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From Biomarker to Mechanism? F2-isoprostanes in Pulmonary Fibrosis

Prostaglandins, thromboxane, and leukotrienes are enzymatically-derived metabolites of arachidonic acid long recognized as pleiotropic mediators of biologic processes. In contrast, isoprostanes are prostaglandin-like substances formed by free radical-mediated oxidation of arachidonic acid. Because such oxidative reactions are spontaneous and undirected, up to 64 isomers may be produced, with the most well-characterized being 8-iso prostaglandin $F_{2\alpha}$, referred to here as F2-isoprostane. The presence of elevated concentrations of F2-isoprostanes in biologic samples has long been recognized as a marker of increased oxidative stress (1). Elevated concentrations of F2-isoprostanes have been found in the blood, BAL fluid, and exhaled breath condensate of patients with idiopathic pulmonary fibrosis (IPF) (2, 3), among many other diseases. In addition, F2-isoprostanes were found to be elevated in the rat bleomycin model, and F2-isoprostanes stimulated myofibroblast differentiation of rat lung fibroblasts (4). The primary receptor for F2-isoprostanes is the TXA2R (thromboxane A2 receptor), although some evidence has suggested the presence of an additional receptor for F2-isoprostanes (5). TXA2 itself is produced enzymatically from arachidonic acid by activated platelets and powerfully promotes platelet activation, aggregation, release of other platelet factors, and clot formation. TXA2R is widely expressed and plays important roles in cardiovascular disease, pulmonary hypertension, asthma and allergic diseases, liver and kidney disease, and cancer cell angiogenesis and metastasis (6).

In this issue of the *Journal*, Suzuki and coworkers (pp. 596–607) conduct a comprehensive examination of the role of TXA2R in pulmonary fibrosis (7). They report that TXA2R was elevated in lung tissues from patients with IPF and the mouse bleomycin model. TXA2R knockout mice were protected from bleomycin fibrosis, supporting a mechanistic role for TXA2R in driving pathogenesis. However, a chemical inhibitor of TXA2

M.P.-G. was supported in part by R35HL144979. T.H.T. does not declare any support for this editorial.

Originally Published in Press as DOI: 10.1164/rccm.202205-0914ED on June 28, 2022

synthesis failed to appreciably protect the mice from bleomycin, indicating that the fibrosis-promoting ligand was probably not TXA2 itself. The authors then showed that synthetic 8-iso-$PGF_{2\alpha}$ promoted proliferation, myofibroblast differentiation, and Smad and AKT (protein kinase B) phosphorylation of human lung fibroblasts in a TXA2R-dependent manner. Finally, they demonstrated that ifetroban, an orally active TXA2R antagonist developed nearly 30 years ago (8), can inhibit murine lung fibrosis elicited by bleomycin and radiation and in a genetic model of the Hermansky-Pudlak syndrome. Importantly, ifetroban treatment was able to attenuate bleomycin fibrosis even when begun as late as 14 days after bleomycin, offering hope for a genuine antifibrotic action that might be able to halt the progression of established disease.

TXA2R is a G-protein coupled receptor that activates Gαq11 and Gα12/13 (9). Gαq signaling leads to calcium flux and activation of protein kinase C and ERK (extracellular-signal–regulated kinase), whereas Gα12/13 signaling leads to activation of RhoA and Rho-associated kinases; both pathways are strongly implicated in IPF (10, 11). These actions offer a plausible mechanism by which F2-isoprostanes could drive the pathogenesis of fibrosis, rather than merely being a biomarker. Of course, their pathogenic relevance will depend, in part, on the relative concentration of F2-isoprostane ligands and the affinity of the TXA2R. The Kd of the receptor for 8-iso-$PGF_{2\alpha}$ has been estimated to be 30–60 nM (12), which is in line with typical values for other lipid mediator G-protein coupled receptors. Reported concentrations of the ligand in biological fluids and tissues are typically in the pM range, but these are, of course, confounded by dilution introduced in sampling and analytical procedures, and it is commonly assumed that the local concentrations of the mediators at their pertinent sites of action are in fact higher. New advances in spatially resolved mass spectrometry, allowing *in situ* metabolomics, may eventually resolve this age-old problem once and for all (13).

What the data with TXA2R knockout mice and antagonism unquestionably demonstrate, though, is that some endogenous ligand (or combination of ligands) of this receptor is present at concentrations sufficient to serve as a driver of fibrosis. Even if the mouse experiments argue against the importance of TXA2 in the bleomycin model, it cannot be entirely dismissed. TXA2 has a short half-life of about 30 seconds, being spontaneously hydrolyzed to the inactive thromboxane B2. Few studies have measured both TXB2 and F2-isoprostanes in the same samples, but one such study reports 10-fold higher concentrations of TXB2 than F2-isoprostanes in exhaled breath condensate (14). Evidence supporting a role for platelets themselves (the major cellular source and target for TXA2) in pulmonary fibrosis include the facts that platelet aggregates and platelet activation proteins in the blood predict disease severity in patients with IPF (15, 16) and that depletion of platelets attenuates fibrosis in the bleomycin mouse model (17). It was also previously reported that TXA2 production was elevated in lung fibroblasts of patients with IPF (18). Clearly, further study is needed to evaluate the relative importance of potential TXA2R agonists in this context.

Ifetroban (CPI211, Pubchem ID: 3,037,233) is a TXA2R antagonist that is orally active and was well-tolerated in a phase I dose–escalation study (8), but which has so far failed to find a clinical use, although phase II trials are ongoing in aspirin-exacerbated asthma, cardiomyopathy associated with Duchenne muscular dystrophy, and other conditions. Despite the impressive and promising data presented by Suzuki and coworkers, a large helping of caution and humility is appropriate in envisioning the clinical impact of these findings. This derives in large part from the increasing recognition that pulmonary fibrosis involves multiple pathways conspiring together to drive fibrosis, and that blockade of individual pathways, whereas effective in animal models, has been much less effective in actual patients (19). For example, RhoA can be activated not just by TXA2R but by multiple upstream pathways, including YAP (yes-activated protein), Wnt, TGFβ (transforming growth factor β), MRTF (myocardin-related transcription factor), and integrins (10). Perhaps combination therapies that block multiple signaling pathways, including TXA2R, can help turn the tide against IPF. Unfortunately, even drugs that have activity in humans, such as the U.S. Food and Drug Administration-approved compounds pirfenidone and nintedanib, have proven incapable of reversing established fibrosis—the clinical holy grail. Finally, it remains possible that achieving this holy grail will require not merely the blockade of individual drivers but the restoration and activation of endogenous antifibrotic "brakes" on fibrosis capable of opposing a myriad of redundant driver pathways yet are disabled in fibrosis. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Misbehaving Guests in the Right Ventricle

Macrophage–NLRP3 Activation in Pulmonary Hypertension

Excessive inflammation has been linked to the development of right ventricle (RV) failure in pulmonary arterial hypertension (PAH) (1, 2). However, mechanisms of inflammation initiation and propagation during RV failure development are not entirely elucidated. The nucleotide-binding domain, leucine-rich–containing family, and pyrin domain-containing protein 3 (NLRP3) inflammasome is a mediator of organ dysfunction in several conditions marked by inflammation or cellular stress (3, 4). On priming by damage-associated or pathogen-associated molecular patterns and activation by a variety of additional stimuli, NLRP3 employs ASC (apoptosis-associated speck-like protein) to form an NLRP3–ASC complex (Figure 1). This complex then recruits and activates caspase 1, which subsequently cleaves pro–IL-1β and pro–IL-18 to activate IL-1β and IL-18, respectively. Activated IL-1β and IL-18 are then released from the cell with the help of the pore-forming protein gasdermin D (also activated and cleaved by NLRP3–ASC–caspase 1) to induce pyroptosis, an inflammatory type of lytic programmed cell death. Although NLRP3 activation and pyroptosis frequently occur during infections with intracellular pathogens, NLRP3 activation may also occur in the setting of sterile inflammation. For example, the NLRP3 inflammasome is activated in left heart failure and has been linked to the development of contractile dysfunction (5).

Emerging evidence suggests that the NLRP3 inflammasome is activated in the pulmonary vasculature in models of PAH (6). This is not surprising because triggers of the NLRP3 inflammasome, such as potassium efflux, calcium influx, and altered mitochondrial reactive oxygen species generation, are common in vascular cells in PAH (7). However, it remains unknown if NLRP3 inflammasome activation also occurs in the RV. In light of data on inflammasome activation in the left ventricle (LV), and given the observation that patients with severe PAH exhibit macrophage infiltrates in the RV (8), it is conceivable that NLRP3 may also be activated in the RV and contribute to RV maladaptation.

In this issue of the *Journal*, Al-Qazazi and colleagues (pp. 608–624) test the hypothesis that RV inflammation, driven by activation of the NLRP3 inflammasome in recruited macrophages, is a contributor to RV maladaptive remodeling in experimental pulmonary hypertension (9). The authors demonstrate that M1-polarized, monocyte-derived, $CCR2^{+}$ macrophages are increased in RVs (but not LVs) of rats with monocrotaline- or sugen/hypoxia-induced pulmonary hypertension and highly express NLRP3. On the other hand, in a rat model of pulmonary artery banding without RV failure, NLRP3 signaling was not upregulated. Cultured monocytes from monocrotaline pulmonary hypertension rats exhibit NLRP3 activation and mediate mitochondrial damage in neonatal rat cardiomyocytes cocultured with these cells. The altered cardiomyocyte phenotype was prevented when the coculture systems were cotreated with the NLRP3 inhibitor MCC950. *In vivo*, MCC950 reduced RV NLRP3 activation and attenuated pulmonary vascular remodeling, hemodynamic alterations, and RV dysfunction. In RV tissues from patients with PAH with decompensated RV function, there was evidence of macrophage NLRP3 pathway upregulation compared with control subjects. Together, these data demonstrate

Supported by the National Heart, Lung, and Blood Institute (5R01HL144727 [T.L.]); U.S. Department of Veterans Affairs (VA Merit Review Award 2 I01 BX002042-07 [T.L.]); and Bayer (PHAB Level 2 Award [R.S.F.]).

Originally Published in Press as DOI: 10.1164/rccm.202205-0977ED on June 15, 2022