Loss of Protein Tyrosine Phosphatase Nonreceptor Type 22 Regulates Interferon-γ–Induced Signaling in Human Monocytes

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BACKGROUND & AIMS: A gain-of-function variation within the locus that encodes protein tyrosine phosphatase nonreceptor type (PTPN)22 is associated with a reduced risk for Crohn's disease (CD), whereas a loss-of-function variant seems to promote autoimmune disorders. We investigated how loss of PTPN22 could contribute to chronic inflammation of the intestine. **METHODS:** Intestinal tissue samples from patients with or without inflammatory bowel disease (controls) were analyzed for levels of *PTPN22* messenger RNA (mRNA) and protein. In human THP-1 monocytes, protein levels were analyzed by immunoblotting, mRNA levels by real-time polymerase chain reaction, and cytokine release by enzyme-linked immunosorbent assay. **RESULTS:** Intestinal tissue samples from patients with CD had reduced levels of *PTPN22* mRNA and protein, compared with samples from controls. In human THP-1 monocytes, interferon-γ (IFN-γ) induced expression and activity of PTPN22. Loss of PTPN22 increased levels of p38-mitogen-activated protein kinase, but reduced phosphorylation of nuclear factor-κB subunits. Increased activity of suppressor of cytokine signaling-1 was accompanied by reduced phosphorylation of signal-transducer and activator of transcription protein 1 and signal-transducer and activator of transcription protein 3 in PTPN22-deficient cells incubated with cytokines. PTPN22 knockdown increased secretion of the inflammatory cytokines interleukin (IL)-6 and IL-17, but reduced expression or secretion of T-bet, intercellular adhesion molecule-1, nucleotide-binding oligomerization domain containing-2, monocyte chemoattractant protein-1, IL-2, and IL-12p40 in response to IFN-γ. **CONCLUSIONS: PTPN22 expression is reduced in intestinal tissues of patients with active CD. PTPN22 regulates intracellular signaling events and is induced by IFN-γ in human monocytes. Knockdown of PTPN22 alters activation of inflammatory signal transducers, increasing secretion of T-helper 17–related inflammatory mediators. Genetic variants that reduce PTPN22 activity might contribute to the pathogenesis of CD by these mechanisms.**

Keywords: SOCS1; ICAM1; MCP1; Th17.

Crohn's disease (CD) and ulcerative colitis (UC) are the most common forms of inflammatory bowel disease (IBD). For both diseases, genetic, bacteriologic, and immunologic factors contribute to disease onset and persistence.[1] In health, the intestinal immune system is tightly controlled by a balance between proinflammatory and anti-inflammatory cytokines.[2] In contrast, in IBD patients, this balance is disturbed, resulting in increased levels of proinflammatory cytokines. CD especially is associated with highly increased levels of interferon-γ (IFN-γ).[3] Although IFN-γ generally is regarded as a proinflammatory cytokine, it also seems to exert anti-inflammatory effects[4] because IFN-γ deficiency aggravates inflammation in a mouse model of colitis.[5]

Upon IFN-γ binding to its receptor, receptor-associated Janus kinase (Jak)1 and Jak2 get phosphorylated (meaning activated), leading to the phosphorylation of IFN-γ receptor and signal-transducer and activator of transcription protein (STAT)1.[6] Phosphorylated STAT1 translocates into the nucleus where it acts as a transcription factor. STAT signaling is regulated by suppressor-of-cytokine-signaling (SOCS)-1 and SOCS3, which suppress Jak1 and STAT1 activity.[7] In addition, IFN-γ also activates mitogen-activated protein kinase (MAPK) isoforms, such as p38.[8]

Functionally, IFN-γ signaling results in enhanced levels of intracellular adhesion molecule (ICAM)-1, nucleotide-binding oligomerization domain-containing protein (NOD)2, and interleukin (IL)-6, all of them relevant for IBD pathology.[9,10] Of interest, IL-6, STAT proteins, as well as SOCS1 and SOCS3, are all involved in the differentiation of naive $CD4^+$ T cells into various T-helper cell populations.[11] Alterations in T-helper cell populations are clearly involved in IBD pathogenesis, with increased T-helper (Th)1 and/or Th17 populations proposed as driving forces for CD.[12,13]

Abbreviations used in this paper: CD, Crohn's disease; ERK, extracellular signal–regulated kinase; IBD, inflammatory bowel disease; ICAM, intracellular adhesion molecule; IFN-γ, interferon γ; IκB, inhibitor of κB; IL, interleukin; Jak, Janus kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein 1; mRNA, messenger RNA; NOD2, nucleotide-binding oligomerization domain-containing protein 2; PTPN22, protein tyrosine phosphatase nonreceptor type 22; siRNA, small interfering RNA; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; T-bet, T-box transcription factor 21; TGF, transforming growth factor; Th, T helper; UC, ulcerative colitis.

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Genome-wide association studies revealed that variations within the gene locus encoding protein tyrosine phosphatase nonreceptor type 22 (PTPN22) are involved in a wide range of autoimmune-associated diseases, such as rheumatoid arthritis, systemic lupus erythematosus, or IBD.[14] Although a gain-of-function mutation in PTPN22 protects from CD, a loss-of-function mutation within the PTPN22 gene is associated with a reduced risk for developing UC.[15] On a functional level, in general, protein tyrosine phosphatases are important regulators of the immune system by interfering with intracellular signaling cascades.[16] In particular, PTPN22 is involved in regulating B- and T-cell–receptor signaling,[17,18] but additional functions in other cell types and pathways are less studied. Disease-associated variations within the PTPN22 gene result in altered natural killer cell proliferation,[19] but only a few studies have addressed its function in myeloid cells.[20]

The aim of this study was to address the relevance of PTPN22 in IFN-γ–activated human THP-1 monocytes. We show that PTPN22 levels are increased in THP-1 cells in response to IFN-γ but reduced in the intestine of CD patients. On a functional level, loss of PTPN22 leads to alterations in STAT activation and crucially interferes with the secretion profile of proinflammatory cytokines, finally triggering the milieu for a Th17 phenotype.

Materials and Methods

Reagents and Antibodies

All reagents were obtained commercially and are of analytic grade. Details on the antibodies and assays used are provided in the Supplementary Materials and Methods section.

Patient Samples

Intestinal tissue specimens were taken by endoscopy from the terminal ileum, colon, or rectum of patients with CD or UC, respectively, as well as from non-IBD control patients. Patients with active CD (for Western analysis: n = 6; age range, 31–75 y; mean, 49 ± 6 y; for mRNA analysis: severe, n = 6; age range, 20–60 y; mean, 33 ± 16 y; moderate, n = 9; age range, 19–66 y; mean, 38 ± 17 y) and active UC (n = 5; age range, 19–53 y; mean, 33 ± 15 y) presented clinically and macroscopically with signs of acute inflammation. Biopsy samples were taken from macroscopically inflamed–appearing regions. Patients with CD and UC in remission (CD: n = 14; age range, 20–72 y; mean, 38 ± 16 y; UC: n = 5; age range, 27–42 y; mean, 32 ± 6 y) presented for surveillance endoscopy and showed no clinical or macroscopic signs of acute or chronic inflammation. Biopsy samples were taken from macroscopically noninflamed–appearing regions. Control patients (n = 7; age range, 37–75; mean, 54 ± 5 y Western analysis; and n = 21; age range, 28–68 y; mean, 54 ± 16 y for polymerase chain reaction analysis) were asymptomatic and presented for colon cancer screening. Written informed consent was obtained before specimen collection and studies were approved by the local ethics committee. Supplementary Tables 1 and 2 provide details on patient characteristics and the Supplementary Materials and Methods provides additional information about sample processing.

Small Interfering RNA Transfection

Transfection of small interfering RNA (siRNA) constructs was performed using the Amaxa Nucleofector Kit (Lonza, Walkersville, MD) as described previously[21] and in detail in the Supplementary Materials and Methods section. Knockdown efficiency was assessed after each experimental set by Western blot or real-time polymerase chain reaction. The knockdown efficiency was always between 30% and 50%. Representative results are shown where appropriate. The used siRNA constructs were commercially obtained from Applied Biosystems (Foster City, CA) and assay IDs are listed in Supplementary Table 3.

Statistical Analysis

Data are presented as means ± standard error of the mean for a series of n experiments. Data are expressed as relative values of the respective control. Statistical analysis was performed by analysis of variance followed by the Student–Newman–Keuls post hoc test or the Mann–Whitney test (Figure 1*E* and *F* and Supplementary Figure 1), where appropriate. *P* values less than .05 were considered significant.

All further techniques were performed according to standard protocols as described previously[21–23] and in detail in the Supplementary Materials and Methods section. Detailed information about the assays used also can be found in the Supplementary Materials and Methods section.

Results

PTPN22 is Increased by IFN-γ in Human THP-1 Monocytes

We first investigated whether the proinflammatory cytokine IFN-γ regulates expression and activity of PTPN22 in human THP-1 monocytes. We stimulated THP-1 cells with a previously validated concentration of 1000 U/mL IFN-γ[21] for different time points and found increased PTPN22 messenger RNA (mRNA) levels after 2, 4, 8, and 48 hours ($P < .05$ and $P < .01$; Figure 1*A*). On a protein level, we found increased PTPN22 by 24 hours of IFN-γ treatment that was increased even further by 48 hours of treatment ($P < .001$; Figure 1*B*). Increased PTPN22 mRNA and protein levels were accompanied by an increase in relative enzymatic phosphatase activity of PTPN22, which already was detectable after 30 minutes of IFN-γ stimulation ($P < .01$; Figure 1*C*). To verify the effect of IFN-γ on PTPN22 expression in primary cells, we treated peripheral blood mononuclear cells from healthy donors for increasing time with IFN-γ. We found enhanced PTPN22 mRNA expression after 24 and 48 hours ($P < .01$ each, Figure 1*D*). Taken together, these data show that IFN-γ treatment increases PTPN22 expression and activity in THP-1 monocytes.

PTPN22 mRNA Expression is Decreased in Crohn's Disease

To show the relevance of our findings for human disease, we next studied PTPN22 expression in intestinal biopsy specimens from a Swiss cohort of non-IBD control and IBD patients. We tested PTPN22 expression in tissue samples obtained from terminal ileum,