Supplemental methods

Mice and ovariectomy

Seven-week-old female C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan). All mice were housed in groups of four to six per cage (21.5×32×13.5 cm^3) under controlled temperature (23 ± 3 °C) and humidity (23 ± 3 °C), with 12-hour light/dark cycle and food and water *ad libitum*. Mice were randomly assigned to either a sham surgery (Sham) or bilateral ovariectomy (OVX) at eight weeks of age, as previously reported.

Reagents and treatment

Mice were injected intradermally with 10 μL of phosphate-buffered saline (PBS) containing IL-31 (100 ng, and 300 ng) (Abcam Inc., Cambridge, UK) at the shaved nape of the neck 4 weeks after ovariectomy or sham surgery. A 10 μL of PBS containing 1 μg anti-IL-4 antibody (R＆D systems (Minneapolis, MN, USA)) and 5 μg anti-IL-13 antibody (R&D systems), corresponding to 3 times the ND_{50} for 7.5 ng/mL IL-4 and 5 times the ND_{50} for 10.0 ng/mL IL-13, or isotype IgG (BioLegend (San Diego, CA, USA)) as a control in 10 μL of PBS, was injected intradermally into the shaved nape 4 hours before IL-31 administration.

Evaluation of scratching behavior by the SCLABA®-NEXT real-time scratch counting system

The frequency of scratching behavior was recorded using the SCLABA®-NEXT real-time scratch counting system (Noveltec Inc., Kobe, Japan). Locomotor activity was measured using image data. During the acclimatization and observation period, each chamber was supplied with DietGel® 76A: water-containing food (ClearH_2O, Westbrook, ME, USA):1 cup per day. Experimental conditions were set as $\theta c = 27$, $\tau 0 = 30$, $\tau 1 = 70$, and $\tau 2 = 150$. After an acclimation period of at least 0.5 h, the behavior of each mouse was recorded for 30 min, 2 h, 24 h, 48 h or 72 h. The number of scratching sessions was counted visually in the video as follows: one scratching session was defined as one instance of lifting the hind paw from the ground, scratching, and returning the paw to the ground, or bringing the paw to the mouth. Long-lasting scratching (LLS) was defined as the number of movements lasting from ≥1.5 seconds to ≤3 seconds each.[1] The total number of behaviours measured was the number of all movements of the mice, including all behaviours such as scratching and jumping.

The IL-31-induced itch-associated scratching behavior observed in the video was prominent within 30 min -2 h after each IL-31 administration, as was the case with other scoring systems. However, the scores obtained for all movements increased and were comparable in mice receiving IL-31 and PBS during the night when the mice are active,

suggesting that the scratching behavior visually observed in the video and LLS may be the reliable scores for evaluating spontaneous or induced itch, as previously shown by Arai et al.[1] We used a score of the scratching behavior visually observed in the video in the early experiments and a score of LLS in the later experiments including 48 h monitoring.

Spontaneous scratching behavior observed prior to IL-31 administration was also evaluated using three types of scores. Interestingly, the levels of LLS, the most reliable score for IL-31 induced pruritus, were comparable between OVX- and sham-mice, although the levels of scratching behavior on the video and LLS were higher in OVX-mice than in sham-mice. This suggests that the LLS may be inappropriate for assessing spontaneous pruritus, in which mild scratching is observed in the video.

REFERENCES

1. Arai I, Tsuji M, Takeda H, et al: A single dose of interleukin-31 (IL-31) causes continuous itch-associated scratching behaviour in mice. Exp Dermatol. 22:669-71, 2013. doi:10.1111/exd.12222