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# Type 1 diabetes patients have significantly lower frequency of plasmacytoid dendritic cells in the peripheral blood

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**Abstract** Dendritic cells uniquely orchestrate the delicate balance between T cell immunity and regulation and an imbalance favoring immunogenic rather than tolerogenic DC is believed to contribute to the development of autoimmune diseases such as type 1 diabetes (T1D). In this study, we determined the frequencies of three blood DC subsets (pDC, mDC1 and mDC2) in 72 T1D patients and 75 normal controls using the Miltenyi blood DC enumeration kit. The frequency of blood pDC was found to be negatively correlated with subject age in both normal controls and T1D patients ($p=0.0007$), while the frequency of mDC1 and mDC2 do not change significantly with subject age. More importantly, the mean frequency of pDC in blood was, after adjusting for age, significantly lower in T1D (mean=0.127%) than controls (mean=0.188%) ($p<6.0\times10^{-5}$), whereas no difference was observed for mDC1 and mDC2 between T1D and controls. Furthermore, T1D patients have a lower proportion of pDC and higher proportion of mDC1 among the total blood DC population than normal controls. These results indicate that the frequency of blood pDC and the pDC/mDC1 ratio are negatively associated with T1D.


## Introduction

The break of tolerance to self-antigens in autoimmune diseases including type 1 diabetes (T1D) is largely due to deficient immune regulation [1,2]. Although recent studies have focused on the roles of various types of regulatory T cells (Treg), antigen presenting cells, especially dendritic cells (DC), have long been recognized to play a pivotal role in the pathogenesis/protection of autoimmune diseases because

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they are capable of either priming effector T cells or activating Treg cells depending on the maturation stimuli and/or DC subsets [3–5]. DC comprises a heterogeneous group of cells and the natural DC population includes two distinct subsets, conventional or myeloid DC (mDC) and plasmacytoid DC (pDC). Analysis of DC subsets in human blood has been difficult due to the lack of specific surface antigens. Recently, a number of novel monoclonal antibodies have been developed and used as blood DC markers [6]. These novel antibodies recognize two subsets of myeloid DC. The mDC1 subset is positive for BDCA1 (CD1c) while the mDC2 subset is positive for BDCA3. pDC were originally identified in human [7] and subsequently identified in mice [8,9]. Human blood pDC are specifically recognized as cells positive for BDCA2 and BDCA4.

pDC can produce vast amounts of type I interferons in response to viruses and other stimuli and thus play an important role in antiviral immunity and potentially in autoimmunity [10,11]. Murine immature pDC, when freshly isolated from mouse secondary lymphoid tissue, are endowed with tolerogenic potential by inducing differentiation of Treg [12]. Immature pDC can also induce allogeneic T cell hyporesponsiveness and prolong heart graft survival when they are derived from bone marrow [13]. Human pDC appear to have an intrinsic capacity to prime naïve T cells to dedifferentiate into regulatory T cells [14].

Although DC are believed to be an important player in T1D pathogenesis, the roles of various DC subsets in T1D has only received some attention in recent years. In the NOD mice, pDC cells are increased after treatment with GCSF, which protects against diabetes [15]. The protective role of pDC has recently been demonstrated in NOD mice [16]. Human studies are more limited due to lack of specific markers and the general difficulties associated with patient-based studies, e.g. high individual variability, ethnic/population differences, variation over time and specimen availability. An early study suggested that T1D patients had higher pDC numbers and secreted more IFN-$\alpha$ than normal controls [17], while a more recent study found no difference in the frequencies of total DC or DC subsets between T1D patients and controls but found reduced IFN-$\alpha$ secretion in T1D patients [18]. These inconsistent results are not surprising as both studies had small sample sizes. In contrast, a recent report using larger sample size suggested that pDC numbers and frequencies were reduced in T1D patients [19]. In order to reconcile these controversial reports, we have undertaken a study on human blood DC subsets in a large cohort of T1D patients using the recently discovered DC surface markers. Our study indicates that the frequency of pDC is significantly reduced in T1D patients while mDC1 and mDC2 subsets are not different between T1D and controls.

## Research design and methods

### Human subjects

A total of 147 Caucasian subjects were recruited from the Augusta area in Georgia. Among these subjects, 72 were T1D patients and 75 were normal controls (NC). The demographic information for these subjects is presented in Table 1. Diagnosis of T1D was made using the criteria of the American Diabetes Association by physician scientists with extensive experience in type 1 diabetes. All patients used in this study unambiguously have type 1 diabetes as questionable cases were not included in this study. These patients have had diabetes for an average of 5.7 years (range 0–35 years). Healthy controls were subjects who had no autoimmune disorders and were negative for the presence of T1D-associated autoantibodies and do not have a family history of T1D. The average age for the control group is 30.6 years. The Medical College of Georgia institutional review board approved the study design and informed consent was obtained from all subjects.

**Table 1** Clinical characteristics of blood DC subsets in healthy and diabetic subjects

| Variable | AbN | T1D |
|---|---|---|
| Total number | 75 | 72 |
| Age (years) [a] | 30.6 ± 9.3 | 17.9 ± 11.4 |
| Age range | (1–65) | (2–61) |
| Male | 39 | 30 |
| Female | 36 | 42 |
| Duration of T1D (years) | N/A | 5.7 (0–35) |
| <30 years of age | 42 | 62 |
| >30 years of age | 33 | 10 |

Age and duration of disease are presented as means (range).
[a] The mean age is significantly different between T1D and AbN groups ($p<0.0001$).

### Analysis of human blood DC subsets using DC enumeration kit

Fresh heparinized blood was obtained through venipuncture. Within 2 h of blood drawing, an aliquot (300 $\mu$l) of the blood sample was stained with 20 $\mu$l of anti-BDCA cocktail (containing monoclonal antibodies specific for the DC markers BDCA1, BDCA2 and BDCA3 along with CD14 and CD19 antibodies) or 20 $\mu$l of control cocktail containing appropriate isotype controls (Miltenyi Blood DC Enumeration kit—Miltenyi Biotec Inc., Auburn CA, USA). Dead cell detector (10 $\mu$l) was added to both tubes. Following 10 min of incubation on ice erythrocytes were lysed. Cells were then washed in PBS containing 1% BSA, 0.1% sodium azide, 2% FCS and 1% human serum. Cells were fixed and analyzed by four-color flow cytometry with the exclusion of B cells, monocytes and dead cells. One million events in a mononuclear gate were collected. With our protocol, the viability of the cells is about 99% and the frequencies of the DC subsets are consistent when the samples are processed within the first few hours of sampling. The relative proportion and the total number of the three DC subsets (mDC1, mDC2 and pDC) were calculated. The total count of each DC subsets could also be calculated for those subjects who had less than 1 million PBMC in 300 $\mu$l of blood.

### Statistical analysis

Seven continuous variables (% of DC in PBMC, % of pDC in PBMC, % of mDC1 in PBMC, % of mDC2 in PBMC, % of pDC in DC, % of mDC1 in DC and % of mDC2 in DC) were analyzed using analysis of covariance (ANCOVA) with diabetes status as