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Evolutionary trade-offs in kidney injury and repair

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ABSTRACT

Evolutionary medicine has proven helpful to understand the origin of human disease, e.g. in identifying causal roles of recent environmental changes impacting on human physiology (environment-phenotype mismatch). In contrast, diseases affecting only a limited number of members of a species often originate from evolutionary trade-offs for usually physiologic adaptations assuring reproductive success in the context of extrinsic threats. For example, the G1 and G2 variants of the APOL1 gene supporting control of Trypanosoma infection come with the trade-off that they promote the progression of kidney disease. In this review we extend the concept of evolutionary nephrology by discussing how the physiologic adaptations (danger responses) to tissue injury create evolutionary trade-offs that drive histopathological changes underlying acute and chronic kidney diseases. The evolution of multicellular organisms positively selected a number of danger response programs for their overwhelming benefits in assuring survival such as clotting, inflammation, epithelial healing and mesenchymal healing, i.e. fibrosis and sclerosis. Upon kidney injury these danger programs often present as pathomechanisms driving persistent nephron loss and renal failure. We explore how classic kidney disease entities involve insufficient or overshooting activation of these danger response programs for which the underlying genetic basis remains largely to be defined. Dissecting the causative and hierarchical relationships between danger programs should help to identify molecular targets to control kidney injury and to improve disease outcomes.

Keywords: regeneration; fibrosis; coagulation; inflammation; healing; repair
Introduction

Evolutionary medicine can provide surprising explanations for pathomechanisms of human disease (Williams and Nesse, 1991). Phenotype adaption along evolution is driven by maintaining diversity in phenotypes and selection via reproductive success. Reproductive success requires reaching the reproductive phase of life and a certain fitness to get selected for mating. Evolutionary medicine has been explored in many directions but there is a relative paucity of concepts regarding kidney diseases and kidney pathology. Homer Smith presented a careful overview on the evolution of the kidney and salt handling (Vize and Smith, 2004). Robert L. Chevalier introduced the concept of evolutionary trade-offs versus mismatches to kidney disease (Chevalier, 2017). Here we approach the aspect of kidney pathology. Kidney injury can produce a large number of histopathological lesions because of the complex diverse cellular composition and the numerous functional compartments inside and outside the nephrons of the kidney. Kidney disease rarely involves mechanical trauma but is rather the consequence of triggers that set off intrinsic injurious pathomechanisms destroying the kidney. Interestingly, also concomitant healing responses trying to stabilize the remaining nephrons contribute to injury and malfunction. However, the number of cellular responses are limited, hence, different triggers can produce similar histopathological lesions such as focal segmental glomerulosclerosis (FSGS), mesangial proliferation, crescents arising from Bowman’s capsule, thrombotic microangiopathy, tubular necrosis as well as interstitial fibrosis. Such nonspecific lesions require further diagnostic work. Here we present the view from the angle of evolutionary medicine on how kidney injury generates distinct histopathological lesions by interactions of a set of danger response programs (Anders, 2012). We start out by presenting such danger response programs assuring survival in multicellular organisms and how they may turn into pathomechanisms of kidney disease in humans as an evolutionary trade-off (Table 1)(Williams and Nesse, 1991). This conceptual approach can help to sort out the hierarchical relevance of certain histopathological lesions and to identify molecular and cellular elements central to the pathogenesis of kidney disease, potentially serving as therapeutic targets in the future.
ASSURING REPRODUCTIVE SUCCESS: RESPONSE PROGRAMS CONTROL DANGERS

Injuries require either regeneration or repair of tissue defects (Gurtner et al., 2008). Injured epithelial barriers involve the risks of fluid loss and microbial infection. From early on evolution positively selected those multicellular organisms equipped with adequate danger control mechanisms providing significant survival benefits that outweigh the trade-offs associated with collateral injuries or tissue remodeling. While such trade-offs can be acceptable for a species as a whole when assuring species survival, they can cause significant morbidity or even mortality for single individuals of that species. By concept, medicine is a cultural human trait that focusses on those individuals of the human species affected by trade-offs turning into disease such as kidney disease. We present four central danger response programs, positively selected by evolution to assure survival upon traumatic or toxic tissue injury, and how their evolutionary trade-offs contribute to the spectrum of kidney diseases listed in medical textbooks (Table 1). These danger response programs are coagulation, inflammation, re-epithelialization, and mesenchymal repair (Velnar et al., 2009; Suarez-Alvarez et al., 2016).

_Coagulation controls bleeding upon vascular injury._ Vascular injury implies a barrier defect that cannot be regenerated in an instant, hence requires an immediate functional sealing to minimize the risk of hemorrhagic shock. Already present in arthropods, e.g. by haemolymph aggregation, the need for a balance between wanted and unwanted clotting evolved in the vertebrate as a complex interplay of endothelial cell activation, plasmatic enzymes and substrates, and platelets (Smith et al., 2015). While wanted clotting minimizes short term risks from bleeding, unwanted clotting presenting as diffuse intravascular coagulation or thromboembolism implies substantial trade-offs manifesting as tissue hypoperfusion, ischemia and fatal embolism or infarcts (Jennewein et al., 2011; Damman et al., 2015).

_Inflammation creates a functional barrier to pathogens._ Survival benefits provided by a balanced host defense against pathogenic threats are the driving element in the evolution of the immune system (Buchmann, 2014). Wounding external barriers holds the risk for infection and is able to rapidly re-install the barrier to limit pathogen entry and spreading. While re-epithelialization takes days, (Abais et al., 2014) local immune responses can create a functional barrier within few hours (Medzhitov, 2008; Hickey and Kubes,
Pathogen-derived *pathogen-associated molecular patterns* (PAMPs) and *damage-associated molecular patterns* (DAMPs) released by dying parenchymal cells activate identical pattern recognition receptors in infectious and sterile forms of inflammation (Anders, 2010; Chan *et al*., 2012; Leventhal and Schroppel, 2012; Kaczmarek *et al*., 2013; McCarthy *et al*., 2014). There is a tight cross-talk between clotting and inflammation, e.g. platelet aggregation releases cytokines and chemokines from platelet granules promoting leukocyte influx and inflammation at the site of injury, i.e. immunothrombosis (Niessen *et al*., 2008; Delvaeye and Conway, 2009; Engelmann and Massberg, 2013; Wu, 2015). Also, the formation of neutrophil extracellular traps (NETs) releases lytic proteases and DAMPs that further fuel local inflammation (Palabrica *et al*., 1992; Semple *et al*., 2011; Martinod and Wagner, 2014). The inflammatory response triggers regulated forms of cell necrosis (Linkermann *et al*., 2014; Mulay, Linkermann, *et al*., 2016), e.g. in pyoderma gangraenosum but the same process can contribute to parenchymal losses in the kidney (Ahronowitz *et al*., 2012). Sepsis-related cytokine storm is the central cause of mortality in early sepsis, whereas the compensatory suppression of innate immunity implies a susceptibility for secondary infections in late sepsis (Hotchkiss *et al*., 2009).

**Re-epithelialization (regeneration) reinstalls a structural barrier against fluid loss and pathogen entry.**

Epithelial injury implies barrier dysfunction, hence, rapid regeneration of the barrier is needed to limit fluid losses and pathogen entry, e.g. in skin burns or ulcerative colitis (Plikus *et al*., 2012; Sun *et al*., 2014). The epithelial layers in the kidney are not exposed to pathogens but anyway serve important barrier functions. For example, podocyte loss implies proteinuria (Reiser and Sever, 2013). While closing small gaps may be achieved by hypertrophy of the neighboring cells larger defects require *de novo* formation of epithelial cells and coordinated migration events initiated by signals acting on intrinsic cells with regenerative properties (Lasagni and Romagnani, 2010; Shankland *et al*., 2017). Already coagulation produces mitogenic factors that initiate cell cycle entry for either hypertrophy or proliferation of surviving epithelial cells (Nurden, 2011). Other mediators linking coagulation, inflammation, and re-epithelialization are growth factors (EGF, FGFs, HGF, TGF-β), cytokines (IL-6, IL-17, IL-22), chemokines (fractalkine, CXCL10) as well as microRNAs (Braun *et al*., 2008; Ishida *et al*., 2008; Pickert *et al*., 2009; Ebihara *et al*., 2011; Kroeze *et al*., 2012; McGee *et al*., 2013). Indeed, miRNAs evolve as important posttranscriptional regulators of the cell cycle machinery in cells that contribute
to cutaneous wound healing (Lai and Siu, 2014). For example, miRNAs modulate human monocyte/macrophage differentiation (miR-424), TLR signaling (miR-147), collagen expression and wound contraction (miR-21) as well as the expression of the cell cycle master regulator p53 (miR-203) during keratinocyte differentiation (Lai and Siu, 2014).

The intrinsic regenerative capacity for re-epithelialization is largely provided by local progenitors committed to differentiate into the specific epithelial lineage (Romagnani, 2009; Plikus et al., 2012; Sallustio et al., 2013; Vaughan et al., 2015). Lack or delay of sufficient re-epithelialization fosters persistent barrier defects and may present as chronic dermal wounds or as proteinuria. Vice versa, over re-epithelialization can lead to epithelial hyperplasia (glomerular crescent formation) or (renal cell) carcinoma (Smeets et al., 2009; Ryu et al., 2012).

**Fibrosis stabilizes functional integrity of remnant parenchyma.** The reconstitution of parenchymal cell losses requires stabilizing scaffolds guiding regenerating elements to rebuild the precedent structural organization (Wynn, 2007). The obvious benefits of such a transient mesenchymal scaffold are well described during reconnecting broken bones, nerves, and blood vessels. Mesenchymal scaffolds are formed by mesenchymal cells surrounding the functional units of the organ, e.g. within muscle sheaths, periosteum, nerve sheaths, vascular pericytes, mesangial cells or interstitial fibroblasts. Upon injury, epithelial growth factors are released together with mesenchymal growth factors driving an early concomitant formation of a mesenchymal scaffold that stabilizes the injured units (Wynn, 2007). Once regeneration concluded these mesenchymal scaffolds disappear by degradation but whenever regeneration remains incomplete the scaffold persists as tissue fibrosis, a histopathological lesion obviously associated with poor outcomes as it indicates persistent parenchymal losses (Wynn, 2007). This association, however, does not necessarily imply that fibrosis causes tissue injury, a popular concept based on little experimental evidence (Zeisberg and Neilson, 2010). Overshooting mesenchymal healing as it presents as keloid of the skin exists in certain individuals and might contribute to further tissue losses. But what are the elements that link insufficient re-epithelialization with tissue fibrosis? Epithelial-mesenchymal transition (EMT) and arrest in the G2/M phase of the cell cycle was reported to be associated with pro-fibrotic TGF-β cytokine secretion (Yang et al., 2010; Zeisberg and
Collagen-producing fibroblasts mainly originate from bone marrow-derived precursors via the circulation or from resident fibroblasts (Niedermeier et al., 2009; Chen et al., 2013; Xia et al., 2014). Instead, the interstitial fibroblasts pool was reported to take little from pericytes or endothelial cell transition (LeBleu et al., 2009; Humphreys et al., 2010; Falke et al., 2015), but the relative contribution could also depend on the type of kidney injury.

THE TRADE-OFF: DANGER CONTROL PROGRAMS AS DISEASE PATHOMECHANISMS

Well-balanced responses solve most incident problems without ending in acute organ failure or chronic damage. However, within a population, biological traits follow bell-shaped patterns. Hence, the human capacity to control dangers is not identical in all members of the population. A bell-shaped distribution of the capacity to balance danger responses implies that some individuals will respond to the same type of insult either with exaggerated or insufficient coagulation, inflammation, re-epithelialization, and scaring, respectively (Figure 1, Table 1). For example, genetic weaknesses in the expression or function of complement inhibitors promotes exaggerated complement-related inflammation, e.g. in atypical hemolytic uremic syndrome (aHUS) or C3 glomerulopathy (Noris and Remuzzi, 2013). It is likely that most kidney diseases result from genetically determined imbalances in danger control, where countless combinations of generic variants explain the wide spectrum of different manifestations of disease (Stokman et al., 2016). As such, genetic variants can exaggerate evolutionary trade-offs and produce drastic phenotypes in such unfortunate individuals. Indeed, our drive to categorize disease entities and classifying stages for medical teaching and administrative processing largely oversimplified a biology driven by countless combinations among genes and epigenetic as well as environmental factors.

Coagulation as pathomechanism

Exaggerated clotting. Unbalanced coagulation is a central element in thrombotic microangiopathies involving microvascular endothelial cell injury, activation of platelets, and plasma coagulation factors (Chapman et al., 2012). In the kidney thrombotic microangiopathies cause ischemic necrosis. Exaggerated
clotting also contributes to crescentic glomerulonephritis. For example, in renal vasculitis complement-driven vascular injuries activate clotting inside glomerular capillaries, which becomes evident by fibrin deposition in capillary loops (Sorensen et al., 2011; Srivastava et al., 2013). In Alport nephropathy, a non-inflammatory form of glomerular vascular injury, progressive GBM disintegration associates with hematuria, fibrinogen conversion to fibrin, and plasma leakage into Bowman’s space, a process that contributes to glomerular pathology (Ryu et al., 2012).

Insufficient clotting. Insufficient clotting manifests by persistent bleeding. Vice versa persistent bleeding can relate to insufficient clotting. Certain glomerular diseases are associated with persistent microhematuria, Microhematuria implies capillary wall injury in few glomeruli (Vivante et al., 2013; Yuste et al., 2015). It remains uncertain if persistent hematuria originates from persistent vascular lesions of always the same glomeruli that do not heal or whether clotting stops vascular leakage in some glomeruli, while others start to bleed with persistent hematuria as a net effect. Episodes of intermittent macrohematuria occur in IgA nephropathy and other renal disorders and can last several days implying insufficient clotting (Gutierrez et al., 2012). The fibrinolytic activity of urokinase that is highly expressed in the urinary tract may contribute to this phenomenon.

Inflammation as pathomechanism

Exaggerated inflammation. Inflammation is a central element of host defense often turning into a predominant pathomechanism underlying organ failure (Medzhitov, 2008). In sterile inflammation the trade-off of inflammation is particularly obvious as there are few and often no benefits associated with this response (Rock et al., 2010). For example, traumatic or toxic sterile injuries still benefit from the release of growth factors from infiltrating leukocytes that contribute to tissue regeneration but inflammation related to crystal formation, e.g. in gout or crystal nephropathies does not share the same benefits (Mulay and Anders, 2016, 2017). Sterile inflammation is the most prevalent form of renal inflammation, e.g. in glomerulonephritis, allograft rejection, ischemic or toxic tubular necrosis, interstitial nephritis as well as during tissue remodeling along progressive CKD (Anders and Schaefer, 2014). Resident and infiltrating macrophages are equipped with
numerous pattern recognition receptors translating PAMPs and DAMPs into local cytokine release and subsequent local inflammation (Anders, 2010; Nelson et al., 2012). Because renal parenchymal cells express only a limited number of such pattern recognition receptors they contribute less to the overall secretion of pro-inflammatory cytokines (Anders, Banas, et al., 2004). For example, renal cells lack TLR7 and TLR9 and, therefore, do not respond to certain forms of immunostimulatory nucleic acids (Anders, 2007; Anders and Schlondorff, 2007). In addition, renal cells produce only little IL-1β by activation of the NLRP3 inflammasome, a response strongly active in macrophages and dendritic cells (Anders and Muruve, 2011; Lichtnekert et al., 2011; Abais et al., 2014; Shahzad et al., 2015; Anders, 2016).

Renal cell necrosis strongly contributes to intrarenal inflammation via the passive release of DAMPs activating pattern recognition receptors (Mulay, Linkermann, et al., 2016). For example, tubular cell necrosis releases the TLR2 and -4 agonist HMGB1, driving inflammation during acute kidney injuries (Wu H., 2010; Mudaliar et al., 2013; Nair et al., 2015). A kidney-specific DAMP is Tamm-Horsfall protein/uromodulin, i.e. exclusively expressed within the cells of the thick ascending limb of the loop of Henle (Garimella and Sarnak, 2017). Upon injury in this area Tamm-Horsfall protein leaks into the interstitial space and activates dendritic cells via TLR4 as well as the NLRP3 inflammasome (Darisipudi et al., 2012). TLRs- and inflammasome-mediated DAMP recognition as a signal of renal cell necrosis is a central element also of rapid-progressive glomerulonephritis, thrombotic microangiopathies, and acute tubular necrosis (Andersen et al., 2014; Leemans et al., 2014; Anders, 2016). The subsequent release of TNF-α induces further regulated cell necrosis, e.g. by triggering RIPK3- and MLKL-mediated necroptosis (Linkermann and Green, 2014). This auto-amplification loop of necrosis-related inflammation and inflammation-related necrosis is referred to as “necroinflammation” (Linkermann et al., 2014; Mulay, Kumar, et al., 2016). The molecular mechanisms of renal necroinflammation are counter-regulated at numerous levels to limit expanding renal cell necrosis (Mulay, Linkermann, et al., 2016). Inhibitors of TLR signaling suppress the activation of immune and non-immune cells and thereby limit immune-mediated kidney diseases (Lech et al., 2007; Noris et al., 2009; Lassen et al., 2010; Lech et al., 2010; Gunthner et al., 2013; Lech et al., 2014). Extracellular histones have a particular role in renal necroinflammation as they promote immunostimulatory as well as cytotoxic effects (Allam et al., 2014). Renal cell necrosis as well as NET formation both release histones into the extracellular space where they elicit
cytotoxic effects on other cells by direct charge-mediated plasma membrane disruption (Allam et al., 2012; Nakazawa et al., 2017). A vicious circle further promotes histone- and DAMP-mediated necroinflammation (Allam et al., 2013; Nakazawa et al., 2017). When dying neutrophils release NETs they become an important source of extracellular histones killing endothelial cells and more tubular cells cells, e.g. in septic acute tubular necrosis or in crescentic glomerulonephritis, respectively (Kumar et al., 2015). Histones are also mediators of remote tissue injuries, e.g. in the lung that occur in the context of acute tubular necrosis (Nakazawa et al., 2017).

Extrarenal infections often trigger flares of chronic glomerulonephritis or renal vasculitis because circulating pathogen products activate pattern recognition receptors on non-immune and immune cells of the kidney (Pawar et al., 2006; Allam and Anders, 2008; Allam et al., 2008; Pawar et al., 2009), which promotes the intrarenal release of cytokines that increase renal inflammation and renal cell loss (Ryu et al., 2011; Brahler et al., 2012). In lupus nephritis (or renal vasculitis) pathogen-derived nucleic acids activate dendritic cells, macrophages, and B cells, a process that can trigger expansion of the pathogenic lymphocyte clones and disease activity of these chronic autoimmune diseases (Leadbetter et al., 2002; Wen et al., 2007; Villanueva et al., 2011). In particular, IFN-α triggers a systemic “pseudoantiviral” immune response that mimics the clinical manifestations of viral infection (Anders, 2009; Migliorini and Anders, 2012). Also, mesangial cells and glomerular endothelial cells recognize such immunostimulatory nucleic acids and secrete type I interferons, a phenomenon linked to podocyte loss and glomerular scaring during viral glomerulonephritis (Allam et al., 2009; Flur et al., 2009; Hagele, Allam, Pawar and Anders, 2009; Hagele, Allam, Pawar, Reichel, et al., 2009; Anders et al., 2010; Migliorini, Angelotti, et al., 2013).

The local expression of chemokines initiates a sequential recruitment of various leukocyte subsets but it is the local microenvironment within different tissue compartments that determines their functional status and phenotype (Anders and Ryu, 2011; Lech et al., 2012). Indeed, macrophages, T cells, and B cells come in functionally distinct subsets that enforce different aspects of danger control (Weidenbusch and Anders, 2012; Kurts et al., 2013; Minton, 2015; Mauri and Menon, 2017). For example, bacterial products induce neutrophils to undergo NET formation86 or activate infiltrating or resident macrophages to acquire a pro-inflammatory (M1) phenotype (Anders, Banas, et al., 2003; Anders, Vielhauer, et al., 2004; Brinkmann et al., 2004). Blocking
the CC-chemokine CCL2 or its receptor CCR2 reduces the intrarenal numbers of classically-activated (M1) macrophages, which can prevent damage and remodeling in glomeruli and the tubulointerstitium (Kulkarni et al., 2007; Ninichuk et al., 2008; Kulkarni et al., 2009; Sayyed et al., 2011; Urushihara et al., 2011; Seok et al., 2013).

Thus, unbalanced inflammation is a common trade-off, especially in sterile forms of renal inflammation, and largely contributes to tissue injury in transient and persistent forms of kidney disease.

Insufficient inflammation. The evolutionary origins of inflammation arise from the benefits associated with host defense (Medzhitov, 2008), which in kidney disease relate to renal infections. For example, PAMP-driven and TLR/inflammasome-mediated immune activation are absolutely required to limit BK virus replication in kidney allografts (Babel et al., 2011; Ribeiro et al., 2012). The same applies to the control of *Escherichia coli* and other uropathogenic bacteria during bacterial pyelonephritis (Anders and Patole, 2005; Abraham and Miao, 2015). Inflammation is designed to enforce local pathogen killing and to limit pathogen spreading from local to systemic infection (Godaly et al., 2015; Behzadi and Behzadi, 2016), as happens in mice that lack pattern recognition receptors such as in TLR4-deficient mice (Patole et al., 2005). The lack of pathogen control involves in turn benefits on collateral tissue injuries such as renal abscess formation, which are absent in TLR4-mutant mice (Patole et al., 2005). However, less immunopathology at the pathogen entry site comes at the cost of fatal gram negative sepsis (Abraham and Miao, 2015). Along the same line, TLR2 recognizes leptospiral outer membrane proteins in proximal tubular epithelial cells with similar implication for leptospirosis (Yang et al., 2006).

Together, the evolutionary trade-off to host defense is that a wide range of triggers initiate sterile inflammation causing unnecessary tissue injury, especially inside the kidney. Insufficient inflammation refers to lack of pathogen control in renal infection in immunocompromised hosts.

Re-epithelialization as pathomechanism
“The kidney” is actually a collection of up to 1 million independent functional units, i.e. the nephrons. Nephron structure and function is largely determined by an epithelial monolayer along the glomerular tuft and all tubular segments up to the collecting ducts opening into the renal pelvis. No matter in which segment it occurs, renal epithelial injury requires rapid re-epithelialization to avoid functional and structural problems (Rabelink and Little, 2013; Romagnani et al., 2013). Re-epithelialization is supported by surviving epithelial cells via cell division (proliferation = regeneration) or via increasing cell size without cell division (hypertrophy = reconstitution), two distinct processes that both imply cell cycle activation and therefore are detected by “proliferation markers” such as immunolabeling (Thomasova and Anders, 2015). Although not formally proven by lineage tracing, clonal analysis it is widely assumed that functional recovery after acute tubular necrosis is associated with sufficient tubular epithelial cell regeneration (Bonventre, 2003; Duffield, Park, et al., 2005). Growth factors like platelet derived growth factor (PDGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF) and bone morphogenetic proteins (BMPs) induce re-epithelialization of injured epithelial monolayers (Werner and Grose, 2003; Sugimoto et al., 2012). Murine double minute (MDM)-2 supports cell cycle entry of surviving tubular cells by inhibiting p53-dependent cell cycle arrest in G1 (Mulay et al., 2012; Mulay et al., 2013). MDM2 as well as numerous other molecules and mediators form close links between inflammation and regeneration (Thomasova et al., 2012; Ebrahim et al., 2015; Mulay, Romoli, et al., 2016). Re-epithelialization requires first a resolution of inflammation because many pro-inflammatory elements suppress epithelial healing (Anders, 2014). The phenotype switch from “pro-inflammatory” (M1) to “wound healing” (M2) macrophages is important in this process. M2 macrophage-related CSF-1 secretion drives local M2 macrophage proliferation in an autocrine manner (Duffield, Forbes, et al., 2005; Alikhan et al., 2011; Iwata et al., 2012). A tight regulation of re-epithelialization is essential because both overshooting as well as insufficient epithelial healing can contribute to aberrant kidney remodeling and organ failure.

Exaggerated re-epithelialization. Pro-mitotic signals without concomitant signals for differentiation promote cellular hyperplasia rather than epithelial healing (Lasagni and Romagnani, 2010), conceptually like in myeloproliferative disorders. For example, in crescentic glomerulonephritis renal progenitors within the parietal epithelial cell (PEC) layer of the Bowman’s capsule no longer differentiate into podocytes to replace
lost podocytes but rather produce hyperplastic lesions creating cellular crescents that obstruct the Bowman`s space (Smeets et al., 2009; Bollee et al., 2011; Ryu et al., 2012). Not only inflammatory signals drive crescent formation. Indeed, crescent formation from PEC hyperplasia can occur also only upon plasma leakage from broken glomerular capillaries, conceptually similar to the mitogenic effect of serum components on cultured epithelial cells in vitro (Ryu et al., 2012). Indeed, under homeostatic conditions glomerular epithelial cells are never exposed to serum apart from in vascular injury.

**Insufficient epithelial regeneration.** Insufficient re-epithelialization upon podocyte loss is a common cause for aging- or injury-related CKD (Hodgin et al., 2015). Differentiated podocytes lack the capacity to complete mitosis to replace lost podocytes next to them, because podocytes put all their cytoskeleton into the foot processes (Lasagni et al., 2013). Simplifying cell shape and recruiting the cytoskeleton to form the mitotic spindle is no option for differentiated podocytes as both processes would be incompatible with maintaining the filtration barrier (Lasagni et al., 2013). Thus, podocyte loss can only be compensated by hypertrophy of remnant podocytes or by de novo production of podocytes from local progenitors (Hagen et al., 2016). Under certain circumstances strong mitogenic signals force podocytes to bypass cell cycle restriction points, as evident from some multinucleated podocytes, but such podocytes rapidly detach and die, i.e. mitotic catastrophe (Liapis et al., 2013). Hypertrophy of remnant podocytes can compensate a loss of up to 20-30% of podocytes but losses exceeding this number often result in focal segmental glomerulosclerosis (FSGS) (Fukuda et al., 2012). De novo podocytes formation was clearly demonstrated by lineage tracing using the podocyte specific expression of the tomato-GFP reporter (Wanner et al., 2014). The source of such new podocytes remains an area of intense research activities (Shankland et al., 2017). Some studies suggest that bone marrow-derived progenitor cells are able to replace lost podocytes (Prodromidi et al., 2006; Sugimoto et al., 2006; Zoja et al., 2012), whilst others propose that podocytes originate from local Pax2+ podocyte progenitors residing among the PECs or migrating onto the glomerular tuft from a pool of renin+ cells at the juxtaglomerular apparatus (Shankland et al., 2013; Lasagni et al., 2015; Lichtnekert et al., 2016). The capacity of podocyte progenitors to replace lost podocytes in the adult kidney seems very limited. Notch and Wnt signaling, EGF and SDF-1/CXCL12 regulate the behavior of PECs in glomerular injury (Lasagni et al., 2010;
Darjisipudi et al., 2011). Histone H3K9, H3K23 (acetylation), H3K4 (dimethylation) and H3K4 phosphorylation at serine 10 are associated with incomplete podocyte recovery and glomerulosclerosis (Gaikwad et al., 2010; Sayyed et al., 2010).

Repetitive or persistent injuries together with persistence of classically-activated (M1) macrophages impair tubular re-epithelialization after acute tubular necrosis (Duffield, Forbes, et al., 2005; Anders, 2014; Lech et al., 2014). Tubular cells secrete the cytokine CSF-1 that enforces the expansion of macrophages inside the kidney but also enforces re-epithelialization of tubule segments (Menke et al., 2009). Severe injuries also eradicate those tubular cell progenitors more resistant to cell death and found scattered along the tubule, re-epithelialization may no longer be possible (Angelotti et al., 2012). The concept that bone marrow stem cells invade the injured tubulus to replace tubular cells by differentiation was experimentally excluded, but such cells may still provide paracrine support to the regenerating tubule cells from outside (Humphreys et al., 2011; Romagnani, 2011). Inability to rebuild the tubular epithelial monolayer leads to atrophy of the entire nephron.

In summary, both insufficient and exaggerated re-epithelialization contribute to kidney disease. Identifying the predominant pathomechanism in a clinical setting and identifying drugs that have the capacity to correct the abnormal response remains a challenge.

**Fibrosis/Sclerosis as pathomechanism**

Mesenchymal structures stabilize the different compartments of the nephrons. Mesangial cells produce the mesangial matrix that stabilizes the glomerular tuft of capillaries. Similarly, interstitial fibroblasts produce the interstitial matrix that stabilizes the tubules. Pericytes stabilize intrarenal blood vessels especially upon injury by secreting TIMP3 and ADAMTS1 (Schrimpf et al., 2012; Smith et al., 2012). Nephron injury or microvascular damage activate such mesenchymal elements to expand and to produce more matrix enforcing the stabilization of the nephron compartments and to support the scaffold for parenchymal regeneration (Gurtner et al., 2008). Once nephrons undergo atrophy and are irreversibly lost during injury, these mesenchymal elements fill the space with extracellular matrix, a process required to assure the functional
integrity of the kidney as a whole. Nevertheless, nephron loss in CKD is associated with a declining kidney mass, because scar tissue does not exceed parenchymal losses.

*Exaggerated mesenchymal healing.* Mesangial cells lost during injury are usually rapidly replaced from several sources including potentially remnant mesangial cells, renin-producing cells from the extraglomerular mesangium, or mesangial cell precursors from the bone marrow (Imasawa et al., 2001; Ikarashi et al., 2005; Daniel et al., 2008; Starke et al., 2015). Mesangial repair upon mesangiolysis can be conveniently studied using the rat anti-Thy1.1 model (Eitner et al., 1997; Hugo et al., 1997). In contrast, mesangial hyperplasia is found in “mesangioproliferative” and “membranoproliferative glomerulonephritis” (Sethi and Fervenza, 2012). The stabilizing function of scar formation also becomes obvious looking at insufficient podocyte regeneration. Whenever podocyte loss cannot any more be compensated by remnant podocyte hypertrophy or de novo podocyte formation, focal adhesions between the glomerular tuft and Bowman’s capsule allow PECs to migrate onto the tuft (Smeets et al., 2011; Peired et al., 2013). There they lay down extracellular matrix and generate lesions referred to as FSGS (Smeets et al., 2011). Thus, whenever PECs are unable to contribute to re-epithelialization, e.g. by a lack of differentiation into podocytes they instead contribute to mesenchymal repair (Shankland et al., 2013; Shankland et al., 2017). Like other scars FSGS lesions stabilize the rest of the glomerular tuft and minimize protein loss across the denudated GBM (Kriz and Lemley, 1999). However, the dynamics of hyperfiltration-related additional stress on the remaining podocytes can foster further podocyte loss and drive the progression to global glomerulosclerosis (Kriz and Lemley, 1999; Helal et al., 2012).

The well-documented association between interstitial fibrosis and poor renal function has led to the assumption that fibrosis drives nephron loss and CKD progression rather than simply being a marker of preceding nephron loss (Bohle et al., 1992; Zeisberg and Neilson, 2010). There is little evidence on a causal role of fibrosis for GFR decline and further nephron loss. There are no obvious genetic diseases causing primary fibrosis of the kidney, not even diffuse scleroderma presents with renal fibrosis (Bussone et al., 2011), and also genome-wide association studies on CKD cohorts did not identify risk genes pointing towards fibrogenesis (Boger et al., 2011). Indeed, ubiquitous presence of renal fibrosis in CKD implies it to be mesenchymal healing
of secondary nature related to precedent insufficient epithelial healing (Anders and Schaefer, 2014). For example, tubular epithelial cells unable to regenerate were reported to be arrested in the G2/M phase and to produce tumor growth factor-beta as occurs in Chinese herb nephropathy (Yang et al., 2010). Leukocytes contribute to mesenchymal healing of the kidney demonstrated by interventions inhibiting leukocyte influx (Anders et al., 2002; Anders, Vielhauer, et al., 2003; Vielhauer et al., 2004; Ninichuk et al., 2005; Anders et al., 2006; Furuichi et al., 2006; Ninichuk et al., 2007; Clauss et al., 2009). Indeed, inhibition, deletion or depletion of alternatively-activated (M2) macrophages abrogates intrarenal fibrogenesis (Lee et al., 2011; Braga et al., 2012; Chen et al., 2012; Cao et al., 2015). In addition, Ly6G+ collagen-producing “fibrocytes” from the bone marrow invade the kidney via CCL21-CCR7 and contribute to local collagen secretion (Sakai et al., 2006; Reich et al., 2013). Finally, pericyte-derived fibroblasts produce collagen and contribute to renal interstitial fibrosis . (Lin et al., 2008; Smith et al., 2012; Kramann et al., 2015; Kramann et al., 2017).

Insufficient mesenchymal. Insufficient scaring is a less prevalent problem in kidney disease. Mesangiolysis noted on a kidney biopsy implies insufficient regeneration from mesangial cell progenitors, e.g. in atypical hemolytic uremic syndrome, immune complex glomerulonephritis or C3 glomerulopathy (Bomback and Appel, 2012; Migliorini, Ebid, et al., 2013). Hyperglycemia in diabetes is another state known to compromise mesangial healing. For example, Kimmelstiel-Wilson nodules in diabetic nephropathy are thought to result from mesangiolysis that cannot be restored from cellular sources and is rather filled by nodular matrix deposits (Stout et al., 1993).

In summary, mesenchymal repair serves an important function, i.e. to stabilize remnant parenchymal tissue to to serve as a scaffold during tissue regeneration. By contrast, insufficient mesenchymal healing is a rare problem in the kidney.

CONCLUDING REMARKS

Diseases are often manifestations of evolutionary trade-offs. Evolution selected danger responses to cope with potentially life-threatening risks but such danger responses need to be balanced to avoid collateral
damage implying other morbidities and even fatal consequences. Clotting, inflammation, epithelial and mesenchymal healing are excellent examples of life saving danger response programs that under certain circumstances can cause disease (Hagemann et al., 2013). Overshooting or insufficient responses can occur due to genetic or environmental insults and can become predominant pathomechanisms of disease. Identifying the predominant pathomechanism in individual patients and identifying specific drugs to correct the abnormal tissue response are the central challenges for translational research in the following years.

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Conflict of Interest

The authors declare no conflict of interest.
REFERENCES


<table>
<thead>
<tr>
<th>Kidney disease</th>
<th>Based on ancient evolutionary benefits on reproductive success</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension-related kidney injury</td>
<td>Bipedal lifestyle on arid land</td>
</tr>
<tr>
<td>Gout, uric acid nephropathy</td>
<td>Loss of uricase activity in great apes and humans to increase serum uric acid levels for higher blood pressure, bipedal lifestyle on land when climate change created more arid biotopes, pseudogenization of uricase allows conversion of fructose into fat</td>
</tr>
<tr>
<td>Obesity-/T2D-related nephropathy</td>
<td>Energy storage for periods of food shortage</td>
</tr>
<tr>
<td>Accelerated CKD progression in patients with APOL-1 risk alleles</td>
<td>APOL-1 risk allele-related better host defense against Trypanosoma infection</td>
</tr>
<tr>
<td>Ischemic nephropathy (sickle cell disease)</td>
<td>Hemoglobin mutation-related benefits during Plasmodium infection</td>
</tr>
<tr>
<td>Thrombotic microangiopathy</td>
<td>Limiting blood loss upon trauma via coagulation</td>
</tr>
<tr>
<td>Renal abscess in pyelonephritis</td>
<td>Host defense to microbes, local barrier to avoid sepsis,</td>
</tr>
<tr>
<td>Immune complex glomerulonephritis</td>
<td>Host defense to pathogens</td>
</tr>
<tr>
<td>Interstitial nephritis, allograft rejection</td>
<td>Host defense to pathogens</td>
</tr>
<tr>
<td>Crescentic formation</td>
<td>Re-epithelialization in wound healing</td>
</tr>
<tr>
<td>Renal cyst, cell adenoma, carcinoma</td>
<td>Re-epithelialization in wound healing</td>
</tr>
<tr>
<td>Glomerulosclerosis, interstitial fibrosis</td>
<td>Stabilization of remnant parenchyma in wound healing</td>
</tr>
</tbody>
</table>

T2D = type 2 diabetes, CKD = chronic kidney disease, APOL = apolipoprotein
Figure 1. 3D Gaussian distribution of danger response programs in kidney disease. Danger responses are well-balanced in the majority of the population and can usually help to regain homeostasis upon minor insults in the majority of the population (illustrated by the y-axis as being the peak of bell-shaped curves). However, a subset of the population being affected either by stronger injurious triggers or by genetic alterations resulting in exaggerated or insufficient danger responses suffer from a spectrum of different kidney diseases. The numerous combinations of genetic profiles and diverse environmental factors explain the broad spectrum of clinical manifestations. Current definitions of disease entities as well as guideline medicine often fall short in matching with the complexity of these biological processes. FSGS = focal segmental glomerulosclerosis.
HISTOLOGY AND HISTOPATHOLOGY

- Infective pyelonephritis
- Hematuria
- Mesenchymal healing
- Glomerulosclerosis Interstitial fibrosis
- Epithelial healing
- Renal cyst Carcinoma
- Exaggerated responses
- Mesangiolysis
- Tubular atrophy
- Clotting
- Insufficient responses
- Inflammation
- Thrombotic microangiopathy
- Glomerulonephritis Interstitial nephritis Allograft rejection