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The effect of the autoimmunity-associated gene, PTPN22, on a BXSB-derived model of lupus

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Abstract A single nucleotide polymorphism in PTPN22 is linked to increased disease susceptibility in a range of autoimmune diseases including systemic lupus erythematosus (SLE). PTPN22 encodes the Lyp phosphatase that dampens TCR signaling and is necessary for signaling downstream of toll-like receptors in myeloid cells. To understand these dual functions in disease, we examined the impact of deficiency in PTPN22 on a spontaneous murine model of SLE. Male PTPN22 KO mice carrying BXSB chromosome 1 and the *Yaa* disease accelerating factor developed disease at a similar rate and severity as PTPN22 WT. In contrast, although female mice showed no differences in survival in the absence of PTPN22, autoantibody production was significantly increased and splenic populations associated with pathogenesis in this model were expanded in the PTPN22 KO group. These findings support the notion that when coupled with other predisposing autoimmunity genes, PTPN22 deficiency contributes to a predisposition to lupus pathogenesis.

1. Introduction

The autoimmunity associated allele of PTPN22, R620W (C1858T), has been linked to a number of autoimmune conditions in humans such as type I diabetes (T1D) [1], rheumatoid arthritis (RA) [2] and systemic lupus erythematosus (SLE) [3]. To investigate the role PTPN22 plays in these diseases numerous mouse models of autoimmunity in which PTPN22 has been deleted, overexpressed, knocked down or mutated are beginning to emerge [4–8].

For SLE, multiple studies have shown increased risk associations between the C1858T SNP in PTPN22 [3,9–11] over a range of ethnic populations with odds ratios (OR) for the T allele varying between 1.32 and 2.56. Interestingly,

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one study showed a stronger relationship between the PTPN22 risk variant in pediatric-onset SLE in Mexican populations than in adult-onset SLE in Caucasians [12]. Recent reports have even suggested correlation of this SNP with distinct disease subclasses, for example those patients with anti-cardiolipin antibodies [13].

PTPN22 encodes a phosphatase known as lymphoid tyrosine phosphatase (LYP) in humans or PEST-enriched phosphatase (PEP) in mice. Its function is best characterized in T cells in which it functions to dephosphorylate proximal TCR signaling molecules (Lck, ZAP-70, Src family kinases) that regulate TCR driven activation [4,14,15]. Loss of PTPN22 on the B6 background results in accumulation of memory T cells and increased germinal centers and serum IgG although these mice do not exhibit more autoantibodies or autoimmunity, possibly due to increased Treg numbers and function, or the lack of other factors that contribute to autoimmune disease [4,16,17]. Studies have suggested a role for PTPN22 in B cell signaling [18–22], although the extent to which this may be a consequence of increased T cell help is unresolved [4,23]. Recently a novel, non-phosphatase role for PTPN22 in myeloid cell activity has been described downstream of TLR signaling which is necessary for efficient type I IFN production [24].

Two recent reports in which mice were engineered to express a mutation (R619W) analogous to the human R620W variant have described a phenotype similar to that of the PTPN22 KO mouse [5,19]. The most recent of these papers describes the breaking of tolerance in these mice manifesting in systemic autoimmunity when on a mixed 129/B6 genetic background, a phenotype that is lost with successive backcrosses to B6 [19]. PTPN22 KO or mutation is not sufficient to develop spontaneous autoimmunity so the use of established autoimmune models or multiple gene knock-outs/mutations have been used to study this gene in a disease specific context [6,7,17,23,25].

The BXSB mouse, a recombinant inbred strain derived from C57BL/6 and SB/Le mice, develops SLE [26,27]. This disease is characterized by B cell hyperplasia in peripheral lymphoid organs. Male mice develop more rapid and severe disease compared to females due to the presence of a Y chromosome linked accelerating factor (*Yaa*) that is an X chromosome translocation resulting in duplication of at least 16 genes including the *Tlr7* gene [28,29]. Males typically die around 4–5 months of age and pathology includes immune complex mediated renal disease. Females typically have a 50% survival of approximately 19.4 months of age but develop detectable levels of autoantibodies earlier [26,27]. BXSB susceptibility regions aside from the *Yaa* locus can be found on chromosomes 1, 3 and 13 [30]. Regions on chromosome 1 that have been shown to confer lupus phenotypes include *Bxs1–4* [31,32]. The *Yaa* locus alone is insufficient to cause a disease on non-autoimmune prone backgrounds but accelerates disease on the lupus prone backgrounds through a TLR7/type I IFN mechanism [28,29,33]. Type I IFN is crucial to disease in both mouse models and human lupus [34,35].

To investigate the effect of PTPN22 on SLE we introduced regions from chromosome 1 of BXSB on PTPN22 KO, this report is the first to describe the effect of PTPN22 on a classical, spontaneous mouse model of lupus.

2. Materials and methods

2.1. Mice

Experimental procedures were carried out according to the National Institutes of Health Guide for the care and use of laboratory animals and approved by the Scripps Institutional Animal Care and Use Committee. PTPN22$^{-/-}$ (KO) mice were obtained from Dr. Andrew Chan (Genentech, San Francisco, CA) and have previously been described [4]. BXSB/Scr mice were obtained from Scripps breeding colony and bred to PTPN22$^{-/-}$ mice. Male BXSB-*Yaa* were crossed to female PTPN22$^{-/-}$ mice and the F1 mice were then bred to female BXSB mice until all selected microsatellite regions on chromosome 1 were homozygous for BXSB. The resulting mice, known as BXSB;B6 c1(19.8–174.9 Mb)$^{BXSB/BXSB}$–*PTPN22*$^{+/-}$ were then interbred to yield BXSB;B6 cl(19.8–174.9 Mb)$^{BXSB/BXSB}$–*PTPN22*$^{+/+}$ and BXSB;B6 c1(19.8–174.9 Mb)$^{BXSB/BXSB}$–*PTPN22*$^{-/-}$ and used in subsequent assays. Microsatellite markers used to track BXSB desired regions were *D1mit3*, *D1mit21*, *D1mit387* and *D1mit206* (this includes chromosome 1 regions between 19.8 and 174.9 Mb) as described in the first report of this mouse strain [32]. The resulting mouse strain is homozygous for BXSB chromosome 1 and a mixture of BXSB and B6 in the remainder of the genome. For clarity we will refer to these mice as BXSB;B6 in this manuscript.

2.2. Flow cytometry

Cells to be stained were resuspended in FACS buffer (HBSS containing 1% FCS) and incubated with the indicated antibodies for 15 min on ice. The cells were then washed in FACS buffer before acquisition on an LSR-II flow cytometer (BD Biosciences, Franklin Lakes, NJ) and analysis using FlowJo (Treestar). Antibodies (BioLegend, San Diego, CA unless otherwise stated) used were anti-mouse CD4 PerCP-Cy5.5, CD8 Pacific Blue/APC-cy7, PD-1 FITC, CXCR5-biotin (BD Biosciences), CD44 Pacific Blue, GL-7 FITC, FAS PE, CD138 APC, CD19 APC-cy7, CD23 PE, CD21 PerCP-Cy5.5, CD11b-biotin, CD11c Pacific Blue/APC, B220 PE, PDCA-1 Pacific Blue and streptavidin APC/FITC/PerCP. For intracellular staining of markers, an intracellular staining kit (Fix/Perm, eBioscience, San Diego, CA) was used together with anti-mouse Foxp3 PE (eBioscience).

2.3. ELISA

Serum was collected from the mice at the stated time points. MaxiSorp plates (Nunc, Rochester, NY) were coated with 3.6 μg/ml of chromatin overnight at 4 °C. Plates were blocked in 1% gelatin (Sigma Aldrich) for an hour at 37 °C. Plates were washed three times with wash buffer (HBSS with 0.1% Tween-20 (Sigma Aldrich)). Sera were diluted accordingly following optimization for each experiment in the reagent buffer (HBSS containing 1% BSA, 0.1% Tween-20) and incubated on the plate in duplicate for 1 h at 37 °C. The plates were washed three times. Anti-mouse IgG alkaline phosphatase (AP) was then diluted and added to the wells for a further hour at 37 °C (Jackson ImmunoResearch). The plates were washed and then incubated with a pNPP AP substrate (Sigma Aldrich). The plates were read using a VersaMax Plate Reader (Molecular Devices, Sunnyvale, CA) at 405 nm.