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The green tea polyphenol (−)-epigallocatechin-3-gallate ameliorates experimental immune-mediated glomerulonephritis

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The unchecked overproduction of reactive oxygen and nitrogen species by inflammatory cells can cause tissue damage, intensify inflammation, promote apoptosis, and accelerate the progression of immune-mediated glomerulonephritis (GN). Here we tested whether the anti-inflammatory and antioxidant properties of the green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) favorably affect the development of immune-mediated GN. Pretreatment of 129/svJ mice with EGCG from 2 days before to 2 weeks after the induction of GN led to reduced proteinuria and serum creatinine, and marked improvement in renal histology when compared with vehicle-pretreated diseased mice. This pretreatment reduced oxidative stress, and normalized osteopontin, p65/nuclear factor-κB, inducible nitric oxide synthase, nitric oxide metabolites, p-Akt, phosphorylated extracellular signal-regulated kinases 1 and 2, p47phox, and myeloperoxidase, all of which were elevated in vehicle-pretreated diseased mice. Levels of glutathione peroxidase and peroxisome proliferator-activated receptor-γ (PPARγ), both reduced in the vehicle-pretreated diseased mice, were normalized. This renoprotective effect was reversed by concomitant administration of the PPARγ antagonist GW9662 throughout the EGCG pretreatment period. Importantly, mortality and renal dysfunction were significantly attenuated even when the polyphenol treatment was initiated 1 week after the onset of GN. Thus, EGCG reversed the progression of immune-mediated GN in mice by targeting redox and inflammatory pathways.

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Anti-glomerular basement membrane antibody-induced glomerulonephritis (anti-GBM-GN) is pathologically and clinically the most severe form of immune-mediated GN. It is caused by circulating antibodies directed to the GBM components, non-collagenous-1 domain of the α3 or α5 chain of type IV collagen, leading to an inflammatory reaction in the glomerular capillaries.[1–4] The treatment of anti-GBM-GN aims to modulate the injury-causing immunologic process with high-dose corticosteroids, cytotoxic drugs, and plasmapheresis. However, end-stage renal disease develops in 40–70% of patients.[4,5]

Reactive oxygen species (ROS) are products of normal cellular metabolism that modulate physiological functions and affect innate immunity in infectious and noninfectious inflammation.[6] However, unchecked overproduction of ROS, reactive nitrogen species, and reactive chlorine species by inflammatory cells can cause further tissue damage, intensify inflammation, promote apoptosis, and accelerate progression of many diseases including anti-GBM-GN.[7,8] In physiologic condition, superoxide dismutase (SOD) catalyzes superoxide to molecular oxygen and hydrogen peroxide (H_2O_2). H_2O_2 is degraded to water and molecular oxygen by catalase or by glutathione peroxidase (GPx) in the presence of reduced glutathione. However, in the presence of electron donors such as iron, H_2O_2 is converted to hydroxyl radical, which is the most reactive and cytotoxic ROS. Additionally, myeloperoxidase (MPO), expressed in neutrophils and macrophages,

converts H_2O_2 to hypochlorous acid, a highly cytotoxic reactive chlorine species. Finally, H_2O_2 is an activator of nuclear factor-κB (NF-κB), a master regulator of pro-inflammatory cytokines, chemokines, and fibrogenic factors. Superoxide reacts with nitric oxide (NO) to produce peroxynitrite, a highly reactive nitrogen species. Excessive production and impaired capacity of the antioxidant system to inactivate superoxide and H_2O_2 play a central role in the pathogenesis of glomerular injury, hyperpermeability, and worsening of inflammatory glomerular disease.[9]

The green tea catechins, particularly (−)-epigallocatechin-3-gallate (EGCG), are potent anti-inflammatory and antioxidant agents shown to inhibit leukocyte chemotaxis, quench free radicals, chelate transition metals, and interrupt lipid peroxidation chain reaction.[10–13] EGCG is estimated to be 25 times more potent than vitamin E and 100 times more potent than vitamin C.[14] However, the effect of EGCG on immune-mediated renal injury has not been investigated. We tested the hypothesis that the anti-inflammatory and antioxidant properties of EGCG favorably affect the course of immune-mediated GN using a murine model of anti-GBM-GN. Here, we show that the EGCG administration significantly improves the laboratory and histopathological features of anti-GBM-GN.

RESULTS

EGCG attenuated the development of anti-GBM-GN

General data and biochemical/immunological measurements. At the end of the 2-week observation period, the vehicle-pretreated mice with anti-GBM-GN developed weight loss, renal insufficiency, and proteinuria in contrast to the normal controls. No mice died of disease during the course of the study. EGCG pretreatment led to significantly less proteinuria and normalized serum creatinine compared with the vehicle-pretreated mice (Table 1). The levels of immunoglobulin G (IgG) mouse anti-rabbit antibodies were comparable between the two groups of mice with anti-GBM-GN, indicating that the reduced renal disease in EGCG-pretreated mice was not caused by a decreased xenogenic immune response to the injected immunoglobulin (Figure 1).

Renal histology. The vehicle-pretreated mice showed moderate to severe renal injury characterized by crescent formation with marked intracapillary hypercellularity, obliterated capillary lumens, and thickened capillary walls (Table 1 and Figure 2). Tubular atrophy and dilation with hyaline casts and interstitial fibrosis were also noted. In comparison, the EGCG-pretreated mice exhibited milder renal injury with occasional crescent formation, and focal tubulointerstitial injury. No lesions were seen in the normal control group. In addition, both glomerular and interstitial

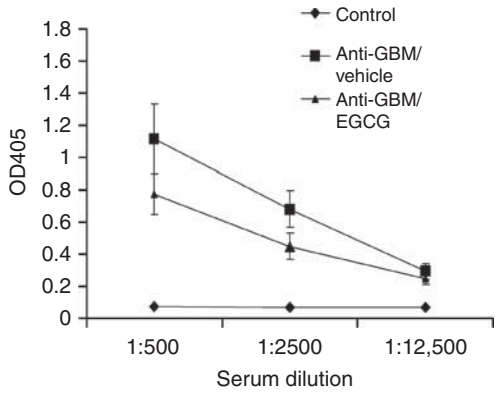

Figure 1 | Anti-rabbit immunoglobulin (Ig) response in (−)-epigallocatechin-3-gallate (EGCG)- or vehicle-pretreated mice with anti-glomerular basement membrane antibody-induced glomerulonephritis (anti-GBM-GN). Depicted are the serum levels of IgG mouse anti-rabbit antibodies (measured on day 14) assayed in serial dilutions. Values are expressed as mean ± s.e.m. $n = 7$ in each group.

Table 1 | General data and renal injuries in EGCG- and vehicle-pretreated mice with anti-GBM-GN and in normal controls

	Control	Anti-GBM/vehicle	Anti-GBM/EGCG
Body weight (g)	20.2 ± 0.4	17.0 ± 0.5**	19.1 ± 0.5##
Serum creatinine (mg/dl)	0.09 ± 0.01	0.15 ± 0.01**	0.10 ± 0.01##
Proteinuria (mg per 24 h)	0.78 ± 0.08	12.68 ± 1.45**	5.11 ± 1.01**,##
Glomerulonephritis score (0–4)	0 ± 0	3.5 ± 0.2**	2.0 ± 0.4*,#
Crescents (%)	0 ± 0	22.8 ± 4.2**	6.5 ± 3.3**,##
Tubulointerstitial injury score (0–5)	0 ± 0	2.4 ± 0.2**	1.2 ± 0.3**,##
Macrophage infiltration			
Glomeruli (cells per 50 glomerular cross-section)	1.4 ± 0.8	112.0 ± 20.6**	8.7 ± 3.8*,##
Interstitial (cells per 10 high-power field)	50.5 ± 7.2	520.3 ± 82.7**	132.3 ± 30.0*,##
Lymphocyte infiltration			
Glomeruli (cells per 50 glomerular cross-section)	1.9 ± 0.6	19.2 ± 8.4**	3.2 ± 0.8##
Interstitial (cells per 10 high power field)	3.8 ± 1.1	45.1 ± 10.2**	7.5 ± 3.6##

Abbreviations: Anti-GBM/EGCG, anti-glomerular basement membrane antibody-induced glomerulonephritis pretreated with (−)-epigallocatechin-3-gallate; anti-GBM/vehicle, anti-glomerular basement membrane antibody-induced glomerulonephritis pretreated with vehicle; GN, glomerulonephritis.
Values are mean ± s.e.m.; n=14 in each group.
*$P < 0.05$, **$P < 0.01$ vs control.
#$P < 0.05$, ##$P < 0.01$ vs anti-GBM/vehicle.