JAKs are a family of nonreceptor tyrosine kinase and consists of four members: JAK1, JAK2, JAK3, and Tyk2 (**1**-Johnston-1994). All four members mediate signals initiated by cytokines through interactions with receptors for IL-2, IL-5, IL-7, IL-9, and IL-15 via the common γ chain (**2**-Witthuhn-1994). Different studies have shown that JAK3 is widely expressed in different organs (**2**-Witthuhn-1994). Previous studies with IL-2Rγ-null mice showed that JAK3 is related to the development of spontaneous inflammatory bowel disease (IBD) symptoms (**3**-Miyazaki-1994). Moreover, abnormal activation of JAK3 was associated with human hematological (**4**-Ihle-1997), indicating that a tight balance of its activity was essential for normal hematopoietic development.

Although JAK1, JAK2, and Tyk2 are each widely expressed, JAK3 is predominantly expressed in hematopoietic cells and is known to associate only with the common γ (γc) chain of the IL-2, IL-4, IL-7, IL-9, and IL-15 receptors (**5**-Nosaka-1995). IL-4 is a very well-known cytokine that plays a crucial role in the polarization of naïve T cells to type 2 helper T cells. It plays a major role in the growth and proliferation of many immune cells such NK and T cells (**6**-Dhupkar-2017). Homozygous mutant mice in which the JAK3 gene had been disrupted were generated by gene targeting. JAK3-deficient mice had profound reductions in thymocytes and severe B cell and T cell lymphopenia similar to severe combined immunodeficiency disease (SCID), and the residual T cells and B cells were functionally deficient. Thus, JAK3 plays a critical role in γc signaling and lymphoid development.

Domain of Applicability

?福山先生　human or mouse をこのパラに入れてください～。

This proposed AOP involves inhibition of JAK activity leading to suppression of TDAR and is not dependent on life stage, sex, or age. Since JAK3 inhibitors (PF-06651600, RB1) are currently under a phase 2 clinical evaluation to treat rheumatoid arthritis, the AOP appears to be applicable to all life stages. Since JAK3 inhibitor-induced outcomes in humans are mimicked by similar responses in a variety of animal models, including non-human primates and rodents, immunosuppression induced by inhibition of JAK3 activity is considered to occur across a variety of mammalian species. For example, PF-06651600 reduces paw swelling with an unbound EC50 of 169 nM in the rat adjuvant-induced arthritis.

Similarly, PF-06651600 significantly reduces disease severity in the experimental autoimmune encephalomyelitis (EAE) mouse model at 30 or 100 mg/kg or prophylactically at 20 and 60 mg/kg. Then, PF-06651600 is going to clinical trials (**7**-Telliez-2016).

Essentiality of the Key Events

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MIE and later events: JAK3-knockout (KO) mice

JAK3 was initially identified (**1**-Johnston-1994,**2**-Witthuhn-1994) in studies to identify the JAK family member that was involved in the signaling of a group of cytokines that shared in common the utilization of the γc chain first identified in the interleukin 2 (IL-2) receptor complex. It was subsequently demonstrated that JAK3 physically associates with the γc chain and is activated in a receptor complex that also contains JAK1, which associates with the ligand specific alpha or beta chain of the receptors (**3**-Miyazaki-1994). JAK3 is somewhat unique within the JAK family in that it is predominantly expressed in hematopoietic cells and is only activated in the responses to cytokines that use the γc chain (**4**-Ihle-1997). The phenotype of the JAK3 deletion mice was quite striking and consisted of a range of deficiencies which collectively constituted SCID (**5**-Nosaka-1995,**8**-Thomis-1995). At the same time, two groups identified individuals that lacked JAK3 and exhibited somatically acquired SCID (**9**-Macchi-1995,**10**-Russell-1995). One of the most striking components of the phenotype is the dramatic reduction seen in both the T and B cell lineages. Comparable reductions are seen in mice that lack IL-7 (**11**-von Freeden-Jeffry-1995), the IL-7 receptor alpha chain (**12**-Peschon-1994), or the γc chain. In spite of the reduced numbers, the cells that do develop are phenotypically normal. These results are consistent with the hypothesis that activation of JAK3 give it a critical role in the expansion but not the differentiation of early lymphoid lineage-committed cells. In addition to the reduced numbers, the differentiated lymphoid cells that are generated fail to respond to the spectrum of cytokines that utilize the γc chain and activate JAK3 normally.

B6.Cg-Nr1d1tm1Ven/LazJ mouse

Primary immunodeficiencies (PIDs) are inborn errors that cause developmental and/or functional defects in the immune system (**13**-Picard-2015). Most frequently rare and monogenic, PIDs present clinically with a broad array of phenotypes including increased susceptibility to infection. One of the most deadly categories of PID is SCID, which is invariably caused by severe developmental and/or functional defects of T lymphocytes, but may also present with variable defects of B and/or Natural Killer (NK) cells. The B6.Cg-Nr1d1tm1Ven/LazJ mouse line harbors a spontaneous mutation in JAK3, which generates an SCID phenotype (**14**-Robinette-2018).

KE1: STAT5-KO mice

STAT5 plays a major role in regulating vital cellular functions such as proliferation, differentiation, and apoptosis of hematopoietic and immune cells (**15**-Rani-2016,**16**-Wittig-2005). STAT5 is activated by phosphorylation of a single tyrosine residue (Y694 in STAT5) and negatively regulated by dephosphorylation. A wide variety of growth factors and cytokines can activate STAT5 through the JAK-STAT pathway. The activation of STAT5 is transient and tightly regulated in normal cells (**17**-Quezada Urban-2018).

The following phenotypes are observed in STAT5-KO mice:

The transcription factor STAT5 is expressed in all lymphocytes and plays a key role in multiple aspects of lymphocyte development and function (**18**-Owen-2017). STAT5 was initially identified as a transcription factor activated by prolactin in mammary gland epithelial cells (**19**-Schmitt-Ney-1992,**20**-Wakao-1992). Subsequent studies identified STAT5 binding activity in T cells (**21**-Beadling-1994), and it was later established that STAT5 was expressed in multiple cell types and activated by a number of cytokines, including the common γc-dependent cytokines interleukin 2 (IL2), IL4, IL7, IL13, and IL15 (**22**-Lin-1995).

STAT5 in T-cell development

The observation that STAT5 is activated by multiple cytokines in T cells suggested that it might play a critical role in the development or function (or both)

of these cells. Disruption of Stat5a or Stat5b genes alone resulted in relatively modest phenotypes; for example, Stat5a-/- mice had defects in mammary gland development and lactation while Stat5b-/- mice had defects in response to growth hormone in male mice and natural killer cell proliferation (**23**-Imada-1998,**24**-Liu-1997). To determine whether combined deletion of Stat5a and Stat5b might result in more profound immunodeficiencies, subsequent studies deleted the first coding exons of both Stat5a and Stat5b. This intervention resulted in the production of truncated forms of STAT5a and STAT5b that acted as functional hypomorphs. These mice too had surprisingly mild defects in lymphocyte development, although T cells were grossly dysfunctional, as they could no longer proliferate in response to IL2 (**25**-Moriggl-1999,**26**-Teglund-1998). Finally, complete deletion of Stat5a and Stat5b using Cre-LoxP approaches demonstrated that STAT5a and STAT5b are absolutely required for lymphocyte development, as Stat5a/b-/- mice had profound blocks in lymphocyte development, which mimicked that observed in Il7r-/- mice (**27**-Cui-2004,**28**-Yao-2006). These studies definitively demonstrated that the STAT5 hypomorph mice retained significant STAT5 function.

Evidence Assessment

Biological Plausibility

T-cell development is mainly regulated by JAK-STAT system, and JAK3 deficiency in T cells is known to induce multiple types of immunosuppression, including TDAR.

JAK3-deficient mice had profound reductions in thymocytes and severe B cell and T cell lymphopenia similar to SCID disease, and the residual T cells and B cells were functionally deficient (**12**-Peschon-1994).

Mice lacking JAK3 also showed a severe block in B cell development at the pre-B stage in the bone marrow. In contrast, although the thymuses of these mice were small, T cell maturation progressed relatively normally. In response to mitogenic signals, peripheral T cells in JAK3-deficient mice did not proliferate and secreted small amounts of IL-4. These data demonstrate that JAK3 is critical for the progression of B cell development in the bone marrow and for the functional competence of mature T cells (**5**-Nosaka-1995).

Furthermore, the abnormal architecture of lymphoid organs suggested the involvement of JAK3 in the function of epithelial cells. T cells developed in the mutant mice did not respond to either IL-2, IL-4, or IL-7 (**29**-Ito-2017).

Specific JAK3 inhibitor PF-06651600 or RB1, which selectively inhibited JAK3 with an over 100-fold preference over JAK2, JAK1, and TYK2 in the kinase assay, displayed reduced inflammation and associated pathology in collagen-induced-arthritis mice. Importantly, with PF-06651600 or RB1 administration, pro-inflammatory cytokines and JAK3 and STATs phosphorylation decreased in mice, suggesting that the inhibition of JAK3/STAT signaling was closely correlated with induction of multiple types of immunosuppression, including TDAR.