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Macrophage depletion ameliorates nephritis induced by pathogenic antibodies

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ABSTRACT

Kidney involvement affects 40–60% of patients with lupus, and is responsible for significant morbidity and mortality. Using depletion approaches, several studies have suggested that macrophages may play a key role in the pathogenesis of lupus nephritis. However, "off target" effects of macrophage depletion, such as altered hematopoiesis or enhanced autoantibody production, impeded the determination of a conclusive relationship. In this study, we investigated the role of macrophages in mice receiving rabbit anti-glomerular antibodies, or nephrotoxic serum (NTS), an experimental model which closely mimics the immune complex mediated disease seen in murine and human lupus nephritis. GW2580, a selective inhibitor of the colony stimulating factor-1 (CSF-1) receptor kinase, was used for macrophage depletion. We found that GW2580-treated, NTS challenged mice did not develop the increased levels of proteinuria, serum creatinine, and BUN seen in control-treated, NTS challenged mice. NTS challenged mice exhibited significantly increased kidney expression of inflammatory cytokines including RANTES, IP-10, VCAM-1 and iNOS, whereas GW2580-treated mice were protected from the robust expression of these inflammatory cytokines that are associated with lupus nephritis. Quantification of macrophage related gene expression, flow cytometry analysis of kidney single cell suspensions, and immunofluorescence staining confirmed the depletion of macrophages in GW2580-treated mice, specifically within renal glomeruli. Our results strongly implicate a specific and necessary role for macrophages in the development of immune glomerulonephritis mediated by pathogenic antibodies, and support the development of macrophage targeting approaches for the treatment of lupus nephritis.

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1. Introduction

Systemic lupus erythematosus (SLE) is characterized by the production of autoantibodies and systemic inflammation, which collectively result in damage to various end organs. Kidney involvement, known as lupus nephritis (LN), is seen in up to 60% of SLE patients, and is associated with increased morbidity and mortality. Even with current immunosuppressive regimens, chronic renal insufficiency develops in about a quarter of patients. In a two year follow up of patients with LN, the complete remission rate with current treatment regimens was less than 50%, and the relapse rate was as high as 30% [1]. A better understanding of disease pathogenesis could lead to the discovery of more targeted therapies, which may prevent or suppress relapses and improve long term kidney prognosis.

Macrophages are innate immune system cells that are present in every tissue [2] where they represent 5–15% of the cells [3]. They have a very high degree of plasticity, and the ability to assume different functions ranging from trophic, to immune suppressive or tissue destructive phenotypes [2]. Macrophages derive from three known lineages within mice: the embryonic yolk sac, the fetal liver, and the adult bone marrow. Most tissue resident macrophages are thought to derive from the yolk sac, whereas macrophages originating from precursors in the bone marrow seed tissues mostly under inflammatory conditions [2,4,5].

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Macrophages are believed to contribute to the pathogenesis of LN. In human lupus, renal mononuclear phagocyte infiltration is associated with poor outcomes [6]. Furthermore, studies have shown that unique, activated populations of macrophages are present during active murine LN [1,7,8]. Additionally, depletion studies have sought to demonstrate that macrophages are not just present during disease, but also actively contribute to pathogenesis. However, major limitations of the methods employed for macrophage depletion have seriously hindered a definitive determination of whether or not macrophages are truly involved in disease progression [8–14].

In this study, we sought to deplete macrophages in an inducible model of LN. Our aim was to minimize any potential confounding factors, so as to conclusively determine the role of macrophages in the pathogenesis of renal disease mediated by pathogenic antibodies. We utilized a classic model known as nephrotoxic serum transfer, or NTS, in which non-autoimmune mice are passively transferred with heterologous serum containing pre-formed, nephrotoxic antibodies. The immune response initiated by these antibodies mimics the glomerulonephritis seen in SLE patients, including immune complex deposition, complement activation, and inflammatory cell infiltration [15,16].

GW2580 is a selective inhibitor of the CSF-1 receptor (CSF-1R) kinase [17], which is expressed on all macrophages [18–20]. GW2580's monospecificity for the CSF-1R has been validated against every kinase studied to date [17,21]. By blocking signaling through CSF-1R, which is important for macrophage proliferation, survival, activation, and recruitment [20,22,23], GW2580 treatment selectively reduces macrophages in several tissues including the kidney [22,24], and in response to inflammatory stimuli [17]. This particular kinase inhibitor is superior to other depletion methods due to its specificity for CSF-1R. Using GW2580, we determined whether macrophage depletion ameliorates disease within this inducible murine model of LN.

2. Methods

2.1. Mice

DBA/1J and B6 mice were purchased from The Jackson Laboratory and housed in the animal facility at the Albert Einstein College of Medicine (Bronx, NY). All animal studies were approved by the Institutional Animal Care Committee.

2.2. Nephrotoxic serum transfer

Nephrotoxic serum nephritis was induced as described [25]. In brief, on Day 0, 10 week old female DBA/J mice were immunized with rabbit IgG in CFA via intraperitoneal injection. On day 5, mice received either an intravenous injection of nephrotoxic serum (NTS) or PBS. Blood and urine were collected at baseline (day 0) and at subsequent time points throughout the experiment.

Three separate groups of mice were included in each experimental cohort. The first group (control mice) was immunized with rabbit IgG as described above on day 0, but not given the NTS transfer. The second group (NTS/GW2580 mice) was immunized with rabbit IgG on day 0, and injected with NTS on day 5 of the experiment. In addition, this group received a daily oral gavage, starting on day 0 and continuing until day 9, of 100 mg/kg of GW2580 (LC Laboratories, Woburn, MA) suspended in 0.2 ml of PBS. This dose was selected based upon the known pharmacokinetics of GW2580, and produces peak drug levels which effectively deplete macrophages [17]. The third group (NTS/PBS mice) was similarly immunized with rabbit IgG and injected with NTS. In addition, mice in this group were orally gavaged with 0.2 ml of PBS alone as a control, using the same schedule. Two independent trials were performed, with trial #1 consisting of 5 control mice, 10 NTS/PBS mice, and 10 NTS/GW2580 mice, and trial #2 including 4 control mice, 8 NTS/PBS mice, and 8 NTS/GW2580 mice.

Separate experiments were performed that assessed the therapeutic potential of GW2580. In these studies GW2580 treatment was initiated later in the disease model (day 5), to insure that peak macrophage depletion would occur after the onset of proteinuria. GW2580 was given via oral gavage at the same dose as above, 100 mg/kg in 0.2 ml of PBS per day for 10 days. As before, three groups of mice were utilized for the study, including a total of 9, 18, and 18 mice in each of the control, NTS/PBS, and NTS/GW2580 groups, respectively.

2.3. Assessment of proteinuria and renal function

Levels of proteinuria were determined by Uristix test strips (Siemens Healthcare Diagnostic, Tarrytown, NY). Albumin levels were measured by the Mouse Albumin ELISA Quantitation Set (Bethyl Laboratories, Montgomery, TX). Serum blood urea nitrogen (BUN) was determined by the Quantichrom DIUR 500 kit (BioAssay Systems, Hayward, CA). Serum and urinary creatinine were measured by the QuantiChrom Creatinine Assay Kit (BioAssay Systems).

2.4. Renal histopathology

Paraffin kidney sections were deparaffinized and stained with hematoxylin and eosin (H&E) and periodic acid Schiff (PAS). Kidney histology was analyzed and quantified by an experienced nephropathologist (L.H.), who was blinded to the treatment assignments. The slides were scored for the presence of endocapillary hypercellularity, extracapillary proliferation, immune deposits, tubular atrophy, tubular casts, tubular dilation and interstitial fibrosis and inflammation. A scale of 0–5 was used, where 0 is absent, 1 denotes involvement of 1–5% of glomeruli or cortical area, 2 is 6–10%, 3 is 11–20%, 4 is 21–50%, and 5 is greater than 50%.

2.5. Mouse anti-rabbit IgG and rabbit anti-mouse glomerular basement membrane (GBM) ELISA

Serum titers of mouse anti-rabbit IgG and IgG rabbit anti-mouse GBM antibodies were measured by ELISA, as described in detail [25].

2.6. Renal IgG deposition

Glomerular immunoglobulin deposition was assessed by immunohistochemistry on paraffin embedded kidney sections, as described [26].

2.7. Flow cytometric analysis

Following systemic perfusion, kidneys and spleens were harvested for analysis. Single cell suspensions of kidneys were generated by a 30 min incubation of sliced kidney tissue in 2 mg/ml of collagenase IV (Worthington, Lakewood, NJ) at 37 °C, followed by serial pipetting. Spleens were mashed through a 70 μm filter with the back of a syringe in order to create a single cell suspension. Following red blood cell lysis, cells were Fc-blocked for 30 min on ice with anti-CD16/CD32 (BD Pharmingen) diluted 1:200 in 3% FBS in PBS. Cells were then washed three times with FBS/PBS and stained in the dark with the following antibodies for 30 min, on ice: APC-CD11c, PE-CD11b, PerCP-F480, and FITC-GR1 (all purchased from BioLegend, San Diego, CA). After gating out neutrophils

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