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Of Mosaicism and Mechanisms: How a JAK1 variant goes awry

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Summary

Personalized medicines require understanding the molecular causes of disease. In this issue of *Immunity*, Gruber *et al.* identify mosaic expression of a gain-of-function JAK1 genetic mutant that drives multi-organ immune dysregulation via kinase dependent and independent mechanisms. The work highlights how biochemistry can inform therapies to resolve immune disorders

Main Text

The development of effective treatments for many autoimmune diseases is frequently hindered by a lack of understanding of the molecular processes and primary cellular perturbations that cause the immune dysregulation. However, the advent of next generation DNA sequencing has opened-up the capacity to diagnose the genetic causes of rare complex immune disorders which then allows the design of personalized and effective clinical management strategies (Lee et al., 2014). However, one challenge for understanding how mutations in signaling molecules can drive immune dysfunction is that immune cell populations are highly heterogeneous and need to be analysed at the level of rare populations or at the single-cell level to determine the molecular processes that drive immune phenotypes. In this issue of *Immunity*, Gruber et al. (2020) present strategies that uncovered the molecular basis of an undiagnosed complex primary autoinflammatory and atopic syndrome in a patient, when standard genetic analysis pipelines failed to find evidence for causal germline mutations.

The authors hypothesized that the physical asymmetry of disease symptoms in the patient reflected that the underlying cause was a *de novo* mosaic mutation of a key molecule for immune cell function (Figure 1). They then applied lower-frequency reads from whole-exome-sequencing analysis of the peripheral blood cells of the patient to identify DNA mutations that may be present in only a subpopulation of immune cells. This DNA sequencing work identified a new variant of the tyrosine kinase, JAK1 (JAK1c2108GT>T) as a potential disease-causing molecule. However, once identified, it was the clever and combined use of *in vitro* biochemical analyses of the mutant JAK1 protein and quantitative, single-cell analyses of the patient's immune cells that then provided the key insights necessary to understand how cells with the JAK1c2108GT>T variant caused immune dysfunction. Importantly, these insights proved crucial for the successful selection of a drug to resolve the clinical disease caused by the JAK1 mutation.

JAK1 was first identified in an unbiased discovery program that exploited the existence of highly conserved sequence elements within the catalytic domains of tyrosine kinases to generate degenerate oligonucleotide primers that would identify novel kinases (Wilks et al., 1991). We now know that there are four JAK kinases (JAK1, JAK2, JAK3 and TYK2), and within cells, the major substrate for JAKs are the STAT family transcription factors, of which there are seven (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6) (O'Shea et al., 2015). JAK1 is expressed ubiquitously, and is activated by a broad range of cytokine families including common gamma chain cytokines (γc), gp130 chain cytokines, interferons and IL-10-family cytokines (O'Shea et al., 2015; Schwartz et al., 2017). The STAT-mediated transcriptional programmes coupled to JAK1 are highly diverse, as a consequence of the formation of specific combinations JAK partners and STATs that associate with distinct cytokine receptors. The ability to understand the molecular implications of JAK1 mutations for immune cells stems from the pioneering work of scientists in the early 1990s who characterised a series of human fibrosarcoma cell lines with chemically induced mutations that blocked IFN-α/β and IFN-γ signaling (Müller et al., 1993). One of these was the JAK1-null UC4 cell line which is completely defective in interferon responses, despite expression of TYK2, which partners with JAK1 to mediate IFN-α/β signaling, and JAK2, which couples with JAK1 to induce signaling by the IFN-γ receptor. Critically, re-expression of JAK1 in the UC4 cell line can restore IFN-α/β and IFN-γ signal

transduction pathways (Müller et al., 1993). The UC4 cell line is thus an established model system for analysis of JAK1 function, and, importantly allows rapid assessment of the impact of JAK1 mutations on the activity of TYK2 and JAK2.

Gruber *et al*. exploited the U4C system to compare the signaling responses induced by either *WT JAK1* or the JAK1c2108GT>T variant. The intriguing findings from the reconstitution study were JAK1c2108GT>T variant produced a gain of function JAK1 protein. The JAK1c2108GT>T variant but not *WT JAK1*, thus stimulated cytokine independent phosphorylation of STAT proteins and STAT-induced transcription (Figure 1). Moreover, cells expressing the JAK1c2108GT>T variant were hyperresponsive to IFN-α, IFN-γ and IL-6 stimulation and showed increased phosphorylation of other JAK kinases, JAK2, JAK3 and TYK2 (Figure 1). The increase in basal and stimulated STAT phosphorylation induced by the JAK1 mutant was confirmed by the authors in an elegant approach that took advantage of the mosaicism of JAK1c2108GT>T variant expression in B-cells to derive clonal immortalized B cell lines either expressing WT or mutant JAK1.

However, it was the *in vitro* re-constitution experiments that allowed the authors to uncover that the JAK1c2108GT>T variant produced a gain of function JAK1 mutant that could transactivate partnering JAK kinases, JAK2, JAK3 and TYK 2 independently of its catalytic domain. In this respect, the JAK1c2108GT>T variant produces a protein with an S703I mutation within a highly conserved region of the pseudokinase domain of JAK1. These JAK pseudokinase domains are thought to keep JAK monomers catalytically inert while they are associated with cytokine receptors in the absence of ligands (Babon et al., 2014). However, it is unclear whether the pseudokinase-mediated inhibition occurs through an intramolecular interaction between the kinase and pseudokinase within the same protein (*cis* regulation), or intermolecular between two JAK kinases associated with different receptor chains (*trans* regulation) (Babon et al., 2014). Hence, a critical observation by Gruber *et al.* was that many of the gain-of-function changes induced by JAK1 S703I were not dependent on JAK1 S7031 catalytic activity (Figure 1), nor were they completely blocked by selective inhibitors of JAK1 enzymatic activity. Rather, pan-JAK inhibitors, which simultaneously target the partnering JAKs trans-activated by the JAK1 mutant, were more effective at blocking signaling changes induced by JAK1 S703I in cells. Accordingly, Tofacitinib, which was

developed to target JAK3 but which does have activity against JAK1 and JAK2, has proved to be a drug which could effectively treat the clinical disease in the patient with the JAK1c2108GT>T variant. These data are, thus, consistent with a model whereby the JAK1 S703I mutation in allows the JAK1 pseudokinase domain to regulate JAK partners in *trans.* The salient point is that the reconstitution experiments allowed rapid identification of the best drugs to block the functional activity of the JAK1 variant; this information was then translated into a treatment that lead to rapid resolution of clinical disease in the patient.

From a translational perspective, the present study illustrates the importance of fully understanding the biochemical consequences of kinase mutations for trans-regulation of other kinases and other signaling pathways as a basis for choosing effective drugs for precision medicine. In this respect, it is perhaps important to consider that JAKs do not just phosphorylate STATs but trigger serine and threonine kinase cascades to promote phosphorylation of regulators of mRNA translation, chromatin, GTPases, vesicle trafficking, and the actin and microtubule cytoskeleton (Ross et al., 2016). Moreover, JAKs do not signal in isolation but frequently operate alongside signaling pathways mediated by other protein kinases (Ross et al., 2016). Hence, for a patient with mosaicism of the JAK1c2108GT>T variant, the observed immune dysregulation may be determined by a multitude of factors. JAK1 is expressed ubiquitously, but for an individual cell, the ultimate outcome of expressing the JAK1 S7031 variant will be determined by the relative abundance of the other JAK and STAT family members in that cell. Indeed, the Gruber *et al*. (2020) study provides clear glimpses of the divergent outcome of JAK1 S730I expression for different cytokines and for different cells. For example, IL-2 and IL-4 both signal via JAK1 and JAK3 but expression of JAK1 S730I caused hyperphosphorylation of STAT5 in response to IL-2 but there was no hyperphosphorylation of STAT6 associated with IL-4 stimulation. However, it was observed that IL-2 and IL-4 induced phosphorylation of STAT1 occurred in patient and not control cells, indicating to the authors that the JAK1 S703I mutant could activate non-canonical JAK signaling pathways. One caveat to these observations is that while JAK mediated tyrosine phosphorylation is a key driver of STAT mediated transactivation, STAT activation may involve additional STAT phosphorylation on serine residues (Stark and Darnell, 2012), and there is not a full understanding of how STAT serine phosphorylation pathways are co-ordinated. Additionally, the control of

cell metabolism and protein synthesis is often rate limiting for execution of a cells transcriptional program (Wolf et al., 2020) and one question not addressed by Gruber *et al.* is whether expression of the JAK1 S703I variant had any cytokine dependent or independent effects on leukocyte metabolism. In particular, there was no assessment of the ability of the JAK1 S703I variant to drive protein synthesis. It, thus, remains to be determined whether the transcriptional changes initiated by the JAK1 S703I variant are alone sufficient to change cell phenotypes.

In summary, the present study demonstrates how an in-depth characterisation of a variant of JAK1 in multiple different immune cells can be used to inform clinical treatment. The study discovered a gain-of-function mutation in JAK1 that potentiated JAK-dependent signaling pathways, independently of its catalytic activity. The salient point is that, it was necessary to block this aberrant signaling to fully reverse the impact of the JAK1 mutation on cell phenotype. Together, this highlights the importance of molecular immunology, and basic discovery biochemistry for optimised treatments of immune disorders.

Figure legend

Figure 1: Expression and function of JAK1 S703I

JAK1 S703I resulted from a variant, JAK1 c2108GT>T allele that arose as a *de novo* mosaic mutation within the first 12 divisions between fertilization and gastrulation. Consequently, the patient had cells that were either homozygous for *WT JAK1* or heterozygous for *S703I JAK1*. Adding a further layer of complexity, expression of JAK1 in cells is monoallelic. Hence not all cells heterozygous for *S703I JAK1* allele express the JAK1 S703I protein. The S703I mutation occurred within a highly conserved region of the pseudokinase domain of JAK1. This resulted in a gain-of-function in JAK1 signaling. JAK1 S703I expression increased the autophosphoylation of JAK1 (yellow circles), and the phosphorylation of other JAKs and the JAK substates, STAT proteins (yellow circles) basally and in response to cytokines. Interestingly, kinase dead (KD) JAK1 S703I was capable of transactivating other JAK proteins and, thus, could potentiate aberrant cytokine signaling in cells independently of its kinase activity. The JAK1 S703I mutation triggered autoinflammation in the patient. The discovery that a pan-JAK inhibitor, and not just specific inhibition of JAK1, was required to reverse the

biochemical changes intiated by expression of JAK1 S703I was key to identifying a treatment that reversed the clinical manifesations suffered by the patient.

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