Elevated Levels of Mast Cells Are Involved in Pruritus Associated with Polycythemia Vera in JAK2V617F Transgenic Mice

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Elevated Levels of Mast Cells Are Involved in Pruritus Associated with Polycythemia Vera in JAK2V617F Transgenic Mice

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Pruritus occurs frequently in patients with polycythemia vera (PV), and the pathophysiology of PV-associated pruritus is unclear. We have previously demonstrated that transgenic mice expressing JAK2V617F displayed clear PV-like phenotypes. In the current study, we found frequent occurrence of pruritus with aged JAK2V617F transgenic mice and further investigated the underlying mechanisms by studying mast cells, key players in allergic reactions and anaphylaxis. Massive accumulations of mast cells were observed in the skin of pruritic JAK2V617F transgenic mice. In vitro culture yielded much higher mast cell counts from the bone marrow, spleen, peripheral blood, and peritoneal cavity of JAK2V617F transgenic mice than from controls. Cultured mast cells from JAK2V617F transgenic mice exhibited enhanced proliferative signals, relative resistance to cell death upon growth factor deprivation, and a growth advantage over control cells under suboptimal growth conditions. However, these mast cells displayed normal morphology and contained normal levels of mast cell proteases before and after degranulation. Finally, the JAK2 inhibitor G6 effectively reduced mast cell numbers and alleviated pruritus in JAK2V617F transgenic mice. Collectively, these data demonstrate that mast cells are involved in PV-associated pruritogenesis and that JAK2 inhibitors are potential antipruritis drugs. The Journal of Immunology, 2014, 193: 000–000.

Polycythemia vera (PV) is a Philadelphia chromosome-negative myeloproliferative neoplasm (MPN) characterized by an absolute increase in RBC mass accompanied by an increased production of platelets and WBCs (1–4). A classical symptom of PV is pruritus, which occurs in ∼48–65% of patients (5, 6). Characteristically triggered by sudden environmental changes, such as contact with water, PV-associated pruritus is reported to be the top reason for significantly impaired quality of life among patients (5–7). However, the pathophysiology is not clear, and effective treatments of PV-associated pruritus are lacking (7–10).

Mast cells are best known for their involvement in IgE-mediated allergic responses (11–13). A variety of mast cell–derived mediators, including histamines, proteases, lipid mediators, neuropeptides, and various cytokines, have been identified as being involved in the elicitation or modulation of pruritus (14–17). Recent studies demonstrated a significantly increased level of pruritogenic factors in mast cells derived from the peripheral blood of PV patients (18, 19), indicating that functional abnormalities of mast cells may be the cause of PV-associated itching. However, the underlying cellular and molecular mechanism has yet to be defined.

PV is associated with JAK2V617F, a gain-of-function mutant form of tyrosine kinase JAK2, found in >95% of PV patients (2–4). The pathogenesis of JAK2V617F has been demonstrated by transgenic and knockin mouse models (20–26). In earlier studies, we generated JAK2V617F transgenic mice using the vav promoter to drive transgene expression in the hematopoietic system, and these mice recapitulate clear PV-like clinical features, including elevated levels of RBCs, platelets, and WBCs (22). This provides us with an excellent animal model to study PV and complications associated with the disease. In this study, we demonstrated the occurrence of pruritus among these JAK2V617F transgenic mice and provided evidence for the involvement of mast cells in the pathogenesis of PV-associated pruritus. We further identified the JAK2 inhibitor G6 as a potential antipruritis drug.

Materials and Methods

Mice and drug treatment

The generation of JAK2V617F transgenic mice has been described previously (22). The mice were crossed with wild-type C57BL/6 mice for >10 generations (27). Homozygous JAK2V617F mice were used in this study. Nontransgenic sibling or wild-type C57BL/6 mice were used as controls. Animals were housed in ventilated cages under standardized conditions. JAK2 inhibitor G6 was synthesized as previously described, with a purity >99% (28). It was dissolved in DMSO and diluted 4-fold with PBS for the treatment of pruritic JAK2V617F transgenic mice. The drug was administered i.p. at a daily dose of 10 mg/kg for 4 wk. The pruritus status and severity were assessed blinded.

Histamine and cytokine analyses

Skin samples (50–100 mg) were homogenized in PBS and centrifuged at 16,000 × g, 4°C for 10 min, and the resulting supernatants were used to evaluate histamine content and cytokines. Histamine was determined using...
an enzyme immunoassay kit from Cayman Chemical (Ann Arbor, MI), and mouse IL-6 using IL-6 Quantikine ELISA Kit from R&D Systems (Minneapolis, MN).

Culture of mast cells
Mast cells were cultured in IMDM supplemented with 20% FBS in the presence of mouse IL-3 and stem cell factor (SCF), as previously described (29). IL-3 and SCF were obtained in the form of conditioned media of cultured CHO cells overexpressing IL-3 and SCF. Unless otherwise indicated, the concentrations of IL-3 and SCF were 1% conditioned medium. Cell cultures were maintained for ≤5 mo with equal volumes of fresh medium added every 3–5 d. For determination of mast cell concentrations and purity, cells were stained with PE-labeled anti-mouse CD117 and FITC-labeled anti-mouse FceR1 (eBioscience) and then subjected to flow cytometric analyses. Dead cells were excluded by staining with 7-amino-actinomycin D, and CountBright absolute counting beads (Invitrogen) were used as a standard for cell counting. Bone marrow–derived mast cells (BMMCs) were used for further analyses, unless indicated otherwise. For mast cell colony assays, the culture medium also contained 1% methylcellulose. Typically, 2–10 × 10⁶ nucleated cells from bone marrow, spleen, and peripheral blood were plated into 12-well dishes in 0.5 ml medium at 37 °C with 5% CO₂. After 9 d of culture, the number of colonies was enumerated.

Cell survival assays
BMMCs were washed twice with IMDM containing 20% FBS and further incubated in the medium at 37 °C for >20 h. Cells were then stained with Annexin V FITC and propidium iodide followed by analyses with a FACS-2-yl)-2,5-diphenyltetrazolium bromide following standard protocols (30). Cell viability was assessed by MTT assays with 3-(4,5-dimethylthiazol-

Cell stimulation and Western blot analysis
BMMCs were either incubated in IMDM containing 20% FBS in the absence of IL-3 and SCF for 4 h and then stimulated with IL-3 and SCF or charged with anti-DNP IgE (Sigma-Aldrich) overnight in complete medium with SCF and IL-3 and subsequently stimulated with Ag DNP-HSA (Sigma-Aldrich). Cells were collected in and washed with ice-cold PBS. Protein samples were prepared by addition of SDS gel sample buffer directly into the cell pellets. Western blotting analyses were performed with Abs against JAK2, ERK1/2, phospho-ERK1/2 (Thr204/Tyr204), and phospho-MEK1/2 (Ser217/221) (Cell Signaling Technology) followed by HRP-conjugated secondary Abs. Detection and quantification of ECL signals were done using the FluorChem SP imaging system from Alpha Innotech.

Tissue and cell staining
For histological analysis, tissues were fixed in formaldehyde and embedded in paraffin. Tissue sections (5 μm) were deparaffinized and then stained with 0.1% toluidine blue for 20 min to reveal mast cells in the skin. For Wright–Giemsa staining, cells were spun onto glass slides by cytocentrifugation.

Statistical analysis
Statistical analyses were performed using the GraphPad Prism program. Differences between two groups of samples were assessed using t tests. Fisher’s exact best was used to assess the prevalence of pruritus and the response of pruritic mice to drug treatment. A p value < 0.05 (two-tailed) was considered significant.

Results

JAK2V617F transgenic mice display severe pruritus as they age, with a massive accumulation of mast cells in the skin
We previously generated JAK2V617F transgenic mice using the vav gene promoter that drives transgenic expression in all hematopoietic cells (22). One line of these mice (line A) displayed clear PV-like phenotypes in 10 wk, with markedly increased levels of RBCs, platelets, and WBCs (22). They also developed splenomegaly and myelofibrosis as they aged. In tracking the age-dependent progression of this PV-like disease, we noticed that a significant number of JAK2V617F transgenic mice had spontaneous itching beginning at ∼40 wk of age. From the 80 mice at ages 45–55 wk we analyzed, 34 (42%) of them showed clear signs of pruritus, whereas it was completely absent in the 49 control mice of comparable ages (p < 0.0001, Table I). The rate of pruritus in JAK2V617F transgenic mice increased as the animal aged. The pruritic symptom occurred in both male and female mice without significant preference (p = 0.184). The affected mice displayed severe skin problems, including alopecia, inflammation, and even ulceration (Fig. 1A; see also Supplemental Video). Because mast cells are major mediators of allergic responses (11–13), we speculated that they may be involved in induction of itching in these JAK2V617F transgenic mice. Indeed, toluidine blue staining of flank skin and ear pinnae sections revealed massive accumulations of both granulated and degranulated mast cells in the transgenic mice (Fig. 1B, 1C). Note the thickening of the dermis and epidermis as a result of pruritus. Consistent with the presence of mast cells, the histamine content in the skin of pruritic transgenic mice was much increased, which was accompanied by an elevated level of IL-6, an inflammatory cytokine (Fig. 1D, 1E).

JAK2V617F transgenic mice have markedly increased mast cell progenitor cells
The accumulation of mature mast cells in the skin may be caused by increased production of these cells from progenitor cells in JAK2V617F transgenic mice. By performing mast cell colony-forming assays, we first evaluated mast cell progenitor cell numbers in the bone marrow, spleen, and peripheral blood of control and JAK2V617F transgenic mice. In the presence of IL-3 and SCF, bone marrow cells from JAK2V617F transgenic mice produced significantly higher numbers of mast cell colonies compared with control mice (Fig. 2A). Furthermore, the size of colonies was large on average, but individual cells had typical morphologies of mast cells, as revealed upon Wright–Giemsa staining (Supplemental Fig. 1). The large colony size suggests enhanced expansion of progenitor cells. More strikingly, spleen and peripheral blood cells from JAK2V617F transgenic mice also formed mast cell colonies, which were hardly seen with the control mice (Fig. 2A). This suggests a distribution of mast cell progenitor cells in the peripheral tissues, which is consistent with mobilization of hematopoietic stem/progenitor cells, as observed in our earlier studies (22). We further compared the expansion of mast cells in liquid culture. The cells were determined by staining cells with mast cell makers CD117 (cKit) and FceR1. Fig. 2B shows the total number of mast cells obtained after 19-d culture of 1 × 10⁶ nucleated cells from bone marrow, spleen, peripheral blood, and peritoneal cavity. As expected, JAK2V617F transgenic mice produced a significantly higher number of mast cells than did control mice at both 14 and 40 wk of age. Of interest, although control mice produced

| Table I. Prevalence of pruritus in mice 45–55 wk of age |
|----------------|-----------|---------|
|                | Total     | With Pruritis | % Pruritus |
| Tg             |           |            |           |
| Male           | 41        | 18        | 46        |
| Female         | 39        | 16        | 39        |
| Control        |           |            |           |
| Male           | 24        | 0         | 0         |
| Female         | 25        | 0         | 0         |

Tg, transgenic.
comparable numbers of mast cells at young and old ages \( (p > 0.2) \), JAK2V617F transgenic mice produced more mast cells at old age, especially with the cells from peripheral blood and spleen \( (p < 0.01) \), and this appeared to correlate with the onset of pruritus at the older ages. The cultured mast cells from control and transgenic mice reached \( 95\% \) purity after 5 wk of culture, showing typical morphology of mast cells with comparable levels of CD117 and FcεR1 expressions (Supplemental Fig. 2). In liquid culture, the growth rate decreased upon continued culture, especially for cells from the peritoneal cavity. However, at given time points the growth rate of cells from transgenic and control mice are comparable under optimal growth conditions. Together, the data indicate that JAK2V617F transgenic mice contain a markedly elevated level of mast cell progenitors in peripheral tissues as well as in the bone marrow. Conceivably, they have a greatly enhanced ability to produce a large number of mast cells. This is observed in mice with or without onset of pruritus.

**BMMCs from JAK2V617F transgenic mice exhibit enhanced growth factor signaling**

To investigate mechanisms underlying the rapid expansion of JAK2V617F-positive mast cell progenitors, we investigated whether cell signaling important to proliferation of mast cells is altered by expression of JAK2V617F. First, we analyzed the activation status of the MAPK signaling pathway in normal growing BMMCs obtained from bone marrow after 5 wk of culture in liquid medium in the presence of 1% SCF and 1% IL-3. These cells had a purity of 95%. Western blotting analysis with phospho-specific Abs revealed an elevated level of pERK1/2 and pMEK1/2 with cells from JAK2V617F transgenic mice (Fig. 3A). This is also consistent with mobility shift of ERK1/2 revealed by Western blotting with anti-ERK1/2. These findings demonstrated an increased activation of the MAPK pathway in JAK2V617F-positive BMMCs at the basal level, correlating with an increased expression of total JAK2 (Fig. 3A, top panel). We further analyzed activation of ERK1/2 upon stimulation. For this purpose, BMMCs were deprived of IL-3 and SCF starved for 4 h and then treated with 0.1% IL-3 and SCF for different periods. Growth factor deprivation caused decreased phosphorylation of ERK1/2, but the level in JAK2V617F cells remained significantly higher than in control cells. Upon stimulation with IL-3 and SCF, marked induction of ERK1/2 phosphorylation level was observed in both control and JAK2V617F BMMCs, with the latter cells reaching a higher level (Fig. 3B), indicating an enhanced response of JAK2V617F mast cells to growth factors. We also in-

**FIGURE 1.** JAK2V617F transgenic (Tg) mice develop severe pruritus and have massive accumulation of mast cells in the skin. **(A)** Representative photographs of control and pruritic JAK2V617F transgenic mice at age 45–55 wk. The red squares indicate alopecia and inflammation in JAK2V617F transgenic mice. **(B)** Representative toluidine blue staining of paraffin-embedded sections of flank skin and ear pinnae from 45-to 55-wk-old control and JAK2V617F transgenic mice. Arrows point to typical granulated and degranulated mast cells. **(C)** Quantification of mast cells per 0.2 mm² of flank skin and ear sections. **(D and E)** Determination of levels of histamine (D) and IL-6 (E) in flank skin tissues normalized with weights. Error bars denote SD \( (n \geq 5) \). \*\( p = 0.01 \), **\( p < 0.0001 \) in comparison with the correspondent control mice.
investigated signaling following activation of BMMCs induced by ligation of the high-affinity IgE receptor FcεRI via IgE. For this purpose, BMMCs were preincubated with anti-DNP IgE overnight in the presence of SCF and IL-3 and then stimulated with DNP-HSA Ag for various periods. Of note, this produced robust activation of ERK1/2 in control and JAK2V617F transgenic mice at a comparable level. This finding suggests that IgE/Ag-induced activation of ERK1/2 is independent of JAK2V617F. Collectively, our findings demonstrated that activation of the MAPK pathway is enhanced in JAK2V617F-positive BMMCs both at steady state and upon stimulation with growth factors/cytokines, which may lead to enhanced cell survival and proliferation.

BMMCs from JAK2V617F transgenic mice are relatively resistant to apoptosis induced by growth factor deprivation and display growth advantages under suboptimal growth conditions

The increased number of mast cells in JAK2V617F transgenic mice may be caused by increased proliferation and/or reduced cell death. We first evaluated cell survival in the absence of growth factors and cytokines. Following deprivation of IL-3 and SCF, BMMCs underwent rapid cell death, as revealed by positivity in annexin V and propidium iodide staining (Fig. 4A, 4B). However, significantly higher percentages of mast cells from JAK2V617F transgenic mice remained alive after starvation than did those from control mice (70.5% versus 49% after 10 h and 39% versus 13.8% after 20 h, on average). The relative resistance to cell death may be partly responsible for the elevated levels of mast cell progenitors in JAK2V617F transgenic mice. It also predicts growth advantages of JAK2V617F-positive BMMCs in suboptimal environments. When BMMCs were cultured in a medium containing 1% each of SCF and IL-3, cells grew at a comparable rate and doubled in 4 d (Fig. 4C). Further increasing the concentrations of SCF and IL-3 did not affect the growth rate significantly. However, when SCF and IL-3 were reduced to 0.1% each, the growth of control cells was nearly stopped, but cells from JAK2V617F transgenic mice continued to grow at a moderate rate, with 60% increase in cell numbers in 4 d (Fig. 4C). This observation suggests that JAK2V617F mast cells have a substantial growth advantage over control cells under suboptimal concentrations of growth factors. This can potentially explain the accumulation of mast cells in JAK2V617F transgenic mice. It also suggests hypersensitivity of the JAK2V617F cells to growth factors, consistent with the enhanced activation of ERK1/2 upon stimulation with lower concentrations of SCF and IL-3.

JAK2V617F expression does not alter the levels and secretion of mast cell enzymes chymase, tryptase, and β-hexosaminidase

As described above, mast cells from control and JAK2V617F transgenic mice displayed normal morphologies with comparable surface expressions of CD117 and FcεRI (Supplemental Fig. 2). To define further the properties of these cells, we analyzed the activity of chymase and tryptase, two major proteases stored in the secretory granules of mast cells (17). Analyses of BMMC cell extracts revealed no significant difference in the activities of these enzymes between control and JAK2V617F transgenic mice (Supplemental Fig. 3A). We further analyzed β-hexosaminidase, a granule protein and marker for evaluation of lysosome activity in mast cells (31). The enzyme activity of total β-hexosaminidase in JAK2V617F-positive BMMCs was also unchanged (Supplemental Fig. 3A). Degranulation and subsequent secretion of mast cell effectors are major functions of this cell type (11–13). We then assessed secretion of enzymes induced by ligation of the high-affinity IgE receptor FcεRI via IgE. By determining the activity of enzymes released to the medium, we did not see a significant difference in the level of secreted tryptase and β-hexosaminidase (Supplemental Fig. 3B). Taken together, the data indicate that expression of JAK2V617F does not affect the total level of key enzymes in mast cells and their secretion upon degranulation. This observation suggests that mast cells from JAK2V617F transgenic mice are functionally normal but are simply produced in a large quantity. In essence, this parallels the overabundance of functional RBCs in PV patients.

JAK2 inhibitor G6 causes effective inhibition of mast cells and provides therapeutic benefits to pruritic JAK2V617F transgenic mice

Owing to its gain-of-function nature, JAK2V617F represents an obvious target for therapeutic drug development to treat myelo-proliferative neoplasms. Indeed, many potent JAK2 inhibitors have been identified, and some have been applied to treat patients (32–34). In earlier studies, we have identified a potent JAK2 inhibitor, designated G6, that provides significant therapeutic efficacy to the bone marrow in JAK2V617F transgenic mice (28, 35–37). We thought it may be applied to treat JAK2V617F-induced pruritus. We first tested its potency at inhibiting the growth of cultured mast cells in PV-associated pruritus.

**FIGURE 2.** JAK2V617F transgenic (Tg) mice have significantly increased numbers of mast cell progenitors in bone marrow, spleen, peripheral blood, and peritoneal cavity. (A) Nucleated cells from bone marrow (BM), 2 × 10⁶ spleen (SP), 1 × 10⁶, and peripheral blood (PB) of 20-wk-old mice were plated in methylcellulose-based media in the presence of IL-3 plus SCF. Mast cell colonies were enumerated after 9 d of culture. (B) Nucleated cells (1 × 10⁶) from bone marrow, spleen, peripheral blood, and peritoneal cavity (PT) of mice at 14 or 40 wk of age were cultured in liquid media containing IL-3 and SCF. CD117/FcεR1⁺ mast cells were counted on day 19 using flow cytometry with CountBright absolute counting beads (Invitrogen) as a standard. Error bars denote SD (n ≥ 3). *p < 0.01 in comparison with correspondent control (Con) cells, **p < 0.001 in comparison with correspondent control and 14-wk transgenic mouse cells.
cells. Fig. 5 shows the results obtained from semisolid phase colony assays and liquid medium cultures. Under both conditions, G6 significantly inhibited the growth of both control and JAK2V617F mast cells at submicromolar to micromolar concentrations. Of interest, when cultured under the optimal concentrations of SCF and IL-3, control and JAK2V617F cells responded equally to G6 treatment (Fig. 5A, 5B, *p* > 0.2), but at the suboptimal concentrations of SCF and IL-3, JAK2V617F cells appeared to be more sensitive than the control cells (Fig. 5C, *p* < 0.01). This occurred because G6 mainly halted cell growth without causing major cell death, and control cells hardly grew under suboptimal concentrations of growth factors, in contrast to the continued growth of JAK2V617F cells under the same suboptimal conditions. We further applied the inhibitor to treat pruritic JAK2V617F transgenic mice and examined its efficacy in improving skin manifestations. For this purpose, JAK2V617F transgenic mice (47–52 wk) displaying mild or moderate pruritus were randomly assigned to one of two groups (four mild and four moderate in each group). These mice then received either 10 mg/kg per day of G6 or DMSO vehicle control solutions for 28 d. Along with the treatment, pruritic symptoms were evaluated based on four criteria (negative, no itching with or without alopecia;...
mild, itching with alopecia; moderate, itching with alopecia and inflammation; severe, itching with alopecia, inflammation, and ulceration). We found that G6 provided significant therapeutic improvement in itching symptoms by reversing or at least stopping the progression of pruritus, corresponding to a response rate of 100% (Fig. 6A, 6B, \( p = 0.022 \)). In contrast, mice receiving vehicle control solution progressed to more severe skin disorders during the treatment. Toluidine blue staining of ear pinnae sections revealed a significant reduction of mast cells in the pruritus-affected area of JAK2V617F transgenic mice after treatment with G6 (Fig. 6C, 6D). In conclusion, G6 inhibited mast cells and showed clear efficacy in alleviating the pruritus symptoms in JAK2V617F transgenic mice. This finding may further promote development of antipruritus drugs to treat PV patients.

Discussion
In the current study, we demonstrated the occurrence of pruritus in JAK2V617F transgenic mice, which is associated with increased numbers of mature mast cells and mast cell progenitors. Mast cells are granular immune effector cells best known for their role in IgE-mediated allergic responses (11–13). They develop from hematopoietic stem cells in the bone marrow, circulate as committed progenitors, and mature in their target tissues. Mast cells are distributed in many tissues, including the skin, gut, brain, and peritoneal cavity. They are the central source of histamine, trypsin, PGs, and leukotrienes, all of which are mediators of the inflammatory response that can generate pruritus. Associations of increased mast cells with the occurrence of pruritus have been observed in other types of mast cell–related diseases. For example, patients with mastocytosis, which is characterized by hyper-
proliferation of mast cells caused by gain-of-function mutation of cKit, usually display the complication of itching (38). In addition, atopic dermatitis patients are distinguished by markedly increased numbers of mast cells that release mediators capable of inducing severe pruritus (39), although histamine is not involved (40). Our study with JAK2V617F transgenic mice suggests that PV-associated pruritus is also associated with more mast cells. This finding is consistent with earlier studies demonstrating a greater number of mast cells obtained from CD34+ cells of MPN patients than from normal controls (18, 19).

A major feature of our pruritic JAK2V617F transgenic mice is the massive accumulation of mast cells in the skin. Involvement of mast cells in PV-associated pruritus has been a debated issue. It has been demonstrated that the numbers of papillary dermal mast cells were increased in pruritic PV patients after exposure to water (41), and the itching symptom correlated strongly with the numbers of skin mast cells, but not with circulating basophils or whole blood histamine (42). Recent studies demonstrated the presence of JAK2V617F-positive mast cell clones in Philadelphia chromosome-negative MPN patients, and increased release of proinflammatory factors in mast cells derived from peripheral blood cells of PV patients (18, 19). However, a major argument against the role of mast cells in PV-associated pruritus is the failure to detect serum tryptase in pruritic PV patients after water exposure (43). Mast cell proteases play an important role in mediating mast cell functions (17). In fact, the serum level of mast cell tryptase is considered the best-established parameter for evaluation of systemic mastocytosis (44). However, it remains to be determined if mast cells from PV patients contain a sufficiently high amount of tryptase to cause a significant elevation in total serum upon degranulation. Our study showed that total and secreted tryptase appeared unaltered in mast cells from JAK2V617F transgenic mice in comparison with control (Supplemental Fig. 3). From this, we conclude that a lack of increased serum tryptase in pruritic PV patients does not necessarily exclude the involvement of mast cells in PV-associated pruritus.

Our study demonstrated that JAK2V617F transgenic mice develop pruritus as they aged, correlating with a greater number of mast cells produced from older mice (Fig. 2B). We found that pruritus occurred spontaneously in JAK2V617F transgenic mice after they reached 40 wk of age, far behind the onset of the PV-like phenotype, which was usually observed in 10 wk. However, development of pruritus coincides with the age-dependent progression of JAK2V617F-induced MPN-like phenotypes. The initial phenotypes of JAK2V617F transgenic mice are manifested in elevated levels of RBCs, platelets, and WBCs in the blood, but other, later symptoms include splenomegaly, myelofibrosis, and mobilization of hematopoietic stem/progenitor cells (22). The occurrence of pruritus apparently represents a further progression of the PV-like phenotype in our JAK2V617F transgenic mice. In humans, PV mainly affects the elderly, and pruritus is usually found at the time of or after diagnosis of PV (4–6). However, in rare PV cases, pruritus can precede the development of hematological manifestations (45, 46). This finding suggests that humans may have a lower threshold level for onset of pruritus with latent PV. JAK2V617F possesses enhanced kinase activity and results in constitutive activation of downstream signaling pathways, including the MAPK pathway, which is important for cell proliferation and survival (47). We showed that JAK2V617F-positive BMPCs retained a higher level of ERK1/2 phosphorylation after growth factor/cytokine starvation and exhibited a stronger response to stimulation by SCF and IL-3 than did control cells (Fig. 3). This characteristic presumably provides JAK2V617F-positive mast cells with resistance to apoptosis and gives them a growth advantage under suboptimal growth conditions, as seen in our cell culture analyses (Fig. 4). We believe that JAK2V617F-positive mast cell progenitors behave similarly in vivo when growth factors and cytokines are limited. Therefore, JAK2V617F transgenic mice are more susceptible to the development of pruritus because they have more mast cell progenitors stored in the peripheral tissues and are able to produce massive numbers of mast cells when triggered. In addition to its effects on mast cell proliferation, JAK2V617F may also enhance the migration of existing mast cells. However, our in vitro migration assays with Boyden Transwell chambers did not reveal any significant difference in the response of these cells to SCF, a well-known chemoattractant of mast cells (Ref. 48; data not shown). Whether they respond differently to other factors is to be investigated. In addition, we observed that only 42% of mice 45–55 wk of age developed pruritus, although they all displayed greatly increased numbers of mast cell progenitors in the bone marrow, spleen, and peripheral blood. It will be important to find out what triggers the onset of pruritus in JAK2V617F transgenic mice.

As a defining feature of PV, pruritus is the major complaint among patients with PV. However, no effective treatment is currently available. A wide array of treatments have been reported, including antihistamines, antidepressants, UV phototherapy, transcutaneous electrical nerve stimulation, IFN-α, phlebotomy, aspirin, iron supplements, and myelosuppressive medications (7–10). Owing to lack of understanding of the mechanism underlying PV-associated pruritus, these treatments are largely experimental and have mixed results. For example, symptomatic treatment with antihistamines is often ineffective (7). Antidepressant medications with selective serotonin reuptake inhibitors are somewhat efficacious, but how they work is not clear (8). Cytotherapeutic therapy with IFN-α is reserved for high-risk patients, and although it has some antipruritic benefit, it also causes major side effects (9). Identification of JAK2V617F in PV has provided an excellent target for drug development. Many potent JAK2 inhibitors have been developed, and one of these inhibitors, namely, ruxolitinib, is in clinical use for treatment of myelofibrosis (32–34). Of note, a recent study demonstrated that ruxolitinib was able to relieve food allergy in mice by preventing mast cell hyperplasia and inhibiting mast cell activation (49). In this study, we showed that the JAK2 inhibitor G6 reduces mast cell numbers and alleviates pruritus in JAK2V617F transgenic mice. In vitro study with cultured mast cells demonstrated that G6 inhibits growth of both JAK2V617F-positive and -negative cells. It suggests that targeting mast cells provides potential therapeutic benefit. Considering the role of cKit in mast cell development, multitarget tyrosine kinase inhibitors that inhibit both JAK2 and cKit may be more effective in treating pruritus associated with JAK2V617F.

In conclusion, we demonstrated the occurrence of pruritus in JAK2V617F transgenic mice associated with increased mast cells. This study thus provides a unique mouse model for the study of pathophysiology of MPN-associated pruritus and functions of mast cells. The increased numbers of mast cells are presumably caused by enhanced proliferating ability, increased numbers of mast cell progenitors in the bone marrow, and mobilization of these cells to peripheral tissues, including the blood, spleen, and peritoneal cavity. Our study also provides a useful model for developing therapeutic drugs for pruritus. Because JAK2V617F plays a crucial role in causing enhanced signaling and cell proliferation, JAK2 inhibitors hold promise as an effective treatment of pruritus. In this regard, our data with the JAK2 inhibitor G6 provide a proof-of-principle and a strong candidate for further drug development.

Disclosures
The authors have no financial conflicts of interest.
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Mast Cells in PV-Associated Pruritus

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