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# The *Idd13* congenic interval defines the number of merocytic dendritic cells, a novel trait associated with autoimmune diabetes susceptibility

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ABSTRACT

When antigens derived from apoptotic cells are presented by conventional dendritic cells (cDC), T cell tolerance is induced. Surprisingly, the presentation of apoptotic cell antigens by an unconventional DC subset, termed merocytic dendritic cells (mcDC), can reverse T cell anergy. The potency of mcDC at breaking T cell tolerance has been demonstrated in the context of tumors and autoimmunity, suggesting that modulating the number of mcDC in vivo may be of clinical interest. To identify the genetic determinants that define the number of mcDC, we performed a linkage analysis between NOD and C57BL/6 mouse strains, where autoimmune-prone NOD mice show an increased proportion of mcDC relative to the non-autoimmune-prone C57BL/6 mice. We identified a locus on chromosome 2 significantly linked to both the proportion and the absolute number of mcDC in the spleen. Interestingly, the dominant interval on chromosome 2 overlaps with a locus previously associated with diabetes protection, namely *Idd13*. Using NOD.*Idd13* congenic mice, we validate the impact of the *Idd13* congenic interval in defining the proportion and number of mcDC in the spleen. These results show that the decreased number of mcDC is conferred by C57BL/6 alleles at the *Idd13* locus, which is linked to diabetes resistance.


## 1. Introduction

Dendritic cells (DC) are professional antigen-presenting cells that actively participate in immune surveillance [1]. In non-inflammatory conditions, however, DC contribute to the maintenance of immune tolerance. Indeed, upon uptake of apoptotic self-cellular debris, the conventional DC (cDC) subsets present self-antigens to T cells inducing either T cell anergy or regulatory T cell differentiation [2]. This mechanism of peripheral tolerance is believed to perpetually eliminate T cell autoreactivity, in order to limit autoimmune reactions when inflammation arises [3].

The identification of a novel DC subset challenges the concept that all DC contribute to immune tolerance in non-inflammatory conditions. Indeed, recent work unveiled a DC population with the unexpected ability not only to prime both CD4 and CD8 T cell responses upon uptake of antigens from apoptotic cells, but also to break T cell tolerance [4–7]. This subset was termed merocytic DC (mcDC) for its unusual ability to store apoptotic antigens in cytoplasmic vesicles (μερος, meros = particle) [4]. Moreover, upon exposure to apoptotic cells, mcDC effectively secrete IFN-α [5,8]. Together, the unique properties of mcDC have conferred them the ability to effectively promote tumor clearance when exposed to apoptotic tumor cells [5,7,8].

The role of mcDC has also been studied in the context of autoimmune diabetes. In fact, normal tissue remodeling of the pancreas leads to apoptosis of insulin-producing pancreatic β cells at weaning and this event precedes lymphocytic infiltration in the autoimmune-prone NOD mice [9,10]. Interestingly, mcDC are found in higher numbers in the NOD mouse strain relative to autoimmune-diabetes resistant strains, including both BALB/c and C57BL/6 (hereafter referred to as B6) [4]. In addition, mcDC isolated from the pancreatic lymph nodes of diabetic NOD mice break T cell tolerance to pancreatic β cell antigens and rapidly induce diabetes upon in vivo transfer in non-diabetic recipients [4]. These findings suggest that the increased number of mcDC in NOD mice leads to an increased presentation of apoptotic β cell antigens to T cells in NOD mice, thus promoting autoimmune diabetes progression. Therefore, the ability to modulate the number of mcDC in vivo by targeting specific molecular determinants responsible for the regulation of their homeostasis might prevent the development of autoimmune diabetes.

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To identify genetic factors contributing to the regulation of mcDC number in vivo, we first validated that the increased proportion of mcDC in NOD mice relative to B6 mice was not due to the autoimmune disease process by evaluating the proportion and number of mcDC in Rag1-deficient B6 and NOD mice, which do not develop autoimmune diabetes. We next performed a linkage analysis on a (NOD × B6) $F_2$ cohort deficient for *Rag1* and observed that both mcDC proportion and number linked to a dominant locus at the distal end of mouse chromosome 2, corresponding to the *Idd13* locus. Using Rag-sufficient NOD.*Idd13* congenic mice, we validated the contribution of the *Idd13* interval in defining both the proportion and number of mcDC in NOD mice. Our findings thus link the *Idd13* locus to a novel immunological phenotype, which may contribute to autoimmune susceptibility.

## 2. Material and methods

### 2.1. Mice

C57BL/6 (hereafter denoted B6), NOD, B6.Rag1$^{-/-}$ and NOD.Rag1$^{-/-}$ mice were purchased from The Jackson Laboratory. The NOD.NOR-*Idd13* mice (denoted as NOD.*Idd13* throughout), for which the *Idd13* locus is of B6 origin [11], were generously provided by David Serreze (Jackson Laboratory). All of these strains were subsequently maintained at the Maisonneuve-Rosemont Hospital animal house facility (Montreal, Canada). F1.Rag (B6 Rag1$^{-/-}$ × NOD Rag1$^{-/-}$) and F2.Rag (F1.Rag × F1.Rag) mice were bred in house from the parental strains. Six to 8 week-old mice were used for all phenotypic analyses. The Maisonneuve-Rosemont Hospital ethics committee, overseen by the Canadian Council for Animal Protection approved the experimental procedures.

### 2.2. Flow cytometry

Spleens were treated with collagenase (1 mg/mL in PBS, Type V from Clostridium histolyticum, Sigma–Aldrich) for 15 min at 37 °C and passed through a 70 μM cell strainer (BD Biosciences) to yield single-cell suspensions prior to staining with antibodies. CD11c PE-Cy7, CD8α PE, CD11b APC-Cy7 and CD11b Pacific Blue antibodies were purchased from Biolegend, while mouse pDC Ag-1 allophycocyanin, staining the Bst-2 antigen, was purchased from Miltenyi Biotec. All samples were acquired using FACSCanto I (BD Biosciences) or BD LSRII (BD Biosciences) and were analyzed using the FlowJo software (TreeStar, Ashland, OR). The gating strategy to select mcDC is based on previous publications [4,5] and the threshold for selecting CD11b$^{-/low}$ cells is based on fluorescence minus one (FMO) staining. Note that we implemented a precise gating strategy to allow for the accurate identification of mcDC proportion in Rag1-deficient mice. Briefly, to remove autofluorescent cells [12], a dump channel is used on the 488 nm laser, namely FL3. After excluding doublets, CD11c$^{high}$ cells are selected. This is followed by the application of a strict size exclusion gate to the FSC/SSC profile, corresponding to live CD11c$^{high}$ cells. Within this gate, mcDCs are selected based on the CD8α$^{-}$ CD11b$^{-/low}$ phenotype.

### 2.3. Linkage analysis

Genomic DNA was isolated from the tails of F2.Rag male and female mice by using the DNeasy blood and tissue kit from Qiagen. 202 single nucleotide polymorphisms were then detected from the F2.Rag mice DNA using the Illumina mouse low density linkage panel serviced through The Centre for Applied Genomics at the Hospital for Sick Children, Ontario, Canada. Marker location (in Mb) was determined using the National Center for Biotechnology Information (NCBI) Build m37. The logarithm of odds (LOD) scores were obtained through a single- or two-dimensional quantitative trait locus model using the R/qtl package [13] for the R software (version 2.11.1). To increase SNP resolution, the Haley-Knott algorithm was applied [14]. LOD scores higher than 3.1 were significant for single-dimensional analysis according to permutation tests ($n$ = 10 000, $p$ = 0.05), and LOD scores between 2 and 3.1 were considered suggestive. Significant LOD scores above a threshold of 9.61 for a two-dimensional analysis were obtained using permutation testing ($n$ = 1000, $p$ = 0.05). A Pearson's $\chi^2$ for allele frequencies confirmed that all the significant and suggestive loci did not deviate from the Hardy–Weinberg equilibrium (Supplementary Table 1 and data not shown).

### 2.4. Statistical analysis

Data for the various experiments were tested for significance using a non-parametric Mann–Whitney test with a minimal threshold of 0.05. Estimation of the interval coordinates was obtained using a 95% Bayes interval test. Significance for the genotype distribution differences was tested with an ANOVA. All statistical analyses and the $F_2$ distribution were obtained using the SPSS 19.0 software.

## 3. Results

mcDC express high levels of CD11c and differ phenotypically from the conventional DC subsets by the lack of expression of CD8α combined with a low to negative expression of CD11b [4,5,7]. First, to clearly delimit the low to negative expression of CD11b on CD11c$^{high}$ cells, we established the baseline expression level of CD11b in comparison to an FMO staining. By gating on CD11c$^{high}$

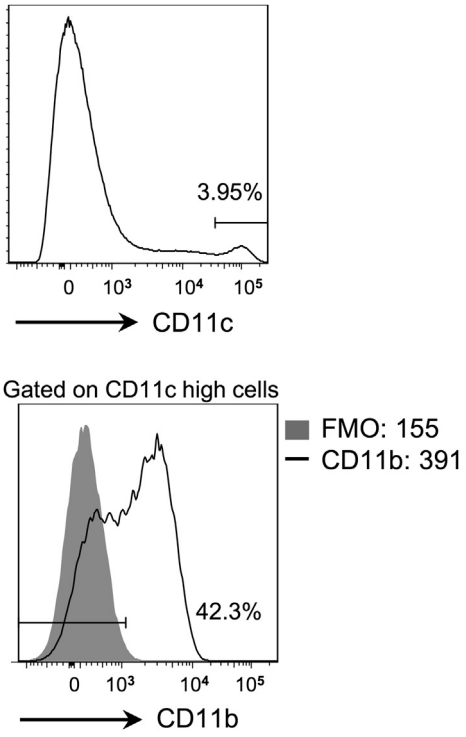


**Fig. 1.** Determining the threshold for selecting CD11b$^{-/low}$ dendritic cells. Spleen cells from B6 mice were stained for CD11c and CD8α without (FMO) or with CD11b. Dendritic cells were selected based on the CD11c$^{high}$ expression (upper histogram). The histogram to the bottom presents an overlay of the APC-Cy7 fluorescence for CD11c$^{high}$ cells stained with anti-CD11b-APC-Cy7 (CD11b) or without the antibody (FMO). MFI levels are indicated.