Bone & Joint Research

Supplementary Material

10.1302/2046-3758.1211.BJR-2023-0101.R1

Table i. Species of animals in each group and treatment received.

Species	Treatment	N
LPS	N/A	8
PBS	N/A	8
WT	LPS	8
	PBS	8
IL-19$^{-/-}$	LPS	8
	PBS	8

IL-19$^{-/-}$, interleukin-19 knockout; LPS, lipopolysaccharide; N/A, not applicable; PBS, phosphate-buffered saline; WT, wild-type.

Table ii. Parameters related to lipopolysaccharide-induced improvement in bone loss and bone resorption in mouse bone trabeculae.

Parameter	L1 vertebrae			Femur trabecular bone			Numbers analyzed
	Mean BV/TV, % (SD)	Mean Tb.N, mm^{-1} (SD)	Mean Tb.Sp, mm (SD)	Mean BV/TV, % (SD)	Mean Tb.N, mm^{-1} (SD)	Mean Tb.Sp, mm (SD)	
LPS	13.63 (1.613)	2.327 (0.2316)	3.377 (0.5006)	4.762 (0.4821)	1.879 (0.2120)	0.7327 (0.04859)	n = 8
PBS	24.02 (2.984)	4.644 (0.6395)	1.761 (0.1477)	7.342 (0.7601)	3.010 (0.5064)	0.5853 (0.06434)	n = 8

BV/TV, bone volume fraction; L1, lumbar #1; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; SD, standard deviation; Tb.N, trabecular bone number; Tb.Sp, trabecular bone separation.

Table iii. Parameters related to interleukin-19 levels in peripheral blood serum in lipopolysaccharide-induced bone loss of mice on Day 0, 3, 6, and 9.

Parameter	Timepoint				Numbers analyzed
	Day 0	**Day 3**	**Day 6**	**Day 9**	
Mean LPS in serum, pg/ml (SD)	254.9 (24.58)	737.7 (62.76)	1205 (99.85)	857.6 (104.6)	n = 8
Mean PBS in serum, pg/ml (SD)	251.9 (32.32)	251.3 (28.08)	232.8 (35.78)	238.3 (37.61)	n = 8
Mean LPS in bone marrow, pg/ml (SD)	35.99 (5.218)	55.52 (4.138)	76.01 (6.828)	61.46 (9.467)	n = 8
Mean PBS in bone marrow, pg/ml (SD)	34.55 (2.425)	36.96 (4.852)	38.40 (4.295)	35.29 (3.859)	n = 8

LPS, lipopolysaccharide; PBS, phosphate-buffered saline; SD, standard deviation.

Table iv. Parameters related to interleukin-19, RANKL, and osteoprotegerin levels in peripheral blood serum and bone marrow aspirates.

Parameter	Peripheral blood serum			Bone marrow aspirates			Numbers analyzed
	IL-19	**RANKL**	**OPG**	**IL-19**	**RANKL**	**OPG**	
Mean WT/LPS, pg/ml (SD)	521.5 (38.41)	87.68 (7.529)	705.8 (98.56)	59.06 (9.332)	21.69 (2.161)	103.1 (9.315)	n = 8
Mean WT/PBS, pg/ml (SD)	247.0 (28.51)	77.39 (10.09)	1038 (126.2)	29.28 (2.725)	20.01 (1.981)	153.4 (15.74)	n = 8
Mean IL-19$^{-/-}$/LPS, pg/ml (SD)	20.73 (1.991)	81.12 (9.802)	823.7 (96.97)	3.634 (0.4007)	19.68 (2.934)	132.6 (8.966)	n = 8
Mean IL-19$^{-/-}$/PBS, pg/ml (SD)	22.21 (2.840)	N/A	N/A	4.248 (0.6264)	N/A	N/A	n = 8

IL, interleukin; LPS, lipopolysaccharide; N/A, not applicable; OPG, osteoprotegerin; PBS, phosphate-buffered saline; RANKL, receptor activator of nuclear factor-κB ligand; SD, standard deviation; WT, wild-type.

Table v. Parameters related to the effects of interleukin-19 and lipopolysaccharide on bone in mice.

Parameter	L1 vertebrae			Femur trabecular bone			Numbers analyzed
	Mean BV/TV, % (SD)	**Mean Tb.N, mm^{-1} (SD)**	**Mean Tb.Sp, mm (SD)**	**Mean BV/TV, % (SD)**	**Mean Tb.N, mm^{-1} (SD)**	**Mean Tb.Sp, mm (SD)**	
WT/LPS	15.42 (2.134)	1.923 (0.3305)	3.227 (0.2767)	3.603 (0.3931)	1.971 (0.1962)	0.8174 (0.06681)	n = 8
WT/PBS	21.40 (2.126)	4.171 (0.4360)	1.644 (0.1961)	6.716 (0.6221)	3.670 (0.3996)	0.6030 (0.08428)	n = 8
IL-$19^{-/-}$/LPS	19.04 (1.448)	3.084 (0.3411)	2.332 (0.3343)	5.716 (0.5904)	2.625 (0.3454)	0.6353 (0.05004)	n = 8
IL-$19^{-/-}$ /PBS	20.10 (1.572)	3.792 (0.2555)	1.848 (0.2601)	6.676 (0.3916)	3.528 (0.3733)	0.5821 (0.06128)	n = 8

BV/TV, bone volume fraction; IL, interleukin; L1, lumbar #1; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; SD, standard deviation; Tb.N, trabecular bone number; Tb.Sp, trabecular bone separation; WT, wild-type.

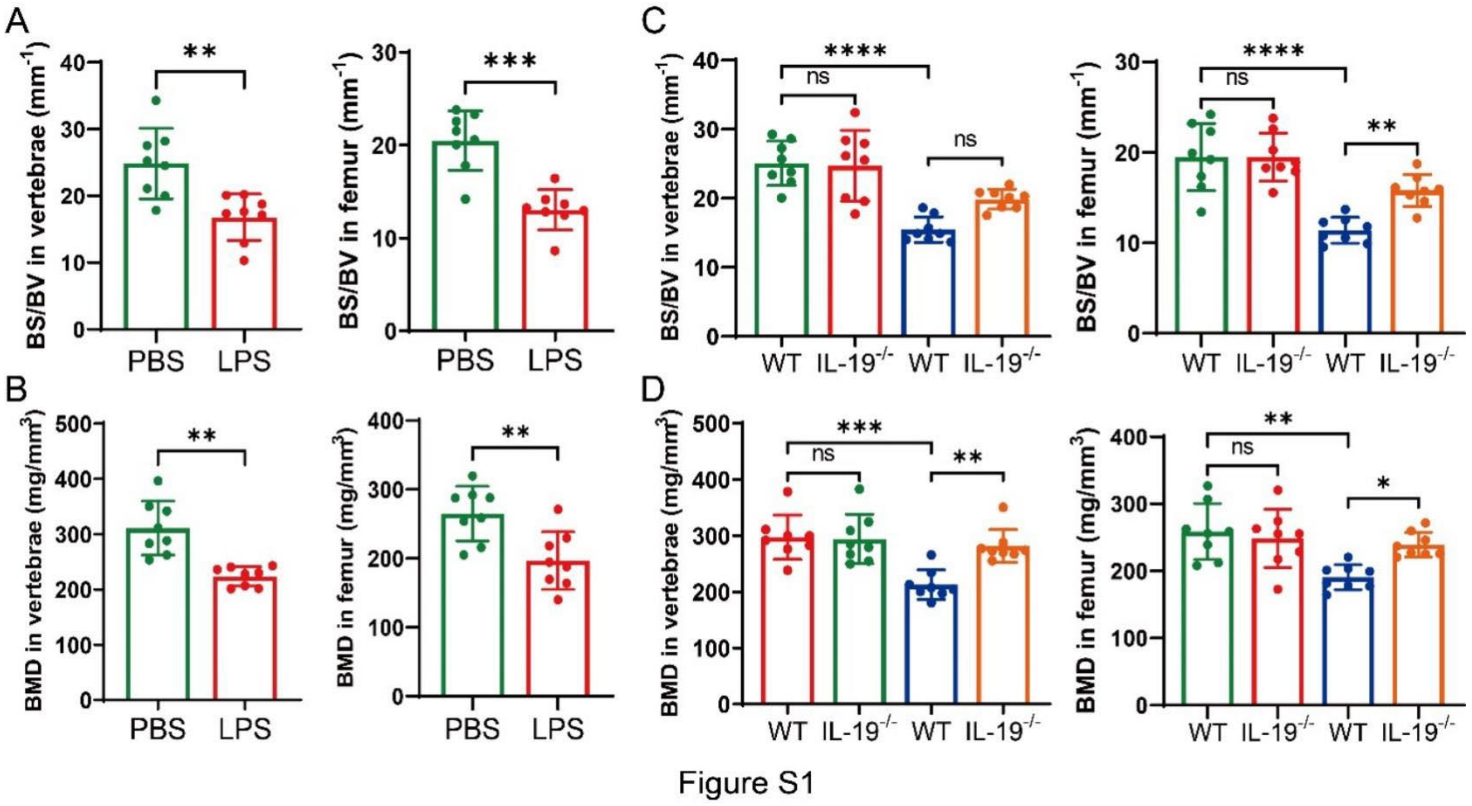

Figure S1

Fig a. Complementary parameters of lipopolysaccharide (LPS)-induced trabecular bone loss and bone resorption improvement in mice. a) Bone surface/volume ratio (BS/BV) of L1 vertebrae and distal femur in LPS-induced bone loss of mice (n = 8). b) Bone mineral density (BMD) of L1 vertebrae and femur trabecular in LPS-induced bone loss of mice (n = 8). c) BS/BV of L1 vertebrae and distal femur in LPS-induced bone loss of interleukin (IL)-19$^{-/-}$ mice (n = 8). d) BMD of L1 vertebrae and distal femur in LPS-induced bone loss of IL-19$^{-/-}$ mice (n = 8). Data are shown as means and standard deviations. P-values were determined by independent-samples *t*-test (a, b) and one-way analysis of variance followed by Tukey's test (c, d). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. BMD, bone mineral density; LPS, lipopolysaccharide; ns, non-significant; PBS, phosphate-buffered saline; WT, wild-type.

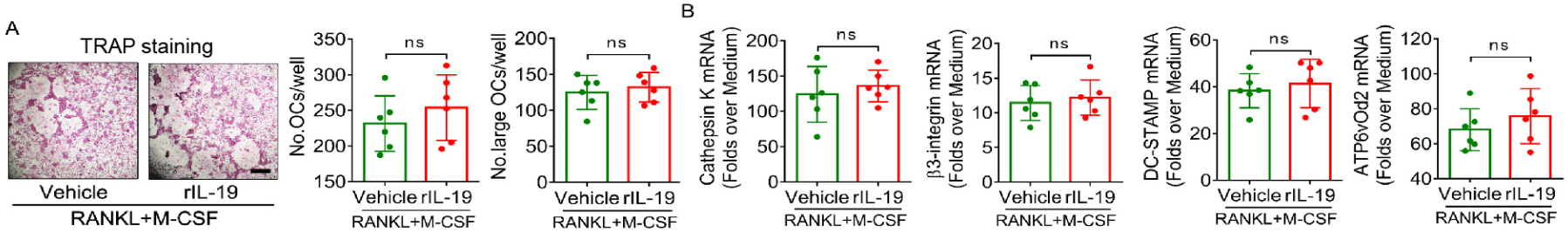

Figure S2

Fig b. In vitro osteoclast (OC) differentiation of bone marrow monocytes (BMMs) in response to interleukin (IL)-19 treatment. a) Tartrate-resistant acid phosphatase (TRAP) staining images and quantification of in vitro bone marrow monocyte culture in response to macrophage-colony stimulating factor (M-CSF)/receptor activator of nuclear factor-κB ligand (RANKL) and recombinant IL-19 (100 ng/ml) treatment. b) Messenger RNA (mRNA) expression of osteoclast differentiation markers including Cathepsin K, β3-integrin, DC-STAMP, and ATP6v0d2 in BMM culture in response to M-CSF/RANKL and recombinant chemokine (C-C motif) ligand 12 (CCL12) (100 ng/ml) treatment determined by quantitative real-time polymerase chain reaction (RT-qPCR) (n = 8). α-tubulin was used as internal control for quantitative polymerase chain reaction (qPCR). Data are representative of three independent experiments, and are shown as means and standard deviations. P-values were determined by independent-samples *t*-test. ns, non-significant; rIL-19, recombinant IL-19.

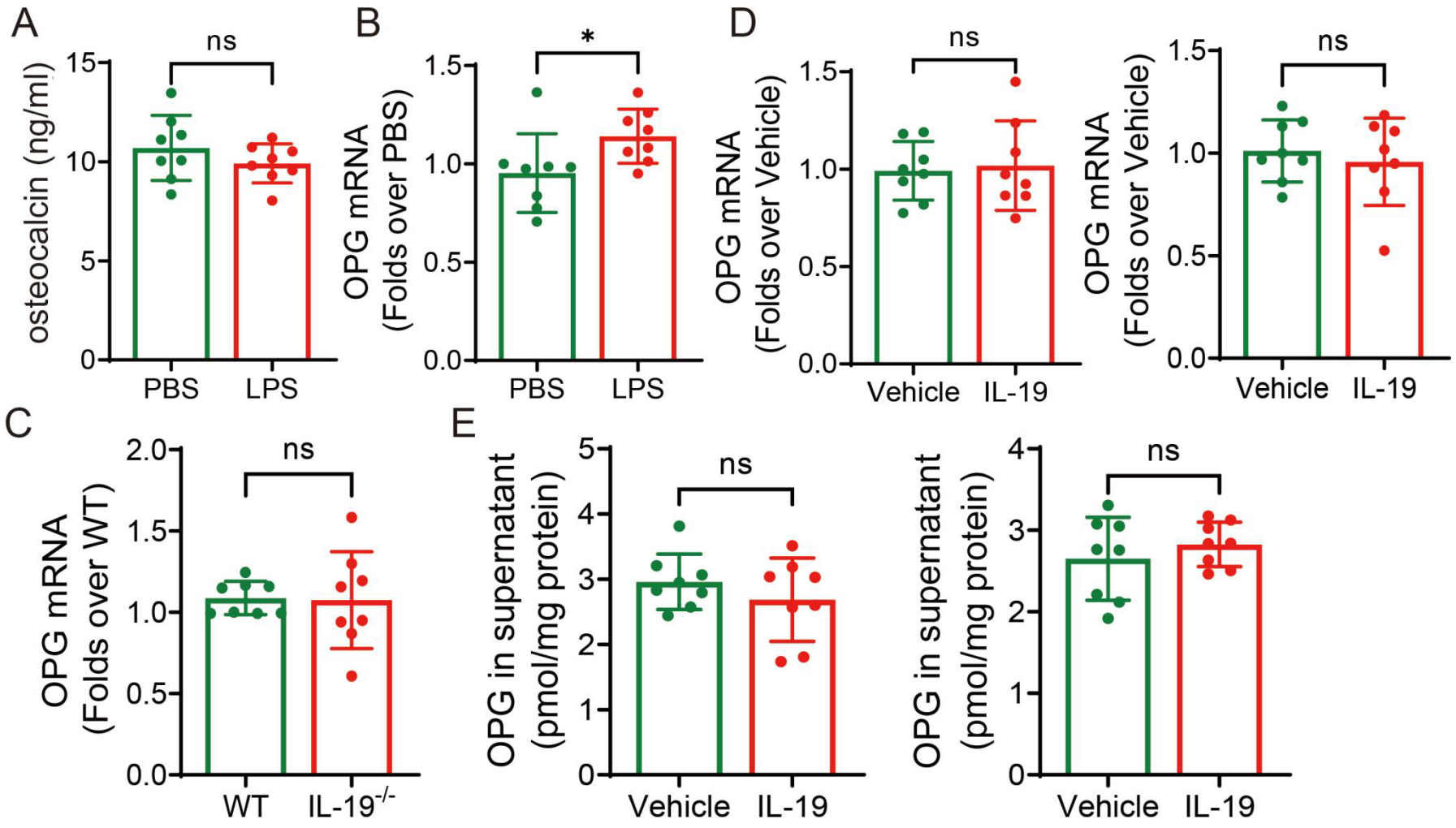
A
osteocalcin (ng/ml)
15
10
5
0
ns
PBS
LPS
B
OPG mRNA (Folds over PBS)
1.5
1.0
0.5
0.0
*
PBS
LPS
D
OPG mRNA (Folds over Vehicle)
1.5
1.0
0.5
0.0
ns
Vehicle
IL-19
OPG mRNA (Folds over Vehicle)
1.5
1.0
0.5
0.0
ns
Vehicle
IL-19
C
OPG mRNA (Folds over WT)
2.0
1.5
1.0
0.5
0.0
ns
WT
IL-19-/-
E
OPG in supernatant (pmol/mg protein)
5
4
3
2
1
0
ns
Vehicle
IL-19
OPG in supernatant (pmol/mg protein)
4
3
2
1
0
ns
Vehicle
IL-19

Figure S 3

Fig c. Interleukin (IL)-19 had no effect on osteocalcin levels or osteoprotegerin (OPG) expression in osteocytes. a) Osteocalcin levels in peripheral blood serum in lipopolysaccharide (LPS)-induced bone loss of mice determined by enzyme-linked immunosorbent assay (ELISA) (n = 8). b) OPG messenger RNA (mRNA) expression of osteocytes in LPS-induced bone loss of mice determined by quantitative real-time polymerase chain reaction (RT-qPCR) (n = 8). c) OPG mRNA expression of osteocytes in LPS-induced bone loss of IL-$19^{-/-}$ mice determined by RT-qPCR (n = 8). d) OPG mRNA expression in in vitro primary osteocytes and cell line MLO-Y4 culture in response to 100 ng/ml recombinant IL-19 treatment for 48 hours determined by RT-qPCR (n = 8). e) OPG secretion levels in the medium of in vitro primary osteocytes and cell line MLO-Y4 culture in response to 100 ng/ml recombinant IL-19 treatment for 48 hours determined by ELISA (n = 8). α-tubulin was used as internal control for quantitative polymerase chain reaction (qPCR). Data are representative of three independent experiments, and are shown as means and standard deviations. P-values were determined by independent-samples *t*-test. *$p < 0.05$. ns, non-significant; PBS, phosphate-buffered saline; WT, wild-type.

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Methods of Abstract Randomization
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Supplementary Table 1 Animals
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	no criteria were set there were no exclusions Supplemental Table 1
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Randomization Randomization
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Blinding/masking
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	LPS-induced trabecular bone loss and bone resorption improvement in mice; IL-19 accumulation in serum and bone marrow in mice with LPS-induced it's not applicable to our study
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Statistical analysis Statistical analysis
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Animals Animals;RNAi;Global deletion of IL-19 counteracts LPS-induced trabecular bone loss and bone resorption improvement
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	LPS-induced bone loss mouse model LPS-induced bone loss mouse model Principal Institution LPS-induced bone loss mouse model
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Supplementary Tables 2-5 it's not applicable to our study