Role of Urinary Osteoprotegerin)OPG (as a biomarker for Lupus Nephritis activity

A Thesis submitted for partial fulfillment of the master degree

in internal medicine

By

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Abstract

since kidney biopsies are not repeated at every flare of lupus nephritis, a noninvasive predictor of renal pathology would be very useful in choosing therapy. A biomarker that can forecast lupus nephritis flare well before thresholds of proteinuria, renal function, and urine sediment that signal clinical flare are reached would be a valuable tool .Osteoprotegerin)OPG(, a member of the tumor necrosis factor)TNF (receptor family, has been identified as a regulator of bone resorption .Increased plasma concentrations of OPG have been found in patients with diabetes mellitus and have been associated with diabetic microvascular manifestations .

Keywords :*Role of Urinary Osteoprotegerin)OPG (as a biomarker for Lupus Nephritis activity*

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Abu -Hashim Ahmed

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ACE	Angiotensin converting enzyme
Ang	Angiotensin II
ARBs	Angiotensin receptor blockers
AT_1	Angiotensin receptor 1
ATP	Adenosine triphosphate
CRP	C-reactive protein
EGF	Epidermal growth factor
ET	Endothelin
GFR	Glomerular filtration rate
ICAM	Intercellular adhesion molecules
IDDM	Insulin dependent diabetes mellitus
IDDM	Insulin dependent diabetes
IGF-1	Insulin-like growth factor-1
IL	Interleukin
LDL	Low density lipoprotein
mRNA	Messenger ribonucleic acid
MCP-1	Monocyte chemoattaractant protein
NEFAs	Non esterified fatty acids
NFKβ	Nuclear factor K beta
NO	Nitric oxide
OPG	Osteoprotegerin
tSLEDAI	Total systemic lupus disease activity index
rSLEDAI	Renal systemic lupus disease activity index

Chapter one

Systemic lupus erythematosus

Introduction

Systemic lupus erythematosus)SLE (is an autoimmune disease with a broad range of manifestations and a great polymorphism in clinical expression***) Appel et al, 1994 (***

The course of the disease is marked by episodes of active inflammation and clinically silent remission .All organ tissues may be involved, but it is the skin, the joints, the heart, the nervous system and the kidney that are most commonly affected .Clinical evidence of renal disease occurs in 35 to 75 %of patients with SLE ***)Estes et al, 1971(***

Probably, the total prevalence of renal involvement may exceed 90 %because of changes of diffuse lupus nephritis)LN (seen in kidney biopsies from SLE patients without clinical evidence of renal involvement***) Leehey et al, 1983(***

In most cases, renal involvement is mild with, for instance, proteinuria seen in 42%, while life threatening renal disease is relatively rare .Rapidly declining renal function remains a subject of special attention which demands early diagnosis, optimal clinical monitoring, and aggressive immunosuppressive therapy .The survival of patients with SLE is influenced both by the severity of renal involvement and by the serious toxic side effects of the immunosuppressive agents used ***)Wallace et al, 1992(***

The pathogenesis of nephritis in SLE

There is overwhelming evidence that LN is caused by glomerular immune deposits, as indicated by the presence of immunoglobulins and complement breakdown products in virtually all kidney biopsy specimens obtained from patients with active LN .A major advance in lupus research has been the discovery that the disease is—at least in part—the result of an autoantigen-driven immune response ***)Datta et al, 1997(***

Anti-dsDNA Antibody Response

Anti-dsDNA Antibody Response is Driven by Histone-Specific T-Helper Cells . On the basis of the classical view that anti-dsDNA antibodies are pathogenic in SLE, T-helper cells from lupus mice and patients have been cloned for their ability to induce the production of anti-dsDNA antibodies when cultured with autologous B cells .Sequencing of the T cell receptor chain genes of these T-helper cell clones revealed a recurrent motif of anionic residues in the junctional region of the CDR3 loop, suggesting that the corresponding autoantigens were rich in cationic residues ***)Adams et al, 1991(***

It therefore was anticipated and proved that the relevant antigens were peptides derived from the nucleosome, the DNA packaging unit, made of a 146-bp DNA loop wound around a cationic histone octamer core ***) Mohan et al, 1993(***

This first set of experiments indicated that the so-called anti-dsDNA antibody B cell response in lupus was actually driven by histone-specific T-helper cells .B cells trap circulating DNA-binding proteins, such as nucleosomes, through their DNA-recognizing membrane-bound Ig .The complex is endocytosed and processed, and cationic peptides are presented in an MHC class II-restricted way to histone-specific T-helper cells .In the presence of appropriate co-stimulatory signals, such as CD40–CD40L)CD154 (or CD28–B7.1/B7.2) CD80/CD86(, this cognate interaction results in B cell activation and proliferation, in particular through the production of cytokines ***) .Desai-Mehta et al, 1995(***

Impaired Clearance of Apoptotic Bodies

The next step was to understand how nucleosomes are released and why they become immunogenic in SLE .Thus, it was demonstrated that blebs that appear on the surface of apoptotic cells, such as keratinocytes exposed to UV light, contained nucleosomes as well as

other lupus target antigens, such as the ribonucleoproteins Ro and La, thereby suggesting that apoptotic waste could be a source of autoantigens ***)Casciola-Rosen et al, 1994(***

Most interesting, the clearance of apoptotic bodies by macrophages or other phagocytes is impaired in lupus patients, and apoptotic material was found to be tightly associated with dendritic cells in lupus lymph nodes .Taken together, these results suggest that autoantigens are processed by professional antigen-presenting cells and presented to autoantigen-restricted T-helper cells .Nucleosome-containing apoptotic material, rather than being engulfed and eliminated by macrophages without inducing immune and inflammatory responses, therefore could become immunogenic in SLE***) Baumann et al, 2002(***

Consistently, defective removal of apoptotic cells in C1q knockout mice or serum amyloid P knockout mice is associated with a lupus-like disorder .The reason that removal of apoptotic material is skewed toward dendritic cells in lupus patients is unknown .In this respect, the recent observation that IFN -present in lupus sera favors the maturation of circulating monocytes in dendritic cells might be relevant***) Robson et al, 2001(***

Nucleosome/Antinucleosome Complexes

On the effector side, the role of nucleosome/antinucleosome complexes has been recently hypothesized .Thus, specific antinucleosomal antibodies)not reacting with dsDNA or with histones but with the complex of the latter and the former (are detected in the sera of LN patients, and their titers correlate with renal disease activity***) Bruns et al, 2000(***

Renal perfusion of nucleosome/antinucleosome complexes induces glomerular immune deposits and proteinuria in mice .Finally, nucleosomal antigens are detected in the glomerular basement membrane) GBM (of LN patients .Among other hypotheses, the cationic histone part of the nucleosome/antinucleosome complexes could bind to the negatively charged heparan sulfate molecule expressed on the GBM .Such nucleosome-

mediated binding of antibodies to the GBM could then initiate glomerulonephritis, through complement activation but also through complement-independent mechanisms induced by Fc/Fc receptors interaction ***)van Bruggen et al, 1997(***

Beyond the Role of Antinuclear Antibodies

The pathogenesis of LN is probably much more complex than the above-mentioned mechanisms .Thus, Waters *et al*., using a very elegant genetic approach, could demonstrate that breaking tolerance to dsDNA, nucleosome, and other nuclear antigens is not required for the development of nephritis in NZM2328 lupus-prone mice .In these animals, the *Cgnz1 locus* on chromosome 1 is linked to nephritis, whereas the *Adnz1 locus* on chromosome 4 is linked to antinuclear antibody production .Congenic mice in which the genetic interval that contains *Adnz1* was replaced by that from C57L/J non-lupus-prone mice, still experienced nephritis, although their serum was negative for antinuclear, anti-dsDNA, and antinucleosome antibodies, thereby indicating that antinuclear antibody production and nephritis are under independent genetic control***) Waters et al, 2004(***

The genetic dissection of SLE will bring new insights into the pathophysiology of LN . Genome-wide screens performed in multiplex SLE families have already allowed the identification of multiple susceptibility loci and of candidate genes, such as PDCD-1 that encodes a protein that plays a role in lymphocyte activation and activation-induced cell death ***)Prokunina et al, 2002(***

In lupus-prone mice, congenic dissection is a powerful strategy to analyze the respective contribution of individual susceptibility loci to a polygenic trait .Congenic animals that bear a given susceptibility locus, such as *Sle1, Sle2, Sle3*, on a resistant background)B6 (have been obtained .Most interesting, although each of the *Sle*-congenic strains displayed immune alterations, none developed fatal LN .Only multicongenic animals experienced full-blown disease .Fine mapping will further allow identification of disease-associated genes or pathways that could be specifically targeted***) Lauwerys et al, 2004(***

The clinical features of lupus nephritis

Nephritis is a common visceral manifestation of SLE .An abnormal urinalysis is present in almost half of the patients at the beginning of the disease and in up to 75 %in the later course ***)Wallace et al, 1992(***

The total incidence of renal involvement might probably exceed 90 %since renal biopsy in patients without any clinical evidence has revealed focal or diffuse proliferative glomerulonephritis .However, severe life threatening renal disease is relatively rare. ***)Leehey et al, 1983(***

Lupus nephritis demonstrates a wide spectrum of glomerular alterations reaching from mesangial through focal proliferative to diffuse proliferative glomerulonephritis with crescent formation .Tubulointerstitial nephritis may also be an additional feature .In order to describe the various lesions in the kidney the WHO standard classification is commonly used .In this classification, type I represents an entirely normal kidney biopsy at light microscopy, on immunofluorescence, and electron microscopy***) Churg et al, 1982(***

Mesangial and sub-endothelial deposits are characteristics of focal or diffuse proliferative immune-complex glomerulonephritis)type II-IV .(Diffuse thickening of the basement membrane with normal glomerular cellularity and electron-dense deposits in the sub-epithelial space of the basement membrane are found in type V LN .Approximately 75 %of renal biopsy specimens reported in several studies have been classified as focal proliferative, diffuse proliferative, or membranous glomerulonephritis ***)Golbus et al, 1994(***

In some patients with slowly progressive renal dysfunction and a relatively normal urinary sediment, only sclerosing glomerulonephritis can be found histologically)type VI .(This form may represent healing from a prior inflammatory injury .In an individual patient, multiple

histopathological findings belonging to more than one WHO class in parallel may be apparent on biopsy***) Kashgarian et al, 1994(***

Since the clinical course in LN is extremely variable, an individual prognosis is difficult to predict .In recent years, a substantial improvement in the survival of patients with SLE has been documented ***)Pistiner et al, 1991(***

Five-year survival rates of 90 %and 10-year survival rates of more than 80 %have now been demonstrated, compared with 5-year survival rates of 25 to 40 %in the decade 1960 to 1970 ***)Appel et al, 1987(***

The improvement has been due to advances in therapeutic options and in general medical care .Survival is still low in SLE patients with severe multi-system disease .Infection is the most common cause of death ***)Hellmann et al, 1987(***

Other leading causes are related to acute vascular neurologic events, renal failure, and coronary artery disease .The presence of severe renal disease and systolic hypertension seems to be associated with a decreased survival in SLE***) Hochberg et al, 1992(***

The prognosis of SLE with renal involvement depends on the activity of the underlying renal disease, and the degree of already existing renal functional impairment .Whether or not renal pathological findings may be of prognostic help still remains controversial .Some studies found that active)hypercellularity, fibrinoid necrosis, cellular crescents (or chronic)glomerulo-sclerosis, fibrous crescents, interstitial fibrosis, tubular atrophy (lesions on kidney biopsy were helpful to discriminate between favorable and worse renal outcome***) Esdaile et al, 1993(***

Especially, the demonstration of chronic lesions have been reported to increase the risk of later development of renal insufficiency .However, others were not able to find a correlation between the chronicity index and renal outcome***) Austin et al, 1994(***

In fact, these lesions might only reflect post-inflammatory scarring without implication for future renal damage .Not surprisingly, histologically documented irreversible nephron damage is unresponsive to therapy ***)McLaughlin et al, 1994(***

Information from kidney biopsy is further limited by the fact that transformation of one morphological pattern to another occurs in 15 to 40 %of rebiopsied patients ***)Lee et al, 1984(***

By contrast, patients with severe active disease indicated by diffuse proliferative lupus glomerulonephritis with endocapillary proliferation or necrosis in more than 50 %of the glomeruli)WHO class IV (have a worse prognosis compared to all other groups ***)Glassock et al, 1996(***

The reported 10-year survival in this group is about 60 .%Prognostic approaches including clinical and histologic parameters such as nephrotic range proteinuria, evidence of systemic disease activity, and histologic evidence of extensive crescents and fibrinoid necrosis seem to indicate a greater likelihood of renal disease progression ***)Schwartz et al, 1987(***

Moreover, hypertension and/or initial serum creatinine have been identified as predictors of outcome .The former is a well known prognostic factor in all kidney diseases. The latter represents a pre-existing decline in glomerular filtration rate and an already reduced renal function prior to diagnosis .In general, kidney survival has considerably improved during the last decade as shown in a multicenter study of 536 patients with lupus nephropathy .About 80 % of patients still retained adequate renal function 10 years after diagnosis***) Sloan et al, 1996(***

Membranous glomerulonephritis in SLE has an unpredictable course and outcome . This variability is related to the extent and degree of glomerulonephritis seen on renal biopsy ***) loan et al, 1996(***

While the course in membranous glomerulonephritis in SLE is reported to be similar to that of the idiopathic variety, the prognosis is poor in patients with superimposed focal or diffuse proliferative glomerulonephritis ***)Radhakrishnan et al, 1993(***