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PTPN22 controls virally-induced autoimmune diabetes by modulating cytotoxic T lymphocyte responses in an epitope-specific manner

Georgia Fousteri*, Tatiana Jofra, Roberta Di Fonte, Mirela Kuka, Matteo Iannacone, Manuela Battaglia

Division of Immunology Transplantation and Infectious Diseases (DITID), Diabetes Research Institute (DRI) Istituto Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute, Milan, Italy

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Abstract *Ptpn22* is one of the most potent autoimmunity predisposing genes and strongly associates with type 1 diabetes (T1D). Previous studies showed that non-obese diabetic mice with reduced expression levels of Ptpn22 are protected from T1D due to increased number of T regulatory (Treg) cells. We report that lack of *Ptpn22* exacerbates virally-induced T1D in female rat insulin promoter lymphocytic choriomeningitis virus (RIP-LCMV-GP) mice, while maintaining higher number of Treg cells throughout the antiviral response in the blood and spleen but not in the pancreatic lymph nodes. GP_{33-41}-specific pentamer-positive cytotoxic lymphocytes (CTLs) are numerically reduced in the absence of *Ptpn22* at the expansion and contraction phase but reach wild-type levels at the memory phase. However, they show similar effector function and even a subtle increase in the production of IL-2. In contrast, $NP_{396-404}$-specific CTLs develop normally at all phases but display enhanced effector function. Lack of *Ptpn22* also augments the memory proinflammatory response of GP_{61-80} CD4 T cells. Hence, lack of *Ptpn22* largely augments antiviral effector T cell responses, suggesting that caution should be taken when targeting Ptpn22 to treat autoimmune diseases where viral infections are considered environmental triggers.

1. Introduction

Ptpn22 encodes for the protein tyrosine phosphatase LYP (in humans, PEP in mice), which is involved not only in T- and B- but also in innate-cell signaling and it is associated with susceptibility to various autoimmune diseases [1,2].

Abbreviations: Ptpn22, protein tyrosine phosphatase non-receptor type 22; RIP, rat insulin promoter; LCMV, lymphocytic choriomeningitis virus; CTL, cytotoxic lymphocyte; Treg, T regulatory; WT, wild-type; KO, knockout; T1D, type 1 diabetes; pancLN, pancreatic lymph node; EM, effector memory; CM, central memory.

* Corresponding author. Fax: +39 022643 4668.
E-mail address: fousteri.georgia@hsr.it (G. Fousteri).

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With regard to type 1 diabetes (T1D), the *Ptpn22* susceptibility allele *C1858T* has emerged as one of the strongest non-HLA (human leukocyte antigen) genetic risk factors [3]. Therefore, understanding the contribution of *Ptpn22* in autoreactive T-cell development and function might provide new ways to treat T1D. Previous studies showed that reduced [4] or increased [5] levels of Ptpn22 can prevent T1D in non-obese diabetic (NOD) mice, the most commonly used model of spontaneous autoimmune diabetes. Here, we tested the role of *Ptpn22* in a virally-induced model of T1D, where an acute viral infection triggers the disease.

The rat insulin promoter (RIP)-lymphocytic choriomeningitis virus (LCMV) transgenic mouse model was developed more than two decades ago [6,7] and has served in elucidating different immunological rules that govern islet autoimmunity triggered by an acute viral infection. The transgenic mice express the LCMV glycoprotein (GP) antigen under control of the RIP in pancreatic β cells. Viral infection with LCMV induces rapid CD8-dependent T1D that initiates right after viral clearance. This model has served to address not only the effectiveness but also the safety of several treatments known to abrogate T1D in NOD mice and has helped in elucidating how specific genetic determinants interact with environmental triggers (i.e., viral infections) [8].

Anti-LCMV-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) can be easily tracked and studied in RIP-LCMV-GP mice by class I LCMV-specific pentamers and intracellular cytokine staining following LCMV-specific peptide stimulation. Pathogenic CTLs express an effector memory (EM) (i.e., $CD44^{hi}CD62L^{lo}$) phenotype and are functionally characterized by co-production of TNF-α and interferon (IFN)-γ [9]. Whereas T1D in RIP-LCMV-GP mice occurs in a CD4-independent fashion [10], CD4-mediated T-cell help is important for efficient memory CTL development [11]. Progression to T1D in RIP-LCMV-GP mice is influenced by several factors and the most important ones regard the generation and function of LCMV-specific CTLs [12], and the production of proinflammatory cytokines, such as type I IFNs [13], IL-12 [14] and IFN-γ [15]. Interestingly, T1D can be also modulated in RIP-LCMV-GP mice with the induction of $FOXP3^+$ T regulatory (Treg) cells [16–18].

It appears that *Ptpn22* modulates several innate and adaptive immune responses that are triggered by LCMV, suggesting that *Ptpn22* might play a role in T1D development in RIP-LCMV-GP mice. For example, lack of *Ptpn22* reduces DC activation and type I IFN production [19], but amplifies T- and B-cell activation [20], germinal center (GC) and T follicular helper (T_{FH}) cell development [21], and Treg [4,22,23] and memory T-cell generation [24,25]. Furthermore, lack of *Ptpn22* enhances the activation and proinflammatory cytokine production of T cells responding to weak but not strong epitopes [25]. Here, we studied the role of *Ptpn22* on Treg cell and LCMV-specific CTL formation during T1D pathogenesis in wild-type (WT) and *Ptpn22* knockout (KO) RIP-LCMV-GP mice. We demonstrate that female RIP-LCMV-$GP^{Ptpn22KO}$ mice contain a higher number of Treg cells but develop T1D with higher incidence as compared to ^{WT} littermates. Moreover, exacerbation of virally-induced T1D in the absence of *Ptpn22* is associated with amplified EM cytokine production by a subset of antigen-specific CTLs and $CD4^+$ T cells.

2. Materials and methods

2.1. Mice

Homozygous *Ptpn22*-deficient mice, referred throughout the manuscript as KO (knockout), were previously described [24]. Mice were interbred with RIP-LCMV-GP transgenic [6] and the heterozygous (HET) offspring were intercrossed to obtain RIP-LCMV-$GP^{Ptpn22WT}$ (wild-type), ^{HET} and ^{KO} littermates that were used throughout the study. All mice were housed under spf conditions in compliance with guidelines of the San Raffaele Institutional Animal Care and Use Committee (IACUC #479).

2.2. Viruses and infection

LCMV Armstrong plaque-purified was prepared by a single passage on BHK-21 cells and used throughout experiments. To induce T1D, 8–12-week-old mice were infected with a single dose of 10^3 PFU i.p. To measure viral load, mice were infected with 2×10^5 PFU i.p.

2.3. Blood glucose monitoring

Blood glucose was monitored with OneTouch Ultra at weekly intervals. Diabetes was defined as blood glucose values over 300 mg/dl.

2.4. Peptides

Peptides used for viral studies were the dominant D^b-restricted LCMV epitopes $GP_{33–41}$, and $NP_{396–404}$ and the I-A^b-restricted epitope $GP_{61–80}$ (all from Proimmune).

2.5. Immunohistochemistry

Tissues were immersed in Tissue-Tek OCT (Bayer) and quick frozen on dry ice. Using cryomicrotome and Superfrost Plus slides (Fisher Scientific), 6-μm tissue sections were cut. Sections were fixed with 100% acetone at room temperature, and after washing in TBS, an avidin/biotin-blocking step was included (Vector Laboratories). Primary and secondary antibodies (Vector Laboratories) were reacted with the sections for 60 min each, and color reaction was obtained by sequential incubation with Vector Blue AP III and AEC (Vector Laboratories) as previously described [26]. Primary antibodies used were rat anti-mouse CD8a (Ly2)-biotin, rat anti-mouse CD4 (L3T4; BD Biosciences)-biotin and guinea pig anti-swine insulin (DAKO). Goat anti-guinea pig AP was used to detect insulin [26].

2.6. Virus titration

LCMV titres of infected spleens were determined in a virus plaque assay as previously described [27]. Cells from the fibrosarcoma cell line MC57 were plated at a concentration of 2.5×10^5 in 24-well plates. Tissue samples (frozen at −80 °C in MEM-0% FCS) were thawed, homogenized, and added to the MC57 cells in 5-fold dilution steps. The virus was allowed to infect MC57 cells for 4 h, then methylcellulose (Methocel, Sigma, Mo., US) was diluted 1:2 in 2× Dulbecco