Supplementary material

Additional methods

RNA preparation and real-time PCR. Isolation of total RNA and reverse-transcription into single-stranded cDNA was carried out as described (27). cDNA was subjected to real-time PCR (iCycler; BioRad) using the SYBR-Green assay with gene-specific primers at a final concentration of 0.2 μM. For characterization of GM-CSF and M-CSF macrophages the following primer sequences were used: IL-6 *forward:* 5'-TTCTCCACAAGCGCCTTCGGT-3', *reverse:* 5'-GTCTCCTTTCTCAGGGCTG AG-3'; IL-1β *forward:* 5'-AGTGCTCCTTCCAGGACCTGGA-3', *reverse:* 5'-CACTCTCCAGCTG TAGAGTGG-3'; TNF-α *forward:* 5'-GAAAGCATGATCCGGGACGTGGA-3', reverse: 5'-TGGCA GGGGCTCTTGATGGCAGA-3'; IL-10 *forward:* 5'-AGAACAGCTGCACCCACTTC-3', *reverse:* 5'-CGCCTTGATGTCTGGGTCTT-3'; TGF-β1 *forward:* 5'-ACCATGCCGCCCTCCGGG-3', *reverse:* 5'-TCAGCTGCACCTTGCAGGAGC-3'; CD163 *forward:* 5'-ACTTGAAGACTCTGGATCT GCT-3', *reverse:* 5'-CTGGTGACAAAACAGGCACTG-3'; GAPDH *forward:* 5'-TCCATGACAA CTTTGGTATCGTGG-3', *reverse:* 5'-GACGCCTGCTTCACCACCTTCT-3'. Data were collected during annealing steps and were further analysed by using the iCycler iQ optical system software (BioRad).

Staining and flow cytometric analysis of macrophages. After differentiation of macrophages in the presence of GM-CSF or M-CSF, cells were stained for CD11b, CD14 and CD163. For this purpose, 1x10⁶ cells of each macrophage population were washed in staining buffer (PBS+ 0.5 % BSA + 2 mM EDTA) before incubation in FcR blocking reagent (1:10 diluted in staining buffer) (Miltenyi Biotec, Bergisch Gladbach, Germany) for 15 minutes in the refrigerator. Then, cells were divided and either incubated with 10 μl of a rat IgG2b PE-conjugated anti-CD11b antibody (Miltenyi Biotech), 10 μl of a mouse IgG2a PE-conjugated anti-CD14 antibody (Miltenyi Biotech), 20 μl of a mouse IgG1 PE-conjugated anti-CD163 antibody (BD Pharmingen, Heidelberg, Germany) or the respective isotype control antibodies (Miltenyi Biotech, BD Pharmingen), each diluted in 100 μl staining buffer

containing FcR blocking reagent and incubated for 10 minutes on ice. After washing in staining buffer, cells were collected in 200 µl staining buffer and analysed by a FACS Calibur (BD Biosciences, Heidelberg, Germany) using the CellQuest Software.