

# Building Rational Models of Humoral Autoimmunity: The Example of Lupus Tubulointerstitial Nephritis

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## Abstract

Mice have proven to be extremely useful tools for simulating pathways involved in humoral autoimmune diseases observed in patients. A relevant animal model can act as a means with which existing drugs can be tested, and new ones rationally designed. With a view to rationally developing animal models, the common features of organ-specific antibody-mediated diseases are briefly recalled herein. Historically however, hurdles have often existed that have thwarted the description of these features, and therefore the development, and testing of hypotheses that explain pathways that yield autoimmune pathologies. The difficulties in assembling these hypotheses from human data have included acquisition of sufficient information regarding antibodies, antigens, and genetics, and the subsequent marrying of these data-sets with particular clinical manifestations. Moreover, once well-assembled hypotheses have been generated, appropriate platforms for their testing are frequently absent. In the mouse, the immunologists' 'go-to' simulation platform, components integral to mounting antigen specific immune responses are often poorly conserved, or even absent. In this review, following a description of some of the cross species inconsistencies, tools such as plasmids for expressing murine monoclonal antibodies with human variable regions, and mice engineered to express human Fcγ receptors, and HLA molecules, often capable of surmounting these issues, are highlighted. By avoiding historical pitfalls, and considering how new technologies could be employed in the future, a rational approach will be devised for the detailed characterization of the recently discovered organ-specific autoimmune phenomenon of lupus tubulointerstitial nephritis (TIN), and an animal model that simulates it.

**Keywords:** Autoimmunity; Nephritis; Renal failure; Pathogenesis; Prognosis; Systemic lupus erythematosus; Tertiary lymphoid neogenesis; Tubulointerstitial inflammation

## Humoral Autoimmunity: The Conventional Model

Irrespective of the order in which their distinguishing characteristics have been identified, most T cell dependent, antibody-mediated autoimmune diseases end up adhering to the same (admittedly oversimplified) conventional model; in individuals, genetically predisposed to being less immunologically tolerant, who are exposed to particular environmental factors (not discussed in this article), and with the capacity to present autoantigens because of carriage of particular HLA alleles, autoimmunity to particular autoantigens ensues (Wegner et al. [1]). Providing sufficient accumulation of target autoantigens, the autoantibodies that develop (often measurable in the serum) perpetuate chronic inflammation in particular tissues. Characterization of autoimmune phenomena in patients often results in the development of clinically useful serological assays for diagnosing particular diseases, and hypotheses regarding pathogenic pathways. However, it is the development of animal models that many researchers often strive most vehemently towards. This is because they not only serve to test our hypotheses regarding disease pathways, they may also be useful as *in vivo* systems, with which drugs can be tested, and/or rationally designed. The conventional model of autoimmunity mentioned above acts as a good skeleton upon which the flesh of disease specific detail can be hung, and therefore serves as a road map which guides toward acquiring the data sets that are likely to be most pertinent when modeling disease. Focusing on obtaining these data sets when characterizing autoimmune pathways during future endeavors may accelerate the development of 1) a detailed model of a particular disease, 2) tools for its prediction, prognosis, and diagnosis, and 3) medicinal regimens, and new drugs to treat them.

## Modeling Lupus Tubulointerstitial Nephritis (TIN): Acquiring the Appropriate Data from Humans, before Developing a New Mouse Model

Some of the historical hurdles that have existed in gaining relevant

data, and reagents, from the relevant group/s of patients are brought to the forefront of mind in this review. Historical issues in translating patient data into animal models of humoral autoimmunity, and testing the pathogenicity of human autoantibodies in mice are then discussed. Minefields experienced in attempting to model antigen-specific, antibody-mediated disease are reviewed with particular attention brought to the erosive (anti-citrullinated protein antibody [ACPA] positive) arthritis witnessed in patients with RA. The erosive arthritis in ACPA<sup>+</sup> RA is a relevant case to TIN in this review. Both are recently described, demonstrate local antibody production at the site of lesion (although the character of the autoantibodies produced in TIN have yet to be characterized), both exist amid other features of systemic autoimmunity, and are features that predict particularly aggressive disease. Also, neither has a robust animal model. However, TIN is a more recently described, less thoroughly researched manifestation than ACPA + arthritis, and lessons can therefore be learned from the research carried out on the modestly older organ-specific autoimmune phenomenon. The methods, and tools that have been developed recently to overcome issues faced in modeling disease in mice rationally follow. Only by avoiding historical landmines in patient data acquisition, and employing new relevant murine technologies will we be able to assemble the best possible strategy for characterizing TIN. It is argued in this review that characterization of the antigen/s that drive

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TIN is the most rational next juncture in what is the early phase of characterization of the disease. To date, little is known about the nature of the autoimmune response that drives TIN. Existing animal models of autoimmune nephritis are focused on Glomerulonephritis (GN), and do not demonstrate the tubulointerstitial infiltrates observed in patients with TIN. Therefore, patient data needs to be acquired, and appropriate animals require developing to understand this important aspect of lupus nephritis. *In situ* B-cell clonal expansion, and other descriptive correlates support the hypothesis that TIN is a *bona fide*, antigen-specific antibody-driven organ-specific autoimmune disease, which occurs amidst systemic autoimmunity [2]. Moreover, Hsieh et al. have demonstrated that the degree of tubulointerstitial lymphocytic infiltration is predictive of End Stage Renal Disease (ESRD), and surprisingly, that Glomerulonephritis (GN) and serum anti-DNA titers (both of which are well characterized systemic autoimmune features) are not [3]. TIN is therefore a manifestation likely to contribute profoundly to renal failure, and to be driven by different autoimmune pathways to GN, which is worthy of thorough characterization.

## Identifying the Pitfalls

### Associating the relevant autoimmune pathway with the relevant disease manifestation

A number of issues have delayed the identification of the factors involved in human autoimmune diseases, and their ultimate validation in animal models. Most are technological. However, some hurdles have, with hindsight, been created by us. The first issue, which has been one of our greatest challenges, has been how best to define a particular autoimmune disease; from autoantibody/autoantigen system to presenting symptoms. By reading the ACR criteria for syndromes such as SLE [4], and RA [5], one can see that two individuals defined as having the same disease may have completely different sites of inflammation, and/or ongoing autoimmune processes as defined by their autoantibody profiles. Clumping these heterogeneous groups of patients together confuses the identification of the components that give rise to particular symptoms. As alluded to above, these components often include HLA alleles (such as DR3, associated with autoimmune hepatitis, and Sjögren syndrome, and DR4 associated with RA), non HLA associated genetic traits (such as those in *ptpn22*, and *ctla4*, associated with numerous autoimmune diseases), and disease specific antibodies (such as anti-TSH receptor, and ACPA, specific for Grave's disease, and RA respectively).

The second issue is that most of the autoantibody/autoantigen processes that have been characterized have been done so using antibodies in serum (often with the aim of developing serum based antibody assays for diagnosis, and prognosis). For any given disease, there is a myriad of autoantibody types present in the serum. These are often disease-associated (i.e. RF), as opposed to being specific for a particular manifestation. Of course there are other autoantibodies that are closely associated with particular manifestations; high anti-Sc170 antibody titers are associated with interstitial lung disease in scleroderma. Generally speaking though, trying to understand which antibodies are disease initiating, perpetuating, or even protective for a particular autoimmune phenomenon, can be incredibly difficult to ascertain. Even if antigens targeted by disease specific autoantibodies are characterized, the idiotypes that are pathogenic are often poorly characterized at best, and the genetic compositions of the variable regions are unknown. Therefore, determining how the parent B-cells of serum autoantibodies have been selected, and what the contributing variable region gene segments that give rise to pathogenic epitope-specific antibodies are, is not possible. More importantly, whether the

B-cells themselves and/or their progeny autoantibodies are actively involved in disease pathogenesis is an incredibly difficult puzzle to solve. Using serum as a source of autoantibodies when performing reproducible serological assays, epitope mapping, and pathogenicity studies is also limited by finite volumes, and variability of antibodies between different blood draws.

## The Limitations of Mice when Modeling Human Autoimmunity

Validation of autoimmune pathways, hypothesized from patient data, is often complicated by the frequent absence of relevant animal models. CD4<sup>+</sup> T cell mediated diseases are generally restricted to a limited set of HLA alleles [6], and analogous MHC molecules encoded by them may not be expressed by mice [7], preventing the simulation of the contexts in which the antigenic peptides are presented to autoantigen-specific T cells. Genetic components (e.g. SNPs in *ctla4*, and the *ptpn22* allele encoding an R620W variant [8]) that appropriately decrease tolerance may also need to be introduced to set the appropriate threshold for lymphocytic activation. Other genetic components which may influence the generation of a pathogenic autoimmune response may involve those which govern clearance of antigens. For example, C1q deficiency (and C1q inhibitory antibodies) leads to the impairment of clearance of apoptotic bodies, which predisposes to GN. Animals may therefore require further genetic engineering so as to mimic appropriate levels of immunological responsiveness.

Testing the pathogenicity of autoantibodies in mice is inherently complicated. One of the reasons for this is that the expression pattern of Fcγ receptors is different between mouse, and man [9]. Ravetch et al. have demonstrated, by comparing the biological activities of the same specificity of human IgG1, murine IgG2a, and IgG2b, that the human IgG<sub>1</sub> constant region does not have comparable biological activity to the afore mentioned murine IgG antibody constant regions [10]. Human monocyte-derived DCs express only FcγRIIa, and FcγRIIB, whereas the corresponding cell type in mice expresses FcγRI, FcγRIIB, FcγRIII, and FcγRIV. What is more, humans express the activation receptor FcγRIIA, and also FcγRIIB, both of which are absent in mice. Therefore, performing human IgG autoantibody transfers, in wild-type mice, to test pathogenicity could yield artifacts, most likely false negatives. Another major issue with autoantibody study of this nature is that, historically, the most relevant, and available source of autoantibodies has been the serum of patients. As mentioned above, the isolation of a sufficient quantity of antigen-specific antibodies can be problematic. Even if purified antigen-specific antibodies are demonstrated to be pathogenic in animal models, further detailed, epitope mapping, and matched passive transfer studies are necessary to confirm the epitope specificities of the pathogenic antibodies.

Arguably, the most fundamental issue in understanding autoimmunity is the nature of the autoantigens themselves. Some antigens, such as fibrinogen (an antigen specifically targeted by autoantibodies in patients with RA when citrullinated), are simply not well conserved in mice, whereas others are not expressed in healthy mice at the site of interest [11]. Examples of these antigens include citrullinated proteins, which only become up-regulated, and exposed in the synovial joint during inflammation [12,13]. Likewise, nuclear antigens only become available during certain cell death processes such as apoptosis, and NETosis [14,15], and may only accumulate when genetic alterations, such as those observed in patients with lupus, result in deficient clearance. Therefore epitopes targeted in the lesion during active autoimmunity may not be exposed at the appropriate anatomical sites in healthy mice when autoantibodies are transferred to them [16].

## Avoiding the Pitfalls

### Characterizing the autoantigens as a starting point when developing an animal model, the example of RA

The characterization of the relevant autoantigens is a critical issue during the characterization of an autoimmune pathway. When rationally developing a murine model of humoral autoimmunity, strategizing acquisition of relevant patient data, and engineering of a mouse model should be dictated by autoantigenic nature. The importance of focusing on the appropriate disease-specific autoimmune pathways was recently highlighted for RA. The genetic factors that most heavily predispose to the development of RA have long been known to be HLA alleles encoding for antigen presenting molecules containing the shared epitope [17]. This HLA class II allelic predisposition supported the idea that RA was an antigen specific, T cell dependent, disease. Also, RA has long been considered an antibody driven autoimmune disease. This has been the case ever since the discovery of RF. Autoantibodies being at the center of the erosive arthritis witnessed in RA was further supported by the subsequent descriptions of other, all be it non-RA-specific, autoantibodies such as anti-GPI (and mouse models demonstrating the arthritogenic nature of anti-GPI antibodies), ANCA, and antibodies to glycolytic enzymes such as  $\alpha$ -enolase in the serum of RA patients [18]. However it wasn't until citrullinated antigens were demonstrated to be targeted by RA-specific antibodies that a robust, statistically sound, antigen-specific, etiopathogenic model for the erosive polyarthritis (the hallmark of RA), could be assembled (Kinloch et al. [19]). Only by serotypic, and immunogenetic, characterization of huge cohorts from Sweden, and the Netherlands has it been possible to reach this hypothesis yielding stage [20]. ACPA are the most specific antibodies for RA, and their frequencies (most commonly detected using variations of anti-CCP ELISA) are almost as high as RF. Because of studies associating HLA types with different autoantibody positivities in patients with arthritis, striking observations were made. Independent studies have demonstrated that the Shared Epitope (SE) does not predispose to RA *per se*. Instead, it predisposes to the development of ACPA<sup>+</sup> disease in a way that RF does not (de Vries et al. [21]). Moreover, there are additive effects of ACPA positivity, and SE positivity, in terms of associations with erosions. These studies support the model that the ACPA response is pathogenic, and that the citrullinated antigen specific immune response presented in the context of the SE is particularly potent, even more so than the ACPA pathway that is mediated through non-SE MHC class II molecules. This may be due to different citrullinated antigens being targeted. Possibly those targeted by SE<sup>+</sup> patients are more exposed, and abundant in the joint. Whatever the subtle differences, both SE<sup>+</sup> ACPA<sup>+</sup>, and SE<sup>-</sup> ACPA<sup>+</sup> patients presumably have the capacity to present peptides from citrullinated antigen bearing complexes, and the autoimmunity to citrullinated autoantigens contributes to chronic inflammation, and the erosion of the joint. Interestingly, further studies have shown that HLA-DR3 predicts anti-CCP negative RA [22,23]. This could be due to activation of Tregs by particular citrullinated antigens that would otherwise activate pathogenic T cells in the context of SE<sup>+</sup> HLA molecules. Longitudinal studies have demonstrated that ACPA are present in patients with early undifferentiated arthritis prior to full satisfaction of the revised ACR criteria [5]. This has had translatable benefits to rheumatologists who have knowledge of SE, and ACPA status, as they now have the chance to monitor particular early arthritis patients more rigorously, and introduce DMARDs at an earlier disease stage, before irreversible joint disability takes hold.

However, what has so far been absent in the ACPA studies for RA is the development of an animal model that recapitulates particular

components of the pathway that results in either the production of ACPA or the arthritis mediated by the antibodies. Some of the reasons for this were addressed above. Other problems include the capacity to marry data regarding ACPA epitope specificity with citrullinated antigen expression *in vivo*. Knowing which of the fine-specificities of ACPA are pathogenic is therefore unknown. The stoichiometry of citrullination in the joint is incredibly low, and many mass spectrometric studies have failed to demonstrate the presence of citrullinated epitopes on candidate antigens (demonstrated to be targeted by ACPA *in vitro* by using synthetic peptides) in the rheumatoid joint [13,19,24]. However, should proteomic data become available that demonstrate which ACPA targets are citrullinated *in vivo* then how best to engineer a relevant animal model will be made clearer. The challenge of characterizing Post-Translational Modifications (PTMs) of low stoichiometry, that constitute epitopes on autoantigens is likely to be ongoing in the field of autoimmunity as a whole (given that so many established antigens are comprised of PTMs). Providing that proteomic methodologies are improved sufficiently to demonstrate PTMs of low abundance in targeted epitopes, then using some of the techniques described in the following section are likely to assist in modeling autoimmune responses to them.

### Humanization of Fc receptors in mice

In order to simulate human IgG-mediated pathways in mice, a number of tools have been engineered. Inclusive are mice transgenic for Fc $\gamma$ RIIa, which is expressed under its own promoter (Hogarth et al. [25]). The expression pattern of this molecule in these transgenic mice therefore mirrors that displayed by humans. Introduction of this transgene confers on mice hypersensitivity to antibody-induced inflammation [26,27], highlighting the significance of Fc $\gamma$ RIIa in autoantibody-mediated pathologies. The pathogenic effects of Fc $\gamma$ RIIa therefore are not mediated in human antibody transfer models using wild-type mice, thus results should be interpreted with the appropriate level of caution. Most impressive of the transgenic mouse strains engineered to reflect human Fc $\gamma$ R function is that developed by Ravetch et al [28]. This strain expresses the full complement of human Fc $\gamma$ Rs (under the control of their respective promoters) on a background where the murine Fc $\gamma$  receptors have been knocked out. These mice are therefore, theoretically, far more pathophysiologically relevant for human IgG antibody transfer studies than wild-type mice. These mice are especially useful for the study of autoantibodies isolated from serum which cannot be subcloned, and made into murine Monoclonal Antibodies (mAbs).

### Murinization of autoantibodies

If the biological activities of human mAbs are to be tested then an alternative approach to using humanized mice is to insert the antibody variable regions into murine expression plasmids. Expressed, and purified murinized antibodies can then be transferred into mice. This is an approach that was elegantly demonstrated by Di Zenzo et al. [29]. In their exploration of Pemphigus Vulgaris (PV), the authors initiated their study by generating anti-desmoglein (anti-DSG) mAbs by screening the supernatants of a panel of mAbs made from the peripheral blood of patients. By engineering murinized versions of these antibodies, and passively transferring them to C57BL/6NCrl mice, the authors were able to determine which clones conferred disease. Also, due to the unlimited mAb source, and being able to ascertain genetic data, by sequencing the variable regions of the mAbs they were able to perform epitope mapping studies, and do genetic analyses of the antibodies to study specificity history. Data generated supported the hypothesis that pathogenic antibodies in PV had arisen from

different combinations of variable region gene segments. Moreover, due to the absence of autoantigen reactivity with the unmutated germline antibody reversion, their data suggested that antibody autoreactivity had been generated following somatic mutation, and affinity maturation in response to an antigen unrelated to DSG3. mAbs can therefore often offer a more plentiful, robust reagent with which antibody pathogenicities can be analyzed, and epitopes mapped in fine detail. They can also provide insight into how the autoimmunity has developed in the first place.

## Humanized Mice for Modeling Antigen Specific T-cells

Humanized mice are also becoming increasingly more common in the study of human autoreactive CD4<sup>+</sup> T-cell responses. To mention but a few, mice are available that express not only human MHC alleles such as 0401, but also human CD4 molecules [7]. These transgenic animals are often better tools for testing hypotheses (generated from patient data) that particular MHC molecules present particular autoantigens in a non-tolerogenic context. However, the problem remains, for both B-, and T-cell receptor targeting, that some autoantigens are not well conserved in mice. In these scenarios double transgenics are called for, which express the relevant human MHC allele, together with a humanized version of the antigen. This approach was successfully adopted by, amongst others, Batsalova et al. [30] in order to study the effects of PTMs in the breach in immunological tolerance to collagen type II, and the development of autoimmune arthritis. In their study, in order to stimulate the appropriate human antigen, and presentation context, they co-expressed the human antigen presentation molecule DRB1\*0401 together with the human version of collagen type II. Before the development of technologies such as these, we could not effectively study T cell specific immune responses to antigens that are not conserved in mice in their appropriate antigen presentation context.

In summary, providing that relevant data, and reagents respectively have been generated from patients, such as disease predisposing-alleles, and SNPs, and mAbs with disease specificity, the appropriate murine tools are now often available, or can be made, for testing hypothesized autoantibody/autoantigen mediated pathways of disease.

## Characterizing, and Modeling TIN

### What we know already

Given the technological arsenal that is now available, we are better positioned to appropriately study partially characterized and recently identified autoimmune features. One such, recently described pathogenic lesion, harboring the features of an autoantibody driven, organ specific autoimmune disease is TIN [2]. TIN is observed in roughly half of lupus nephritis patients at time of diagnostic biopsy [3]. As at the site of lesion in other organ specific autoimmune diseases [31], tertiary lymphoid organs (TLOs) have been demonstrated in the tubulointerstitial during lupus nephritis, varying in stage of development, from diffuse scatterings of lymphocytes, through aggregates of T, and B-cells, to well circumscribed germinal center like structures containing follicular dendritic cells [2]. Local antigen driven selection of B-cells is supported by data generated by laser capture microscopy, followed by sequence analysis of IgG variable region genes. Moreover, higher orders of TLO architecture associate with local Tubular Basement Membrane Immune Complex (TBMIC) deposition. These data support the hypothesis that TBMICs are, at least partly, comprised of locally produced autoantibodies, and that local antigens are inducing proliferation of lymphocytes *in situ*. The worthiness for study of this *in situ* adaptive immune response, as a pathway to better treating lupus nephritis, is given further credence by the correlation

between numbers of CD45<sup>+</sup> lymphocytes in the tubulointerstitium, and the rate of onset of renal failure [3]. Given that the degree of CD45<sup>+</sup> cell presence in TIN is a prognostic indicator, independent of GN, or anti-dsDNA antibody titers, and that tubulointerstitial inflammation can be found in the absence of GN (and vice-versa), the autoimmune pathways that initiate, and/or sustain TIN are likely to be different from those driving GN, and are independently contributing to renal failure.

### How characterization of driving autoantigens will dictate animal modeling

The central issue when characterizing lupus TIN, as it has been for all autoimmune diseases, is the nature of the autoantigens driving the autoimmune response. Although the historic approach has been to screen for targeted autoantigens by using patient serum, as discussed above, this approach can yield a plethora of autoantibodies that are not always relevant to the manifestation in question, effectively generating a haystack before embarking on the inherently difficult pursuit of finding a needle. Given that lupus patients produce a host of different autoantibodies, this haystack creation could be more like a mountain. Instead, we propose that the autoantigens should instead be sought by using mAbs generated from the inflamed tubulointerstitium. Although cloning antibodies from cells sorted from diagnostic biopsies would be a traditional approach, cloning antibodies from areas in the tubulointerstitium, using laser capture, where B-cell expansion is evident, is likely to be the most efficient method for isolating BCRs that are selected due to affinity for local antigens. These studies are currently underway. Only once these autoantibodies have been cloned, and expressed, and their antigens have been characterized can the most appropriate model for validating their pathogenicity be designed. At our present juncture, it is not known whether the autoantigens driving B-cell expansion in the Tubulointerstitial are locally accessible when the tissue is in a basal state of health. Likewise, it is not known whether the targeted epitopes of the autoantigens are conserved between humans, and mice. Providing that the antigens are exposed in the healthy kidney, and that the antigens are in fact conserved, human mAbs can be murinized, and subsequently transferred into wild type mice. Alternatively, they could be transferred as human mAbs to transgenic mice that express human Fcγ receptors. Local antibody deposition, and pathogenicity can subsequently be assayed for. However, should the characterized autoantigens that accumulate in the diseased lesion not accumulate in wild type mice under healthy conditions, then appropriate tubulointerstitial alterations will be necessary before pathogenicity studies can take place on TIN mAbs. Likewise, if epitopes targeted on autoantigens are ill-conserved in mice then transgenic mice will be required that express the human antigenic variants.

### Translating Patient, and Mouse Modeling Data to the Clinic

A serological biomarker for predicting renal failure, beyond elevated serum creatinine is still not available. Characterization of antigens, and the respective epitopes targeted by TIN mAbs is one way in which a panel of candidates could be generated for screening specificities of autoantibodies in the serum of patients with lupus nephritis, in a manner similar to that employed by Di Zenzo et al. [29]. Titers of autoantibodies for respective candidate antigens could then be compared between patients with, and without TIN. However, as has been demonstrated for ACPA, and RF for RA, autoantibodies can predate clinical manifestations, and therefore could predate the development of tubulointerstitial inflammation. Caution will therefore have to be taken when interpreting data from this type of analysis.

The most equivocal data will only become available when longitudinal cohorts of serum samples, and kidney biopsies, such as those that have been generated for arthritis studies in Europe, become available for TIN. At that point, the potential will have been established to associate fine antibody specificities with their predisposing HLA alleles, in the manner described above for RA. Data from these studies will not just aid the development of even more refined murine models of TIN (which will incorporate the appropriate MHC-dependent-T-cell pathways, and other unearthed genetic traits), rheumatologists may be able to generate prognostic autoantibody based tools, which will identify patients who are at increased risk of developing renal failure,, and therefore require closer, and more regular follow up, and tailored therapy.

An exciting prospect for TIN is the following: Should the autoantigens that are driving the tubulointerstitial disease be characterized by using *in situ* derived mAbs from diagnostic biopsy tissue, we will gain insight into the autoantibody/autoantigen systems that are actively driving the disease at the time of diagnosis. This knowledge will help us to understand how current therapies influence the availability of autoantigens at the site of lesion,, and whether the autoantibody/autoantigen pathways are affected by different therapies. This is something that could be applied to a broad range of autoimmune diseases. Using our current rationale,, and empirical strategy, this will be the juncture that will dictate whether specifically focusing on TIN as a pathobiological process is worth inhibiting, with the aim of delaying, and ideally preventing ESRD.

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