The Witschi Hypothesis revisited after 35 years: genetic proof from SP-C BRICHOS domain mutations

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The Witschi Hypothesis revisited after 35 years: genetic proof from SP-C BRICHOS domain mutations. Am J Physiol Lung Cell Mol Physiol 305: L906–L911, 2013. First published October 18, 2013; doi:10.1152/ajplung.00246.2013.—Over 35 years ago, Wanda Haschek and Hanspeter Witschi published a theory for the pathogenesis of lung fibrosis that dared to challenge the longstanding view of lung fibrosis as an “inflammatory disease.” On the basis of considerable experimental evidence, they proposed that lung fibrosis was initiated and propagated by microfoci of epithelial damage that, if unrepaired, upset the normal epithelial-fibroblast balance to create profibrotic microenvironments, without any obligatory contribution of “inflammatory” cells. Unfortunately, this theory was largely overlooked for many years. In the meantime, the repeated failure of attempts to treat idiopathic pulmonary fibrosis with anti-inflammatory regimens has led some investigators to revive the theory referred to, in decades past, as “The Witschi Hypothesis.” This manuscript briefly reviews more recent evidence in support of the “Severity of Epithelial Injury” Hypothesis proposed by Haschek and Witschi. More important, it offers the updated viewpoint that mutations in the BRICHOS domain of surfactant protein C, which cause interstitial lung disease and induce cell death specifically in lung epithelial cells, in effect provide genetic proof that the Witschi Hypothesis is indeed the correct theory to explain the pathogenesis of fibrosis in the lungs.

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The Failure of Anti-Inflammatory Therapies

A remembrance of the Witschi Hypothesis is extremely important not only as an academic exercise, but also in light of the repeated failure of anti-inflammatory/immunosuppressive therapeutic clinical trials to show benefit for the treatment of the most insidious and frequent of the interstitial lung diseases, idiopathic pulmonary fibrosis (IPF). The traditional attempts at treatment of IPF with corticosteroids were ultimately found to provide no survival benefit (24) and to be associated with significant comorbidity (9). Despite promising preliminary data, the addition of azathioprine to the corticosteroid regimen did not produce the expected statistically significant improvement (29). Recently, in a larger meta-analysis, the formerly standard “triple therapy” of prednisone, azathioprine, and N-acetylcysteine was deemed not only ineffective, but indeed harmful, on the basis that IPF patients receiving the triple therapy experienced more hospitalizations, more adverse consequences, and excess deaths compared with those receiving the placebo (46). Clearly, the anti-inflammatory/immunosuppressive approach to the treatment of IPF was failing.

From our viewpoint, the failure of anti-inflammatory therapies in IPF was not entirely surprising; the results of animal model experiments designed to prove that inflammation, or specific cells involved in the inflammatory response, played a causal role in lung fibrosis have not been overly convincing. In fact, two studies have suggested just the opposite of what the authors initially predicted and indeed support the alternate interpretation that inflammation inhibits, not stimulates, subsequent fibrogenesis in the lungs.

One of the earliest was conducted by Thrall and colleagues (37), who tested the theory that the influx of neutrophils (PMNs) into the lungs shortly after administration of bleomycin to rats played a pivotal role in mediating the subsequent deposition of collagens. Using anti-PMN serum developed in rabbits, they first depleted over 90% of circulating PMNs from rats and then administered bleomycin to induce a fibrotic response. To the authors’ surprise, the PMN depletion did not reduce, but rather increased, the deposition of lung collagens significantly, leading them to conclude that the infiltrating “PMN, rather than accelerating or promoting the fibrotic response, may be attempting to restrict it” (37).

Another is the more recent generation of mice with a more robust inflammatory response by knockout of the IL-10 gene by Huaux et al. (17). The mice deficient in IL-10, a primarily anti-inflammatory cytokine, had a more robust inflammatory response after administration of silica to the lungs, as demonstrated by increased numbers of lymphocytes, PMNs, lactate dehydrogenase (LDH), and total protein in bronchoalveolar lavage fluid after the silica challenge, relative to wild-type mice after challenge. Rather than increasing the deposition of collagens, the more robust inflammatory response imparted by IL-10 knockout significantly decreased lung collagen deposition, leading the authors to conclude that “IL-10 is anti-inflammatory but exerts a pro-fibrotic activity” (17).

We suspect that some readers are now frustrated with what was just presented, an admittedly incomplete treatment of the many studies of other inflammatory cell types and processes, cytokines, and other immune/inflammatory factors that suggest positive and causal roles in lung fibrogenesis. On the other hand, we suggest that when considered as a whole, the evidence supporting the view of lung fibrosis as an inflammatory disease is at best conflicting, particularly in light of the failure of anti-inflammatory clinical trials in IPF. Other authors have previously reviewed the evidence against the “inflammation hypothesis” in a clinical perspective and in far more detail than is possible here (11, 31).

Two counterarguments that proponents of the inflammation hypothesis have often raised, at least in the experience of this author (B. D. Uhal), are 1) that the correct combination of anti-inflammatories for use in IPF has just not yet been found, and 2) that IPF patients present late in the course of their disease, and therefore it might be possible that anti-inflammatories could provide benefit if administered earlier. Regarding the latter counterargument, two facts undermine this rationale: first, the histopathology of IPF is heterogeneous both spatially and temporally, often revealing both old and “nascent” lesions, which are believed to reflect “early” pathogenic processes even in patients with more advanced disease (31). If inflammation drove the nascent lesion, anti-inflammatories should still have provided benefit. Second, although it is very difficult, if not impossible, to know what exact events initiate the fibrogenic response in patients with IPF, animal models in which experimental treatments were administered prophylactically gave results that at best conflicted and at worst contradicted a role for inflammation in lung fibrogenesis as discussed above.

Regarding the first counterargument that an optimum anti-inflammatory cocktail has not yet been constructed, a search of the symposia on Clinical Trials in IPF at the annual ATS and ERS meetings over the last 20 years offers evidence that the best clinical researchers have devoted considerable effort to testing the most likely candidate anti-inflammatory regimens in IPF, albeit unsuccessfully. We suggest that the potential role of pharmaceutical firms in perpetuating these efforts should be considered carefully and that caution be taken in the planning of future clinical trials of new anti-inflammatory compounds or cocktails.

Review of the Witschi Hypothesis

Surprisingly to this author, the speculation that lung fibrosis might not be an inflammatory disease continues to raise eyebrows, despite the failure of anti-inflammatory/immunosuppressive therapeutics in IPF and conflicting experimental data just mentioned. Yet more than 35 years ago a theory of lung fibrogenesis that was independent of inflammation was proposed and defended on the basis of cleverly designed toxicological studies in a variety of animal species as well as lung explant and cell culture/coculture models (see Fig. 1). The earliest of these, published in 1979 by Haschek and Witschi (15), studied BALB/c mice exposed to precisely titrated doses of butylated hydroxytoluene (BHT) followed by carefully timed and titrated exposures to hyperoxic gas or room air. The antioxidant BHT damaged exclusively the type I alveolar epithelial cell layer, which, without subsequent exposure to hyperoxia, was replaced by type II pneumocytes proliferating and differentiating into type I cells (verified by electron microscopy autoradiography) without the induction of fibrosis.

If, however, the type II cell division was delayed by additional exposure to hyperoxic gas, lung collagen deposition occurred in regions where the epithelial damage was not repaired. Importantly, the hyperoxia itself was not fibrogenic...
when applied alone, nor if applied together with BHT at times before or after the peak of the type II cell proliferative response; the hyperoxia was fibrogenic only if it was applied at a time and dose that inhibited type II cell proliferation and repair. These observations were the first to lead to the hypothesis that a lack of alveolar epithelial repair was the key event that led to a fibrotic response. The same conclusion was derived from subsequent studies of blood-free lung explants conducted in the following years by Adamson, Young, Bowden, and others at the University of Manitoba (4). Studying a wide range of exposure times and concentrations of hyperoxic gas applied in vivo, these investigators noted that fibrosis in blood-free lung explants occurred only at locations and exposure conditions in which alveolar epithelial repair was incomplete. Detailed electron microscopy and autoradiographic cell kinetic studies supported the hypothesis that the delay of epithelial repair, not inflammation, was what led to the activation of nearby fibroblast proliferation and collagen deposition.

Both the Witschi and Adamson research groups explored this hypothesis further over the subsequent decade and longer, with a variety of in vivo and ex vivo models and injurious agents including but not limited to BHT, hyperoxia, paraquat, bleomycin, silica, and asbestos (1, 3, 14, 16, 20). Those labor-intensive and time-consuming studies, too numerous to review here, consistently supported the same theory that both groups refined in articles published in later years (2, 3, 14, 15, 16, 20, 49). More recently discovered fibrogenic surfactant protein C BRICHOS domain mutations that delay epithelial repair by inducing ATII cell apoptosis now offer genetic confirmation of this hypothesis. See text for details.

Abnormal Epithelial-Fibroblast Balance in Lung

The consideration of lung fibrosis as a disease of epithelial-fibroblast imbalance was revived more recently by Selman et al. (31) and Gauldie et al. (11), both of whom did an excellent job of articulating the growing evidence against the traditional view of lung fibrosis as an inflammatory disease. Both groups of authors also reviewed the still-growing experimental evidence describing how the death of epithelial cells, in itself, could create a profibrotic microenvironment without an obligatory contribution of inflammatory processes. One of the most compelling reviews on this topic, however, was published years earlier by Simon (32), who first discussed the concept of “antifibrotic functions” of intact alveolar type I and II cells that include, among many other roles, 1) robust production of PGE2 that inhibits fibroblast proliferation; 2) constitutive production of plasminogen activators that help to degrade microfoci of fibrin, along which fibroblasts can migrate into the alveolar air spaces; 3) production and deposition of basement membrane components (laminins, fibronectins) that promote

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**Fig. 1.** Summary of the “Severity of Epithelial Injury” or “Witschi” Hypothesis proposed in 1979 by Wanda Haschek and Hanspeter Witschi (15). Microfoci of alveolar epithelial injury, which often impact the more sensitive alveolar epithelial type I cells (ATI), are normally repaired by type II pneumocyte (ATII) proliferation without fibrosis (blue). If alveolar epithelial repair by ATII cells is delayed (red), underlying fibroblasts are released from normal epithelial inhibitions and fibrosis progresses. This theory was supported by robust experimental evidence (1, 2, 3, 14, 15, 16, 20, 49). More recently discovered fibrogenic surfactant protein C BRICHOS domain mutations that delay epithelial repair by inducing ATII cell apoptosis now offer genetic confirmation of this hypothesis. See text for details.
Evidence that AEC Death Is Sufficient to Cause Lung Fibrogenesis

Currently there exists a surge of interest by the lung fibrosis research community in the roles of autophagy in lung fibrogenesis. More than a decade ago, a similar interest centered on the topic of apoptosis, at that time a "new" idea as it related to lung cells. The observation of apoptosis markers in the alveolar epithelium of lung biopsies obtained from patients with IPF (28, 41) led several research groups to test the ideas first offered by Haschek and Witschi, utilizing the potential of pharmacological inhibitors of apoptosis. The caspase inhibitor ZVADfmk, administered in vivo to mice or rats, potently inhibited apoptosis of alveolar epithelial cells after bleomycin administration as expected, but more importantly it also blocked the subsequent accumulation of lung collagens (18, 44) as predicted by the Witschi Hypothesis. Moreover, the discovery that alveolar epithelial cells could express the receptor Fas (Apol, CD95) after lung injury (8) led to the design of experiments to test whether induction of apoptosis, directed toward the epithelium by intratracheal administration of Fas activators, could produce a fibrogenic response. As expected, tracheal administrations of Fas-activating antibodies induced apoptosis mainly in the lung epithelium that was followed by collagen accumulation several weeks later (13). The effect was also dose dependent, since greater induction of epithelial apoptosis with higher doses of Fas activator led to greater collagen accumulation.

One criticism of these studies was the potential nonspecificity of caspase inhibitors or Fas activators. However, two different genetic approaches produced the same result: germ-line knockout of the apoptosis signaling molecule Bid in mice prevented TGF-β1-induced epithelial apoptosis and the deposition of lung collagens that would otherwise follow intratracheal bleomycin administration (6). Another completely different approach, but that again yielded the same outcome, was taken by Sisson et al. (33), who used the surfactant protein C (SP-C) promoter to express the human diphtheria toxin (DT) receptor specifically in the alveolar epithelium of mice. Subsequent administration of DT induced alveolar epithelial cell death and, as predicted by Witschi, increased lung collagen deposition in the mice expressing the epithelial DT receptor in the lungs (but not in wild-type mice). Despite using completely different methods, all these studies strongly support the conclusions that 1) inducing lung epithelial cell death causes lung collagen deposition, and 2) inhibiting lung epithelial cell death blocks the lung collagen deposition that otherwise would follow an injury to the epithelium. All these outcomes were predicted by the Witschi Hypothesis.

The discovery that alveolar epithelial cells could express the receptor Fas (Apol, CD95) after lung injury (8) led to the design of transgenes to be expressed specifically in lung epithelial rather than mesenchymal differentiation signaling; and, more recently discovered, 4) generation of matrix metalloproteinases of the subtypes that degrade interstitial collagens (10). Owing to these and other antifibrotic activities of intact alveolar epithelial cells, death or incomplete repair of a normal epithelial layer could be sufficient to initiate and support fibrogenesis. We refer the reader to those and other excellent reviews for a much more complete treatment of this important topic.

At least 10 pathogenic mutations in the BRICHOS domain of the human SP-C have now been discovered through genetic analyses of DNA samples from infants, children, and/or adults with diffuse interstitial lung diseases (see Table 1). As described in detail by their discoverers (19, 22, 26), the BRICHOS domain is involved in processing of the immature SP-C and, in particular, its proper folding during the intracellular processing steps that eventually, under normal conditions, led to the correct packaging of the mature SP-C together with other surfactant components into the lamellar bodies for secretion. BRICHOS domain mutations can disrupt protein folding.

Table 1. Fibrogenic SP-C BRICHOS domain mutations and alveolar epithelial cell death

<table>
<thead>
<tr>
<th>Mutation in sequence order</th>
<th>G100S</th>
<th>P115L</th>
<th>Exon 4</th>
<th>A116D</th>
<th>Q145H</th>
<th>T187N</th>
<th>L188P</th>
<th>L188Q</th>
<th>C189Y</th>
<th>L194P</th>
</tr>
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<tbody>
<tr>
<td>Causes ILD</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Cause AEC Death</td>
<td>yes</td>
<td>?</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>UPR markers</td>
<td>increased</td>
<td>?</td>
<td>increased</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>increased</td>
<td>?</td>
<td>?</td>
<td>increased</td>
</tr>
<tr>
<td>ER stress Bip, HSPs</td>
<td>increased</td>
<td>reduced (38)</td>
<td>increased</td>
<td>reduced (38)</td>
<td>increased (50)</td>
<td>reduced (38)</td>
<td>increased (19)</td>
<td>reduced (38)</td>
<td></td>
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<td>25, 38, 48</td>
<td>21, 23</td>
<td>38, 50</td>
<td>12</td>
<td>12</td>
<td>19, 22, 38</td>
<td>12, 36</td>
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SP-C, surfactant protein C; ILD, interstitial lung disease; AEC, alveolar epithelial cell; UPR, unfolded protein response; ER, endoplasmic reticulum; HSPs, heat shock proteins.
dye exclusion (40). Therefore, determinations of whether the other fibrogenic SP-C mutants listed in Table 1 also induce apoptosis, or any other mode of AEC death, will be a most important topic in light of the seminal theory of Dr. Haschek and Dr. Witschi. We suggest that these determinations be performed carefully, since LDH release (50) or MTT assay (38) are not capable of detecting apoptosis, and even a seemingly minor stimulation of apoptosis that is nonetheless sufficient to reduce the capacity of the epithelium to repair would compromise reepithelialization over time (39).

Summary and Perspective

The above discussion attempts to articulate our rationale for concluding that epithelial cell death, not inflammation, “causes” fibrosis in the lungs by upsetting the normally anti-fibrotic epithelial-fibroblast cross talk. When comparing the two sides of this debate, every published attempt to initiate lung fibrosis by inducing cell death, as specifically as possible in the epithelium, produced a fibrotic response (13, 33). Moreover, every published attempt to inhibit alveolar epithelial cell death through caspase inhibition (18, 44), genetic deletion of apoptosis signaling molecules (6), or counteracting cell death through administrations of epithelial-specific mitogens (30) resulted in inhibition of lung fibrogenesis. Can a similar argument be made for attempts to test the role of inflammation in the causation of lung fibrogenesis? The answer is clearly no: efforts to deplete individual inflammatory cells (37) or to create a more robust inflammatory response (17) gave results opposite to those expected and led the authors to conclude that the inflammatory cells chosen for study appeared to inhibit, not promote, fibrogenesis as initially hypothesized.

We now know that the BRICHOS domain mutations of SP-C have several features in common that strongly support the “Severity of Epithelial Injury” or “Witschi” hypothesis: 1) SP-C is expressed in only one cell type, the alveolar epithelial type II cell; and 2) in every case in which apoptosis has been evaluated, fibrogenic SP-C BRICHOS mutations induce cell death in the alveolar epithelial cell. On this basis alone, these mutations should be considered genetic proof that the hypothesis proposed in 1979 by Haschek and Witschi was correct. Many years ago, a reviewer of one of my past grants suggested that to prove this hypothesis I needed to induce cell death specifically in the alveolar epithelium and demonstrate a subsequent fibrotic response. Is this not exactly what nature has done in the fibrogenic SP-C BRICHOS domain mutants that induce apoptosis specifically in type II pneumocytes? To supporters of the inflammation hypothesis that still prefer to disagree, responses to this Perspective are invited. However, it is asked that rebuttals written to support the inflammation hypothesis be based mainly on published data, as was done here, rather than speculation or wishful thinking.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

B.D.U. conception and design of research; B.D.U. drafted manuscript; B.D.U. and H.N. edited and revised manuscript; B.D.U. and H.N. approved final version of manuscript; H.N. analyzed data.

REFERENCES


