

### ***RNA purification and RT-PCR***

RNA was purified and semi-quantitative PCR performed, as described previously<sup>1,2</sup>. All PCR reactions were performed with 35 cycles using the following primers: mouse Foxp1, S, atcccagaacgggtccagcgggtggaaccac, and AS, gatctgctgcatttggtgaggagtataac; mouse Foxp1, S, aaggcctggcttctcactgcctgtagtgcc and AS ctggagagacaagaggtgctgctgctgcag; human Foxp1-flag-tag, S, gcagccacgctactgcacacctc and AS, cttgctgctatcgtcctttagtc; mouse c-fms, S, aacaagttctacaaactggtgaagg and AS, gaagcctgtagtctaagcatctgctc; mouse  $\beta$ -actin, S, gtgggccgctctaggcacca and AS, tggccttaggggtcaggggg; mouse cathepsin K, S, gtgggtgtcaagtttctgctgc and AS, gctctcttcagggtcttctcgttc; mouse tartrate-resistant acid phosphatase (TRAP), S, ggattcatgggtggtgctg and AS, tggctaacaatgggtcgaag; and mouse c-Src, S, ctccgactccatccaggctg and AS, cctctccgaagcaacctgg.

### ***Quantitative PCR***

RNA was purified from bone marrow monocytes, blood monocytes, or peritoneal macrophages using Qiagen RNeasy kit and treated by Amplification grade DNase I (Invitrogen) to minimize DNA template contamination. Quantitative PCR was carried out using Brilliant SYBR Green QPCR Mater Mix with Mx3005P instrument (Stratagene, La Jolla, CA). Gene expression level was assessed by equilibration with  $\beta$ -actin level. Primers include 1) mouse endogenous Foxp1: 5'GCCATCCAGAACGGGTCCAGCGGT3', and 5'TGCTGCATTTGTTGAGGAGTGATA3'; 2) mouse  $\beta$ -actin: 5'CATCGTGGGCCGCCCTAGGCACCA3', and 5'GTGTTGAAGGTCTCAAACATGATC 3'; 3) mouse c-fms: 5'TTCACTCCGGTGGTGGTGGCCTGT 3', and 5'GTTGAGTAGGTCTCCATAGCAGCA 3'; 4) human Foxp1: 5'GCAGCCACGCCTACTGCACACCTC 3', and 5'CTTGTCGTCATCGTCCTTGTAGTC 3'. Reaction conditions: 95°C for 10 min, 95°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min for each cycle for total of 40 cycles followed by dissociation cycle 95°C for 30 sec, 55°C for 30 sec, and finally 95°C for 1 min.

### ***Flow cytometry***

Single, 2-color, and 4-color flow cytometry were used to stain neutrophils, monocytes, and macrophages. Cells were incubated with a cocktail of mAbs against T cells (CD90-PE, 53-2.1), B cells (B220-PE, RA3-6B2), NK cells (CD49b-PE, DX5 and NK1.1-PE, PK136), neutrophils (Ly-6G-PE, 1A8), myeloid cells (CD11b-APC, M1/70) and monocyte subsets (Ly-6C-FITC, AL-21), as described previously<sup>3</sup>. F4/80 (BM8)-biotin-strep-PerCP, I-A<sup>b</sup> (AF6-120.1)-biotin-strep-PerCP and CD11c (HL3)-biotin-strep-PerCP mAbs also served to determine macrophage and dendritic cell differentiation. Thioglycolate-elicited and bone marrow cells were also singly or doubly labeled with anti-mouse macrophage/monocyte (FITC-MOMA-2, Serotec), anti-mouse Ly-6G and Ly-6C (PE-Gr-1), PE-F4/80 (Serotec), PE-Mac-3 (M3/80), anti-mouse CD11b (PE-M1/70), and biotinylated ER-MP12 (BMA, Augst, Switzerland) followed by Streptavidin-PE-Cy5. Expression of M-CSFR on peripheral monocytes was assessed by flow cytometry using anti-M-CSFR (AFS98, eBioscience) and enzymatic amplification staining<sup>4</sup>. Monoclonal antibodies were all from BD Biosciences unless indicated otherwise. Flow cytometry was performed on a FACSDiva LSRII (Becton Dickinson) and analyzed using Winlist.

### ***Retroviral transduction of bone marrow-derived monocytes/macrophages***

Wild-type and mutant K614A (kinase-deficient) mouse c-fms cDNA in pZen113 vectors were kindly provided by Dr. Larry Rohrschneider (Fred Hutchinson Cancer research Center, Seattle, WA)<sup>5,6</sup>. cDNA with complete open reading frame from each construct was excised and subcloned into MSCV.GFP retroviral vector (Gift of K. Murphy, Washington University, St. Louis, MO), as previously described<sup>1</sup>. Orientation and authenticity of wild-type and mutant c-fms constructs were verified by sequencing. Retroviruses were generated by transfection of Phoenix packaging cells, as previously described<sup>1</sup>.

Bone marrow-derived monocytes/macrophages were isolated by Percoll gradient centrifugation, re-suspended in DMEM culture medium with 10% fetal bovine serum, and transduced by retrovirus-containing supernatant from Phoenix cells. Cells were cultured for 48-72 hours to induce spontaneous macrophage differentiation and GFP- positive cells were then sorted by BD FACS Aria Flow Cytometer.

### ***Affymetrix microarrays***

Bone marrow-derived monocytes isolated using Percoll gradient centrifugation were cultured on 12-well plate for spontaneous differentiation. RNA was isolated from 9 wild-type and 9 macFoxp1tg mice. To minimize individual mouse differences, RNA from 3 mice per group was pooled, resulting in 3 wild-type and 3 macFoxp1 RNA pools for microarray analysis. After 30 hours, total RNA was isolated from adherent cells using Qiagen RNeasy mini kit. Total RNA (3 µg per sample) was converted into biotin- labeled cRNA probes and then hybridized with Affymetrix GeneChip® Mouse Genome 430 2.0 Array, which includes over 39,000 transcripts. Microarray experiments and data analysis were performed in The Gene Expression and Genotyping Core Facility, Case Comprehensive Cancer Center, Cleveland, OH (<http://gegfc.chosencraft.com/web/>).

### ***Splenocyte isolation***

Spleens were removed, triturated at 4°C with the end of a 3-ml syringe, and filtered through nylon mesh. The cell suspension was then centrifuged, red blood cells lysed, and the resulting single-cell suspension washed. B- and T-lymphocytes were isolated by flow sorting of splenocytes using CD45R/B220-PE and CD3-APC, respectively.

### ***Osteoclast differentiation and enzymatic assay***

Osteoclast progenitor cells were isolated from spleen. Splenocytes ( $3 \times 10^5$ /well) were added to 96-well plates in  $\alpha$ MEM containing 10% FCS and stimulated with RANKL (200 ng/ml) and M-CSF (30 ng/ml) for up to 9 days (media changed every 3 days) to induce osteoclast differentiation. Osteoclasts were identified by tartrate-resistant acid phosphatase (TRAP) staining kit (Sigma). Osteolytic activity was evaluated by measurement of released Eu-labeled collagen fragments using OsteoLyse Assay Kit (Cambrex). For RT-PCR, splenocytes ( $5 \times 10^6$ /well) were cultured in 6-well plates and incubated with RANKL and M-CSF for 7 d prior to extracting RNA.

## REFERENCES

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