Anti-Vascular Endothelial Growth Factor Receptor-2 Antibody Accelerates Renal Disease in the NZB/W F1 Murine Systemic Lupus Erythematosus Model

To the Editor: Recently, Fukasawa and Korc (1) showed that antiangiogenesis therapies are effective in the management of tumors and their metastases. The introduction of the first angiogenesis inhibitor by the U.S. Food and Drug Administration in February 2004 thus offers new promise in cancer treatment. With similar agents currently vying for market approval, physicians and patients are optimistic for the inclusion of these drugs into the therapeutic armamentarium. For the most part, these agents work by antagonizing vascular endothelial growth factor (VEGF) signaling in tumors, the pathway that drives endothelial cell proliferation and migration, with seemingly few adverse effects (2, 3).

VEGF has also been correlated to autoimmune diseases (2), including systemic lupus erythematosus, where elevated VEGF plasma levels have been associated with disease activity and positively correlated the presence of lupus nephritis. These observations have suggested a possible role for VEGF in systemic lupus erythematosus pathogenesis (4). We therefore hypothesized that VEGF blockade might also offer a therapeutic benefit in this disease and studied the effect of anti-VEGF receptor-2 (VEGFR-2) antibody treatment (DC101) on the NZB/W F1 murine model for systemic lupus erythematosus.

We treated a group of twenty 6- to 8-week-old NZB/W F1 mice with 800 μg DC101 (generously supplied by ImClone Systems, Inc., New York, NY) i.p. every 3 days for a total of 24 weeks. The selected dose and route of administration were based on previous murine studies demonstrating the efficacy of DC101 in cancer therapy (5). For comparison, two other groups of 20 mice received an equal volume of PBS (DC101 vehicle) i.p. every 3 days or no treatment. Our controls were chosen in accordance with Ishida et al., who reported NZB/W mice receiving i.p. injections of 1 mg of rat IgG1 anti-mouse antibody twice to thrice per week (a much greater amount than the DC101 given) exhibited survival rates similar to those in PBS-treated and untreated mice (6). Every 6 weeks, two to three animals were randomly selected from each group for urine protein analysis and then sacrificed for histologic evaluation of the kidneys and other organs. Kidney sections were prepared and stained with periodic acid-Schiff as well as a Texas red dye–conjugated AffiniPure F(ab′)/2 fragment donkey anti-rat IgG (H + L, Accurate Chemical, Westbury, NY), reactive against the DC101 antibody. This study was approved by the Animal Care and Use Committee at Johns Hopkins University.

By 10 weeks of therapy, the DC101-treated mice began developing gross ascites and edema followed by death 1 to 2 days later. Contrary to our initial hypothesis, mortality was significantly increased in the DC101-treated animals. After 24 weeks of treatment, 76% of the DC101-treated mice had died compared with 21% in the control mice (Fig. 1). The survival rates in our PBS-treated and no-treatment control mice were 79% after 24 weeks, equivalent to the Ishida et al. (6) isotype control studies.

Fig. 1 Animal mortality rates (% survival) in each study group over time. A, survival at 24 weeks was 79% in the control groups, corresponding to previous studies employing IgG1 isotype control antibodies in NZB/W F1 mice (6). In contrast, survival was 26% at 24 weeks in the DC101-treated group. Direct immunofluorescence staining using a Texas red dye–conjugated anti-Rat IgG reactive to DC101 displayed intense, diffuse mesangial and capillary loop staining in the DC101-treated mice (B) after 12 weeks of treatment but was negative in control mice (C).
over the remaining weeks of the study. Diffuse capillary loop and mesangial staining were noted by direct immunofluorescence (Fig. 1). In contrast, glomerulonephritis and glomerulosclerosis only became evident in the PBS-treated group and no-treatment group after 24 weeks (Table 1).

Although VEGF has been considered a survival factor for vascular endothelium, its role in glomerular functioning and renal disease is not well understood (3). The two most common VEGF isoforms, VEGF121 and VEGF165, both signal through the VEGFR-2. VEGFR-2 is expressed in embryonic and adult mouse glomerular and peritubular endothelial cells (7). VEGF signaling has been implicated in vascular development and tubulogenesis in the developing kidney as well as in the induction and maintenance of capillary fenestrations in mature kidneys (7). The glomerular endothelial cells in mature mice express VEGF-1 and VEGF-2, and VEGF is thought to localize to podocytes in close proximity to these receptors (3).

Sugimoto et al. (3) showed that neutralizing circulating VEGF in mice resulted in glomerular endothelial hypertrophy and damage, detachment from the basement membrane, and occasional disruption/loss of slit diaphragm, causing alterations in the glomerular filtration apparatus. In humans, proteinuria in preeclampsia has been associated with alterations in VEGF signaling, and changes in circulating VEGF levels have been correlated to proteinuria in minimal change disease and focal segmental glomerulosclerosis (2, 8). Furthermore, clinical cancer trials using anti-VEGF antibodies showed increased incidences of proteinuria in patients receiving treatment (9).

Our study showed that inhibition of VEGFR-2 in the NZB/W F1 lupus model leads to an exacerbation in kidney disease and an increase in mortality. The precise mechanism of action remains to be elucidated. DC101 may exacerbate renal disease by disrupting glomerular endothelial functioning and/or facilitate immune complex formation. The results of this study may suggest caution when employing these therapies in patients with underlying renal disease. Prudence may also be needed in those with conditions predisposed to proteinuria or kidney disease and potentially even to patients on concomitant nephrotoxic medications.
REFERENCES


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Hideaki Watanabe, Adam J. Mamelak, Elliot Weiss, et al.