

Supplementary Material

accompanying the manuscript

IL-25 induces alternatively-activated macrophages and reduces renal injury in Adriamycin nephropathy

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Table S1

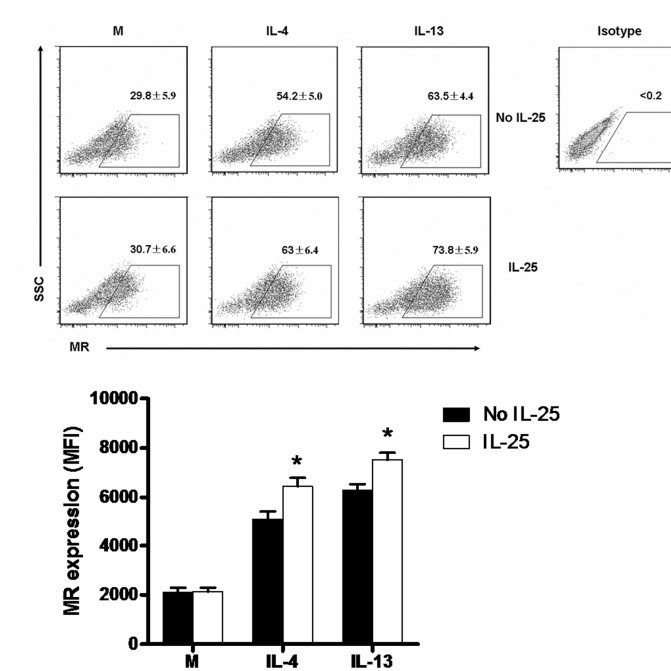


Figure S1. IL-25 increased MR expression in macrophages cultured with IL-4 or IL-13.

The expression of mannose receptor (MR) was assessed by flow cytometry (percentage and mean fluorescence intensity, MFI) in bone marrow macrophages preincubated for 1 hour with medium (M) or IL-25 (100 ng/ml) combined or not with IL-4 or IL-13 for a further 20 hours *in vitro*. The values represent the mean \pm SEM of evaluations from each group (n=4 per group). *P<0.05. vs. No IL-25.

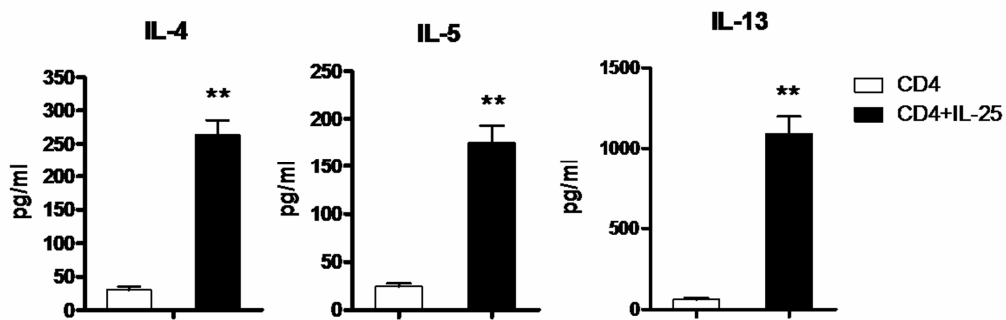


Figure S2. IL-25 promoted Th2 cell differentiation *in vitro*.

Naïve CD4⁺ T cells were stimulated with anti-CD3, anti-CD28, and IL-2 in the presence of recombinant IL-25. After 3 d, cells were restimulated with plate-bound anti-CD3 for 24 h, and cytokine production was measured by ELISA. The values represent the mean \pm SEM of evaluations from each group (n=4 per group). **P<0.01. vs. CD4.

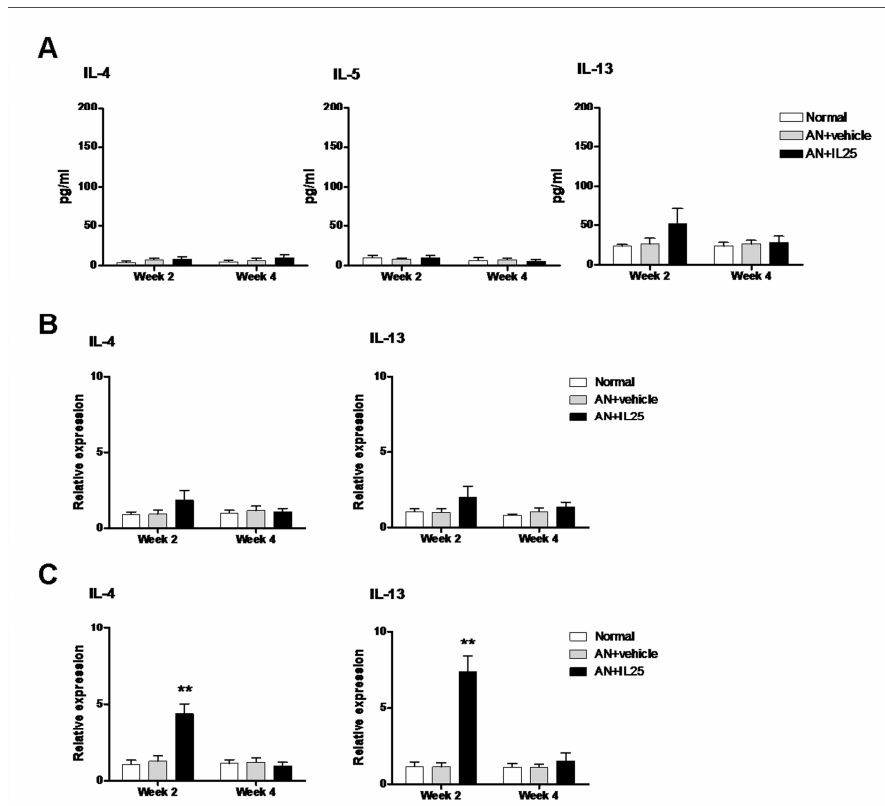


Figure S3. The effects of IL-25 on Th2 immune responses in AN SCID mice. In SCID mice, IL-4, IL-5 and IL-13 levels in serum were assessed by ELISA (A) and the mRNA expression of IL-4 and IL-13 in kidney (B) and renal draining lymph nodes (RDLN) (C) was quantified by real-time PCR in normal, AN+vehicle and AN+IL-25 groups at week 2 and 4. The values represent the mean \pm SEM from each group (n=8 per group). The values represent the mean \pm SEM of evaluations from each group (n=4 per group). **P<0.01. vs. AN+vehicle.

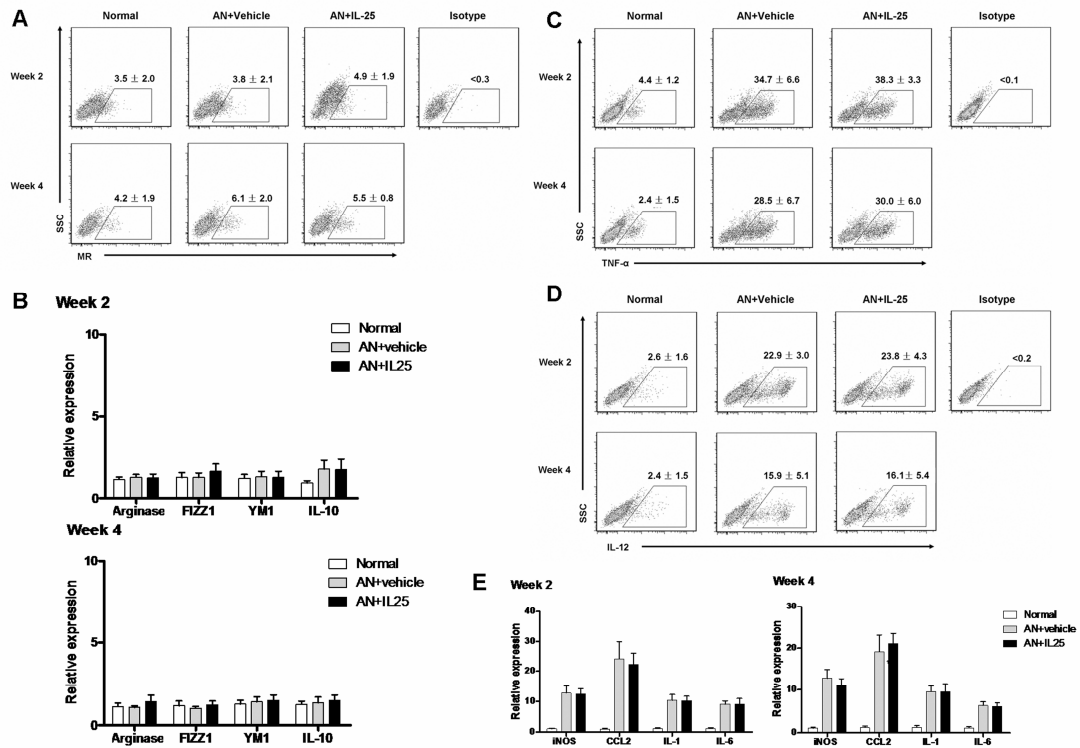


Figure S4. IL-25 failed to induce M2 macrophages and suppress endogenous renal macrophages in AN SCID mice. In SCID mice, the expression of MR, TNF α and IL-12 of endogenous renal macrophages was measured by flow cytometry (A, C and D) and the mRNA expression of arginase, FIZZ1, YM1, IL-10, iNOS, CCL2, IL-1 β and IL-6 was quantified by real-time PCR (B and E). The values represent the mean \pm SEM of evaluations from each group (n=8 per group).

Table S1. Real-time PCR primers

Gene	primer sequence(5'-3')	Product
IL-4 (F)	tcaacccccagctagttgtc	184
IL-4 (R)	tctgtggtgttcttcgttgc	
IL-13 (F)	cagcatggtatggagtgtgg	153
IL-13 (R)	aggctggagaccgtagtgg	
iNOS (F)	caccttgagttcaccagt	170
iNOS (R)	accactgtacttgggatgc	
TNF- α (F)	gctgagctcaaaccctggta	118
TNF- α (R)	cggactccgcaaagtctaag	
MCP-1 (F)	agcaccagccaactctcact	136
MCP-1 (R)	cgtaactgcatctggctga	
IL-1 β (F)	tgccacctttgacagtgatg	136
IL-1 β (R)	atgtgctgctgcgagatttg	
IL-6 (F)	cacaagtccggagaggagac	136
IL-6 (R)	ttgccattgcacaactcttt	
IL-12 (F)	gacatcacacgggaccaaac	160
IL-12 (R)	taccaaggcacagggtcatc	
Mannose receptor (F)	caaggaaggttggcatttgt	111
Mannose receptor (R)	ccttcagtcctttgcaagc	
Arginase (F)	agtctggcagttggaagcat	104
Arginase (R)	ctggttgcaggggagtgtt	
FIZZ1 (F)	tgctgggatgactgctactg	156

FIZZ1 (R)	ctgggtctccacctctca	
YM1 (F)	cagctgggatcttctacca	141
YM1 (R)	attctgcattccagcaaagg	
IL10 (F)	ccagtacagccgggaagaca	121
IL10 (R)	cagctggtcctttggttgaaaga	
CCL17 (F)	tgcttctggggactttctg	147
CCL17 (R)	catcctggaactccact	

F=forward, R=reverse.