CD4+CD25+FOXP3+ T regulatory cells reconstitute and accumulate in the bone marrow of patients with multiple myeloma following allogeneic stem cell transplantation

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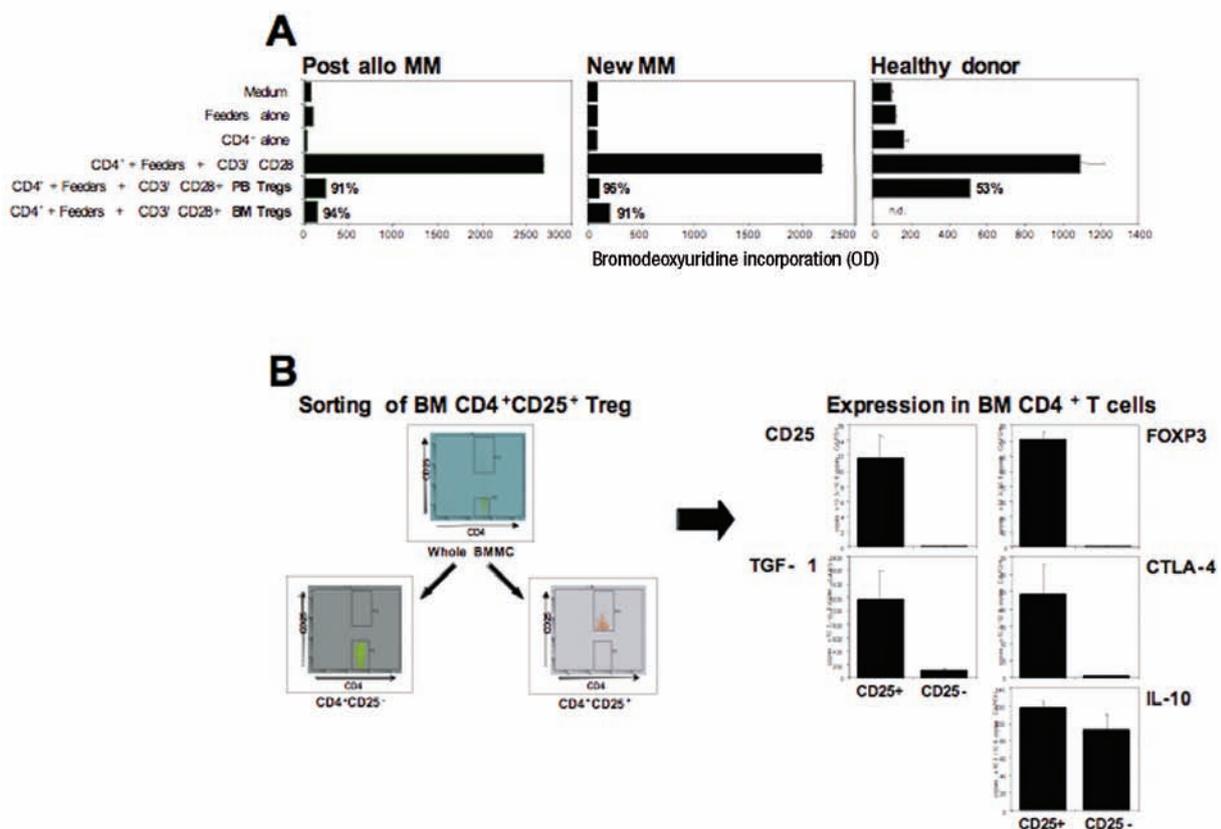
Figure 1

Figure 1. Treg-mediated inhibition of T-cell proliferation and expression of effector molecules by bone marrow-residing Treg (A) CD4+ T cells and CD4+CD25+ Treg were purified from bone marrow and/or peripheral blood and inhibition reperiments were performed as described in the Design and Methods section. Percentages indicate relative inhibition. (B) Bone marrow-residing CD4+CD25+ T cells of three healthy donors were FACS-sorted into CD4+CD25+ and CD4+CD25- subpopulations. RNA expression was analyzed in both populations using real-time polymerase chain reaction. Results are expressed as mean copy number of the target gene in relation to copies of the housekeeping gene *GAPDH*.
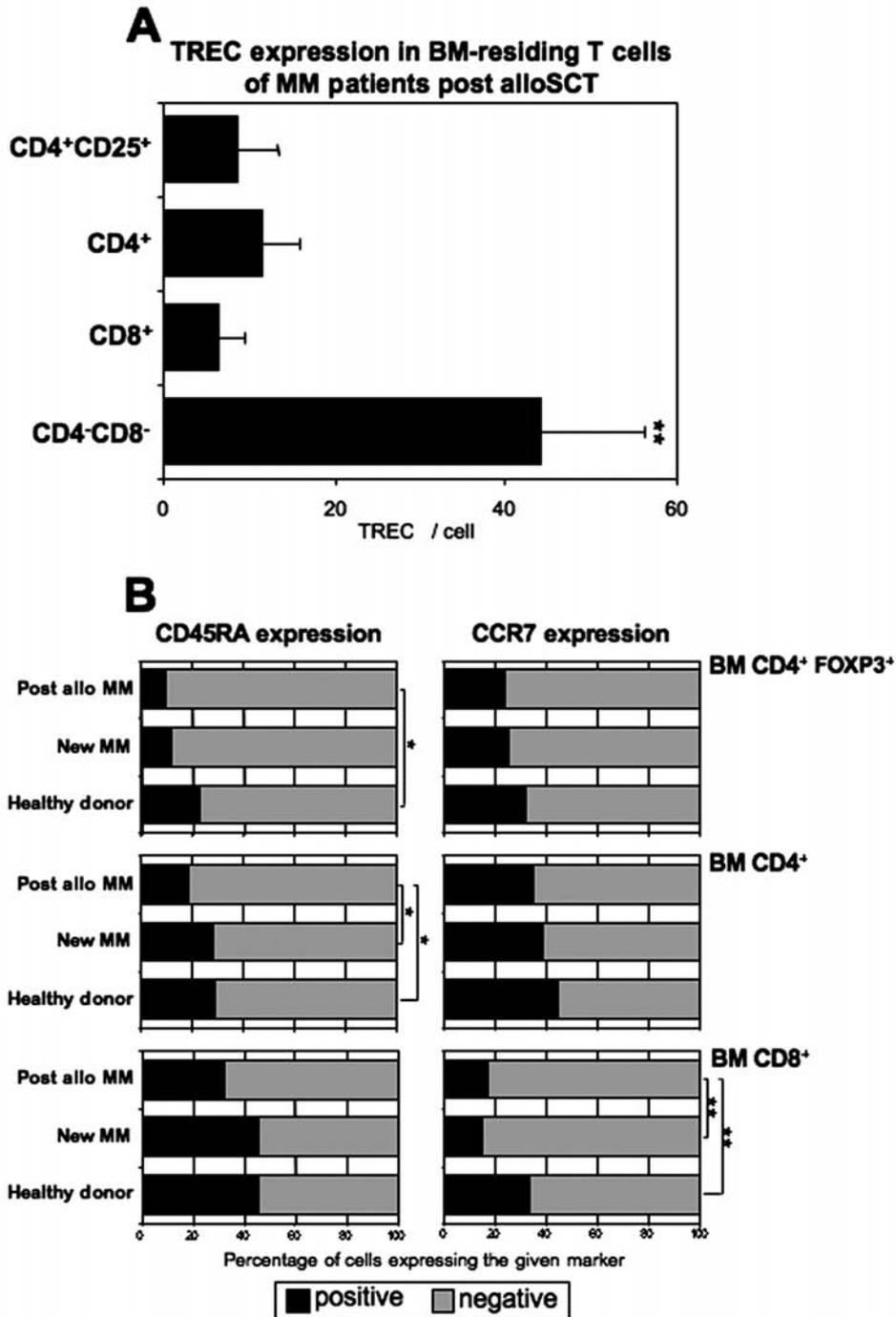
Figure 2

Figure 2. Bone marrow CD4+FOXP3+ Treg of post-allogeneic SCT myeloma patients are characterized by low TREC numbers and display a memory T-cell phenotype. (A) Genomic DNA of eight post-allogeneic SCT myeloma patients was isolated from FACS-sorted bone marrow CD3+ T-cell subpopulations. Copy numbers of TREC were analyzed applying real-time polymerase chain reaction and were normalized for copies of *GAPDH*. Bars show mean values ± SEM and asterisks indicate statistically significant differences between CD3+CD4−CD8− T cells and all three remaining T-cell subpopulations (**$p<0.01$). (B) Expression of CD45RA and CCR7 on bone marrow-residing T-cell subpopulations was determined, using flow cytometry, in 26 post-allogeneic SCT myeloma patients, 15 newly diagnosed myeloma patients, and 10 healthy donors. Bars show mean values, asterisks indicate statistically significant differences between groups (*$p<0.05$, **$p<0.01$).

Supplementary Table 1

Supplementary Table 1. Experimental conditions and oligonucleotide primers used for real-time polymerase chain reaction (PCR). PCR conditions for the analysis of RNA and genomic DNA levels are indicated. F, forward primer; R, reverse primer; Size, size of PCR product; PCR annealing temperature; bp, base pairs.

	Gene		Primer sequence	T (°C)	Size (bp)
RNA	CD25	F:	5'-GAA TTT ATC ATT TCG TGG TGG GGC A-'3	60	398
		R:	5'-TCT TCT ACT CTT CCT CTG TCT CCG-'3		
	Foxp3	F:	5'-GAA ACA GCA CAT TCC CAG AGT TC-'3	61	100
		R:	5'-ATG GCC CAG CGG ATG AG-'3		
	TGF-β1	F:	5'-CTA AAG CAT CAG AGA AGA GAA GC-'3	60	150
		R:	5'-AGA TCT CTT ATT AAT CTT CTC AGA AA-'3		
	CTLA-4	F:	5'-CAC AAG GCT CAG CTG AAC CT-'3	60	295
		R:	5'-AGG TGC CCG TGC AGA TGG AA-'3		
	IL-10	F:	5'-GTG ATG CCC CAA GCT GAG A-'3	60	80
		R:	5'-TCC CCC AGG GAG TTC ACA-'3		
	GAPDH	F:	5'-TGA TGA CAT CAA GAA GGT GG-'3	61	246
		R:	5'-TTT CTT ACT CCT TGG AGG CC-'3		
DNA	TREC (coding)	F:	5'-CAC CTC TGG GCT ACG TGC TAG-'3	58	98
		R:	5'-GAA CAC ATG CTG AGG TTT AAA GAG AAT-'3		
	GAPDH	F:	5'-AAC AGC GAC ACC CAT CCT C-'3	58	81
		R:	5'-CAT ACC AGG AAA TGA GCT TGA CAA-'3		

Supplementary Table 2

Supplementary Table 2. Clinicopathological characteristics of the patients with multiple myeloma (MM). MM patients who had undergone allogeneic SCT (post alloSCT) (N=40) and newly diagnosed MM patients (N=17) were classified according to the clinical characteristics of their disease. Information on the initial stage of disease was available for fewer patients. Data represent mean ± standard error of mean (SEM) or absolute numbers and percentages in brackets.

Characteristics	Number of patients per group	
	MM post alloSCT	New MM
Total	40	17
Male/female ratio	4.0	3.3
Age (years)	53.2 ± 1.6	60.1 ± 3.6
Bone marrow-infiltrating plasma cells (%)	9.3 ± 2.3	25.9 ± 5.5
Heavy chain isotype		
IgG	18 (45.0%)	11 (64.7%)
IgA	16 (40.0%)	4 (23.5%)
Light chain	6 (15.0%)	2 (11.8%)
Light chain isotype		
Kappa	24 (60.0%)	14 (82.4%)
Lambda	16 (40.0%)	3 (17.6%)
Initial Stage (Durie-Salmon)		
I	3 (7.9%)	1 (7.1%)
II	9 (23.7%)	1 (7.1%)
III	26 (68.4%)	12 (85.7%)
Serum albumin (g/dL)	4.4 ± 0.1	4.0 ± 0.1
Serum lactate dehydrogenase (U/L)	187.2 ± 8.4	167.9 ± 15.2