Unique Macrophages different from M1/M2 Macrophages Inhibit T Cell Mitogenesis while Upregulating Th17 Polarization

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Supplementary Informations

1. Supplemental Table S1: Effects of MIS-MΦs on Th17 polarization and RORγt expression by TCR-stimulated T cells under Th17 polarizing condition, when statistically assessed by performing repeated cytofluorometric experiments.

This table indicates the percentage (the mean value ± innterexperimental SEM) of Th17 and RORγt$^+$ T cell populations generated in response to TCR-stimulation, when T cells were cultured under Th17 polarizing condition with or without MIS-MΦs.

2. Supplemental Table S2: Primers Used in PCR Analysis.

This table indicates the primer sets, used for the real-time RT-PCR experiment performed in the present study.

3. Supplemental Figure S1: Effects of MIS-MΦs on T cell differentiation into Treg and Tγδ cell populations.

This figure demonstrates that MIS-MΦs did not up-regulate the

expansion of Foxp3^{+}IL-17A^{-} T cells (iTreg cells) and γδTCR^{+}IL-17A^{+} T cells (IL-17 producing Tγδ cells) under the Th17 polarizing and non-Th17 skewing conditions.

Supplemental Table S1. Effects of MIS-MΦs on Th17 polarization and RORγt expression by TCR-stimulated T cells under Th17 polarizing condition, when statistically assessed by performing repeated cytofluorometric experiments[a]

T cell population counted by flowcytometry	Cultured cells	Ratio of indicated T cells (% ± SEM)[b]	*P* value for the difference between T cell + MIS$^-$MΦ and T cell alone
CD4$^+$IL-17A$^+$	T cell alone	1.5 ± 0.2[c]	
	Tcell+MIS-MΦ	7.6 ± 1.7[c]	0.021
RORγt$^+$IL-17A$^+$	T cell alone	4.2 ± 0.7[d]	
	Tcell+MIS-MΦ	12.9 ± 3.0[d]	0.006
RORγt$^+$IL-17A$^-$	T cell alone	59.6 ± 7.5[d]	
	Tcell+MIS-MΦ	44.9 ± 5.0[d]	0.085
RORγt$^-$IL-17A$^+$	T cell alone	0.4 ± 0.1[d]	
	Tcell+MIS-MΦ	1.4 ± 0.4[d]	0.108

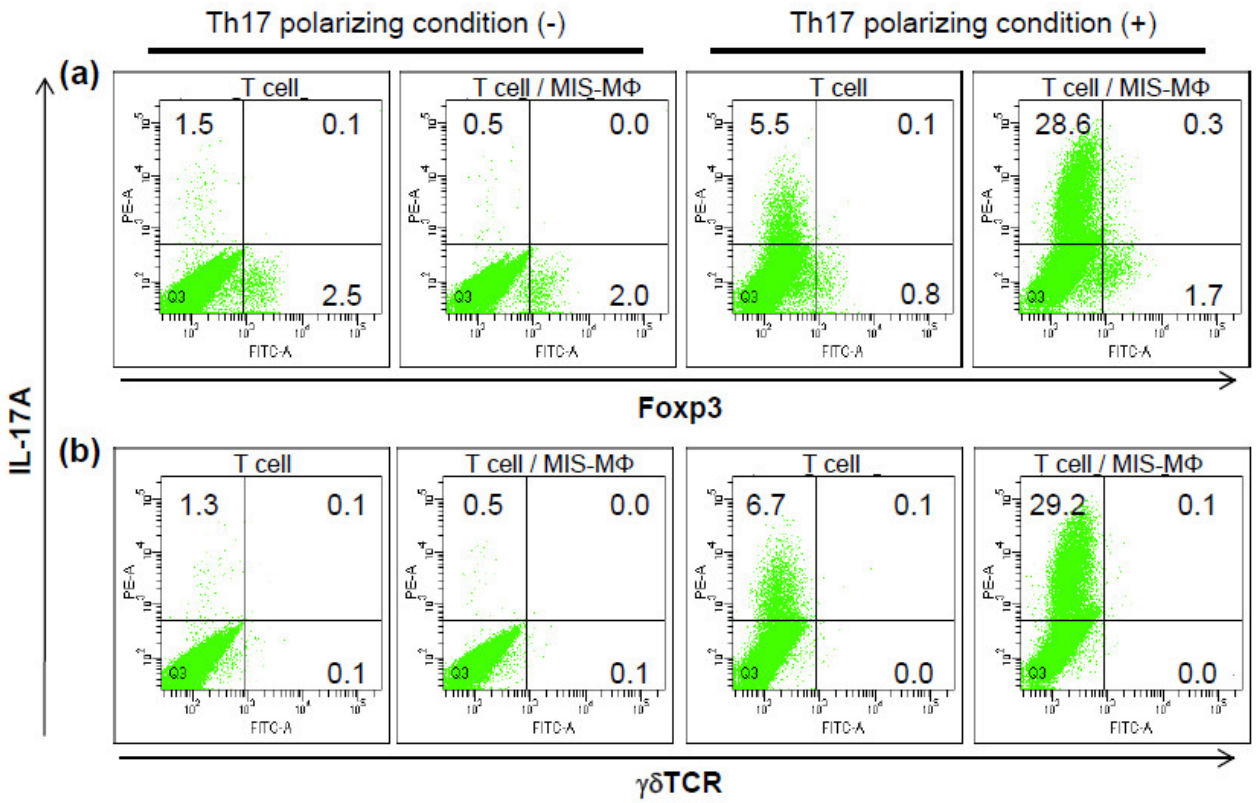
[a]T cell cultivation and subsequent flowcytometry were performed under the same condition as in indicated Fig. 2.

[b]The mean values measured by separate experiments, which were repeated several times, are indicated with interexperimental SEMs.

[c,d] Cytofluorometric experiments were separately repeated four (c) and seven (d) times, respectively.

Supplemental Table S2. Primers Used in PCR Analysis

Gene	Accession number	Primers (5'-3')
IL12p40	NM_008352	GGAAGCACGGCAGCAGAATA
		AACTTGAGGGAGAAGTAGGAATGG
IL1-β	NM_008361	AAGGGCTGCTTCCAAACCTTTGAC
		ATACTGCCTGCCTGAAGCTCTTGT
IL-6	NM_031168.1	TTCCATCCAGTTGCCTTCTT
		CAGAATTGCCATTGCACAAC
TNF-α	NM_013693	TCTCATGCACCACCATCAAGGACT
		TGACCACTCTCCCTTTGCAGAACT
NOS2	NM_010927.3	CCAAGCCCTCACCTACTTCC
		CTCTGAGGGCTGACACAAGG
CCR7	NM_007719.2	AAAGCACAGCCTTCCTGTGT
		AGTCCACCGTGGTATTCTCG
IL-10	NM_010548.2	GGTTGCCAAGCCTTATCGGA
		ACCTGCTCCACTGCCTTGCT
Arg-1	NM_007482.3	CTCCAAGCCAAAGTCCTTAGAG
		AGGAGCTGTCATTAGGGACATC
MR	NM_008625.2	CCACAGCATTGAGGAGTTTG
		ACAGCTCATCATTTGGCTCA
Ym1	M94584	CACCATGGCCAAGCTCATTCTTGT
		TATTGGCCTGTCCTTAGCCCAACT
Fizz1	NM_020509.3	ACTGCCTGTGCTTACTCGTTGACT
		AAAGCTGGGTTCTCCACCTCTTCA
CD163	NM_001170395.1	CCTGGATCATCTGTGACAACA
		TCCACACGTCCAGAACAGTC
GAPDH	NM_008084.2	AACTTTGGCATTGTGGAAGG
		GGATGCAGGGATGATGTTCT



Supplemental Figure S1. Effects of MIS-MΦs on T cell differentiation into Treg and Tγδ cell populations. TCR-stimulated T cells were cultured in the presence or absence of a monolaye culture of MIS-MΦs under the Th17 polarizing or non-Th17 skewing conditions. After 5-day cultivation, cultured T cells were subjected to measurement of intracellular expression of IL-17A, Foxp3 (a), and γδTCR (b) as described in Fig. 2. Data are representative of multiple experiments.