Editorial

The importance of defining which Janus kinases are activated in giant cell arteritis

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The Janus kinases (JAKs) are intracellular tyrosine kinases that transduce signals from cytokine receptors. Four JAKs exist: JAK1, JAK2, JAK3, and tyrosine kinase2 (TYK2). In the human body the most expressed family members at protein level are JAK2 and TYK2, while JAK1 and JAK3 are expressed at medium levels. Concerning the patterns of expression, JAK1, JAK2 and TYK2 proteins show an ubiquitous expression, while JAK3 proteins are expressed mainly in cells of the hematopoietic lineage (data from Protein Atlas) (1). Binding of cytokines, interferons and growth factors to transmembrane type I and II cytokine receptors and the GP130 subunit coupled receptors causes receptor dimerisation, JAK recruitment, autophosphorylation and signalling. Ultimately, Signal Transducers and Activators of Transcription (STATs) bind the phosphorylated sites, become themselves phosphorylated and activated. They form dimers, migrate to the cell nucleus where they bind specific DNA regions, regulating transcription of target genes which tune cell growth, differentiation, immune responses. JAKs function as dimers. Each dimeric JAK regulates specific biological pathways via STATs (2). JAK signalling has a key role in protecting the organisms from infections and tumours. Loss-of-function mutations can lead to severe immunodeficiencies. Instead, gain-of-function mutations can lead to lymphoproliferative and myeloproliferative diseases (3).

JAK1 signals from type I cytokine receptors: IL-2R, IL-4R, IL-7R, IL-9R, IL-15R, IL-21R, containing the common gamma chain; type II cytokine receptors: IL-10R, IL-19R, IL-20R, IL-22R, IL-28R; the GP130 subunit coupled receptors: IL-6R, IL-11R, IL-27R, cardiotrophin-1R, leukaemia inhibitory factor (LIF)-R, ciliary neurotrophic factor (CNTF)-R, oncostatin M (OSM)-R, leptin-R. Furthermore, JAK 1 signals for type I and type II interferons. JAK2 is involved in signalling from receptors for growth hormone, prolactin, erythropoietin, thrombopoietin, IL-3 and granulocyte-macrophage colony stimulating factor. In addition, JAK2 can transduce signals from some type II cytokine receptors and the GP130 subunit coupled receptors. JAK3 transduces the signals from common gamma chain-type I cytokine receptors. TYK2 transduces the signals from common gamma chain-type I and type II cytokine receptors, IFN-type I and IL-12 (3).

Since JAKs transduce signals from several pro-inflammatory cytokines, their inhibition is promising in the treatment of immune-mediated diseases. Giant cell arteritis (GCA) is an immune-mediated disease of large vessels which can cause ischaemic events (*e.g.* vision loss), aortic dissection and aneurysms (4, 5). It is characterised by systemic inflammation and granulomatous immune cell infiltrate in the arteries (mostly large- and medium-sized arteries) triggered by unknown stimuli, leading to arterial remodelling. The mainstay of therapy is based on glucocorticoids, however about half to two third of patients experience flares during glucocorticoid tapering or after glucocorticoid withdrawn. Moreover, up to 85% of patients can have glucocorticoid-related side effects (6). Glucocorticoids did not appear capable of dampening IFNγ-producing Th1 cells, that play a key role in vascular inflammation and remodelling (7). In addition, they did not seem able to clean up immune cells from temporal arteries, also after one year of treatment (8). Only tocilizumab, an IL-6R inhibitor, has been approved for the treatment of GCA by the US Food and Drug Admin-

istration and the European Medicines Agency because it has shown efficacy in randomised controlled trials in increasing rates of remission, decreasing relapses and glucocorticoid requirements in patients with GCA (9, 10). However, about a quarter of patients with GCA treated with tocilizumab still develop flares over a 52-week period (9) and less than half of the patients can maintain tocilizumab-free and glucocorticoid-free sustained remission (11, 12). Therefore, the need to define alternative therapeutic options in GCA is still urgent.

Several cytokines have been documented over-expressed in inflamed temporal arteries from patients with GCA: IL-1β, IL-2, IL-6, IL-7, IL-8, IL-9, IL-12, IL-15, IL-17, IL-21, IL-22, IL-23, IL-27, IL-32, IL-33, IFNα, IFNγ, TNFα, GM-CSF (13, 14), which can lead to pan-JAK activation. Gene expression analysis in inflamed temporal arteries from patients with GCA detected STAT1/2/4 overexpression likely driven by type I and type II IFN signalling, and overexpression of STAT1/2/4-dependent target genes. STAT3/5/6 transcripts showed similar expression between inflamed and normal temporal arteries and their target genes accordingly resulted with low expression (15, 16).

However, to define which JAKs are more likely activated in inflamed arteries, data on the expression of the corresponding cytokine receptors should be integrated. Expression of cytokine receptors has been scarcely investigated in GCA. Receptors for IL-7, IL-9, IL-21, IL-22 have been found up-regulated in inflamed temporal arteries by real-time PCR analysis (IL-7R, IL-9R) and immunohistochemistry (IL-21R, IL-22R) (17-20), suggesting JAK1/3 and TYK2 activation. To infer more data on cytokine receptor expression, we searched in the published high throughput profiling assays. DNA methylation profile in inflamed compared to normal temporal arteries suggests a hypomethylation of CpG sites in the 5'UTR or in the body of IL-2RA, IL-2RG, IL-10RA, IL-12RB1, IL-17RA, IL-22RA and IL-23R, which might lead to receptor overexpression and JAK1/3 and TYK2 signalling [Supplementary Table 1 from Coit *et al*. (19), thresholds: diffscore >|22|, *p*-value <0.05, Δβ >|0.2|]. RNA sequencing on inflamed versus normal temporal arteries indicates an increased expression of IL-12RB1, IL-15RA, IL-17RA, IL-21R, IFNGR2, CSF1R, CSF2RA, CSF3R as well as JAK1, JAK3, TYK2 genes [2-fold change threshold implemented in the eTable 2 gene list from Bubak *et al*. (21)]. RNA sequencing on aortic tissues from inflammatory (GCA plus clinically isolated aortitis) versus non-inflammatory aortic aneurysms has shown an up-regulation of IL-2RA, IL-2RG, IL-5RA, IL-7R, IL-9R, IL-10RA, IL-12RB1, IL-21R, IL-23R as well as JAK3 [2-fold change threshold implemented in the gene list in Supplementary Table 1 from Hur *et al*. (22)]. Finally, a similar, independent, transcriptomic analysis on inflamed aortic tissue from patients with GCA with large-vessel involvement compared to non-inflammatory aortic tissue has revealed the presence of an interferon signature with putative activation of JAK1 and TYK2, associated with STAT1/2/3 signalling (23).

Single cell RNA sequencing experiments performed in normal vascular tissue show that JAK1 mRNA is the most abundant among JAK members and its expression is predominantly in smooth muscle cells and T cells. JAK2 mRNA is mainly expressed by smooth muscle cells, while JAK3 mRNA is mainly expressed by T cells. TYK2 mRNA is the transcript present at lower levels in the vascular tissue and it is expressed by macrophages and T cells (data from protein Atlas) (24).

The above-mentioned data provide guidelines, but mainly concern mRNA expression of JAKs, cytokines and cytokine receptors. JAK pathways are activated by post-translational modifications and protein dimerisation. Therefore, to determine if JAKs are activated in a pathological condition it is necessary to evaluate not only the presence of the JAK family members in specific tissues, but also their phosphorylation status. Nowadays, proof on the overactivation of specific JAKs at protein levels in inflamed arteries from patients with GCA are lacking. Indeed, assays to determine which kind of JAKs are phosphorylated in inflamed arteries should be fostered, because they can be of help for the data-driven selection of the most promising JAK inhibitors for this disease.

What are the available data on the efficacy of JAK inhibitors in GCA? In a mouse model of GCA (chimeric mice engrafted with human arteries and immune cells from patients with GCA), the administration of the JAK1/3 inhibitor Tofacitinib reduced immune cell infiltrating the arteries, inhibited T cell proliferation and cytokine production (*e.g*. IFNγ, IL-17, IL-21), and decreased survival of artery-resident T cells. Moreover, Tofacitinib treatment counteracted neoangiogenesis and intimal hyperplasia in inflamed arteries, limiting inflammation-induced arterial remodelling (16). A prospective, single-centre, uncontrolled, open-label 52-week pilot study on JAK1/2 inhibitor baricitinib in patients with relapsing GCA showed that baricitinib was safe and discontinuation of glucocorticoids was reached in most patients (ClinicalTrials.gov identifier: NCT03026504). Only one patient experienced a serious adverse event, but not attributable to baricitinib. Only one of 14 patients relapsed during the study while the remaining 13 patients stopped glucocorticoid and achieved disease remission, providing preliminary evidence of efficacy of JAK1/2 inhibition (25). Finally, there is an ongoing multicentre, randomised, double-blind, placebo-controlled study to determine the safety and efficacy of the JAK1 inhibitor Upadacitinib in patients with GCA (ClinicalTrials.gov identifier: NCT03725202), which will give more information on the impact of JAK pathways in GCA.

There are several JAK inhibitors approved for the treatment of immune-mediated diseases and hematologic diseases. Since they act on hub proteins which controls pro-inflammatory as well as protective immune responses, their thoughtful use is a must. To move towards precision medicine, data on JAK expression and phosphorylation should be fostered in GCA as well as in other immune-mediated diseases.

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