Journal of Autoimmunity 53 (2014) 105–114

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

PTPN2 restrains CD8$^+$ T cell responses after antigen cross-presentation for the maintenance of peripheral tolerance in mice

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ARTICLE INFO

Article history:
Received 24 January 2014
Received in revised form
12 May 2014
Accepted 23 May 2014
Available online 2 July 2014

Keywords:
PTPN2
Cytotoxic T lymphocyte
Immunity
Autoimmunity
Cross-presentation
Type 1 diabetes

ABSTRACT

Antigen cross-presentation by dendritic cells is crucial for priming cytotoxic CD8$^+$ T cells to invading pathogens and tumour antigens, as well as mediating peripheral tolerance to self-antigens. The protein tyrosine phosphatase N2 (PTPN2) attenuates T cell receptor (TCR) signalling and tunes CD8$^+$ T cell responses *in vivo*. In this study we have examined the role of PTPN2 in the maintenance of peripheral tolerance after the cross-presentation of pancreatic β-cell antigens. The transfer of OVA-specific OT-I CD8$^+$ T cells (C57BL/6) into RIP-mOVA recipients expressing OVA in pancreatic β-cells only results in islet destruction when OVA-specific CD4$^+$ T cells are co-transferred. Herein we report that PTPN2-deficient OT-I CD8$^+$ T cells transferred into RIP-mOVA recipients acquire CTL activity and result in β cell destruction and the development of diabetes in the absence of CD4$^+$ help. These studies identify PTPN2 as a critical mediator of peripheral T cell tolerance limiting CD8$^+$ T cell responses after the cross-presentation of self-antigens. Our findings reveal a mechanism by which *PTPN2* SNPs might convert a tolerogenic CD8$^+$ T cell response into one capable of causing the destruction of pancreatic β-cells. Moreover, our results provide insight into potential approaches for enhancing T cell-mediated immunity and/or T cell adoptive tumour immunotherapy.

1. Introduction

The elimination or inactivation of T cells with auto-reactive potential is a critical task that is synergistically mediated by both thymic and peripheral tolerance mechanisms. The majority of auto-reactive T cells are eliminated in the thymus through negative selection; a process that is facilitated by the ability of the thymic medullary cells to ectopically express peripheral tissue antigens. Nonetheless, the few highly auto-reactive T cells that might escape this selection are subsequently eliminated by peripheral tolerance mechanisms [1–5].

A series of elegant experiments have established that immature dendritic cells (DCs) play an essential role in this context. Immature DCs acquire self-antigens from non-inflamed tissues, transport them to lymph nodes (LNs) and present them in a way that induces limited T cell expansion and finally the deletion of T cells that strongly react to the tissue-derived antigens [5–10]. In contrast, the induction of a cytotoxic T lymphocyte (CTL) immune response to an invading pathogen begins in the LNs that drain the infection site and requires the processing and presentation of exogenous antigens by mature antigen-presenting cells (APCs). Notably, both the initiation of a CTL response and the tolerisation of auto-reactive T cells often depend on the capacity of DCs to acquire exogenous antigens and to channel peptide derived from these antigens onto their own MHC-1 molecules; a process referred to as cross-presentation [6–12].

CD4$^+$ T cells have been shown to impact the cross-presenting and T cell stimulatory ability of DCs, as they are able to induce the maturation of DCs; a process known as T cell licencing [12–14]. The latter is thought to be particularly critical in the absence of strong pro-inflammatory stimuli. Moreover, CD4$^+$ T cells have been shown to mature self-antigen presenting DCs and to thereby transform their tolerising potential into auto-immunity promoting cells.

When naive CD8$^+$ and CD4$^+$ T cells engage peptide antigen presented by major histocompatibility complex (MHC) molecules, the emanating T cell receptor (TCR) signal strength determines whether T cells progress past the G_1 restriction point and commit to cellular division, produce interleukin-2 (IL-2) and undergo clonal expansion and differentiate and acquire effector functions [15]. TCR

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http://dx.doi.org/10.1016/j.jaut.2014.05.008
0896-8411/

signalling is reliant on tyrosine phosphorylation mediated by the Src family protein tyrosine kinases (PTKs), Lck and Fyn, and the Syk family PTK ZAP-70 [15]. Engagement of the TCR allows for Lck to phosphorylate the immunoreceptor tyrosine-based activation motifs of the TCR that result in ZAP-70 recruitment and activation and the phosphorylation of adaptor proteins such as LAT. This in turn allows for the nucleation of signalling complexes and the phosphorylation and activation of multiple effector pathways [15]. Upon TCR engagement, the activation and/or functions of Lck are regulated by the localisation of Lck and its substrates, as well as the abundance, activity and segregation of regulatory molecules within the immunological synapse [15,16]. Such regulatory molecules include protein tyrosine phosphatases (PTPs) that regulate the phosphorylation of the Lck Y505 inhibitory site, as well as the Lck Y394 activating site [17]. PTPs have been proposed to act as gatekeepers determining both the threshold and extent of T cell activation to affect T cell development, homoeostasis and immunity [18,19].

PTPN2 (also known as T cell PTP) is a ubiquitous phosphatase that is expressed abundantly in haematopoietic cells, including T cells [20,21]. Genome-wide association studies have linked *PTPN2* single nucleotide polymorphisms (SNPs) with the development of several human autoimmune diseases including type 1 diabetes, rheumatoid arthritis, Crohn's disease and celiac disease [22-26]. In particular, an intronic *PTPN2* variant, rs1893217(C), has been linked with the development of type 1 diabetes [24,25]. This SNP is associated with an approximate 40% decrease in *PTPN2* mRNA in the $CD4^+$ T cells of healthy individuals that carry the risk allele [27]. PTPN2 is a key regulator of TCR signalling in naive $CD4^+$ and $CD8^+$ T cells, serving to dephosphorylate and inactivate Lck and Fyn [28,29]. PTPN2 also dephosphorylates Janus-activated kinases (JAK)-1/3 and signal transducers and activator of transcription (STAT)-1/3/5/6 to attenuate cytokine signaling [20,21,30,31]. T cell-specific PTPN2-deficiency in mice enhances $CD4^+$ and $CD8^+$ T cell responses *in vivo* and lowers the threshold for TCR-instigated responses so that $CD8^+$ T cells respond more efficiently to peptide antigens with suboptimal TCR affinity [28,29]. Moreover, PTPN2-deficiency in mice results in the development of widespread inflammation and spontaneous autoimmunity with age [28,29]. Recently we reported that PTPN2 is instrumental in attenuating TCR-self-peptide-MHC-mediated homoeostatic T cell expansion [32]. PTPN2-deficiency promotes rapid $CD8^+$ T cell proliferation in lymphopenic mice and the development of antigen-experienced effector T cells that promote autoimmunity [32].

In this study we observed that PTPN2-deficiency converts the recognition of self-antigen on immature DCs from the normally occurring elimination of the responding cells, into a stimulatory condition that supports the differentiation into effector T cells, and promotes β cell destruction and the development of a severe type of autoimmune diabetes. Strikingly, this occurs in the absence of CD4 T cell help and in the presence of a functional regulatory T cell compartment. Thus, PTPN2 is crucial in restraining $CD8^+$ T cell responses after antigen cross-presentation to maintain peripheral tolerance.

2. Material & methods

2.1. Mice

Ptpn2$^{fl/fl}$ (C57BL/6/J),*Lck*-Cre;*Ptpn2*$^{fl/fl}$ (C57BL/6/J) and RIP-mOVA (C57BL/6/J) mice were maintained on a 12 h light–dark cycle in a temperature-controlled high barrier facility with free access to food and water. Age- and sex-matched 6–10 week old female or male recipient mice and 3–6 week old male or female donor mice were used for adoptive transfer experiments. For *ex vivo* experiments either male or female mice were used. *Ptpn2*$^{fl/fl}$ and *Lck*-Cre;*Ptpn2*$^{fl/fl}$ mice and the corresponding OT-I or OT-II TCR transgenic mice were described previously [32]; mice were backcrossed onto the C57BL/6/J background every 5-6 generations. RIP-mOVA mice have been described previously [7] and were maintained by breeding syngenic, non-transgenic C57BL/6/J mice with RIP-mOVA transgenic mice. Experimental mice were generated by mating OT-I;*Ptpn2*$^{fl/fl}$ and OT-I;*Lck*-Cre;*Ptpn2*$^{fl/fl}$, OT-I;*Lck*-Cre;*Ptpn2*$^{fl/fl}$ and C57BL/6/J, or OT-II;*Ptpn2*$^{fl/fl}$ and OT-II;*Lck*-Cre; *Ptpn2*$^{fl/fl}$ mice. RIP-mOVA transgenic mice were provided by William Heath (University of Melbourne, Australia).

2.2. Materials

Recombinant mouse IL-2 was purchased from PeproTech. SIINFEKL peptide was purchased from JPT Peptide Technologies. Hamster α-mouse CD3ε (145-2C11), α-mouse CD28 (37.51) and the FITC-Annexin V Apoptosis Detection Kit I were purchased from BD Biosciences. Mouse α-tubulin (Ab-5) was purchased from Sigma–Aldrich and mouse α-PTPN2 (6F3) was provided by Michel Tremblay (McGill University, Canada). The FoxP3 Staining Buffer Set and the Cell Stimulation Cocktail (plus protein transport inhibitors) were purchased from eBiosciences. Fetal bovine serum (HyClone) was purchased from Thermo Scientific and Dulbecco-Phosphate Buffered Saline (D-PBS) and 1640 RPMI from Invitrogen.

2.3. Flow cytometry

Single cell suspensions from freshly dissected LNs were processed for flow cytometry as described previously [32]. For surface staining, cells (1×10^6/10 μl) were resuspended in D-PBS/2% FBS and stained in 96-well microtiter plates (Falcon, BD Biosciences) for 20 min on ice. For sorting, cells were stained in 15 ml Falcon tubes (BD Biosciences) for 30 min on ice. Cells were washed and resuspended in D-PBS/2% FBS and analysed using a LSRII (BD Biosciences) or purified using an Influx sorter (BD Biosciences). Purified $CD8^+CD62L^{hi}CD44^{lo}$ T cells were routinely tested for purity (>99%). Data was analysed using FlowJo7 (Tree Star Inc.) software.

2.4. Antibodies

The following antibodies from BD Pharmingen (San Jose, CA) were used for staining: Fluorescein-isothiocyanate (FITC)-conjugated or BD Horizon™ V450-conjugated α-CD44 (IM7), phycoerythrin (PE)-conjugated or allophycocyanin (APC)-conjugated α-CD62L (MEL-14), Pacific Blue-conjugated or Alexa Fluor 647-conjugated α-CD8 (53-6.7), PE-conjugated α-CD25 (PC61), PE-conjugated TCR-Vα2 (B20.1), APC-conjugated or PE-conjugated TCR-Vβ5.1/5.2 (MR9-4) and PE-cyanine dye 7 (Cy7) α-CD69 (H1.2F3). PE-conjugated α–IFNγ (XMG1.2) and FITC-conjugated granzyme B (GB11) were purchased from Biolegend.

2.5. Assessment of CTL-activity

Naive $CD8^+$ ($CD62L^{hi}CD44^{lo}$) LN T cells (20×10^5) from 3 to 4 week old OT-I mice were purified by FACS and intravenously injected into the tail vein of RIP-mOVA mice. At day 9 post-transfer pancreatic LNs were harvested and homogenised. Cells were resuspened in 1640 RPMI complete and stimulated with the Cell Stimulation Cocktail [containing phorbol 12-myristate 13-acetate (PMA), Ionomcyin, Brefeldin A and Monensin] for 5 h at 37 °C. Cells were fixed and permeabilised with the FoxP3 Staining Buffer Set according to the manufacturers' instructions. Cells were stained with fluorochrome-conjugated antibodies against CD8, TCR-Vα2, TCR-Vβ5, IFNγ and granzyme B and analysed by flow cytometry.