Differences in immune cell profiles around the time of islet autoimmunity seroconversion in children with and without type 1 diabetes

Immune cell profile changes in islet autoimmunity

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Abstract

Seroconversion (SV) marks the initiation of islet autoimmunity (IA) and pre-clinical phase of type 1 diabetes (T1D), yet the contributions of immune cells beyond cytotoxic T cells remain unclear. We applied high-resolution immune cell-type deconvolution using peripheral blood DNA methylation data from nested case-control samples of the Diabetes Autoimmunity Study in the Young (DAISY; n=151) and The Environmental Determinants of Diabetes in the Young (TEDDY; n=166) to estimate immune cell proportions before SV (pre-SV) and after SV (SV) and construct functional ratios, such as the neutrophil-to-lymphocyte ratio (NLR). Using linear models, we evaluated differences between T1D cases and controls at pre-SV, SV, and the change across timepoints. Pre-SV, T1D cases had higher NLR and lower CD4T/CD8T cell ratios. At SV, the combined B-CD4T-CD8T memory/naïve ratio was reduced in cases. From pre-SV to SV, T1D cases also showed attenuated increases in NLR, B-memory/naïve, and B–CD4T–CD8T memory/naïve ratios. These patterns may reflect delayed or disrupted immune maturation, with persistence of naïve cells or impaired transition to memory subsets following antigen exposure. Our findings suggest that changes in innate and adaptive immune dynamics emerge early in the disease process, and that peripheral immune cell ratios may serve as informative biomarkers for risk stratification and mechanistic insight for development of T1D.

Article Highlights

1. Why did we undertake this study?
    a. We undertook this study to examine immune cell profiles around a critical time window of IA seroconversion for children at an increased risk of developing T1D.
2. What is the specific question(s) we wanted to answer?
    a. We wanted to answer questions regarding immune cell ratio differences between T1D cases and controls in three specific instances: i) pre-SV, ii) SV, and iii) the change pre-SV to SV.
3. What did we find?

    a. We found differences in immune cell ratios between T1D cases and controls before, during, and between IA periods, which have potential etiological and pathophysiological implications.
4. What are the implications of our findings?
    a. Our findings highlight the complexity of immunodynamics around the time of IA seroconversion and the potential for immune cell ratios to aid in T1D risk stratification and intervention.

Introduction

Type 1 diabetes (T1D) remains one of the most common chronic autoimmune diseases affecting children globally[1]. It is characterized by immune-mediated destruction of insulin-producing β-cells in the pancreas, ultimately leading to hyperglycemia, ketoacidosis, and a lifelong dependency on exogenous insulin therapy [1]. Current global estimates point to increasing incidence rates, especially among children and adolescents, with estimated average annual increases of approximately 3-4% over the last three decades, highlighting a significant public health burden [2-4]. Prior to developing T1D, islet autoimmunity (IA) occurs, defined by the presence of autoantibodies targeting pancreatic islet cells, such as insulin (IAA), glutamic acid decarboxylase (GADA), insulinoma antigen-2 (IA-2A), and zinc transporter 8 (ZnT8A). IA signifies early faltering in immune tolerance and marks the beginning of a variable progression towards T1D, although some people do not progress to disease [5, 6]. Despite established associations between IA and T1D, gaps remain in our understanding of the underlying immune processes.

Immune cell dynamics that occur around the time of IA seroconversion and during progression from IA to T1D have not been fully elucidated. Recent advances in immunophenotyping have emphasized the utility of peripheral blood-based immune cell ratios – such as neutrophil-to-lymphocyte (NLR), CD4T/CD8T ratio, and memory/naïve lymphocyte ratios – as potential biomarkers of systemic inflammation, immune

activation, and immune maturation status [7, 8]. Specifically, the NLR indicates systemic inflammation and adaptive immunity, the CD4T/CD8T ratio reflects immune senescence and activation, and the B and T-cell mem/nv ratios reveal levels of balance between the cells of the adaptive immune system [7-10]. From these ratios, we can glean insight into the balance between immune cell subsets rather than focusing on absolute or relative abundance alone, which can help to characterize underlying immune dynamics, such as shifts between innate and adaptive immune responses or naïve and antigen-experienced states. Differential ratios of immune cell populations, therefore, can reflect underlying disease processes and may predict onset or worsening of disease. Ratios, rather than percentages, offer modeling options that address the inherent compositional nature of cell proportion data (i.e., the sum-to-one constraint, whereby a change in one cell proportions affects all other proportions), thereby capturing biologically meaningful contrasts while avoiding potential spurious associations driven by interdependence among components [11, 12]. However, few studies have longitudinally characterized these immune parameters in the context of IA and subsequent progression to T1D among pediatric populations.

This study aims to bridge these knowledge gaps by evaluating immune cell type heterogeneity and dynamics during critical time windows before and after IA. Leveraging two well-established prospective cohorts — the Diabetes Auto Immunity Study in the Young (DAISY) and The Environmental Determinants of Diabetes in the Young (TEDDY) — we estimated immune cell ratios derived from DNA methylation (DNAm) in peripheral blood to better understand the immunological shifts occurring in children genetically susceptible for T1D during a critical stage of T1D pathogenesis.

Research Design and Methods

DAISY study population

The prospective DAISY cohort recruited and followed 2,547 children based around Denver, Colorado. They were recruited either as part of population-based newborn screening at St. Joseph's Hospital for

specific human leukocyte antigen (HLA) genotypes, known to have increased genetic risk of T1D, or from first-degree relatives of T1D patients, then followed for the development of IA and T1D. Additional information pertaining to the study design and follow-up is detailed elsewhere [13]. Clinic visits with blood sample collection occurred at age 9, 15, and 24 months, then annually thereafter until the development of IA. An accelerated visit schedule for participants who developed IA included visits every 3-6 months until physician diagnosed T1D, based on American Diabetes Association (ADA) clinical guidelines. Most participants identified as Non-Hispanic White among the race-ethnicity categories. The study protocol was approved by the Colorado Multiple Institutional Review Board (COMIRB) and adhered to all relevant research regulations. Informed consent was obtained by the parents or primary caretakers of all participating children, and assent was obtained from children seven years of age and older.

A nested case-control subset of DAISY study participants was selected to evaluate epigenetics – specifically, DNAm – and other omics measurements in the context of IA and T1D. Frequency matching occurred relative to T1D cases based on age at IA seroconversion, race-ethnicity, and sample availability across five timepoints throughout the progression of disease – birth, infancy (age 9-16 months), pre-IA, onset of IA, and prior to diagnosis of T1D. This analysis included T1D cases and controls with good quality DNAm data available pre-IA, at IA onset, or both (n = 151).

One of the main outcomes in the DAISY study was IA, where autoantibodies –IAA, GADA, and IA-2A – were measured in blood samples by radioimmunoassay performed at the Barbara Davis Center for Diabetes, currently within the University of Colorado Anschutz Medical Campus (Aurora, CO, USA), and has been described previously [14]. An additional test was performed later for ZnT8A on all samples from children who tested positive for the previous autoantibodies or who developed T1D [15]. We defined IA as the persistent presence of one or more autoantibodies (noted above) detected across two consecutive

visits, or when the participant tested positive for at least one autoantibody at one visit and was then diagnosed with T1D at the next visit, per previous work [16].

TEDDY study population

TEDDY represents a multi-national prospective cohort study of children followed from birth with an increased genetic risk of T1D, based on their HLA genotype, and included 8,676 participants that were followed through the development of IA until T1D diagnosis. More specific study design and follow-up details are described elsewhere [17, 18]. Recruitment occurred across six clinical research centers: three in the USA (Colorado, Georgia/Florida, and Washington) and three in central and northern Europe (Germany, Finland, and Sweden). Visits were conducted every three months until age 4, then every six months until age 15. Similar to DAISY, an accelerated schedule was used after the development of IA, which included visits every three months until age 15 years or diagnosis of T1D based on ADA clinical criteria. The study protocol was approved by local Institutional Review Boards for each of the six clinical centers and adhered to all relevant research regulations. An external review committee formed by the National Institutes of Health in Bethesda, Maryland monitored the study. Informed consent was obtained by the parents or primary caretakers of all participating children, and assent was obtained from children seven years of age and older.

From the TEDDY cohort, a T1D risk-set based on a nested case-control cohort with DNAm data was selected. T1D cases were matched 1:1 with controls based on age, sex, T1D family history, and clinical center. Study participants are primarily composed of persons identifying as Non-Hispanic White, and a majority were recruited from the European clinical centers. We included T1D cases where age at developing IA was known and who had good quality DNAm data available pre-IA, onset of IA, or both, along with samples from matched controls (n = 166).

For the IA outcome in TEDDY, blood samples were analyzed via radioimmunoassay performed at two different laboratories: one at the Barbara Davis Center for Diabetes in Colorado and another at the University of Bristol in Bristol, UK. All autoantibody positive and 5% of negative samples were reanalyzed in the other laboratory, where concordant results confirmed positivity. Similar to DAISY, the TEDDY study defined IA as testing positive for one of IAA, GADA, or IA-2A across two consecutive visits [19].

DNA methylation

DNAm measures were obtained from whole blood samples on the Infinium Methylation450 (450K) and MethylationEPIC (EPIC) BeadChip arrays (Illumina, San Diego, CA, USA), which interrogate over 450,000 and 850,000 methylation sites, respectively, across the genome at single-nucleotide resolution. The DAISY participants had samples from both 450K and EPIC arrays while TEDDY participants only included samples from the EPIC array. Samples of low quality or with discordant values for sex were removed. Additionally, we filtered each DAISY participant down to a single microarray platform for consistency, since some of the participants had samples on both platforms. We prioritized EPIC over 450K arrays to increase resolution where possible.

Cell deconvolution

We performed cell deconvolution using the estimateCellCounts2 function within the FlowSorted.Blood.EPIC package and the cell references from the FlowSorted.BloodExtended.EPIC package in R, utilizing the recommended parameters for CpG selection and normalization [20, 21]. The latter package included an expanded reference for 12 immune cell proportions: neutrophils (Neu), eosinophils (Eos), basophils (Baso), monocytes (Mono), naïve and memory B cells (Bnv, Bmem), naïve and memory CD4+ and CD8+ T cells (CD4Tnv, CD4Tmem, CD8Tnv, CD8Tmem), natural killer (NK), and T regulatory (Treg) cells. If any individuals had zero values in their cell proportions, we imputed them via substitution by taking the minimum value within each cell type then dividing it by two (i.e.,

minimum value/2) [22]. We also used previously published immune cell references to gauge whether our participants were within expected ranges for certain subsets [23]. Compared to published immune cell references, TEDDY participants had slightly lower immune cell proportions, although they overlapped between the 10th and 90th percentiles. **Supplemental Table 1** provides a snapshot of several immune cell subsets (B, CD4T, CD8T, and NK) for TEDDY samples collected between 1-2 years of age, which includes the average age before and during seroconversion.

Study timepoints

Though the DAISY and TEDDY studies collected multiple samples prospectively as part of their designs, for this analysis, we selected one sample per subject for each of the timepoints of interest: the time before IA (pre-SV) and at the time of IA seroconversion (SV) where participants had converted from seronegative to seropositive with the detection of islet cell autoantibodies. The pre-SV timepoints in DAISY and TEDDY included 84 and 122 samples, respectively. The SV timepoints in DAISY and TEDDY included 140 and 160 samples, respectively.

Statistical approach

We tested whether immune cell ratios differed between T1D cases and controls at pre-SV, SV, or changed differently from pre-SV to SV using linear modeling of immune cell ratios as the outcome and an interaction between T1D status and time as the primary covariates of interest. For the outcomes, we used log-transformed immune cell ratios, which included the following: i) neutrophil/lymphocyte (NLR), ii) monocyte/lymphocyte (MLR), iii) B-memory/B-naïve (B-mem/nv), iv) CD4T-memory/CD4T-naïve (CD4T-mem/nv), v) CD8T-memory/CD8T-naïve (CD8T-mem/nv), vi) CD4T/CD8T, and vii) B-CD4T-CD8T-memory/B-CD4T-CD8T-naïve (B-CD4T-CD8T-mem/nv). Lymphocytes consisted of CD4+ and CD8+ T cells, B memory and naïve cells, natural killer (NK) cells, and T regulatory cells.

T1D status (case vs. control) served as our primary explanatory variable, since we were interested in immune cell ratio differences between these groups. A covariate for time was included to distinguish between the two different seroconversion measurements (pre-SV vs. SV). An interaction term between T1D status and time was included to consider how the immune cell ratios varied by T1D status across the two different sample collection timepoints. In DAISY, additional covariates for age, DR3/4 HLA status, and platform were included to either adjust for confounding or increase precision in the models. For TEDDY, DR3/4 HLA status was included as a fixed effect while the case-control index variable (comprised of age, sex, T1D family history, and clinical center) was included as a random intercept to account for the matching strata. In this case, T1D family history was captured as a dichotomous variable (yes vs. no). For reporting, each immune cell ratio was exponentiated to return to the original scale, yielding the immune cell proportion ratio between T1D cases and controls, and presented as percentage differences. The lm base function in R and the lmer function from the lme4 package were used to construct the linear and linear mixed-effects models, respectively [24]. We used an alpha level of 0.05 to determine statistical significance across all analyses.

We estimated the effects of interest separately by study. Then, given the similarities in study design (eligibility, follow-up, IA and T1D estimation), we combined them with inverse variance-weighted, fixed effects meta-analysis using the meta package in R [25]. We evaluated heterogeneity based on Cochran's Q and I^2, following through with the meta-analysis in all instances of low estimated variability between study effects. To evaluate the robustness of our findings, we performed two sensitivity analyses – one that excluded samples with imputed cell proportion values and another which assessed differences by T1D family history. A combination of *a priori* knowledge, Akaike information criterion (AIC), and consistency of effect size and direction were examined to determine the most appropriate models.

Data and Resource Availability

The data used for analysis in this study are available from the authors upon reasonable request. Cohort specific data from DAISY and TEDDY are not publicly available due to privacy concerns. All analyses were performed using R Statistical Software 4.4.0 (R Core Team, 2024) [26]. This research was enabled, in part, by the use of the FlowSorted.BloodExtended.EPIC software package developed at Dartmouth College, which is provided under a free academic license as specified here: https://github.com/immunomethylomics/FlowSorted.BloodExtended.EPIC/blob/main/SoftwareLicense.FlowSorted.BloodExtended.EPIC%20to%20sign.pdf. The software is subject to the licensing terms made available by Dartmouth Technology Transfer and is provided "AS IS" without any warranties, express or implied.

Results

The participant characteristics for both the DAISY and TEDDY cohorts are summarized in **Table 1**, along with the sample characteristics in **Table 2**. Participants are distinguished based on their T1D case or control status, while the samples are also categorized across the pre-SV and SV timepoints. In DAISY, a total of 151 children were included in the analysis, where 76 developed T1D. Values for the frequency-matched variables (age at seroconversion, race/ethnicity, and sample availability) remained similar between groups. T1D cases had a higher proportion of first-degree relatives with T1D as well as DR3/4 HLA genotypes compared to controls. Across the 224 participant samples in the DAISY analysis, there were 111 samples from T1D cases and 113 from controls. These consisted of 84 and 140 samples for the pre-SV and SV time points, respectively, as noted in **Table 2**. In TEDDY, a total of 166 children were included for analysis with 83 developing T1D. The matching variables (age, sex, T1D family history, and clinical center) were closely aligned between groups. T1D cases had a higher proportion of DR3/4 HLA genotypes compared to controls. A total of 282 participant samples were included in the analysis, comprised of 141 samples from T1D cases and 141 from matched controls without T1D. These were composed of 122 and 160 samples for the pre-SV and SV time points, respectively, per **Table 2**. The

average age at which DAISY cases developed T1D was higher than TEDDY at 9.48 years compared to 2.86 years, respectively.

The results from the inverse variance-weighted fixed effects meta-analysis are depicted in **Figure 1** and summarized below. **Figure 1** captures the consistency between the effects (immune cell ratios) of the DAISY and TEDDY studies, as well as the combined effect. This approach allowed us to detect the B-mem/nv and B-CD4T-CD8T-mem/nv ratios that we might have missed if we had only considered the studies independently. In the pre-SV period, the NLR was 15% higher (Ratio: 1.15, 95% CI: 1.00, 1.33; p = 0.0442) while the CD4T/CD8T was 9% lower (Ratio: 0.91, 95% CI: 0.83, 1.00; p = 0.0448) in T1D cases compared to controls after adjusting for covariates. The remaining immune cell ratios were null for the pre-SV timepoint. In the SV period, the B-CD4T-CD8T-mem/nv ratio was estimated to be 26% lower (Ratio: 0.74, 95% CI: 0.57, 0.98; p = 0.0325) among T1D cases compared to controls, after adjustments. The other immune cell ratios did not yield statistically significant effects. Results for the pre-SV to SV change are displayed across **Figure 1** and expanded upon in **Figure 2**. For the pre-SV to SV change analysis, we found that the B-mem/nv, B-CD4T-CD8T-mem/nv, and NLR ratio trajectories were reduced by 35% (Ratio: 0.65, 95% CI: 0.43, 0.96; p = 0.0324), 38% (Ratio: 0.62, 95% CI: 0.41, 0.95; p = 0.0273), and 21% (Ratio: 0.79, 95% CI: 0.66, 0.95; p = 0.0119), respectively, among T1D cases compared to controls after adjusting for covariates. **Figure 2** reveals that the T1D cases started with higher immune cell ratios for the B-mem/nv, B-CD4T-CD8T-mem/nv, and NLR, but the controls had more pronounced increases at the time of seroconversion. No other significant associations were found for the additional immune cell ratios during the pre-SV to SV change. Results were robust to sensitivity analyses, and we did not observe immune cell ratio differences by T1D family history, though, we were likely underpowered for the three-way interaction.

Discussion

Among children who developed T1D, we identified early and dynamic changes in immune cell ratios compared to controls. Some of these included evidence of increased innate immune activation, altered T cell balance, and delayed adaptive immune maturation during the period surrounding IA seroconversion. Specifically, we observed elevated NLR and reduced CD4T/CD8T cell ratios prior to seroconversion, followed by lower B-CD4T-CD8T-mem/nv ratios during seroconversion and attenuated increases in the NLR, B-mem/nv, and B-CD4T-CD8T-mem/nv ratios over time. Since these immune cell ratios were estimated around the time of seroconversion to IA, they may provide insights into the etiology and pathophysiology of the T1D disease process.

We found that T1D cases started with higher NLR pre-SV, but they did not change as much pre-SV to SV as did T1D controls. The NLR is considered a biomarker of systemic inflammation or activation of the innate immune response, and has previously been utilized as such for other health outcomes related to cancer, infections, cardiovascular disease, and type 2 diabetes (T2D) in which a higher NLR typically underscored clinical worsening [7, 27-30]. In the context of T1D, a higher NLR has been associated with diabetic ketoacidosis and carotid artery atherosclerosis [31, 32], while a lower NLR was associated with macroalbuminuria (diabetic nephropathy) [33]. Salami et al. (2018) found reduced neutrophil counts among Swedish boys in the TEDDY study with detected islet autoantibodies [34], though, they studied children 4-12 years of age while we included younger children prior to IA and examined ratios rather than absolute counts, which might explain the difference in our effect direction. With our findings, the elevated NLR at pre-SV in T1D cases may be capturing an innate immune response to an environmental stimulus or otherwise detecting neutrophil activation which could be contributing to the autoimmune process, and thus could see potential use as an early marker of T1D risk prior to IA. The reduced NLR change from pre-SV to SV in T1D cases may suggest several physiological developments after the initial inflammatory response. Specifically, they could suggest that the decreasing neutrophils in blood reflect infiltration of the pancreas and contribution to insulitis, the increasing number of lymphocytes indicate B and T cell

recruitment as part of the autoimmune process, or that a general imbalance is occurring between the innate (neutrophil) and adaptive (lymphocyte) immune responses.

Our study found the CD4T/CD8T ratio lower at pre-SV among T1D cases compared to controls. The ratio between CD4+ and CD8+ T cells has been identified as a marker of immune senescence and activation, the balance between helper and cytotoxic T cell functions of the adaptive immune system, and has seen predominant use in HIV and other infectious disease studies [35, 36]. In the case of HIV, people with lower CD4T/CD8T ratios had reduced activity of CD4+ T helper cells and augmented activity of CD8+ cytotoxic T cells, reflective of their susceptibility to infection and ongoing viral replication [35]. On the other hand, a higher CD4T/CD8T ratio was associated with acute respiratory distress syndrome and mortality during SARS-CoV-2 infection [36]. These differences may not be fully elucidated but are likely explained by the nuanced immune responses between HIV and SARS-CoV-2, with the former suppressing it and the latter activating it. With regard to T1D, previous work from Al-Sakkaf et al. (1989) observed reductions in the CD4T/CD8T ratio among participants who developed T1D [9]. One study in adult onset T1D also found decreased CD4+ and increased CD8+ T cell proportions, suggesting a similar relationship between helper and cytotoxic T cells [8]. Ferreira et al. (2010) also uncovered a quantitative trait loci (QTL) located within the major histocompatibility complex (MHC) for the CD4T/CD8T ratio, which was also associated with class II variants in T1D [37]. In our results, the decreased CD4T/CD8T ratio corroborates the previous work and reveals its potential role as an early indicator of disease risk prior to IA. This early shift in helper vs. cytotoxic T cells could reflect some level of functional impairment of the CD4+ cells, including regulatory T cells, and subsequent triggering of CD8+ cells into autoimmunity of the β-cells in the pancreas.

We found that T1D cases had lower B-CD4T-CD8T-mem/nv ratio at SV and attenuated changes for B-mem/nv and B-CD4T-CD8T-mem/nv ratios compared to controls. B and T-cell memory/naïve ratios serve as relative measures of balance between the cells of the adaptive immune system, and similar ratios

(naïve/memory) have been investigated in diseases such as cancer, where higher relative ratios for CD4+ and CD8+ cells indicated poorer overall survival and a lower relative CD8+ naïve-to-memory ratio had longer progression-free survival [10]. In T1D, the role of CD4+ and CD8+ T cells in autoimmunity is well established, though, B cells also take part via antibody secretion, antigen presentation, and pro-inflammatory cytokines [38]. Luo et al. (2025) found an increased level of naïve B lymphocytes among people with newly diagnosed T1D [39]. People with adult onset T1D also seemed to exhibit an increased level of naïve T cells and decreased level of effector memory subsets [8]. Our findings for the lower B-CD4T-CD8T mem/nv ratio at SV and reduced trajectories pre-SV to SV for the B and B-CD4T-CD8T mem/nv ratios among T1D cases compared to controls may indicate such an expansion or persistence of naïve cells or perhaps an inability to activate into memory subsets after antigen exposure. In this way, people who go on to develop T1D may possess an immature immunologic phenotype which predisposes them to autoimmunity and subsequent T1D.

A major strength of our study includes longitudinal DNA methylation data around the time of IA, which allowed this novel look at immune cell profiles just before and during seroconversion. Meta-analysis of TEDDY and DAISY improved our power to detect these changes and establishes generalizability to high-risk children in Europe and the U.S. Use of the newest high-resolution cell deconvolution reference panel provides greater granularity over the common six-cell panel, including allowing us to distinguish between memory and naïve cell populations.

Our study also possessed several limitations. Since we did not have access to direct flow cytometry data, we utilized cell deconvolution of peripheral blood samples for immune cell estimates with a reference panel derived from healthy adult donors, which included both sexes and several ethnic and ancestral backgrounds. Despite the unavailability of flow cytometry data, we leveraged DNAm data to estimate these proportions with a highly accurate, reference-based immune profiling method that has been previously used for similar tasks in cancer, cardiovascular disease, and neurological disease [40-42].

Regardless of the difference in age for the reference, our immune cell proportions seemed to correspond to previously reported reference ranges for this age group [23]. Despite differences in age at T1D and IA onset across DAISY and TEDDY, immune profiles changes were robust across studies, independent of age. Future research should consider the creation and utilization of a peripheral blood reference panel for DNAm data specific to children and adolescents, since immunological development and subsequent changes occur with age.

In this epidemiologic analysis leveraging two well-characterized, prospective cohorts, we identified distinct alterations in immune cell ratios that precede and accompany islet autoimmunity in children who progress to T1D. The findings herein suggest potential roles for the NLR and CD4T/CD8T ratio in serving as early biomarkers for T1D risk stratification, even before the onset of IA. Having the ability to identify these high-risk individuals provides a unique opportunity for public health intervention. In addition, children who develop T1D appeared to have lower B-CD4T-CD8T mem/nv ratios and decreased trajectories for the NLR, B mem/nv, and B-CD4T-CD8T mem/nv ratios, and may serve as indicators of immune dysfunction or immaturity and T1D progression. Together, these findings underscore the importance of temporal dynamics in immune cell profiles as novel biomarkers of early disease progression and offer potential targets for improved risk stratification and timely intervention aimed at delaying or preventing T1D. Moreover, high-resolution cell deconvolution represents a cost-effective, scalable approach for observational studies with DNA methylation data to profile immunodynamics specific to disease in the absence of flow cytometry. Future work will need to verify these results through functional analyses to determine the biological relevance of the variations in the DNAm-derived immune cell proportions and immune cell ratios.

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Author Contributions (CRediT – Contributor Role Taxonomy)

KRH: Conceptualization, Formal analysis, Methodology, Software, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing

PMC: Conceptualization, Methodology, Writing - Review & Editing

SDS: Data Curation, Writing - Review & Editing

CTR: Data Curation, Writing - Review & Editing

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MR: Funding acquisition, Investigation, Project administration, Resources, Writing - Review & Editing

JMN: Funding acquisition, Investigation, Project administration, Writing - Review & Editing

RKJ: Conceptualization, Funding acquisition, Methodology, Validation, Resources, Supervision, Writing - Review & Editing

Guarantor Statement

R.K.J. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of Interest

The authors declare that they have no conflicts of interest related to the study.

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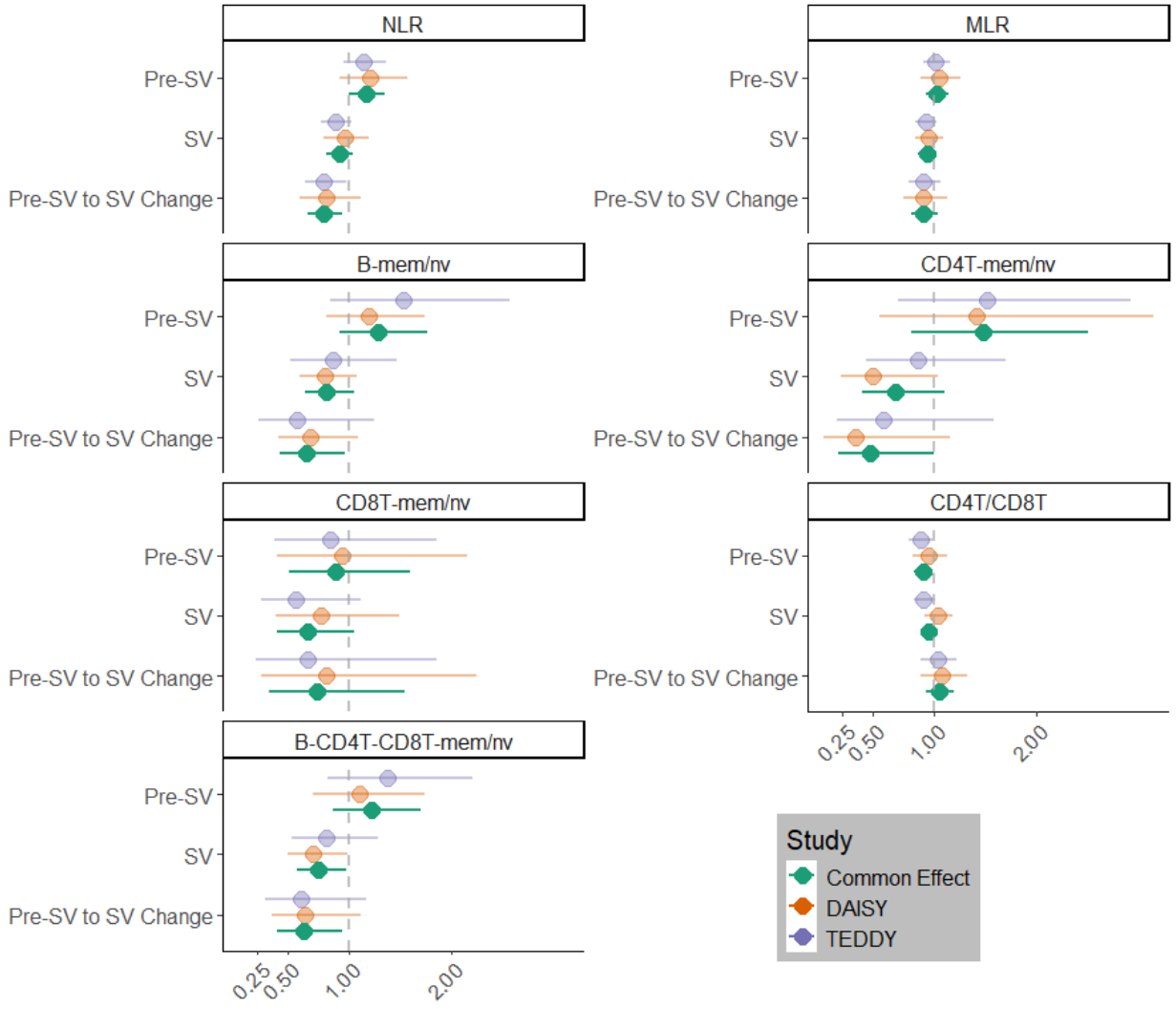
Table 1. Participant Characteristics	DAISY			TEDDY		
	T1D Control N=75	T1D Case N=76	Total N=151	T1D Control N=83	T1D Case N=83	Total N=166
	N (%) or Mean (SD)			N (%) or Mean (SD)		
Sex (female)	30 (40.0)	37 (48.7)	67 (44.4)	36 (43.4)	36 (43.4)	72 (43.4)
Non-Hispanic White (yes)	68 (90.7)	68 (89.5)	136 (90.1)	57 (68.7)	55 (66.3)	112 (67.5)
T1D family history (yes)	41 (54.7)	54 (71.1)	95 (62.9)	27 (32.5)	27 (32.5)	54 (32.5)
Age at IA onset (years)	-	3.78 (3.12)	-	-	1.34 (0.72)	-
Age at T1D onset (years)	-	9.48 (4.79)	-	-	2.86 (1.37)	-
HLA-DR3/4 (yes)	14 (18.7)	34 (44.7)	48 (31.8)	32 (38.6)	48 (57.8)	80 (48.2)
Platform (450K)	37 (49.3)	38 (50.0)	75 (49.7)	0 (0)	0 (0)	0 (0)
Clinical Center						
Georgia/Florida				5 (6.0)	5 (6.0)	10 (6.0)
Washington				5 (6.0)	5 (6.0)	10 (6.0)
Colorado	75 (100)	76 (100)	151 (100)	12 (14.5)	12 (14.5)	24 (14.5)
Sweden				25 (30.1)	25 (30.1)	50 (30.1)
Germany				10 (12.0)	10 (12.0)	20 (12.0)
Finland				26 (31.3)	26 (31.3)	52 (31.3)

Table 2. Number of Samples Across Seroconversion Timepoints By T1D Status	**DAISY**			**TEDDY**		
	T1D Control N = 113	**T1D Case N = 111**	**Total N = 224**	**T1D Control N = 141**	**T1D Case N = 141**	**Total N = 282**
	N (%) or Mean (SD)			N (%) or Mean (SD)		
Time of Sample Collection						
Pre-SV Samples	42 (37.17)	42 (37.84)	84 (37.50)	61 (43.3)	61 (43.3)	122 (43.3)
Age at Sample Collection	3.42 (3.17)	3.32 (2.86)	3.37 (3.00)	1.00 (0.57)	1.01 (0.58)	1.01 (0.57)
SV Samples	71 (62.83)	69 (62.16)	140 (62.50)	80 (56.7)	80 (56.7)	160 (56.7)
Age at Sample Collection	3.79 (3.19)	3.78 (3.12)	3.80 (3.14)	1.35 (0.74)	1.34 (0.72)	1.34 (0.73)

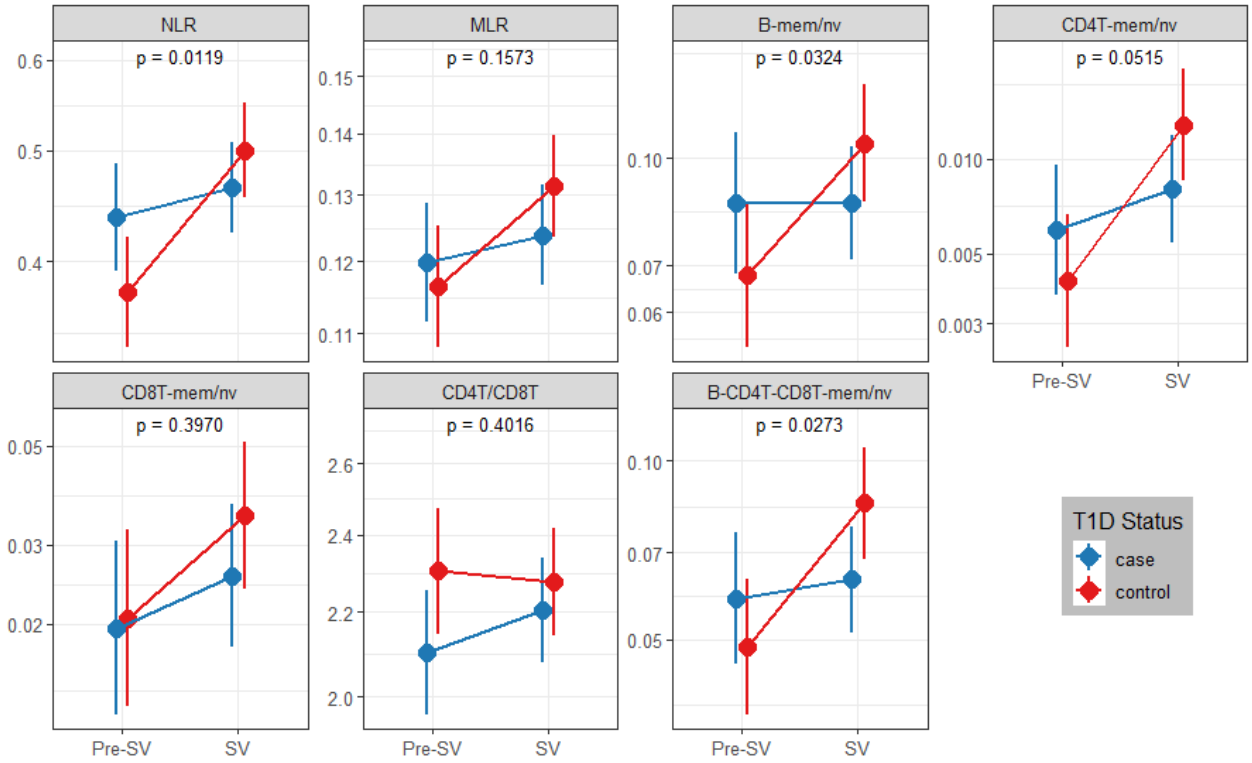
* Note: Not all participants contributed samples for both pre-SV and SV timepoints.

Figure 1. Meta-analysis: Differences in mean immune cell ratios between T1D cases and controls



* Abbreviations: NLR (neutrophil/lymphocyte ratio), MLR (monocyte/lymphocyte ratio), B-mem/nv (B-memory/B-naïve ratio), CD4T-mem/nv (CD4T-memory/ CD4T-naïve ratio), CD8T-mem/nv (CD8T-memory/CD8T-naïve ratio), CD4T/CD8T (CD4T/CD8T ratio), B-CD4T-CD8T-mem/nv (B-CD4T-CD8T-memory/B-CD4T-CD8T-naïve ratio), Pre-SV (pre-seroconversion), SV (seroconversion), T1D (type 1 diabetes). Interpretation: an immune cell ratio < 1 indicates a lower immune cell ratio among T1D cases compared to controls, and vice versa for an immune cell ratio > 1. In the pre-SV to SV change, an immune cell ratio < 1 indicates a reduced trajectory among T1D cases compared to controls, and vice versa for an immune cell ratio > 1.

Figure 2. Immune cell ratios between T1D cases and controls around seroconversion



* Abbreviations: NLR (neutrophil/lymphocyte ratio), MLR (monocyte/lymphocyte ratio), B-mem/nv (B-memory/B-naïve ratio), CD4T-mem/nv (CD4T-memory/CD4T-naïve ratio), CD8T-mem/nv (CD8T-memory/CD8T-naïve ratio), CD4T/CD8T (CD4T/CD8T ratio), B-CD4T-CD8T-mem/nv (B-CD4T-CD8T-memory/B-CD4T-CD8T-naïve ratio), Pre-sero (pre-seroconversion), SV (seroconversion), T1D (type 1 diabetes). Interpretation: immune cell ratio changes between T1D cases and controls across the pre-SV and SV time points.