Supplementing Information

Supplementary Methods

Isolation of rat peritoneal mast cell (RPMC)

Sprague-Dawley rats (8-week-old, weighing 250 - 300 g) were anesthetized by ether, and injected with 30 ml of PMC buffer (1% FBS and PBS) containing 0.1% heparin (JW pharmaceutical, Seoul, Republic of Korea) into the peritoneal cavity; the abdomen was gently massaged for about 3 min. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells was aspirated by pasteur pipette. Then the peritoneal cells were sedimented at 150 × g for 10 min at room temperature and resuspended in PMC buffer. Mast cells were separated from the major components of rat peritoneal cells. In brief, peritoneal cells suspended in 1 ml of PMC buffer were layered onto 2 ml of 45% percoll (Sigma Chemical Co., St. Louis, MO, USA) and centrifuged at room temperature for 10 min at 190 × g. The cells remaining at the buffer-percoll interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 3 ml α-MEM media containing 50% FBS. MC preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypan blue uptake. Purified RPMCs were resuspended in α-MEM media containing 50% FBS for the treatment with compound 48/80 or RANKL. RPMC suspensions (2×10^5 cells) were preincubated for 10 min at 37°C before the addition of compound 48/80 or RANKL for stabilization. The cells were stimulated with the compound 48/80 or RANKL for 20 or 60 min. The reaction was stopped by cooling the tubes in ice.

Prostaglandin D_2 (PGD_2) assay

PGD_2 from RPMC was measured using a PGD_2 assay kit according to manufacturer's protocols (Cayman Chemical Co., Ann Arbor, MI, USA).

Leukotriene C_4 (LTC_4) assay

LTC_4 from RPMC was measured using a LTC_4 assay kit according to manufacturer's protocols (Cayman Chemical Co., Ann Arbor, MI, USA).

Supplementary Figures

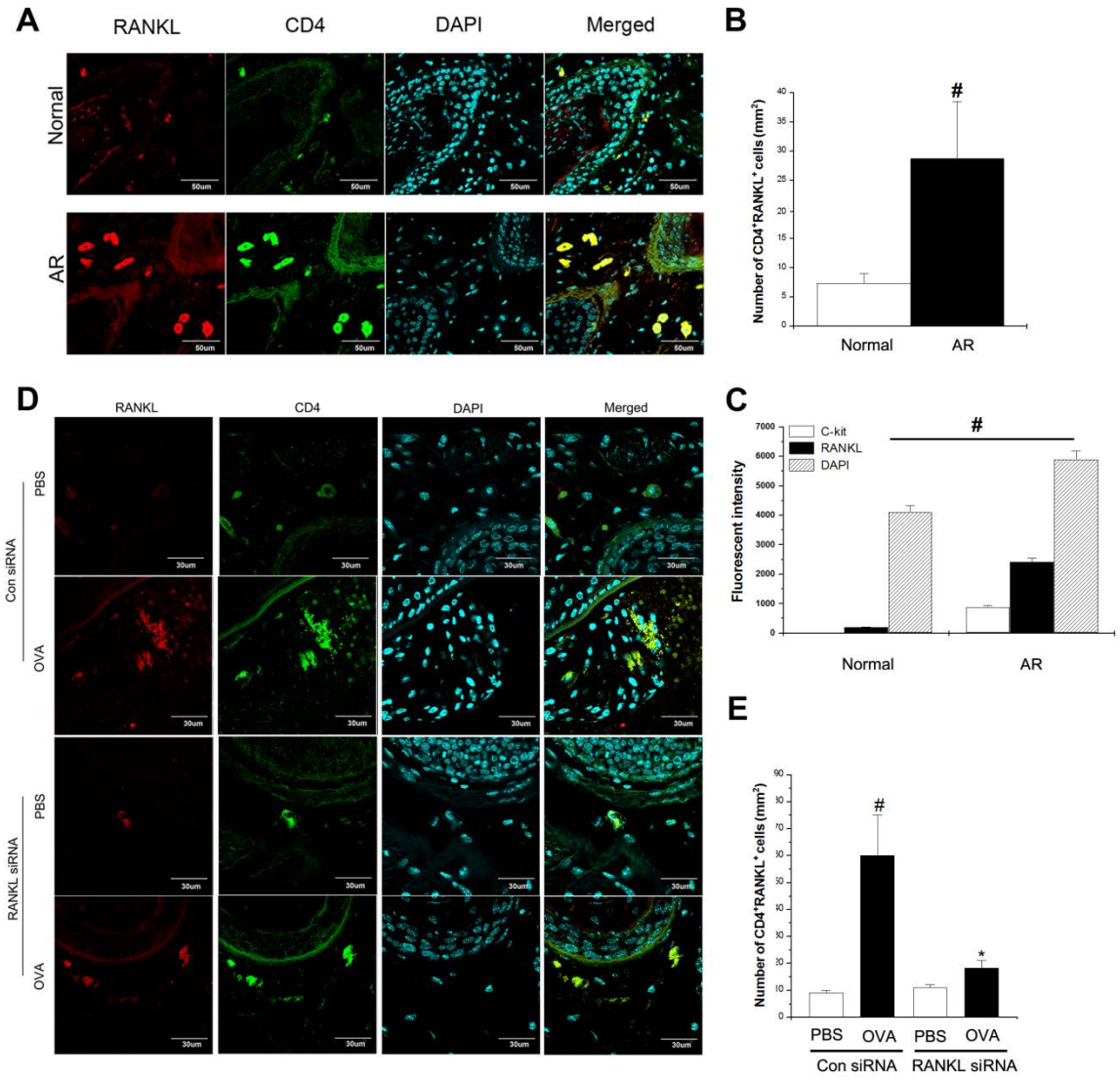

Figure S1.

RANKL is up-regulated in T cells. (A) Nasal mucosa tissues from AR patients were double stained by immunofluorescence with anti-CD4 (green) and anti-RANKL antibody (red) (magnification, × 60). (B) T cells were counted by three individuals in human tissues. Data are represented as the mean ± S.E.M. $^{\#}P < 0.05$; significantly different from the normal. Mice were sensitized on days 1, 5, and 14 by intraperitoneal injections of 100 μg of OVA emulsified in 20 mg of aluminum hydroxide and then received local injections of RANKL

siRNA and control siRNA, or PBS alone into the nasal cavity, and challenged with intranasal OVA for 10 days. (C) Fluorescence intensities in nasal mucosa tissues of normal mice and AR mice were determined as threshold values. (D) Nasal mucosa tissues from normal mice and AR mice were double stained by immunofluorescence with anti-CD4 (green) and anti-RANKL antibody (red) (magnification, × 60). (E) T cells were counted by three individuals in animal tissues. Data are represented as the mean ± S.E.M. $^{\#}P < 0.05$; significantly different from the PBS. $^{*}P < 0.05$; significantly different from the control siRNA OVA.

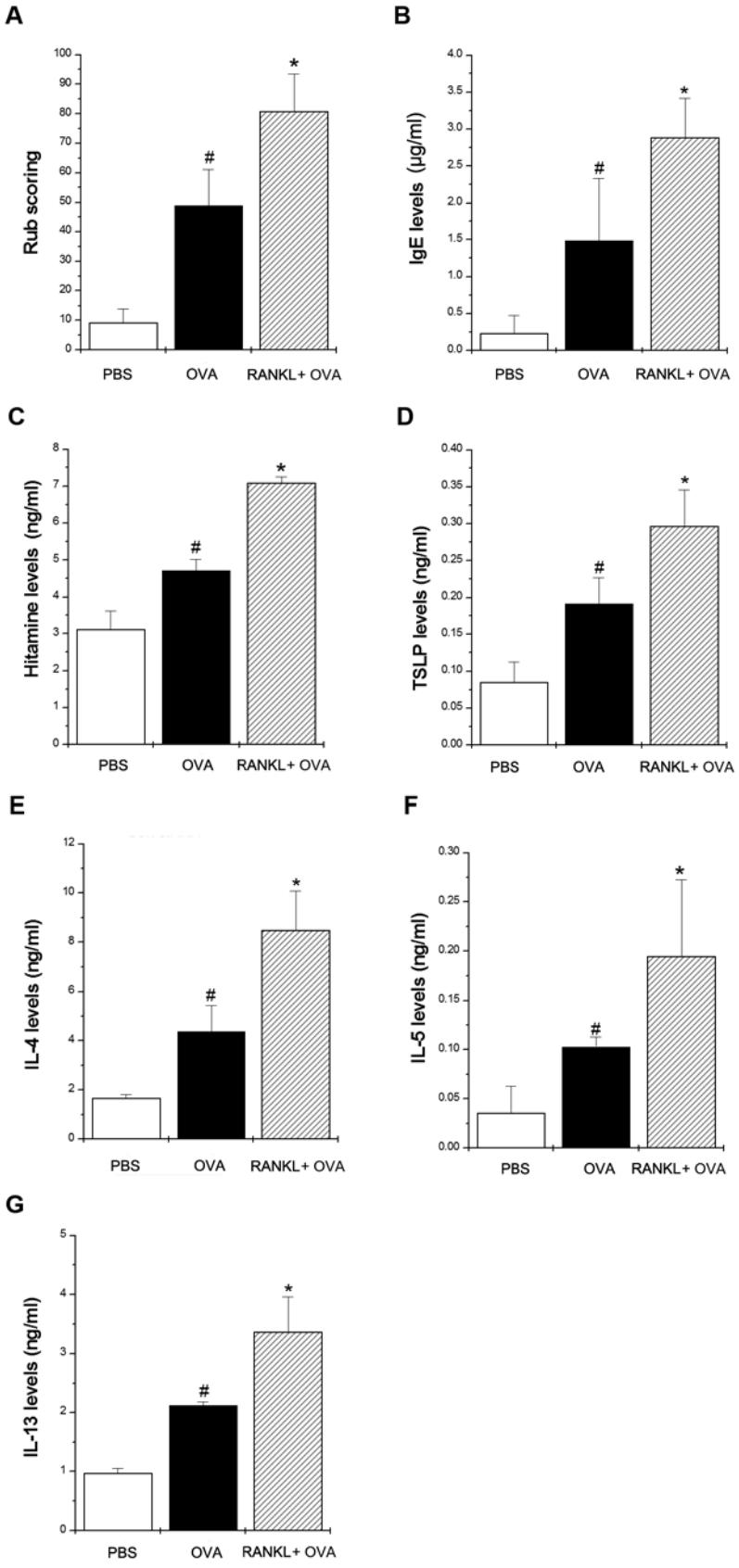
A
Rub scoring
PBS
OVA
RANKL+ OVA
B
IgE levels (μg/ml)
C
Hitamine levels (ng/ml)
D
TSLP levels (ng/ml)
E
IL-4 levels (ng/ml)
F
IL-5 levels (ng/ml)
G
IL-13 levels (ng/ml)

Figure S2.

Co-challenge of RANKL and OVA synergistically increased AR responses. We sensitized mice on days 1, 5, and 14 by intraperitoneal injections of 100 μg of OVA emulsified in 20 mg of aluminum hydroxide and we challenged mice with 1.5 mg of OVA. Mice received rmRANKL before the intranasal OVA challenge for 10 days. (A) The rub number of the nose that occurred 10 min after OVA intranasal provocation. Levels of (B) IgE, (C) histamine, and (D-G) cytokine in the serum. Data are represented as the mean ± S.E.M. with n = 5 mice per group. $^{\#}P < 0.05$; significantly different from the OVA-unsensitized mice. *P < 0.05; significantly different from the OVA-sensitized mice.

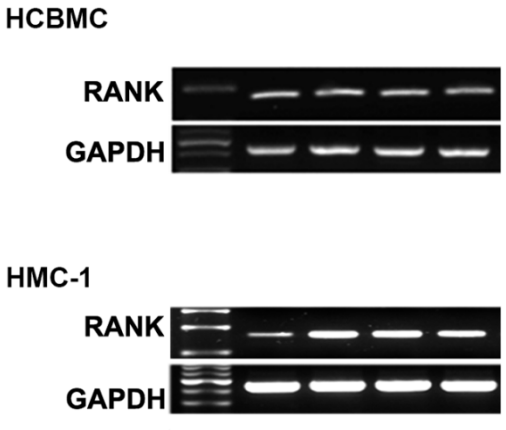

Figure S3.

RANK was expressed on human mast cell. RANK mRNA expressions in human cord blood-derived mast cells (HCBMC) and HMC-1 cells were analyzed by the RT-PCR.

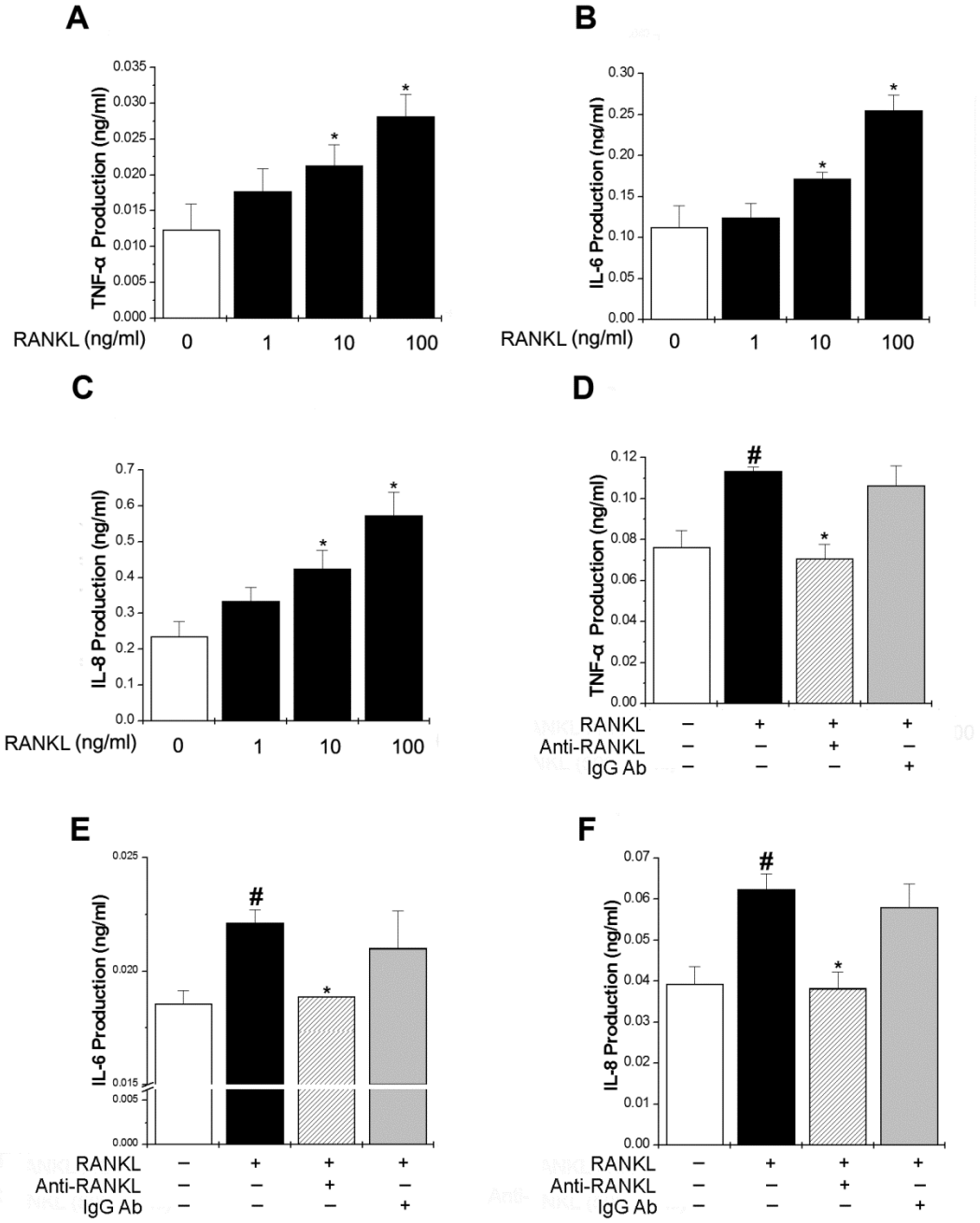

Figure S4.

RANKL induces production of pro-inflammatory cytokines from HMC-1 cells. (A-C) HMC-1 cells (1×10^5) were stimulated with RANKL (1, 10, and 100 ng/ml) for 24 h. The production of pro-inflammatory cytokines in the supernatant was measured by the ELISA method. (D-F) HMC-1 cells were treated with RANKL (10 ng/ml), RANKL neutralizing

antibodies (0.5 μg/ml), and/or IgG isotype for 24 h. The production of pro-inflammatory cytokines in the supernatant was measured by the ELISA method. Each datum represents the mean ± SEM of five independent experiments. $^{\#}P < 0.05$; significantly different from unstimulated cells. $*P < 0.05$; significantly different from RANKL.

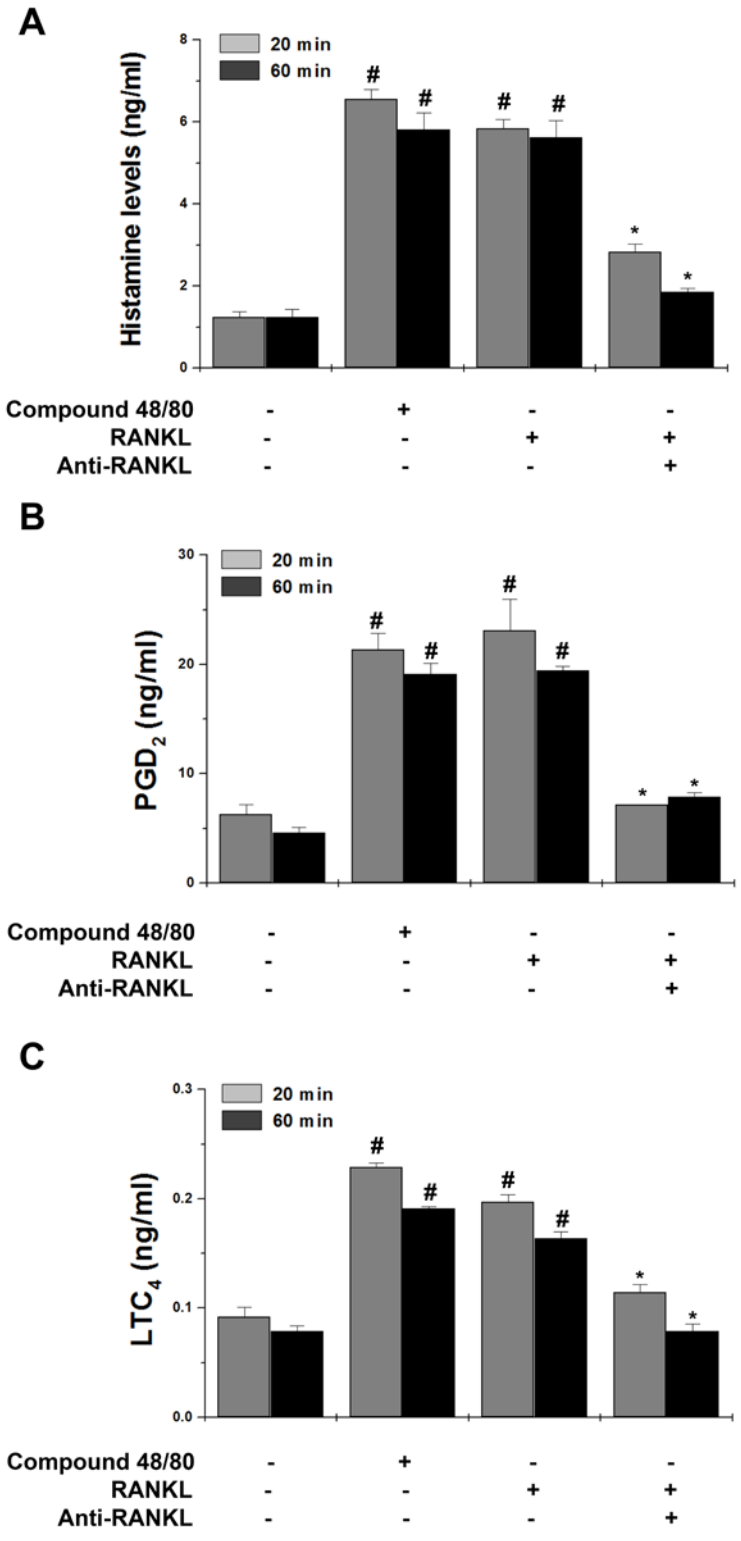

Figure S5.

RANKL induces degranulation of rat peritoneal mast cells (RPMCs). RPMCs were stimulated with RANKL (10 ng/ml), compound 48/80 (6 μg/ml), RANKL + Anti-RANKL Ab

(Anti-RANKL) for 20 and 60 min. The levels of (A) histamine, (B) PGD_2, and (C) LTC_4 were measured from cell supernatant. Data were expressed as mean ± SEM of five independent experiments with duplicate samples. $^{\#}P < 0.05$; significantly different from unstimulated cells. $^{*}P < 0.05$; significantly different from RANKL' value.

Table S1 Baseline characteristics for the normal and AR groups

Sample	Group	Sex profile	Age (y), Mean±SD
Serum (n=80)	Normal (n = 40)	20M, 20F	43.3±18.7
	AR (n = 40)	20M, 20F	33.0±15.5
Nasal mucosa (n=40)	Normal (n = 20)	10M, 10F	37.7±14.9
	AR (n = 20)	14M, 6F	31.2±13.2

F, Female; M, Male

Sensitivity to allergens was assessed by detection of serum specific IgE.

Table S2 RANKL correlation with inflammation in the serum

Parameters	*R* value	*P* value
Inflammation	0.449	0.004

The level of RANKL in the serum was correlated with severity of AR symptoms of the biopsies. Correlations are expressed by using Spearman's rank correlation coefficient. $P < 0.005$