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Renal macrophage activation and Th2 polarization precedes the development of nephrotic syndrome in Buffalo/Mna rats

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Background. At 8 weeks, Buffalo/Mna rats spontaneously develop a nephrotic syndrome associated with focal segmental glomerulosclerosis (FSGS). We have previously demonstrated that this glomerulopathy recurs after renal transplantation, thus supporting the relevance of this rat model to human idiopathic nephrotic syndrome [1]. In this study, we describe renal immune abnormalities which appear in parallel to the initiation and progression of the spontaneous Buffalo/Mna nephropathy.

Methods. Buffalo/Mna rat kidney samples were harvested before (4 weeks) and after the occurrence of proteinuria (at 10, 18, and 24 weeks, and at 12, 15, 18, and 24 months). Renal immune cell populations [total lymphocytes, macrophages, T, B, and natural killer (NK) cells] and the expression kinetics of various related cytokine [transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin (IL)-1, IL-2, IL-4, IL-6, IL-10, IL-12, and IL-13], chemokine [regulated upon activation, normal T cell expressed and secreted (RANTES) and monocyte chemoattractant protein-l (MCP-1)] and T-cell receptor β (TCR β) chain transcripts were studied serially during the course of the disease.

Results. In the Buffalo/Mna kidneys, in parallel to the proteinuria, the focal and segmental glomerular lesions began to develop at 10 weeks (affecting 2.4 ± 0.8% of glomeruli), increased in number, then in intensity (10.4 ± 0.8% at 24 weeks, 14.6 ± 2.3% at 12 months, and 28.9 ± 7.4% at 18 months). Before the onset of the disease, at a nonproteinuric stage, the transcript expression analysis revealed a strong production of some macrophage-associated cytokines, particularly TNF-α (350-fold higher than control levels), which was corroborated by monocyte infiltration. A minor T-cell infiltrate (associated with an increase in Cβ TCR transcripts), with a predominantly Th2 profile and the down-regulation of Th1 cytokines was also observed. These abnormal macrophage and T-cell patterns remained stable after the onset of the disease. No changes in chemokine and TGF-β transcripts were observed during the initial stages of the disease.

Conclusion. Our data suggest that the Buffalo/Mna rat disease may be the result of an immunologic disorder, involving macrophages and Th2 lymphocytes. We hypothesize that this modified environment could result in the production of a factor deleterious to the glomeruli. Thus, this rat strain could provide a new model for the study of human nephrotic syndrome.

Key words: FSGS, idiopathic nephrotic syndrome, rat, Buffalo/Mna, immunologic disorders.

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Idiopathic nephrotic syndrome with primary focal segmental glomerular sclerosis (FSGS) is a disease of unknown etiology, whose symptoms include a selective proteinuria and nonspecific lesions with a hyalinosis and synechia between the floculus and Bowman's capsule. Immunosuppressive regimens such as corticoids, cyclosporine A, and cyclophosphamide can influence the disease outcome to some extent (for review see [2]) but at least 20% of patients ultimately require hemodialysis and/or kidney transplantation for end-stage renal failure (ESRF) [3]. In addition, in 25% to 40% of transplanted patients, the initial disease immediately relapses, leading to graft loss in 50% of cases [4]. This immediate recurrence strongly suggests the presence of an albuminuric plasmatic factor(s), a hypothesis that has been strengthened by the beneficial effect of plasmatic exchanges [5–7] and immunoadsorptions [8, 9].

Despite significant recent progress in the understanding of the genetic abnormalities associated with idiopathic nephrotic syndrome [10], the disease mechanisms, particularly those at the disease onset, are unknown. Several animal models of proteinuria have been described, including age-associated nephropathy [11], nephron reduction [12, 13], and toxic-induced nephrosis [14, 15]. However, although these experimental models can help to identify the mechanisms involved in glomerular sclerosis progression, they are not pertinent as models for the initial stages of the human disease. For this reason, we have been studying the Buffalo/Mna rat strain which, at 8 weeks of age, spontaneously develops a selective

proteinuria associated with hypoalbuminemia, hyperlipidemia, and glomerular epithelial cell alterations with foot process flattening and cytoplasmic vacuolization at the ultrastructural level [16, 17]. The Buffalo/Mna rats were first reported because they present a spontaneous thymoma [18] regulated by an autosomal-dominant gene [19] associated with muscular weakness and demonstrated to be linked to plasmatic antiryanodine receptor antibodies [20]. Following analysis of genetic segregation, two autosomal-recessive genes were proposed to determine susceptibility to glomerular sclerotic lesions [21]. Both genes are located on chromosome 13, but are separate from the proteinuria gene Pur 1 located on the long arm of human chromosome 1 [22]. Moreover, neonatal thymectomy experiments have shown that nephrotic syndrome is functionally unrelated to the thymic disease [23]. In the same way, in our hands, thymectomy in adult Buffalo/Mna rats has no effect on their proteinuria (unpublished personal data), suggesting the absence of a direct interaction between the renal disease and that of the thymoma.

In addition to a genetic susceptibility to develop proteinuria [21, 22], the involvement of an extrarenal factor has been demonstrated by our group in a previous study [1]. We showed that the disease recurs on normal kidneys after renal transplantation into Buffalo/Mna recipients whereas the glomerulopathy regresses when a Buffalo/Mna kidney is transplanted into a normal recipient [1].

In this study, we have attempted to characterize the development of Buffalo/Mna nephropathy by analyzing the histologic lesions, the cells infiltrating the diseased kidneys, and the renal cytokine transcript accumulation. We report here that the prealbuminuric stage of the disease is characterized by an early macrophage infiltration and a Th2 polarization. We suggest that these immunologic disorders could be involved in the Buffalo/Mna nephropathy and that these observations may help to understand this rat disease. Such observations may also be related to the human disease and used to elucidate the human pathologic mechanisms.

METHODS

Animals

The Buffalo/Mna rat line, maintained in our laboratory, was originally kindly provided by Dr Saito (Central Experimental Institute, Nokawa, Kawasaki, Japan). All animals were born from a unique couple and bred for at least 10 generations. Five Buffalo/Mna rats were sacrificed at each time point: 4, 10, 18, and 24 weeks and 12, 15, and 18 months. Five inbred, age-matched Wistar-Furth rats [with the same major histocompability complex background (MHC) as Buffalo/Mna], obtained from an established colony (Janvier, Le Genest Saint Isle, France), were used as controls and sacrificed at 4, 10, 18, and 24 weeks. All animals were fed with standard laboratory food. The animal care was in strict accordance with our institutional guidelines.

Proteinuria measurement

The animals were placed in metabolic cages for 24 hours before measurement with free access to drink but without food to avoid contamination of urinary samples. The total urinary protein concentration (g/L) was measured by a colorimetric method using a Hitachi autoanalyzer (Boehringer Mannheim, Grenoble, France). Urinary creatinine (mmol/L) was measured by the Jaffé method. Proteinuria was expressed according to the following formula: proteinuria (g/mmol) = (urinary protein) (g/L)/(urinary creatinine) (mmol/L).

Light microscopic examination

Kidney samples were fixed for 20 minutes in Carnoy solution and then in 10% buffered formalin and embedded in paraffin. Three micro meter sections were stained with hematoxylin and eosin, periodic acid-Schiff (PAS), Masson trichrome, or periodic acid-silver methenamine (PAM). Slides were analyzed in a blind fashion by an independent pathologist. Lesions were estimated and counted in five fields at a 100× magnification.

Immunohistology and quantitative analysis of cellular populations

Kidney pieces from Buffalo/Mna and Wistar-Furth rats were embedded in optimal cutting tissue compound (Tissue Tek) (Miles, Elkhart, IN, USA), snap-frozen in precooled isopenthane and stored at −80° C until use. Frozen 6 μm tissue sections were fixed in acetone, permeabilized with a solution of methanol 10% H_2O_2, incubated with a Biotin Blocking System (Dako Corporation, Carpinteria, CA, USA), then saturated with rat serum diluted 1/10 in phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA) and stained using a three-step indirect immunoperoxidase technique [20]. The primary antibodies were mouse IgG antirat monoclonal antibodies: Ox1-Ox30 (a mix of two anti-CD45 antibodies, a pan-leukocyte marker), R7.3 [anti-T-cell receptor αβ (anti-TCRαβ)], Ox33 [anti-CD45 receptor antagonist (anti-CD45 RA)], ED-1 (anti-CD68), or 3.2.3 [(anti-CD161 or natural killer receptor (NKR)]. All of these monoclonal antibodies were obtained from the European Collection of Animal Cell Cultures (ECACC), then purified in our laboratory and pretested on healthy rat splenocytes to assess their optimal dilution. Nonspecific staining was taken into account by omission of the first antibody. The secondary antibody used was a rat adsorbed (negligible cross-reactivity) horse biotinylated antimouse IgG (Vector Laboratories, Burlingame,