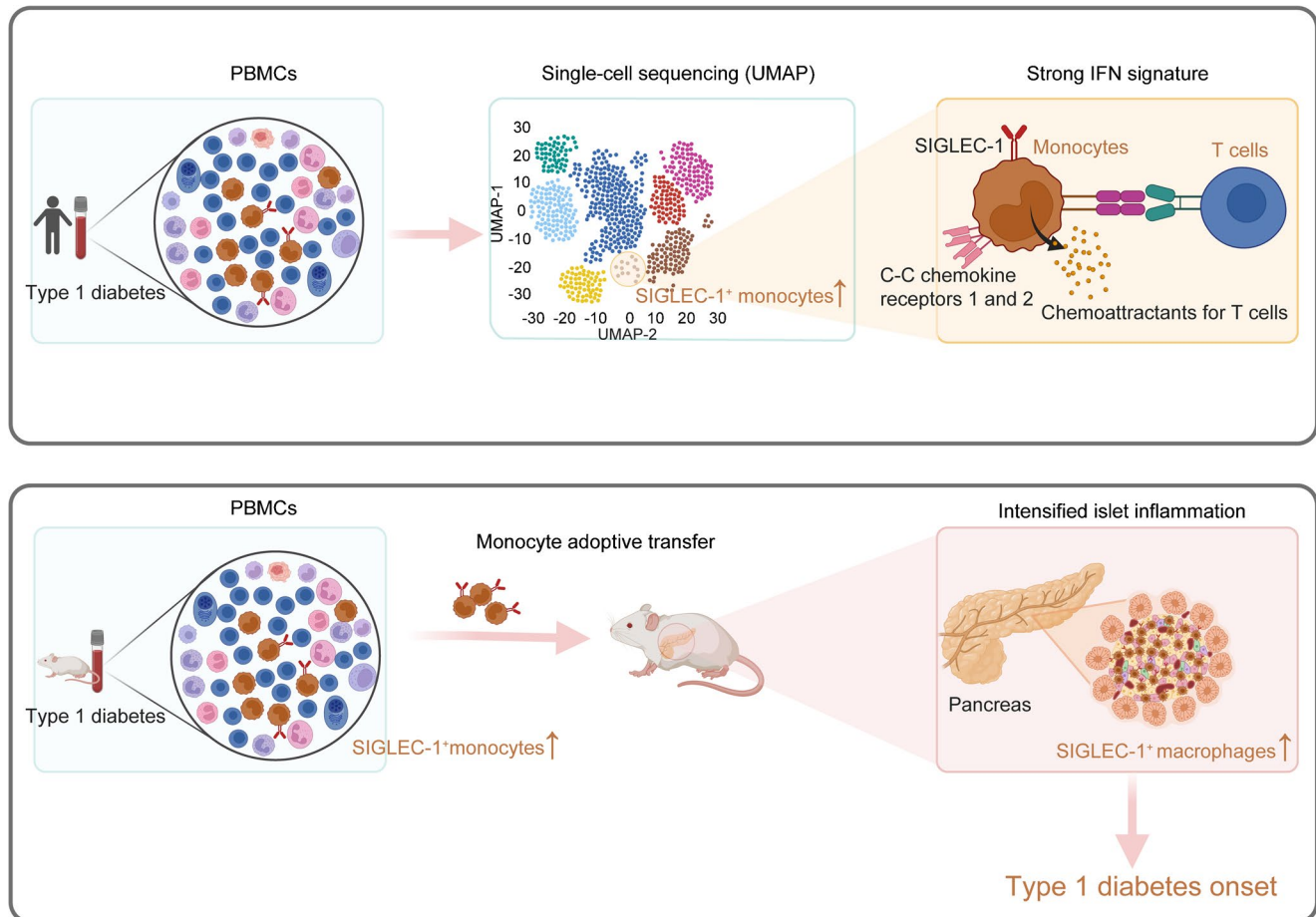


Fig. 7 In individuals with early-stage type 1 diabetes, a significant increase in classical monocytes expressing SIGLEC-1 is observed, accompanied by a robust IFN signature. These SIGLEC-1⁺ monocytes express elevated levels of chemoattractants for T cells, engaging in strong interactions with them. Furthermore, these SIGLEC-1⁺ monocytes demonstrate heightened expression of C-C chemokine receptors 1 and 2, which are known for their role in myeloid infiltra-

tion into the pancreas. In NOD mice, the proportion of SIGLEC-1⁺ monocytes/macrophages in peripheral blood and pancreatic tissues is greater than that in ICR mice. Adoptive transfer of SIGLEC-1⁺ monocytes into NOD mice significantly accelerates diabetes onset, underscoring the pivotal role of recruitment of SIGLEC-1⁺ monocytes to the islets in initiation of destructive insulinitis and the onset of type 1 diabetes

Our study focuses on SIGLEC-1, an IFN-inducible protein and monocyte-specific receptor playing a key role in lymphocyte interactions. [38, 39]. Elevated SIGLEC-1 expression is noted in the early stages of various viral infections [35, 36, 40] and autoimmune diseases [41, 42], making it a dependable marker for IFN hyper-responsiveness. Ferreira et al performed an insightful study that showed transient upregulation of IFN-stimulated genes in preclinical diabetes, as indicated by increased *SIGLEC-1* on CD14⁺ monocytes, but did not specifically identify the SIGLEC-1⁺ CD14⁺ cell subpopulation [11]. Our research, however, successfully isolated and characterised these SIGLEC-1⁺ monocytes in individuals with type 1 diabetes, identifying them as a distinct cluster of classical monocytes.

Affandi et al recently performed a study that showed an increase in SIGLEC-1-expressing CD14⁺ monocytes in

COVID-19 patients, notably within the intermediate CD14⁺ CD16⁺ subset, which exhibited elevated co-stimulatory and HLA molecule expressions compared with our study's findings [39]. Furthermore, Affandi et al demonstrated that treatment with IFN α significantly upregulated SIGLEC-1 expression on CD14⁺ monocytes isolated from COVID-19 patients, enhancing their CD8⁺ T cell-activating capacity. It is noteworthy, however, that SIGLEC-1⁺ monocytes in our study displayed lower expression levels of genes associated with antigen presentation and co-stimulation in comparison with other monocyte subsets. These findings suggest the existence of a distinct cell population that potentially functions through alternative pathways, rather than relying on co-stimulatory and HLA molecules.

In relation to our RNA-seq data, we observed that cluster 4 monocytes exhibit the capacity to engage with all subsets

of T cells and NKs, including Tregs and CD4⁺ naive T cells, through the PLXNB2–Sema4D interaction. Sema4D has previously been reported to play a pivotal role in fostering CD4⁺ T cell proliferation and promoting Th17 cell differentiation, while concurrently suppressing differentiation of Tregs [43, 44]. Interestingly, when comparing the T cell subsets between the LADA and control groups in our study, we did not observe any statistically significant variations. This outcome differs from the findings presented by Tan et al [45]. One plausible explanation for this discrepancy may be the short disease duration observed in the participants with LADA included in our study. It is possible that a more extended timeframe is necessary to detect alterations in T cell subsets within the context of this specific autoimmune condition.

We performed further studies to investigate the role of IFNs and their signalling pathways. IFNs signal by activating Janus protein tyrosine kinases associated with IFN receptors, leading to a cascade of tyrosine phosphorylation and activation of STAT transcription factors [46]. In cluster 4 monocytes, STAT1 emerged as the dominant transcription factor, binding to the *SIGLEC-1* gene promoter. Interestingly, a previous study linked a heterozygous mutation in *STAT1* with the development of type 1 diabetes and suggested that Janus kinase inhibitors could be effective in resolving type 1 diabetes in patients with *STAT1* gain of function [47]. However, the mechanism by which STAT1 causes type 1 diabetes remains unclear.

In conclusion, our study has revealed a novel monocyte subpopulation characterised by a robust IFN signature marked by SIGLEC-1 in the early stages of type 1 diabetes. We have also demonstrated the critical function of SIGLEC-1⁺ monocytes and the underlying molecular mechanism involved in islet destruction and type 1 diabetes onset. These findings have the potential to inform future preventive and therapeutic strategies for type 1 diabetes.

Supplementary Information The online version contains peer-reviewed but unedited supplementary material available at <https://doi.org/10.1007/s00125-024-06098-4>.

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Data availability RNA-seq data have been deposited in the GSA human database (<https://ngdc.cncb.ac.cn/gsa-human/>) under accession number HRA003649.

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Authors' relationships and activities MQG, FW (Fei Wang), YJD, FW (Fang Wang), LLX, YC, RL, YGW and SFL are current employees at the Affiliated Hospital of Qingdao University, China. The other authors

declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

Contribution statement SFL, YGW and JZ were responsible for study conception and design. JNC, CW, YJD, FW (Fei Wang), FW (Fang Wang), LZ, HG, JJZ, RL and SKL were responsible for data acquisition and analysis. MQG, LLX and YC performed the data interpretation. MQG, LLX, YC and FW (Fei Wang) drafted the article. SFL, YGW, JZ, JNC, CW, YJD, FW (Fang Wang), LZ, HG, JJZ, RL and SKL critically reviewed the article. All authors approved the final version of this manuscript to be published. SFL is responsible for the integrity of the work as a whole.

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