Polycystic kidney disease, fungi, and bacterial endotoxin: shifting paradigms involving infection and diet

J. Thomas Hjelle, Marcia A. Miller-Hjelle, Deborah M. Nowak, Mary Ann Dombrink-Kurtzman* and Stephen W. Peterson†

Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine, *Mycotoxin Research and †Microbial Properties Units, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, Peoria, Illinois, USA

The effort to understand the significance of ever-more numerous observations of fungal and bacterial components in tissues and fluids from patients with polycystic kidney disease (PKD) is the focus of this review. Could this second most common genetic disease in man be promoted or even caused by microbes or their components/toxins found in PKD patients? Findings include fungal glucans, fungal antigens, immunoglobulin E reactive with fungal antigens, fungal DNA, bacterial endotoxin from at least three genera, and a newly discovered class of bacteria, Nanobacterium. A new species of fungus, Penicillium pimiteouisense, has been isolated from PKD kidney cells in vitro. What are the sources of these microbes or microbial parts and by what mechanism(s) do they alter those few cells that become the progenitors of all phenotypically cystic cells? Hypotheses concerning the interactions of microbial components with PKD biology are presented along with strategies to confirm and exploit therapeutically these ideas. The study of microbes and their parts in this prominent chronic, genetic disease may provide insights into other polymicrobial, multifactorial diseases.

Keywords: Polycystic kidney disease, glucan, endotoxin, fumonisin, shingolipids.

INTRODUCTION

New methods and concepts are changing the fundamental framework for analysis of chronic diseases potentially caused by microbes [1,2]. Koch's postulates [1-4], rules developed in the 1890s to assess the contribution of microbes to disease causation, are virtually outdated. While useful in understanding acute infections, Koch's postulates become unattainable when applied to many chronic diseases that involve concepts such as i) hit-and-run pathogenesis, and ii) co-pathology driven by synergy between one or more microbes and/or microbial toxins derived from the environment. This expanded awareness of infectious disease causality has changed fundamental research and, in some cases, treatment of a growing number of chronic diseases [1-4] (e.g., stomach ulcers, atherosclerosis, Alzheimer's disease, polycystic ovary disease, sarcoidosis, forms of inflammatory bowel disease, autism, schizophrenia, multiple sclerosis, cerebral palsy, systemic lupus erythematosus, Wegener's granulomatosis, diabetes mellitus,
primary biliary cirrhosis, tropical sprue, Kawasaki disease, and may forms of cancer).

As for genetic diseases, it was recognized over 100 years ago that the genetic background of the patient and microbe(s) interact to influence disease initiation and progression [1,2]. Thus, a microbe may have a profound effect in genetically vulnerable individuals, but have little effect or effects distributed differently in time and appearance in tissues of individuals not carrying the mutation responsible for the vulnerability to disease. Investigation of the concept that microbes are provocateurs of genetic disease would logically start with the most prevalent heritable diseases.

Autosomal dominant polycystic kidney disease (ADPKD), the most common monogenetic disease in humans (one out of every 200–1000 live births), is classically described as a genetic disease that results in abnormal structures and function in kidney, liver, gut, vasculature and other organs [5-9]. Progressive multiple cyst formation in both kidneys ultimately leads to end-stage renal disease (ESRD) in 50% of individuals who have PKD by age 50 years. ADPKD accounts for approximately 8–10% of all ESRD and afflicts 12–15 million individuals worldwide. Kidney dialysis and transplantation are the only life support therapies useful in end-stage PKD.

CURRENT VIEWS OF PKD BIOLOGY

There are two views of PKD aetiology. The genocentric view holds that all ADPKD biology can be explained by gene mutation and resultant aberrations in cell biology independent of environmental factors. Under this approach, the research focus is definition of the underlying genetic anomalies in PKD with the ultimate goal of correcting the genetic mutation responsible for PKD pathology. Alternatively, the vulnerability view posits that ADPKD represents a mutation-based vulnerability to environmental factors, such as microbes and their toxins, chemical toxins, and dietary content. Here the approach is to understand the contributions of environmental factors to PKD that will ultimately allow prevention of mutations to PKD genes, slow the progression of cyst growth, and preserve kidney function throughout a normal lifespan. Experimental treatments for PKD may directly alter the behaviour of PKD cells and/or diminish the influence of microbial and dietary factors on PKD cells and tissues.

Genetic anomalies in PKD

Mutations in three separate genes yield phenotypically indistinguishable forms of ADPKD: PKDI on chromosome 16 and PKD2 on chromosome 4, which normally code for proteins polycystin 1 and polycystin 2, respectively, and a yet-to-be mapped gene [8,9]. About 90% of ADPKD is due to mutations of the PKD1 gene. For both ADPKD types 1 and 2, mutations are dispersed over the entire gene suggesting that no single region of these genes is more vulnerable to mutation. Most families examined exhibit a different mutation within PKD1 and -2 genes suggesting that there is a high first-time mutation rate (PKD → PKDM). In ADPKD (heterozygote) individuals, one normal and one mutated copy of the PKD gene (PKD:PKDM) are present in all cells. However, kidney epithelial cells lining the larger sized cysts demonstrate a loss of heterozygosity, meaning that mutation of the remaining normal allele occurred (PKD:PKDM → PKD M:PKD M) [10]; this second mutation is proposed to be responsible for the complete loss of any remaining functional polycystin leading, by as yet unknown mechanisms, to rapid cyst formation and progression to ESRD. If true, then both ADPKD parent and offspring must each separately experience mutagenesis. This two-hit model of cystogenesis is derived from a similar model for carcinogenesis and is supported by pathological descriptions of PKD cysts as fluid-filled tumours [6,9,10].

The cause(s) of the first and second mutations is/are unknown. It has been proposed that the presence of three polypyrimidine tracts facilitates triple helix formation in the PKD1 gene, thereby making it highly vulnerable to mutation that results from exposure to mutagens, oxidative stress and/or errors in DNA methylation, especially during rapid duplication of DNA that occurs during wound healing [8,9]. A vulnerability to microbe-induced stress contrasts with the concept of an intrinsic instability of the PKD genes that yields mutations independent of environmental factors.

PKD biology

Normal polycystins 1 and 2 are thought to associate to form a transmembrane heterodimer
that transduces information from the extracellular environment into the cell [8,9]. With the possible exceptions of neuronal tissue and skin, adult tissues that express polycystins also show alteration in PKD. Thus, it is inferred that PKDM:PKDM kidney cells lacking functional polycystins ultimately cause kidney cysts due to their inability to sense their local environment, exhibit normal functioning of the cytoskeleton and regulatory pathways that are linked to it, and regulate expression of genetic information. Once cells become PKDM:PKDM they exhibit an enhanced rate of proliferation, an immature phenotype, dysregulation of apoptosis and anomalies in numerous other secretory pathways and formation of extracellular matrix. The ADPKD kidney exhibits interstitial inflammation, altered extracellular matrix, kidney stones, and focal clonal growth of tubular epithelial cells yielding numerous cysts of various volumes [5-9]. Because microbes and their toxins are known to cause mammalian cell damage and induce tissue repair processes, produce mutagens/carcinogens, induce oxidative stress and tissue inflammation, induce apoptosis, alter DNA, affect immunomodulation, and are linked to kidney stone formation [11-23], it is reasonable to examine the concept that microbes initiate and/or provoke cystogenesis.

**Germ-free experiments and human infections in PKD**

The first evidence that microbes contribute to kidney cystogenesis was obtained by Werder et al. [24] in genetically cystic mice. Germ-free conditions essentially eliminated kidney cysts in these mice and increased survival to a normal lifespan; cystic littermates raised under ambient conditions developed kidney cysts and died early in life. Gardner et al. [25,26] found that normal rats made reversibly cystic by a chemical cystogen developed kidney cysts at a much slower rate under germ-free conditions than when raised in ambient conditions. Chemical-cystogenesis could be increased by injection of endotoxin1 to the germ-free rats or moving the germ-free rats to ambient conditions. In both cases, renal interstitial inflammation was a common finding in the highly cystic groups. Indeed, kidney inflammation is common in animal models of PKD, where reduction of tissue inflammatory reactions by drugs and diet are reported to diminish cystic lesions [9].

In humans, multiple kidney infections are correlated with more rapid progression of PKD to ESRD [5]. Occasional findings of fungi in PKD urine have been reported [27,28]. The greater incidence of infections and resultant greater morbidity and mortality in PKD individuals than the general population has prompted speculation that PKD may involve an as-yet uncharacterised defect in microbe clearance mechanisms [29]. In addition 80% of PKD patients are reported to have colonic anomalies, described in diverticula, which may allow enhanced entry of microbial materials from the gut [6], as occurs in leaky gut syndrome [30]. If one or more microbes or their toxins promote cystogenesis, then such microbes must be readily available to the PKD population; that is to say, a high rate of genetic vulnerability is matched by high rate of exposure to cystogenic microbe(s) or their toxins.

**DETECTION OF MICROBES AND MICROBIAL COMPONENTS IN PKD**

**Endotoxin and fungal (1 → 3)-β-D-glucans**

Endotoxin is regarded as the most potent and ubiquitous toxin to which man is exposed and is found in high levels in the human gastrointestinal flora. Even under normal conditions, small amounts of endotoxin are shed from bacteria in the gut and appear in the blood [31]. Early methods for the detection of endotoxins in quantities relevant to human biology required bioassays, such as the rabbit pyrogen test and Limulus Amebocyte Lysate (LAL) assay (Fig. 1). The classical (c)LAL assay reagents contain the components of two separate biochemical pathways that lead to gelation or colour formation, the positive result. One pathway is activated by endotoxin or purified LPS and a second pathway activated by 1 → 3-β-D-glucans (βDG). βDG are shed from the surface of fungi and distributed into body fluids. In the absence of positive fungal cultures and serology or fungal DNA, a finding of βDG is commonly associated with

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1In disease, lipopolysaccharides (LPS) are usually associated with other bacterial components, which collectively are called endotoxin; LPS is the principal biologically active component of endotoxin. LPS purified from cell walls of Gram-negative bacteria, such as Escherichia coli, are used experimentally to minimise pharmacological variability.
deep mycoses or fungaemia [32]. Elevated levels parallel the development and progression of disease and can precede positive blood cultures by days or weeks. Similarly, an associated decrease in βDG levels occurs with clinical improvement in common fungal infections, such as aspergillosis and with emerging pathogens such as *Fusarium* and related hyaline fungi [33].

The cLAL assay does not distinguish between endotoxin and fungal glucans. Unfortunately, some researchers have interpreted positive LAL findings as evidence that fungi produce LPS, which they do not. More recently, reagents developed to inhibit the glucan pathway in the LAL assay (e.g., CM-curdlan) allow for a differential (d)LAL assay that does distinguish endotoxin from fungal glucans (Fig. 1). Although the glucan pathway in the dLAL assay does not identify the genus of fungus involved, therapeutic intervention is not impeded due to the limited choice of available antifungal agents. βDG is not detected in certain situations, such as fungal colonisation and allergic disorders [32]. In contrast to serum and urine, PKD kidney cyst fluid has a slower turnover rate and thus may be viewed as an archive of fungal components accumulated during dietary, airborne, and colonising/infectious exposures. Measurement of these glucans in human specimens has proved useful in detecting generic fungal infections [33].

**Fig. 1.** Diagram of *Limulus* Amebocyte Lysate assay and its component pathways.
Using the cLAL assay we found that 80% of PKD males were positive for putative endotoxin in their urine, but none of the normal healthy male volunteers showed endotoxiuria [34]. In females, the PKD patients had higher levels than did normal female volunteers, where simple introital contamination might account for the low endotoxiuria observed [34,35]. None of the PKD patients exhibited evidence of current infection by symptomatology or routine urine cultures. By cLAL assay Gardner et al. [36] found putative endotoxin in 12% of the human PKD kidney cysts examined without evidence of culturable bacteria. They also reported substantial inhibition and interference with the cLAL assay of cyst fluid and inflammatory cytokines known to be induced by endotoxin (e.g., tumour necrosis factor α).

In contrast, we found that >25% of cyst fluids obtained from 12 PKD kidneys were positive for endotoxin when tested with dLAL assay modified with heating of the specimens or use of serial dilutions to minimise false-negative reactions caused by inhibitory substances, and increased incubation time to improve sensitivity [35,37]. Kidney cyst fluids from two out of 12 ADPKD patients and one out of two autosomal recessive PKD patients contained detectable fungal glucans by this dLAL assay. Values ranged from 40-120 pg βDG/ml cyst fluid; 10 pg/ml was the limit of sensitivity of this assay. Plasma βDG levels have been reported to be less than 10 pg/ml serum in healthy volunteers. Recent reports have indicated cutoff value of 20 pg βDG/ml proved satisfactory for sensitivity, specificity, negative predictive values and efficacy of this test. Applying this cutoff value to the PKD patients with detectable βDG yields clinically relevant results [32,37]. Although a number of commercial immunodetection tests are available, they have yet to be proven reliable in the respective clinical settings. An exception is detection of cryptococcal capsular material by latex agglutination or enzyme immunoassay [37]. Thus, concomitant use of culture and nonculture diagnostic methods is recommended.

Using experimental immunological methods, a small number of human PKD kidney cyst fluids were assayed for fungal antigens and human antibodies reactive with fungal antigens. Antigens from Fusarium, Aspergillus and Candida were found along with immunoglobulin (Ig) E, but not IgG reactive with Fusarium and Candida [39]. The biological half-life of microbe-specific IgG antibodies is only several weeks [40]. Although a larger and longitudinal study is required to define the incidence and speciation of the fungal components present in PKD fluids and tissues, these limited findings are consistent with exposure of PKD kidney to fungal components.

The gold standard for fungal diagnosis remains culture/histopathology. Fusarium spp, as with other hyaline fungi (Penicillium, Paecilomyces spp), are under-represented in the field of serodiagnostics. The identification of hyphae of hyaline fungi by histological diagnosis has limitations due to similar morphology, even to Aspergillus, and non-pathognomonic in situ manifestations [41,42]. Kaufman et al. [42] have demonstrated immunohistological separation
and identification of these hyaline filamentous fungi using a series of polyclonal fluorescent antibodies; their very promising clinical utility, however, has not been evaluated.

Fungal DNA

A small number of PKD cyst fluids were tested for fungal DNA by PCR methods using universal fungal primers [39]. Fungal DNA was found in six out of six cyst fluids from 3 PKD patients and PKD kidney tissue samples from two patients. Use of DNA methods to identify fungal colonizations/infections is especially important because it allows speciation of the organism and also overcomes the lack of specificity and sensitivity of current immunological fungal identification tests. To date, PCR methods have proven no more useful than classical serological and glucan tests for detecting fungal infections [37,43].

Culture of microbes from fluids and tissue

Over the years, we and others have attempted and largely failed to grow microbes from PKD kidney cyst fluids using routine microbiological methods. In contrast, we have encountered fungal growths when PKD kidney epithelial cells isolated from the cyst walls were propagated in cell culture [39]. Taxonomic characterisation of this fungus revealed it to be a heretofore-unrecognised species of Penicillium that we named P. piniteouense [44].

More recently, we have collaborated with E. O. Kajander and N. Ciftcioglu to culture nanobacteria from human PKD cyst fluids, urine, and blood [35]. This newly-discovered, culture-resistant Gram-negative bacterium was also found by electron microscopy and/or immunological methods in PKD kidney and liver cyst fluids. Nanobacteria were also found in aspirated cyst fluid from a patient with simple cystic kidney disease. Based on our findings of nanobacteria in PKD fluids and tissues and current knowledge of nanobacteria pathogenicity, we have proposed that nanobacteria contribute to PKD pathogenesis.

SOURCES OF MICROBES IN PKD

The gastrointestinal tract is the most likely source of microbial components in PKD because, first, it is the single most available pool of microbes and their components to which humans are exposed and, secondly, 80% of PKD patients exhibit anomalies of the colon. Thirdly, sentinel markers of gastrointestinal microflora (e.g., LPS of Bacteroides fragilis) [35] appear in kidney and liver cysts. The intact intestinal mucosa usually excludes antigen(s) of fungi from the general circulation although faecal concentrations of 1–38 μg/g have been reported; cell walls or exopolysaccharides (MWt 20000–75000) also are not absorbed in appreciable amounts [45]. The fungal antigens found in human PKD kidney (Fusarium, Aspergillus and Candida) are either present in the diet or colonise the human gastrointestinal tract [11,12]. Thus, dysfunction of the intestinal mucosal barrier in PKD may be sufficient to allow chronic or more frequent episodic entry of microbes and their components with their subsequent accumulation in renal cyst fluids. As wound healing is proposed to be altered in PKD [6], PKD gut may be more vulnerable to damage by microflora or microbial toxins in the diet.

Not to be underestimated is the potential contribution to PKD of foodborne microbial toxins, especially mycotoxins [11,12]. The World Bank has reported that 40% of human disease is impacted by mycotoxins [46]. Although Fusarium, Penicillium and Aspergillus are found in nature, we use fumonisins of Fusarium as an example of mycotoxins with a wide occurrence in the human diet. Fumonisins are known to alter the gastrointestinal tract and show kidney and liver toxicities [11,12,15,47,48]. Not discovered until about 1988, fumonisins are reported to be present in corn, grain, rice, sorghum and other agricultural products in commerce world-wide. Utilisation of agricultural products containing fumonisins is increasingly under regulatory scrutiny with import restrictions already in evidence. The need to reduce fumonisin content in food [49] has resulted in genetically engineered corn species that are resistant to insect damage, which in turn decreases the infiltration of Fusarium yielding much lower levels of fumonisins in corn kernels [50]. Even research in laboratory animals can be impacted by mycotoxins because fumonisins are reported to be present in variable levels in all batches of rodent chow tested to date [48]. Thus, experimental modification of diet may carry the unintended effect of increasing or decreasing exposure to microbial products.
COMPONENTS IN HUMAN PKD TISSUES AND PKD BIOLOGY

Three points need to be made about assumptions of microbial content and test systems. First, because survey methods for the detection of fungal components at levels relevant to chronic diseases are largely unavailable, we have taken the view that finding fungal species by DNA and/or antigen in PKD tissue and fluids is presumptive of the presence of all components of that fungus. This potentially errs on the side of inclusion, but frames issues for future research and respects the polymicrobial nature of human exposures.

Second, numerous reports describe the biology of PKD in humans and experimental animals. In contrast, the effects of microbes and their components and toxins are described in genetically normal animals or in general human populations. Thus, tests of microbial toxins in non-vulnerable populations tend to exclude or minimise potential findings of procystogenic activity. In addition, little is known of the differences in pathobiology and susceptibility to microbial toxins of kidney cells with only one mutated PKD allele compared to cells with hits to both alleles (i.e., PKD:PKDM versus PKD^1:PKDM).

Third, endotoxin from Gram-negative bacteria and fungal toxins/components are known to act synergistically to cause toxicity to mammalian systems [31]. Thus, low levels of multiple toxins yield effects seen with much higher levels of a single toxin. Because all fungal infections and mycotoxicoses occur in the presence of daily exposures to endotoxin, there is an emerging view that the effects of endotoxin and fungal components on human biology are highly intertwined and perhaps inseparable in most clinical settings.

Contribution of Fusarium, Penicillium and Aspergillus to PKD pathophysiology

Occasionally, kidney cysts in normal animals have been reported following exposure to toxins from Fusarium, Penicillium and Aspergillus [51, 52]. All produce carcinogens/mutagens [11,12], which may come into play in the mutagenesis of PKD genes. In addition, toxins and other products of these fungi may alter other cellular processes and reactions that also impact functioning of PKD kidney epithelial cells with one or two mutated alleles. To illustrate these points, we have listed the effects of fumonisins as a prototypical mycotoxin with detailed anomalies reported for PKD, and the effects of endotoxin, which is known to act synergistically with fumonisin to alter a number cellular processes (Table 1). The kidney is the most sensitive organ to fumonisin toxicity [48].

Endotoxin and fungal (1 → 3)-β-D-glucans

Endotoxin production is the single property shared by the bacteria (nanobacteria) cultured from or tentatively identified immunologically (E. coli, Bacteroides, Chlamydia and Bartonella) in PKD kidney tissue and fluids [35]. Because Nanobacterium sanquineum reacts with anti-Chlamydia LPS and Bartonella henselae hyper-immune sera, and is related by 16S rDNA sequence to Bartonella, findings of putative Chlamydia or Bartonella components in PKD may be due to the presence of nanobacteria. As noted in Table 1, endotoxin is known to affect, either directly and/or in synergy with other microbial components, many of the processes reported to be abnormal in PKD. The structure of LPS influences its biological activity. In turn, the structure of LPS in mammalian fluids is determined by the genus/species of the bacteria that produced it, the metabolic status of the bacteria at the time of LPS synthesis, and the subsequent degree of metabolism or degradation of LPS by mammalian cells. Much is yet to be learned of the relationship between LPS structure and ability to act synergistically with other microbial products to influence mammalian biology. LPS from E. coli is reported to enhance inflammation caused by fungal glucans [53,54].

Alteration of sphingolipid biology in fumonisin and endotoxin toxicity

Sphingolipids play an essential role in the regulation of cellular activity [15]. Alteration of sphingolipid biology was one of the first effects of fumonisin to be defined [12]. Subsequent research has shown that fumonisin, its metabolites and related mycotoxins inhibit, and in some cases are metabolised by, enzymes important to sphingolipid formation [55] (Fig. 2). Because fumonisin inhibits ceramide synthase leading to accumulation of sphinganine, a precursor to ceramide formation, the ratio of levels of free sphinganine (SA) to free sphingosine (SO) in
Table 1. Effects of fumonisins and endotoxin (ET) compared with anomalies of polycystic kidney disease (PKD)

<table>
<thead>
<tr>
<th>Anomalies in PKD</th>
<th>Fumonisin(s) effects</th>
<th>Endotoxin and glucan effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diversy cytokine formation (e.g., TNF-α, caspase activation)</td>
<td>Acts via TNF-α cascade and caspases: acts synergistically with ET to induce TNF-α</td>
<td>Induces cytokines and multi-potent cellular responses</td>
</tr>
<tr>
<td>Altered sphingolipid levels</td>
<td>Blocks acylation of sphingoid bases; alters sphingolipids, especially sphingoid base levels</td>
<td>ET alters FA composition of sphingolipids and phospholipid metabolism; mimics ceramide oxidation of phospholipids</td>
</tr>
<tr>
<td>EGF involved in cyst formation</td>
<td>Peroxidation of membrane lipids. Oxidised lipids react with EGF receptor to cause receptor activation</td>
<td></td>
</tr>
<tr>
<td>CAF present in cyst fluid</td>
<td>Current description of CAF is similar to known chemistry of fumonisins(s)</td>
<td>Chemistry of endotoxin, sphingolipids and fumonisins remarkably similar</td>
</tr>
<tr>
<td>Cell proliferation</td>
<td>Alters cell growth</td>
<td>Alters cell growth</td>
</tr>
<tr>
<td>Dysregulation of apoptosis</td>
<td>Alters apoptosis</td>
<td>Alters apoptosis</td>
</tr>
<tr>
<td>Cell differentiation</td>
<td>Alters activity of protein kinases and phosphatases; altered sphingoid base levels cause loss of cell maturity in kidney cells in vitro</td>
<td>ET alters activity of protein kinases and phosphatases, and signal transduction pathways; nephron formation altered during in-utero exposure</td>
</tr>
<tr>
<td>Fluid secretion and location of Na⁺K⁺-ATPase on membrane; may involve cAMP</td>
<td>Alters calcium fluxes and distribution of Na⁺K⁺ATPase in MDCK cells in vitro; activates cAMP response elements</td>
<td>ET alters calcium fluxes and signal transduction cascade involved in osmoregulation</td>
</tr>
<tr>
<td>Intersitial inflammation</td>
<td>Alters immune system</td>
<td>Classical inflammatory molecules</td>
</tr>
<tr>
<td>Angiosclerosis</td>
<td>Alters vascular cells in vitro and disrupts vascular integrity</td>
<td>Alters vascular reactivity; found in atherosclerotic plaques</td>
</tr>
<tr>
<td>Tubular obstruction</td>
<td>Causes sloughing of renal epithelia</td>
<td>ET is nephrotoxic</td>
</tr>
<tr>
<td>Pro-oxidative metabolism</td>
<td>Oxidation of lipids, DNA, and kidney cells in vitro; increases LPS-induced nitric oxide formation in macrophages</td>
<td>Oxidative bursts resulting in cell damage. Enhances toxicity of diverse drugs and microbial toxins</td>
</tr>
<tr>
<td>Susceptibility to infection</td>
<td>Alters immune system</td>
<td>Alters immune system</td>
</tr>
<tr>
<td>Mutagenesis</td>
<td>Carcinogen; may act via multiple mechanisms including disrupted uptake of folate resulting in hyperhomocysteinemia. In-utero exposure alters embryogenesis in rodents; in vivo, such mycotoxins target kidney, liver, and vasculature</td>
<td>ET reported to be genotoxic in animals and causes DNA strand breaks in vitro. At low doses, exhibits antitumor activity in some cells</td>
</tr>
<tr>
<td>Anomalies of colon reported in 80% PKD patients. A high percentage of dialysis patients have acquired form of PKD independent of a family history of PKD; bowel dysfunction is commonly seen during dialysis</td>
<td>Alters gut integrity. Leaky gut may promote absorption of microbial parts during dialysis and in PKD. Hyperhomocysteinemia reported to be risk factor for cardiovascular disease during dialysis</td>
<td>Bacterial and fungal infections occur during dialysis. Synergy of ET with glucans, drugs and mycotoxins at the cyst is likely. ET in cysts may be derived from gut bacteria</td>
</tr>
</tbody>
</table>

TNF, Tumour necrosis factor; FA, fatty acid; EGF, epidermal growth factor; CAF, cyst-activating factor; MDCK, Madin Darby canine kidney cell line.

tissue and body fluids has been used as a biomarker for fumonisin-mediated toxicity in experimental animals and man [12,15]. If fumonisin plays a role in PKD biology, then alterations in sphingolipid levels should be observed; at this time, there is no evidence that defective polycysts impact sphingolipid biology. Deshmukh et al. [56] reported lower total sphingolipid content in kidneys from phenotypically cystic mice compared with their phenotypically normal littermates. There is a paucity of human tissue data in fumonisin intoxication, fungal infection, and in the early stages of PKD pathogenesis.

Table 2 lists the content of free and total SA and SO in normal human and end-stage PKD kidney tissue. Although the sample size is small, we observed the highest ratio of free SA:SO in cyst walls dissected from PKD kidneys showing evidence of fungal glucans and fungal antigens.
from *Fusarium* [39]. This elevated ratio was not observed in homogenates of kidney cross sections, which is consistent with a focal derangement of sphingolipid metabolism within cysts. In addition, there is a clear trend toward lower free and total sphingoid base content in PKD kidney compared to normal kidney. In PKD kidney cyst fluid, free SA and SO values ranged from not detected (ND) to 0.44 and ND to 5.8 nmol/ml, respectively; total SA and SO values ranged from 0.25 to 55.9 and 8.8 to 378 nmol/ml cyst fluid, respectively. Free sphingoid bases are reported to exhibit antibacterial and antifungal activity [57], which might ac-

**Fig. 2.** Overview of sphingolipid metabolism as impacted by endotoxin, fumonisin and related mycotoxins.
### Table 2. Sphinganine (SA) and sphingosine (SO) levels in human kidney specimens

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Kidney sample</th>
<th>Free SA (pmol/mg)</th>
<th>Free SO (pmol/mg)</th>
<th>Ratio Free SA:SO</th>
<th>Total SA (pmol/mg)</th>
<th>Total SO (pmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 40-60 years; normal kidney</td>
<td>Cross-section</td>
<td>45.1</td>
<td>934.2</td>
<td>0.04</td>
<td>2048</td>
<td>40465</td>
</tr>
<tr>
<td>37 years; ADPKD</td>
<td>Cross-section</td>
<td>10.9</td>
<td>210.7</td>
<td>0.05</td>
<td>1025</td>
<td>17571</td>
</tr>
<tr>
<td>Kidney positive for endotoxin by dLAL and immunodot-blot, glucan by dLAL, fungal antigens and DNA</td>
<td>4.8</td>
<td>75.9</td>
<td>0.06</td>
<td>648</td>
<td>11157</td>
<td></td>
</tr>
<tr>
<td>Cyst wall</td>
<td>2.9</td>
<td>39.4</td>
<td>0.07</td>
<td>643</td>
<td>8917</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.6</td>
<td>16.7</td>
<td>0.34*</td>
<td>305</td>
<td>5138</td>
<td></td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 40-60 years; normal kidney</td>
<td>Cross-section</td>
<td>16.8</td>
<td>237.9</td>
<td>0.07</td>
<td>1244</td>
<td>12681</td>
</tr>
<tr>
<td>1 day old; normal kidney</td>
<td>Cross-section</td>
<td>19.0</td>
<td>114.2</td>
<td>0.17</td>
<td>948</td>
<td>11395</td>
</tr>
<tr>
<td>45 years; ADPKD</td>
<td>Cross-section</td>
<td>2.6</td>
<td>19.9</td>
<td>0.13</td>
<td>578</td>
<td>8467</td>
</tr>
<tr>
<td>Kidney positive for endotoxin by dLAL and immunodot-blot</td>
<td>4.1</td>
<td>42.9</td>
<td>0.09</td>
<td>802</td>
<td>12597</td>
<td></td>
</tr>
<tr>
<td>Cyst wall</td>
<td>12.5</td>
<td>53.2</td>
<td>0.23</td>
<td>720</td>
<td>10441</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>22.0</td>
<td>0.17</td>
<td>512</td>
<td>8332</td>
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<td>52 years; ADPKD</td>
<td>Cross-section</td>
<td>5.3</td>
<td>19.4</td>
<td>0.28</td>
<td>417</td>
<td>6010</td>
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<td>1.7</td>
<td>25.5</td>
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<td>8488</td>
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<tr>
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<td>22.1</td>
<td>0.07</td>
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<td>11800</td>
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<tr>
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<td>4.2</td>
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<td>0.12</td>
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<td>0.26</td>
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<td>41 years; ADPKD</td>
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<td>5925</td>
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<tr>
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<td>10.2</td>
<td>19.0</td>
<td>0.54*</td>
<td>748</td>
<td>6549</td>
<td></td>
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<tr>
<td>43 years; ADPKD</td>
<td>Cross-section</td>
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<td>27.2</td>
<td>0.12</td>
<td>386</td>
<td>7370</td>
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<td>Kidney positive for endotoxin by dLAL, immunodot-blot and fungal DNA</td>
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<td>0.10</td>
<td>325</td>
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<tr>
<td>Cyst wall</td>
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<td>0.12</td>
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<td>4411</td>
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<tr>
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<td></td>
<td>2.7</td>
<td>15.8</td>
<td>0.17</td>
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<td>6241</td>
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</tbody>
</table>

*Highest free SA:SO ratios. Normal human kidneys obtained within 3 h of surgery and dissected into kidney cross sections and frozen at −70°C until assayed. For PKD kidneys, cyst fluids were aspirated, cyst walls and kidneys dissected within 2 h of nephrectomy. Levels of free and total sphingoid bases were determined by the method of Yoo et al. [67]. Data are represented as area under HPLC peak quantified via SO and SA external standard curves, adjusted for recovery of C:20 sphinganine internal standard (gift of A. H. Merrill Jr, Emory University, Atlanta, Georgia, USA), and normalised by tissue protein content [68]. Mean recovery of internal standard from kidney cross-section and cyst wall was 58% and 60%, respectively. ADPKD, Autosomal dominant polycystic kidney disease; dLAL, differential Limulus Amebocyte Lysate assay.
count for the failure to recover common bacteria and fungi from cyst fluid.

Males are known to have higher levels of kidney sphingolipids than females [58], which is also observed in PKD individuals (Table 2). In PKD, males exhibit a higher incidence and severity of kidney lesions [5,6], responsiveness to the anticystic effects of lovastatin [59], and excretion of nanobacterial antigens than females. In normal animals, males excrete greater amounts of sphingolipids and are more sensitive to the nephrotoxic effects of fumonisin than females [48].

Alteration of sphingolipid biology has been proposed as important to the integrity of the gastrointestinal tract during fumonisin intoxication [15]. Supplementation with dietary sphingolipids was reported to reduce colon carcinogenesis while broader associations between cancer, sphingolipid intake, infectious disease and exposure to fumonisin and related mycotoxins were beginning to be recognised [15,58]. Fumonisin B1 is reported to alter the activity of the folate binding protein (FBP) [60], which may result in a functional folate deficiency and subsequent vulnerability to mutagenesis and cancer [61]. Because kidney FBP is responsible for recovery of >95% of filtered folate [62] and thus preservation of stores of folate, fumonisin-induced disruption of the folate recovery pathway may indirectly affect extra-renal tissues also.

As shown in Fig. 2, endotoxin is reported to alter sphingolipid metabolism, exhibit ceramide-mimetic properties, and share transport mechanisms between tissues and intracellular compartments [63]. In PKD cells, the Golgi apparatus, the intracellular site of complex sphingolipid formation and sulphation reactions, shows altered transport of sphingolipids [64] and diminished sulphation of glucosaminoglycans [65]. Taken together, a plausible case for the actions of microbial toxins on PKD sphingolipid biology can be made.

**Therapy of PKD as an Infectious Disease and/or Microbial Toxicosis**

Remarkably, many PKD treatments that attempt to correct defects in mammalian cell biology also exhibit antimicrobial effects and/or diminish the absorption of microbes and their toxins from the gut (Table 3) [8]. For example, reduction of tissue inflammation can occur by decreasing the reactivity of the PKD kidney to inflammatory

<table>
<thead>
<tr>
<th>Promising therapies</th>
<th>Proposed mammalian targets</th>
<th>Possible microbial targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral potassium citrate/citric acid solution</td>
<td>Alkalising agent that decreases ammonia production and intracellular acidosis leading to slower rates of cell proliferation; antioxidant action decreases tissue inflammation, and reduces kidney stone formation by chelating calcium Decreases kidney inflammatory response thereby reducing tissue damage and resultant poorly regulated tissue repair</td>
<td>Optimum pH for most fungal growth is 6.5; alkalining body fluids would diminish fungal growth. Inhibits growth of nanobacteria, which may result in diminished stone formation and reduced tissue inflammation Decreases entry of microbial components from gut due to anti inflammatory actions on gut enterocytes Exhibits antifungal activity</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>Blocks synthesis of lipid-linked regulatory proteins yielding increased cell growth Increased excretion of urinary citrate Diminished production of pro-inflammatory lipids Inhibits synthesis of glycosylation of ceramide thereby partially correcting imbalances in sphingolipid subtypes in PKD kidney</td>
<td>Citrate inhibits growth of nanobacteria in vitro. EPA reported to protect against bacterial infections Nearly all fungi and some bacteria produce complex sphingolipids, which are required for their growth and survival</td>
</tr>
<tr>
<td>Lovastatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flax seed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish oil (EPA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Phenyl-2-decanoyl amino-3-morpholino-1-propanol</td>
<td></td>
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</table>
stimuli and by decreasing the absorption of endotoxin and fungal glucans from the gut by decreasing inflammation of the gastrointestinal tract. The role of diet and drugs [65] in altering the growth of colonising/infecting microbes and the production of microbial toxins is an emerging area of research not only in food safety [15], but in both chronic and genetic diseases.

REFERENCES

MICROBIAL FACTORS IN POLYCYSTIC KIDNEY DISEASE


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