1-090

INDUCTION OF PAUCI-IMMUNE NECROTIZING AND CRESCENTIC GLOMERULONEPHRITIS (NCGN) BY INTRAVENOUS ADMINISTRATION OF ANTI-MYELOPEROXIDASE (ANTI-MPO) ANTIBODIES TO RECOMBINASE ACTIVATING GENE-2 DEFICIENT (RAG-2 -/-) MICE

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Background: Association of antineutrophil cytoplasmic autoantibodies (ANCA) with pauci-immune NCGN raises the possibility that they are pathogenic. We report an animal model that should facilitate understanding the pathogenesis of ANCA disease.

Methods: MPO knockout mice were immunized with murine MPO purified from WEHI cells, or with bovine serum albumin (BSA). Development of anti-MPO and anti-BSA was monitored by ELISA and by indirect immunofluorescence microscopy assay (IFA) using mouse neutrophils as substrate. IgG was isolated from serum of mice immunized with MPO or BSA by 50% ammonium sulfate precipitation followed by protein G affinity chromatography. 50 µg/g mouse weight IgG in PBS was injected i.v. into RAG2-/- mice, which lack B and T lymphocytes. Induction of circulating anti-MPO and anti-BSA was monitored by anti-MPO and anti-BSA ELISA and by ANCA IFA. Serum creatinine, BUN, proteinuria and hematuria were monitored. At the termination of the experiments on day 6 or day 14, tissue was obtained for pathologic examination by light and immunofluorescence microscopy.

Results: Passive transfer of anti-MPO and anti-BSA resulted in an immediate peak in circulating antibodies that declined progressively during observation. At the time of the first urine analysis at day 3, mice that received anti-MPO IgG (n=7) already had developed hematuria and proteinuria, but not mice that received anti-BSA IgG (n=3). Mice sacrificed at day 6 that had received anti-MPO (n=5) all had focal necrotizing glomerulonephritis (mean 18% glomeruli with necrosis) and crescents (mean 11% crescents), whereas mice that received anti-BSA (n=3) had no histologic lesions. Anti-MPO mice had a mean BUN of 47.4 compared to a mean of 22.7 in anti-BSA mice. Mice sacrificed at day 14 that had received anti-MPO had an average of 1.5% glomerular necrosis, 11% crescents, and 34% glomerular sclerosis. Immunofluorescence microscopy demonstrated only a paucity of glomerular staining for immunoglobulins and complement, most pronounced at sites of injury.

Conclusions: In mice with no T lymphocytes, circulating anti-MPO (MPO-ANCA) causes pauci-immune necrotizing and crescentic glomerulonephritis. Necrotizing lesions progress to sclerotic lesions in less than a week.

2-091

INDUCTION OF NECROTIZING AND CRESCENTIC GLOMERULONEPHRITIS (NCGN) AND SMALL-VESSEL VASCULITIS (SVV) BY ADOPTIVE TRANSFER OF ANTI-MYELOPEROXIDASE (ANTI-MPO) LYMPHOCYTES INTO RECOMBINASE ACTIVATING GENE-2 DEFICIENT (RAG-2 -/-) MICE

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Background: Association of antineutrophil cytoplasmic autoantibodies (ANCA) with NCGN and SVV raises the possibility that they are pathogenic. We report an animal model that should facilitate understanding the pathogenesis of ANCA disease.

Methods: MPO-knockout mice were immunized with murine MPO from WEHI cells or bovine serum albumin (BSA), or received no immunization. 1×10^8, 5×10^7, or 1×10^7 splenocytes from these mice were injected into RAG2-/- mice, which lack functional lymphocytes. Anti-MPO, anti-BSA, creatinine, BUN, proteinuria and hematuria were monitored. At termination, tissue was obtained for pathologic examination.

Results: Mice that received anti-MPO splenocytes developed circulating anti-MPO within 3 days, and the titer rose until sacrifice at 13 days. Mice that received anti-BSA splenocytes developed circulating anti-BSA. Mice that received MPO-ANCA splenocytes developed renal failure, hematuria and proteinuria, whereas mice that received anti-BSA or control splenocytes did not. All mice that received 1×10^8 (n=12) or 5×10^7 (n=4) anti-MPO splenocytes developed severe NCGN, 3 developed pulmonary capillaritis, 1 had necrotizing vasculitis in spleen and lymph nodes, and 1 had necrotizing granulomatous inflammation in spleen. Mice that received 1×10^7 anti-MPO (n=4), or 1×10^8 anti-BSA (n=14) or control (n=9) splenocytes developed no crescents or renal failure. They did have mild glomerular hypercellularity and rare segmental necrosis. All mice that received anti-MPO, anti-BSA or control splenocytes developed moderate glomerular localization of mouse immunoglobulin and complement.

Conclusions: All RAG2-/- mice that receive splenocytes from immune-competent mice develop low-level, functionally insignificant, glomerular immune complex localization. Only mice that receive splenocytes that produce MPO-ANCA developed NCGN and SVV. Thus, circulating MPO-ANCA cause NCGN and SVV, possibly by synergistic interactions with another minor inflammatory stimulus.

3-049

CONTRIBUTION OF ACTIVATED NEUTROPHILS AND MPO-ANCA TO THE DEVELOPMENT OF CRESCENTIC GLOMERULONEPHRITIS IN SCG/Kj MICE

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A role of activated neutrophils in the development of nephritis was investigated in the correlation between neutrophil functions and renal failure of SCG/Kj mice that spontaneously develop crescentic glomerulonephritis from early phase of life. MPO release, superoxide generation of peripheral neutrophils, MPO-ANCA titer in serum and histology of glomeruli were examined in young and aged mice. Neutrophil number in peripheral and glomeruli increased depend on the development of nephritis showing proteinuria. Particularly, in the early phase of nephritis, spontaneous release of MPO from peripheral neutrophils of SCG/Kj mice increased, but superoxide generation stimulated with fMet-Leu-Phe and cytochalasin B decreased. MPO-ANCA titer, a marker for vasculitis, in serum of SCG/Kj mice was higher than that of normal mice. While the comparison of neutrophil functions with histological findings, spontaneous release of MPO was correlated to activity index, crescent formation score and chronicity index. On the contrary, superoxide generation was negatively correlated to crescent formation score. Moreover, the number of neutrophils infiltrated in glomeruli was well correlated with MPO-ANCA titer in sera, activity index, crescent formation score and chronicity index. These findings suggest that spontaneously activated neutrophils of SCG/Kj mice may contribute to nephritis development. Aseanostatin (ai-15:0), an inhibitor of human MPO release from neutrophils, was inhibited spontaneous MPO release from peripheral neutrophils in SCG/Kj mice, but not neutrophils of C57BL/6 mice. From these findings, it is strongly suggested that activated neutrophil could trigger the development of crescentic glomerulonephritis in SCG/Kj mice. This mouse is believed to be a good model of crescentic glomerulonephritis.