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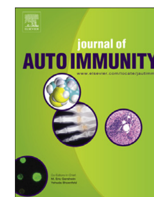

Constitutively active Stat5b signaling confers tolerogenic functions to dendritic cells of NOD mice and halts diabetes progression

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ABSTRACT

Defects in dendritic cells (DCs) development and function lead to autoimmune disorders. Autoimmune diabetes in humans and NOD mice results from a breakdown of self-tolerance, ending in T cell-mediated β-cell destruction. DCs dysfunction in NOD mice results in part from a defect in the JAK-STAT5 signaling pathway associated with the *idd4* susceptibility locus. The involvement of Stat5b in DCs tolerogenic functions remains unknown. We have generated transgenic mice (NOD.CD11c$^{Stat5b-CA}$) expressing a constitutively active form of the Stat5b gene (Stat5b-CA) under control of CD11c promoter. All NOD.CD11c$^{Stat5b-CA}$ mice were protected against diabetes. Protection was associated with an increased in the pool and suppressive function of Tregs, a promotion of Th2 and Tc2 immune response and a decreased percentage of CD8$^+$ T cells. Splenic DCs of NOD.CD11c$^{Stat5b-CA}$ mice acquired a mature phenotype, promoted and induced better conversion of CD4$^+$CD25$^-$Foxp3$^-$ T cells into Tregs (CD4$^+$CD25$^+$Foxp3$^+$ T cells) than DCs of NOD mice. Stat5b-CA.DC-educated CD4$^+$CD25$^-$ T cells delayed diabetes onset whereas Stat5b-CA.DC-educated Tregs blocked ongoing diabetes in 8–10 weeks old NOD recipient mice. Importantly, injection of Stat5b.CA.DC to 8–10-week old NOD mice halted diabetes progression and educated their splenocytes to loose their diabetogenic potential when transferred to NOD.SCID mice. Our work is the first to report that an active form of Stat5b restored DCs tolerogenic functions that re-educated Tregs to re-establish and to sustain long-term protective immune response against diabetes in NOD mice.

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1. Introduction

Dendritic cells (DCs) are professional antigen-presenting cells that play a central role in T-cell immune response and homeostasis [1]. DCs contribute to peripheral tolerance by promoting homeostasis of peripheral regulatory T cells (Tregs) and/or by induction of T cell unresponsiveness. These tolerogenic properties are mediated essentially by their functional state. It has been reported that the absence of activation of immature DCs (iDCs) or low activation state of semimature DCs induce and maintain peripheral T cell tolerance. In contrast, hyperactivated mature DCs efficiently induce the development of effector T cell immunity [2]. Type 1 diabetes (T1D) is a multigenic disease resulting from a breakdown of self-tolerance that leads to T cell-mediated pancreatic β-cell destruction [3–5]. Several lines of evidence have indicated that inappropriate activation of DCs and defects in the number and function of tolerogenic DCs and Tregs contribute to the breakdown of self-tolerance leading to T1D pathogenesis in humans and in the non obese diabetic (NOD) mouse model [6–9].

We have reported that bone marrow-derived DCs of NOD mice conditioned with granulocyte-macrophage colony-stimulating factor (GM-CSF) or thymic stromal lymphopoietin (TSLP) acquired the signature of tolerogenic DCs and that treatment of NOD mice with GM-CSF or TSLP or, TSLP-treated DCs protected from diabetes

Abbreviations used: DCs, Dendritic cells; Stat5, Signal Transducer and Activator of Transcription 5; Tregs, regulatory T cells; NOD, nonobese diabetic; T1D, type 1 diabetes.

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by increasing and maintaining IL-10-producing tolerogenic DCs and Tregs [6,10,11]. GM-CSF and TSLP are known to signal through the JAK1/2 pathway to activate the STAT transcription factor [12,13]. Stat5 consists of two proteins, Stat5a and Stat5b that are encoded by two distinct genes and that share 95% homology in humans and mice [14]. Upon tyrosine phosphorylation, pStat5a and pStat5b form homo or heterodimers which translocate to the nucleus where they bind to specific DNA sequences in the promoter region of various genes, resulting in specific gene activation or repression [15]. Stat5 plays important roles in many responses including orchestration of immunoregulation and immune cell development [16] such as development and maintenance of Treg homeostasis and function [17–20], Th2 differentiation and $CD8^+$ T cell homeostasis [21]. $Stat5^{-/-}$ mice displayed impaired proliferation and/or survival of myeloid cells, mast cells, peripheral T cells, NK and B cells [22–24]. In addition, $Stat5^{-/-}$ mice failed to generate Tregs [20,25] whereas mice expressing constitutively active form of Stat5b (Stat5b-CA) restricted to T and B cell had a marked increase in Treg number [18,25]. Conditional Stat5 deficiency in hematopoietic compartment leads to increased number of plasmacytoid (pDCs) and decreased number of conventional cDCs [26,27]. DC-specific depletion of Stat5 has underlined the importance of Stat5 activation in DCs during TSLP-induced Th2 immune response [28]. In autoimmune diabetes, genes encoding Stat5a and Stat5b map to chromosome 11 within the insulin-dependent diabetes interval 4 (*idd4)* [29,30]. It has been reported that NOD.B6.*Idd4* murine congenic strains carrying the B6-*idd4* locus showed a significant reduction of diabetes incidence [31]. Furthermore, sequencing studies have revealed a unique single mutation within the binding domain of S Stat5b DNA and, consequently, Stat5b had weak DNA binding and reduced expression of downstream genes [32]. The weak DNA binding of Stat5b in the GM-CSF signaling pathway has been reported in T cells and macrophages in NOD mice. However, the role of Stat5b in homeostasis and tolerogenic function of DC in autoimmune diabetes remains largely unknown.

To investigate the role of Stat5b-CA in tolerogenic function of DCs that are crucial to induce and maintain protective immune response against diabetes, we have generated transgenic NOD mice that express a constitutively active form of Stat5b specifically in DCs (called $NOD.CD11c^{Stat5b-CA}$ mice here). We found that $NOD.CD11c^{Stat5b-CA}$ mice were protected from diabetes. The protection was associated with increased Treg pool and a drastic decrease of $CD8^+$ T cells in secondary lymphoid organs. Stat5b-CA-expressing DCs (Stat5b-CA.DCs) acquired a mature phenotype, produced increased amounts of TGFβ but lesser quantities of IL-12p70, induced antigen specific Treg differentiation, enhanced Treg suppressive activity and promoted Th2/Tc2 immune deviation. We also showed that Stat5b-CA.DCs educated Tregs were crucial to inhibit ongoing diabetes. Importantly, a single injection of $CD11c^{Stat5b-CA}$ DCs to 8–10-week old NOD mice protected from diabetes and educated their splenocytes to loose their diabetogenic potential when transferred to NOD.SCID mice. To the best of our knowledge, our study is the first to highlight the important role of the Stat5b transcription factor in DCs tolerogenic function in autoimmune diabetes.

2. Research design and methods

2.1. Mice and diabetes monitoring

$NOD.CD11c^{Stat5b-CA}$ mice were generated as follows. Stat5b-CA cDNA obtained from pMX-puro-Stat5b-CA (a gift from Dr. T Kitamura, University of Tokyo, Japan) was subcloned into pMYc-pIRES2-EGFP (Clontech Laboratories Inc., Mountain View, CA) to generate the Stat5b-CA-IRES2-EGFP construct. Stat5b-CA-IRES2-EGFP cDNA was subcloned into a vector containing the CD11c promotor (pCD11c) (a gift from Dr. K Karjalainen, Nanyang Technological University, Singapore). The transgene DNA sequence pCD11c-Stat5b-CA-IRES2-EGFP was separated from the vector, purified, sequenced and used to generate transgenic NOD mice at the JDRF Center of Immunological Tolerance in T1D facilities (Harvard Medical School, Boston, MA).

NOD, NOD-BDC2.5, and NOD.SCID mice were purchased from the Jackson Laboratory (Bar Harbor, ME). NOD-BDC2.5-Foxp3-GFP mice were obtained by backcrossing C57BL/6-Foxp3-GFP with NOD mice for more than 10 generations. 8.3-NOD mice obtained from Dr. P. Santamaria (University of Calgary, AB) have been described [33]. Mice were housed under pathogen-free conditions and all experiments were performed according to the University of Sherbrooke institutional animal care committee all experiments. Animals were monitored for diabetes by the urine glucose test Uristix (Bayer, Minneapolis, MN) and blood glucose with an Advantage Accu-Check glucometer (Roche Diagnostics, IN) as described [6].

2.2. Antibodies and flow cytometry

mAb against, CD4 (RMA-5), CD25 (3C7), CD11c (clone HL3), CD11b (clone M1/70), B220 (clone RA3-6B2), $I-A^d$ (clone 39-10-8), CD80 (clone 16-10A1), CD86 (clone GL1) and CD40 (clone 1C10), CD8α (clone 53–6.7), CD3 (clone 145-2C11), Vβ 8.1, 8.2 TCR (clone MR5-2) and streptavidin-PerCP were from BD Biosciences (Mountain View, CA). PE-Cy5-anti-CD11c (clone N418), PE-anti-CD8a (clone 53–6.7), Alexa488-and PE-Cy7-anti-CD25 (clone PC61.5), APC-anti-CD4 (clone GK1.5), PE-anti-Foxp3 (clone FJK-16s), PE-anti-CTLA4 (clone UC10-4B9), and CD28 (clone 37.51) antibodies were from eBioscience (San Diego, CA). Anti-Stat5b and anti-pStat5 mAbs were from Cell Signaling technology (Beverly, MA). Splenic DCs were left unstimulated or stimulated with LPS (1 μg/ml, 48 h), washed with PBS and stained with anti-CD11c-PE in combination with PE-Cy5 anti-CD80, PE-Cy5 anti-CD86, PE-Cy5 anti CD40 or Biotin anti-MHC II. Conjugated matched isotypes (Armenian hamster IgG2, Rat IgG2a, or mouse BALB/c IgG3) were used as negative controls. For intracellular staining, Tregs were fixed, permeabilized and stained with anti-CD4, anti-CD25 and anti-Foxp3 mAbs using Foxp3 Staining Kit (eBioscience). Cells were analyzed on a FACS-Calibur or a FACSCanto instrument (BD Biosciences, CA), FACS data were analyzed using the CellQuest Pro (BD Biosciences) or FlowJo (Tree Star, Ashland, OR) software.

2.3. Dendritic and T cell isolation

Splenic $CD4^+$ and $CD8^+$ T cells and DCs were purified using Ab-coated magnetic beads (Miltenyi Biotec, San Diego, CA) as described (Gaudreau et al., 2007). Splenic $CD4^+Foxp3^+GFP^+$ Tregs and $CD4^+Foxp3^-GFP^-$ T cells from NOD-BDC2.5-Foxp3-GFP mice were purified using a FACSAria III cell sorter (BD Biosciences). Tregs and $CD4^+CD25^-$ T cells were purified from spleens of NOD and $NOD.CD11c^{Stat5b-CA}$ mice using a Treg isolation kit (Miltenyi Biotec). Bone marrow-derived DCs were generated as described [11]. Purity was >95% for DCs and >98% for T cells.

2.4. Western Blots

Splenic DCs were purified from NOD and $NOD.CD11c^{Stat5b-CA}$ mice, whole lysates prepared, proteins extracted and Western Blots carried out as described [11].

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