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4. KIDNEYS AND AUTOIMMUNE DISEASE

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4.1 Autoimmune diseases

The human immune system limits invasion of foreign organisms and eliminates foreign cells. Discrimination between self and foreign structures is essential in this process. Ability to recognize “self” and limit “auto”-immune responses against self-antigens is defined as tolerance. In many situations, the mechanisms either inducing or maintaining tolerance are disrupted. This breakdown leads to activation of autoreactive cells which, in turn, may initiate overt autoimmune disease.

In breaking tolerance to self-structures several underlying mechanisms act alone or in combination, including apoptosis, defective clearance of apoptotic cells, molecular mimicry and, certainly, genetics.

In order to develop autoimmune disease, an individual may possess a variety of susceptibility genes which lead to abnormalities in a number of biological pathways.

It is important to appreciate that dysfunction in multiple processes occurs simultaneously. Thus a genetic polymorphisms leading to a variety of immunological abnormalities will be molded by environmental and hormonal factors to produce a particular clinical disease phenotype.

Once an uncontrolled immune response is directed to self-structures, the consequences may be devastating. Approximately 3% of the population suffers from a so far described autoimmune disorder. An additional number of diseases may not yet have characterized autoimmune causes.

Cells of the innate and adaptive immune system participate in the development of autoimmunity. It has been observed that the majority of self-reactive immune cells are normally deleted or inactivated during development. This process has been termed central tolerance. There are also checkpoints that regulate the emergence of autoreactive cells during adult life (e.g., during immune responses versus foreign antigen); this process has been termed peripheral tolerance. Nevertheless, some cells escape both checkpoints, and their activation may lead to autoimmunity.

The generation, maintenance, and proliferation of autoreactive B and T-cells and emergence of autoimmune disease, involves the simultaneous breakdown of multiple

central and peripheral checkpoints involved in the maintenance of tolerance. It is well established that the mere presence of autoreactive B or T-cells is insufficient. For example, in lupus patients autoantibodies have been detected long before the onset of clinical disease (e.g., nephritis).

4.2 Kidneys in autoimmune disease

Renal involvement in autoimmunity has many facets. Glomerular, tubular and vascular structures are targeted and damaged as a consequence of autoimmune processes.

Autoimmunity resulting in renal injury occurs as a systemic disturbance of immunity with the central feature being loss of tolerance to normal cellular and/or extracellular proteins. Some of the target autoantigens are now identified in autoimmune diseases where tissue injury includes the kidney .

In most cases, the autoantigens are non-renal and become renal targets because of the physiological properties of the high flow, high-pressure perm-selective filtration function of the glomerulus. Circulating autoantigens can deposit in glomeruli as part of circulating immune complexes or become a “planted” target antigen by their physico-chemical properties that predispose to their glomerular fixation.

A potentially unique model of deposition of a non-renal antigen in the kidney is seen in anti-neutrophil cytoplasmic antibody (ANCA)-associated small vessel vasculitis, where target autoantigens originating in neutrophil cytoplasmic granules and expressed in the cell membrane (including proteinase-3 [PR3] and myeloperoxidase [MPO]) are targeted by ANCA. These ANCA-activated neutrophils have altered flow characteristics resulting in their lodging in small vessels, particularly glomeruli, resulting in renal injury.

Inflammatory renal disease in the context of autoimmunity occurs because the kidney is targeted by effector responses. The effectors of autoimmunity in the kidney are many, but most often disease is initiated either by antibody deposition or infiltration of immune cells. Once antibodies are deposited, their exposed Fc (fragment crystalline) regions activate and recruit inflammatory cells, and initiate complement activation. This process leads to further cellular infiltration, and secretion of inflammatory mediators by both infiltrating and endogenous cells. Infiltrating cells, which include neutrophils, T-cells and macrophages, and platelets also secrete soluble mediators and directly interact with renal cells and each other to perpetuate the disease process.

Within the kidney, the local response of resident cells plays an important role in determining the severity of inflammation. If severe and/or unlimited, these events may lead to fibrosis and organ failure. The intensity and severity of inflammation and fibrosis are also influenced by genetic factors (e.g., that determine the fibrogenic response).

As mentioned, one can envision several ways by which the kidneys become involved. Among the possibilities, renal tissue may harbour a self-antigen (e.g. the "Goodpasture antigen"). In addition, the kidneys may become affected by antibody-mediated mechanisms where the autoantigen resides outside the kidney. Deposition of resulting immune-complexes within the kidneys subsequently triggers tissue damaging events (e.g. lupus nephritis). Third, antigen and antibodies are neither derived nor deposited within the kidneys. However, the interaction of antibodies with the antigens, or with antigen-bearing cells, causes the disease (e.g. ANCA vasculitis and glomerulonephritis).

4.2.1 Anti-GBM disease

Anti-glomerular basement membrane (anti-GBM) disease is the best-defined renal organ-specific autoimmune disease. The disease is strongly associated with autoantibody formation to a specific target found in the glomerular and alveolar basement membranes and is characterized by a rapidly progressive glomerulonephritis (RPGN) which is often associated with pulmonary hemorrhage, though either may occur alone.

Collagen IV is a major component of the GBM. Six alpha chains of type IV collagen are known and these chains form triple helical molecules (protomers). The major antigen of the circulating and deposited anti-GBM antibodies is the non-collagenous domain of the type IV collagen alpha-3 chain($\alpha 3(\text{IV})\text{NC1}$).

Diagnosis is based on the demonstration of anti-GBM antibodies, either in the circulation or fixed to basement membrane of affected organs on biopsy.

Probably the best test for anti-GBM is the renal biopsy with the detection of linear IgG depositions along the GBM. However, most patients also have circulating anti-GBM antibodies in their plasma detected by enzyme-linked immunosorbent assay (ELISA) or Western blotting. The majority of these antibodies are of the IgG1 subtype, with only few IgG4 antibodies. Very rarely, patients have no detectable anti-GBM IgG, but IgA or IgM antibodies instead.

4.2.2 Lupus nephritis

Systemic lupus erythematosus (SLE) is the prototypic systemic autoimmune disease with widespread clinical manifestations. The prevalence of renal involvement depends strongly on the definition. Almost 100% of the patients will have renal manifestation if immunoglobulin deposition is the criterion, whereas the percentage is approximately 50% if proteinuria is applied. Renal involvement is one of the most serious complications, since nephritis may progress into end stage renal disease (ESRD) and is associated with increased mortality. Changing classifications were applied over past decades. More recently, the ISN/RPS 2003 classification was introduced. The most severe lesions are found in Class IV, with diffuse proliferative GN.

Several autoantibodies are generated in lupus patients (anti-nuclear antibodies (ANAs) and anti-double stranded DNA antibodies (dsDNA) included in diagnostic criteria).

Not all of these antibodies seem to mediate renal damage or indicate renal involvement. For nephrologists, antibodies to anti-C1q and to nucleosomes are of particular interest. Nucleosomes consist of DNA and histones. Anti-nucleosome antibodies may occur even before the development of anti-DNA antibodies and were found in patients as well as in murine disease models.

Nucleosomes are generated during apoptosis as a consequence of linker DNA cleavage between the nucleosomes. Nucleosomes are then presented in membrane blebs that are characteristic of apoptotic cells. Presentation of nucleosomes within blebs results in T-cell-driven B-cell stimulation. It is suggested that complexes of nucleosomes and the resulting antinucleosome antibodies bind to heparan sulphate-rich glomerular structures and induce the inflammatory reactions leading to glomerulonephritis.

4.2.3 ANCA-associated vasculitis and glomerulonephritis

The most frequent subgroup of primary systemic vasculitis is that associated with circulating autoantibodies to neutrophil cytoplasmic antigens (ANCA), with involvement of microscopic blood vessels without immune deposits in the vessel walls, "pauci-immune micro-vasculitis". They are also the most frequent autoimmune diseases that affect the kidneys in a rapidly progressive manner. Glomerulonephritis, with fibrinoid necrosis and crescent formation, is common.

ANCA are autoantibodies that are directed to neutrophil and monocyte constituents. ANCA are found in sera of patients with Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), Churg-Strauss syndrome (CSS) or a renal-limited form presenting with necrotizing crescentic glomerulonephritis (ANCA-GN).

ANCA are detected by indirect immunofluorescence on ethanol-permeabilized neutrophil preparations. A fixation artefact actually leads to the fact that a cytoplasmic ANCA pattern (c-ANCA) can be distinguished from a perinuclear pattern (p-ANCA).

Detailed studies identified proteinase 3 (PR3) and myeloperoxidase (MPO) as the major ANCA antigens. ANCA specificity to these antigens is tested by the use of enzyme-linked immunoassays (ELISA). The c-ANCA mainly recognizes PR3, whereas p-ANCA bind to MPO. However, p-ANCA also recognizes non-MPO molecules, including elastase, lactoferrin, lysozyme and cathepsin G. The perinuclear staining pattern results from distribution of cationic MPO along the negatively charged nuclear membrane after ethanol treatment of the neutrophils.

The p-ANCA pattern becomes a cytoplasmic pattern when MPO-ANCA is tested on formalin fixed neutrophil. An ANCA work-up should always include IF and PR3 and MPO ELISA. Over the past two decades, ANCA has become an important diagnostic tool. However several issues need to be considered when employing ANCA testing. These points include pretest patient selection, technical issues and consideration of the clinical context.

In addition to being a clinical tool, ANCA are causal for the disease induction. The central mechanism in inducing vasculitis is the interaction of ANCA with the neutrophil that contains the ANCA antigens. The majority of MPO and PR3 are stored in neutrophil granules. This granule pool is mobilized to the cell membrane during cytokine-mediated neutrophil priming. PR3 and MPO translocation is controlled by p38 MAPK. ANCA bind to cell surface-expressed ANCA antigens, resulting in subsequent neutrophil activation. The activation process involves cross-linking of ANCA antigens on the cell surface and Fc-gamma receptor signals. ANCA-activated neutrophils respond by generation of reactive oxygen species, degranulation of proteolytic enzymes and up-regulation of adhesion molecules. PI3-K/Akt signaling is central to the activation process.

ANCA-activated neutrophils adhere to and damage endothelial cells. Interestingly, this neutrophil-endothelial cell interaction results in suppression of ANCA-stimulated superoxide production, whereas degranulation of toxic molecules is accelerated.

In the most likely scenario, neutrophils, once rolling over the endothelial surface, become primed, express PR3/MPO, and interact with ANCA. This interaction leads to firm adhesion, transmigration, and also local endothelial damage, all compatible with necrotizing vasculitis and glomerulonephritis.

4.3 Conclusions - what the future might hold

Numerous human and animal studies support the hypothesis that for example lupus nephritis is an immune complex disease and signal the potential therapeutic benefit of suppressing autoantibody production.

The clinical utility of testing for autoantibodies is immediately apparent but even robust associations between specific immunoglobulins and particular autoimmune diseases or patterns of organ involvement do not guarantee a causal link.

Anti-double stranded DNA antibodies were first characterized 50 years ago and it is 25 years since anti-neutrophil cytoplasm antibodies were discovered. Anniversaries coincide with a growing enthusiasm for the use of B-cell targeted therapies in proliferative lupus nephritis and systemic ANCA-vasculitis, the diseases with which these autoantibodies are respectively linked.

Recommended literature:

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