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CD3 mAb treatment ameliorated the severity of the cGVHD-induced lupus nephritis in mice by up-regulation of Foxp3$^+$ regulatory T cells in the target tissue: Kidney

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ABSTRACT

Teff/Treg imbalance orchestrated the onset and the progression of the lupus nephritis in a DBA/2→B6D2F1 murine model with cGVHD. In this paper, we first used 145-2C11 Ab to treat these human SLE-like diseased animals. The results showed that short-term low-dose anti-CD3 antibody treatment induced a significant remission of established proteinuria, production of autoantibodies, immune complex deposition and renal parenchyma lesions in lupus nephritic mice. Of note, we found a robust up-regulation of Foxp3 mRNA expression in the target tissue: kidney from mice with anti-CD3 antibody treatment compared to those with control IgG treatment. Likewise, an increased renal mRNA abundance for IL-10 was also observed in anti-CD3 antibody treated mice. In contrast, genes associated with inflammation and fibrosis as well as cytokines related to effector T cell responses were down-regulated by anti-CD3 mAb treatment. These findings suggested that short-term low-dose anti-CD3 antibody treatment might induced an IL-10-secreting Foxp3$^+$ regulatory T cells in this cGVHD target tissue: kidney, that suppressed the activation of effector T cells (Th1, Th2 and Th17), thus ameliorating the severity of the lupus nephritis in mice.

1. Introduction

System lupus erythematosus (SLE) is an autoimmune disease caused by loss of tolerance, production of autoantibodies, and deposition of complement-fixing immune complexes (ICs) in tissues leading to multisystem injury, including nephritis, arthritis, serositis, dermatitis, and blood dyscrasias [1]. Lupus nephritis is a key manifestation, which is considered to be the proximate cause of death in SLE patients due to late-phase renal failure [2,3].

Injection of DBA/2 lymphocytes to C57BL/6-DBA/2 F1 hybrids leads to a chronic GVHD characterized by a persistent lymphoid hyperplasia producing hypergammaglobulinemia and a systemic lupus erythematosus (SLE)-like disease [4–6] with splenomegaly, B cell expansion, autoantibodies, and severe immune complex-mediated glomerulonephritis.

The hamster 145-2C11 Ab (anti-CD3) binds and cross-links the CD3ε chains of the mouse TCR [7] resulting in costimulation-dependent

Table 1
Primer sequences used for qPCR.

Genes	Primer 1 (Forward, 5′→3′)	Primer 2 (Reverse, 5′→3′)
RANTES	atgaagatctctgcagctgccctc	tgctggtgtagaaatactccttgac
IP-10	atccctctcgcaaggacggtccgctg	tcttagattccggattcagacatc
BLC	atgaggctcagcacagcaacgctgcttc	tccgatctatgatgtttagaccgacaacag
Ltn	atgagacttctcctcctgactttc	ctcccagatgatataggtcttg
MCP-1	atgcaggtccctgtcatgcttctg	ggtgatcctcttgtagctctccag
MIG	atgaagtccgctgttcttttcctc	gaggtctttgagggatttgtag
IL-6	ttccctacttcacaagtccggagaggag	ctgcaagtgcatcatcgttgttc
TNF-α	attcgagtgacaagcctgtagcccac	ctgggagtagacaaggtacaacccat
IL-1β [20]	gcccatcctctgtgactcat	aggccacaggtattttgtcg
TGF-β1	ataccaactattgcttcagctccacag	gtactgtgtgtccaggctccaaatat
CTGF	gcctggtgctggacggctgcggctgct	catctttggcagtgcacactccgatct
α-SMA	agcgtgagattgtccgtgacatcaaggag	gttcgtttccaatggtgatcacctgcccg
Collagen IV α1	agctttccataatggcacatacca	gcttcatcctgtcaccccagatgtg
Collagen I α1	aggcaacagtcgcttcacctacagc	caatgtctagtccgaattcctggtct
IFN-γ	gcacagtcattgaaagcctagaaagtc	ggtagaaagagataatctggctctg
IL-4	atggatgtgccaaacgtcctcacag	gagtctctgcagctccatgagaacac
IL-10	tgccttcagccaggtgaagactttc	cttgatttctgggccatgcttctctg
IL-23	aggatccgccaaggtctggctt	tgatcctctggctggaggagttgg
IL-17	ctacctcaaccgttccac	tctcaggctccctcttc
IL-17 [21]	gggagagcttcatctgt	gaccctgaaagtgaaggg
Foxp3	atgcccaaccctaggccagccaag	tgggccccacttcgcaggtcccgac
GAPDH	tgaaggtcggtgtgaacggatttg	gttgaatttgccgtgagtggagtc

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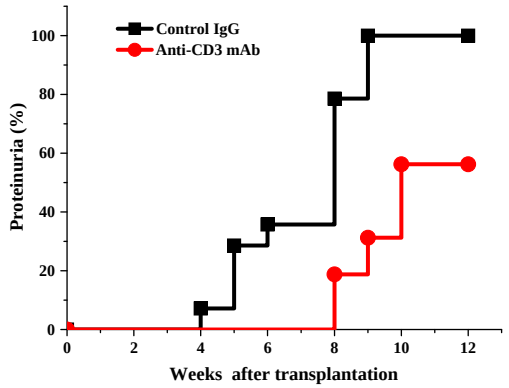

Fig. 1. Anti-CD3 mAb treatment induced remission of established proteinuria in nephritic mice.

$CD4^+$ T cell activation in vitro. Because in vivo administration anti-CD3 Ab induces TCR down-regulation, T cell tolerance, and $CD4^+$ T cell depletion [8], previous studies have used this Ab as an immunosuppressive agent to treat various autoimmune disorders [9–15] and transplant rejection [16–19]. Treatment of nonobese diabetic (NOD) or EAE mice with either mitogenic conventional monoclonal antibodies (5 μg/day i.v. for 5 consecutive days) or nonmitogenic antibody (50 μg/day i.v. for 5 consecutive days) to CD3 results in disease remission with long-term immune tolerance.

In this paper, we tested the effect of 145-2C11 Ab on this established human SLE-like model. The results showed that short-term low-dose anti-CD3 antibody treatment induced a significant remission of established proteinuria, production of autoantibodies, immune complex deposition and renal parenchyma lesions in lupus nephritic mice.

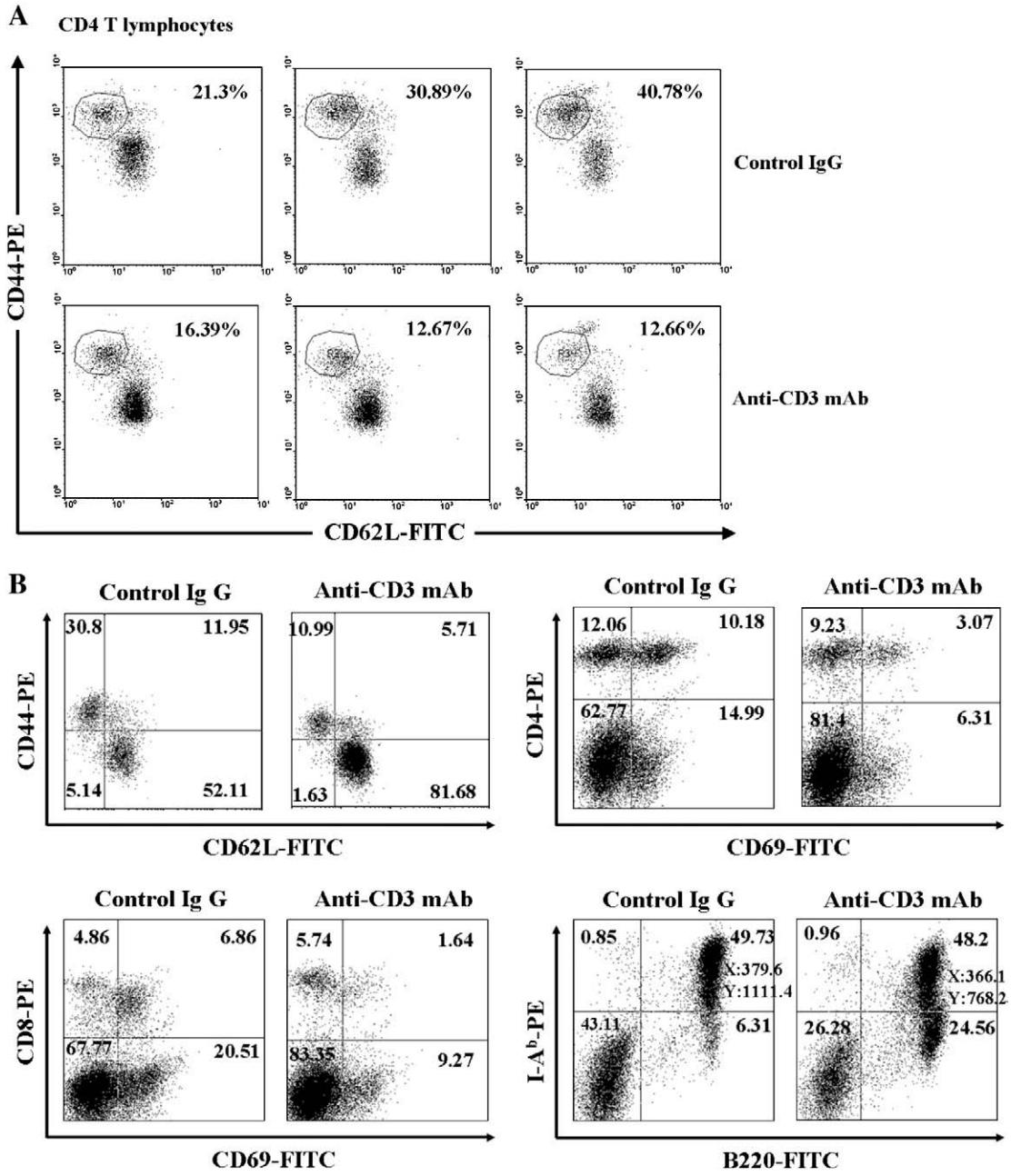

Fig. 2. Anti-CD3 mAb treatment reduced lymphocytes activation. A: The percentage of $CD4^+CD44^{high}CD62^{low}$ population in whole blood from CD3 mAb-treated group mice (13.91% ± 0.022) were largely reduced in contrast to control IgG-treated group mice (30.99% ± 0.097); B: CD3 mAb treatment markedly inhibited the activation of splenic lymphocytes.