AUTOINFLAMMATORY DISEASE

Targeting G-CSF to treat autoinflammatory disease

Matilda Katan and Tom D. Bunney

Institute of Structural and Molecular Biology, Division of Biosciences, University College London, London, UK.

*e-mail: m.katan@ucl.ac.uk

APLAID is a very rare autoinflammatory disease thought to be caused by mutations in *PLCG2*. A mouse model of APLAID recapitulates clinical features of the disease and identifies a critical function for G-CSF that can be targeted therapeutically.

Inflammation is a complex and strictly regulated defence mechanism, triggered to protect an organism from harmful stimuli. However, an excessive innate immune response can lead to self-directed inflammation and autoinflammatory disease, a distinct type of immune dysregulation first described nearly 25 years ago in which typical features of broadly defined autoimmune diseases are often lacking[1]. Since that time, advances in genetic sequencing have revealed that, in many cases, these diseases have a strong genetic background, with mutations in single genes linked to inflammation. The current list of monogenic autoinflammatory disorders includes more than 40 conditions, extending our understanding of the range of genes involved, pathway perturbations and clinical features[2]. The monogenic nature of many autoinflammatory diseases has also prompted the generation of mouse models that have provided important insights into autoinflammatory pathology[3]. In this issue of *Nature Immunology*, Mulazzani *et al.*[4] describe a new mouse model of a monogenic autoinflammatory disease and demonstrate that such models can identify unexpected drivers of inflammation and lead to feasible targets for therapeutic intervention.

The disease condition modelled by Mulazzani *et al.*[4] was designated as APLAID (autoinflammation with phospholipase C γ2 (PLCγ2)- associated antibody deficiency and immune dysregulation) following the discovery of a missense gain-of-function PLCγ2 mutation (p.Ser707Tyr) in patients with dominantly inherited autoinflammation, also affected by immunodeficiency[5]. Physiological functions for this second-messenger-generating phospholipase, highly expressed in B cells and cells involved in innate immunity, have been well documented and linked to stimulation of various ITAM-associated receptors[6]. With respect to immune dysregulation, PLCγ2 was initially linked to autoimmunity and autoinflammation through an N-ethyl-N-nitrosourea (ENU) mutagenesis screen in mice[7] and the first link to complex immune disorder in humans (PLCγ2-associated antibody deficiency and immune dysregulation, or PLAID) revealed by studying families with specific dominantly inherited symptoms[8]. In all identified clinical cases of APLAID (summarized by Mulazzani *et al.*[4]), different missense mutations in PLCγ2 have a similar effect on structure and function of this enzyme (Figure 1). By compromising autoinhibitory, intramolecular

interactions these mutations result in enhanced basal and stimulated PLC activity[9]. Further studies of signalling connectivity and mechanisms, important for understanding consequences of the APLAID PLCγ2 mutations for cellular functions implicated in autoinflammation, suggested a link to upregulation of NLRP3 and IL-1β release when assessing PBMCs from patients with APLAID, compared with healthy individuals[10,11]. However, in the clinical setting IL-1 inhibitors (such as the IL-1 receptor antagonist anakinra) have been mostly ineffective. Similarly, other cytokine inhibitors, including TNF inhibitors, only partially suppress the disease. These observations suggest that unknown pathways and mediators are critical for the pathogenesis of APLAID.

In their search for inflammatory drivers of APLAID, Mulazzani *et al.*[4] first demonstrated that the majority of APLAID symptoms can be recapitulated in PLCγ2$^{S707Y/+}$ mice. They used this mouse to assess factors previously implicated in autoinflammation and, importantly, to search for new mediators responsible for the development and progression of APLAID (Figure 1). Patients with APLAID caused by a missense monoallelic p.Ser707Tyr mutation present with recurring blistering skin lesions, interstitial lung disease, joint pain, ocular inflammation, enterocolitis and immunodeficiency. Comprehensive analysis of the PLCγ2$^{S707Y/+}$ phenotype revealed most of the expected characteristics, including neutrophilic skin and lung inflammation; however, inflammatory eye diseases and a pronounced immunoglobulin reduction were not observed. Next, to analyse whether autoinflammation in APLAID is mediated by IL-6, TNF or the inflammasome, PLCγ2$^{S707Y/+}$ mice were crossed with IL-6, caspase-1 or TNF KO strains. Despite some differences compared with PLCγ2$^{S707Y/+}$ mice, these crosses resulted in a marginal rescue of the APLAID phenotype, with inflammatory infiltrates in the skin and lungs only partially decreased in some crosses.

Information about other factors that could have important inflammatory effects in APLAID came from analysis of an extensive panel of cytokines in samples from PLCγ2$^{S707Y/+}$ mice and their crosses. The most notable differences were in the G-CSF levels, with the highest increase relative to wildtypes detected in plasma from PLCγ2$^{S707Y/+}$ mice. These mice also had increased G-CSF levels in skin and lung lysates. Interestingly, cytokine profiles from the treatment phase of two APLAID patients with different PLCγ2 mutations also revealed high and fluctuating levels of G-CSF. On the basis of these findings, the PLCγ2$^{S707Y/+}$ APLAID mice were treated with anti-G-CSF antibodies. Notably, by suppressing excessive myelopoiesis in the bone marrow (BM) and the spleen, this treatment reduced the neutrophil count and other autoinflammatory manifestations in PLCγ2$^{S707Y/+}$ mice, reversing the APLAID phenotype. In follow up experiments to specifically assess the contribution from the BM haematopoietic compartment, PLCγ2$^{+/+}$ BM cells were transferred to irradiated PLCγ2$^{S707Y/+}$ APLAID mice and, conversely, the PLCγ2 $^{S707Y/+}$ BM cells to PLCγ2$^{+/+}$ mice as well as to G-CSF KO mice. These BM transplantations normalized G-CSF levels and rescued the APLAID phenotype such that G-CSF deficient mice were protected from APLAID. These data suggest that the APLAID mutation critically affects BM cells, which in turn triggers the production of G-CSF in other tissues. However, the mechanistic link between cells affected by the PLCγ2 mutation and a trigger for increased production of G-CSF is elusive and requires further investigation. Nevertheless, these experiments highlight G-CSF, one of the first identified and extensively studied cytokines with clinical application[12], as the key driver of inflammation in this

case of APLAID. Furthermore, two options for new APLAID treatments emerged from this work, G-CSF neutralization by antibody treatment and allogeneic BM transplantation. Considering that targeted, anti-cytokine strategies have already been successful in the clinic and that BM transplantation is an established medical procedure, both options might be further developed and implemented. Whether these main findings and proposed treatment strategies are relevant for any other autoinflammatory disease remains to be investigated.

In summary, the study by Mulazzani *et al.*[4] is an important step towards understanding the diversity of mediators involved in autoinflammation. Specifically, the role of G-CSF in APLAID is strongly supported by several lines of experimental evidence. These new data also outline directions that are needed to facilitate advancement of proposed new treatments. These future directions include more extensive cytokine profiling (including G-CSF) of APLAID patients with different PLCγ2 mutations and profiling of patients with other related autoinflammatory diseases. Also crucial will be case studies showing the effect of G-CSF reduction on inflammatory manifestations in these patients.

Competing interests

The authors declare no competing interests.

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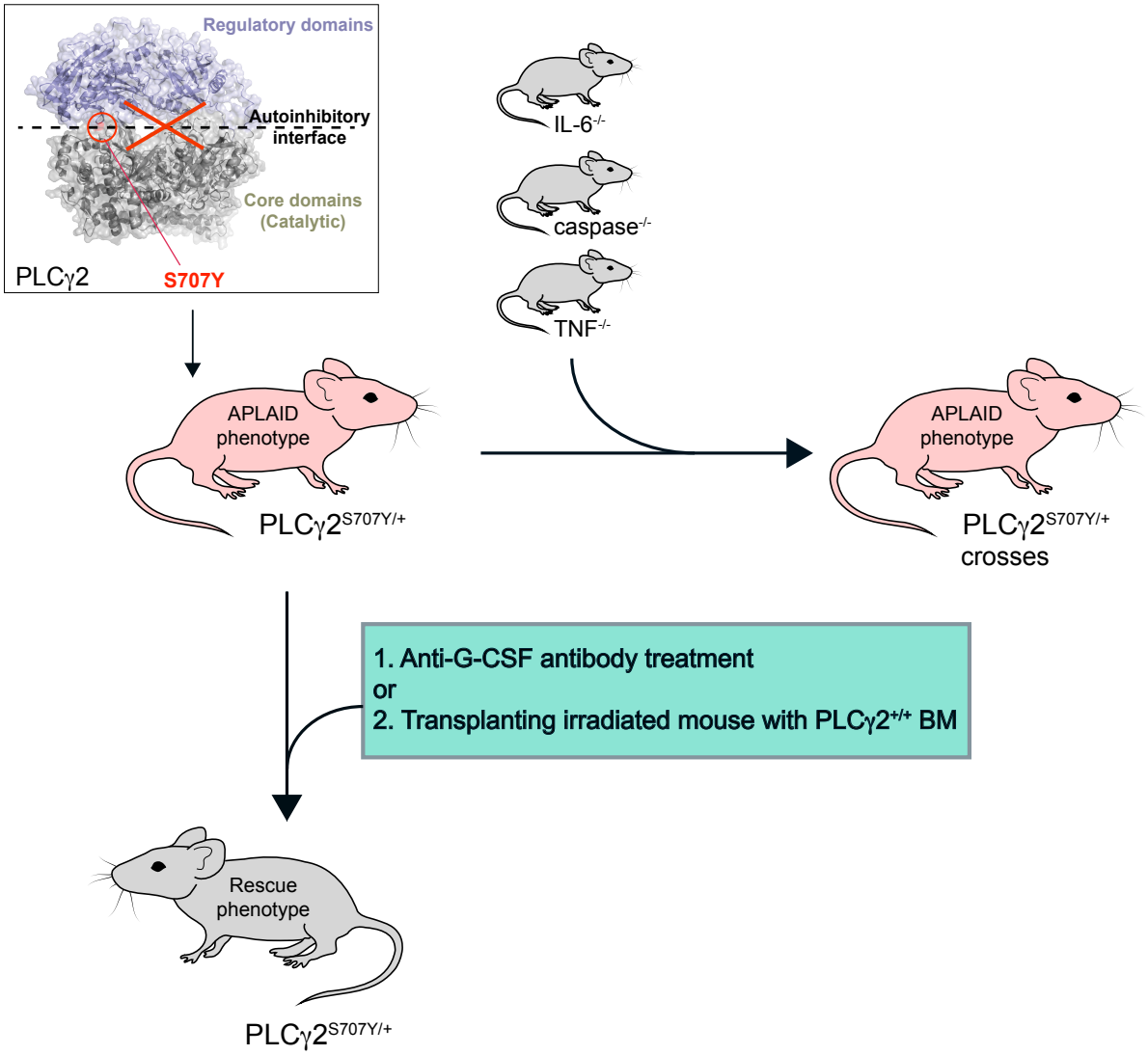
Figure 1. A new model of autoinflammation and emerging treatment options.
A mouse model has been generated for a rare autoinflammatory disease known as APLAID [autoinflammation with phospholipase C γ2 (PLCγ2)- associated antibody deficiency and immune dysregulation]. In the structural model of PLCγ2 (inset), the APLAID mutation p.S707Y localises to the autoinhibitory interface and is proposed to weaken the contact between the regulatory and catalytic-core domains leading to the enzyme becoming more active. A new mouse model carrying this mutation (PLCγ2$^{S707Y/+}$ mice) replicates most symptoms identified in patients with APLAID. Deleting some of the possible mediators of inflammation, IL-6, caspase-1 or TNF in PLCγ2$^{S707Y/+}$ crosses showed only a partial rescue of some APLAID symptoms (top right). By contrast, APLAID symptoms were reversed by treating PLCγ2$^{S707Y/+}$ mice with anti-G-CSF antibodies, which normalised highly increased levels of this cytokine, or by performing a bone marrow (BM) transplant from healthy (PLCγ2$^{+/+}$) donors (bottom left).


Regulatory domains
Autoinhibitory interface
Core domains (Catalytic)
PLCγ2
S707Y
IL-6-/-
caspase-/-
TNF-/-
APLAID phenotype
PLCγ2S707Y/+
APLAID phenotype
PLCγ2S707Y/+ crosses
1. Anti-G-CSF antibody treatment
or
2. Transplanting irradiated mouse with PLCγ2+/+ BM
Rescue phenotype
PLCγ2S707Y/+