ONLINE METHODS

Study subjects and skin samples. Skin biopsies of DPCP-challenge reactions and placebo-treated sites were obtained from 11 volunteers (ages 29–58, 8 males and 3 females) under a protocol approved by The Rockefeller University's institutional review board[22]. We obtained written informed consent from all subjects and the study adhered to the Declaration of Helsinki principles. Consent to publish was obtained for all subjects who were photographed. Immunization with DPCP (Hapten Pharmaceuticals), recall and sampling have been described before[22]. Briefly, we sensitized healthy individuals to DPCP and 2 weeks later challenged them with two applications (0.04%) of DPCP on their left thigh and two placebo applications on their right thigh. We took skin biopsies at day 3, 14 and month 4 after challenge, and stored them at −80 °C until processing. For three subjects (one female, two males, age range 46–55, selected randomly), HTS was performed on all biopsy samples.

Animal experiments. We purchased C57BL/6 mice (5–7 weeks of age, females, $n = 302$ total) from The Jackson Laboratory, Bar Harbor, ME. Purchased mice had at least 1 week of acclimation in the animal facility of Harvard Institutes of Medicine at Harvard Medical School, before the initiation of any experiment. We bred and housed C57BL/6J *Thy1.1 Rag1*$^{-/-}$ Tg(TcraTcrb)1100Mjb/J (better known as OT-I) mice at the same facility. We performed all animal experiments in accordance with the guidelines set out by the Center for Animal Resources and Comparative Medicine at Harvard Medical School. For all experiments, animals were randomized into the different experimental groups by the animal facility staff, so that the investigator did not select groups based on size or appearance, but the investigator was not blinded once the experiments were initiated. Groups of three mice for naive or simple DNFB-positive ear-swelling control experiments were sufficient as ear swelling is highly reproducible (pooled naive of positive control in this paper, 36 mice). For other conditions, we chose groups of five mice because prior publications had identified this as the correct number in regard to reaching statistical power with the minimal number of animals. The sole exclusion criterion was death of the subject animal (one occurrence of unknown cause, five others due to complications linked with the surgical procedure).

Antigen challenge to skin. We sensitized C57BL/6 mice by topical applications of 20 µl of 0.25% DNFB diluted in acetone:olive oil(aOO) (3:1 v/v) at the indicated time points (day 0 and 1, or 0, 1 and 7). Challenged was performed at the indicated time points with 20 µl of DNFB–aOO (0.25%). We measured ear thickness with a digital thickness gauge (Mitutoyo) before and after DNFB challenge. For OVA + CT immunization, mice were immunized epicutaneously as previously described[8]. Briefly, we used scotch tape (3M) to gently remove the cornified layer of ear skin, and then treated the skin with acetone and cholera toxin adjuvant (List Biological Labs) before administrating chicken ovalbumin + cholera toxin. For OT-I experiments, we transferred 1×10^4–1×10^6 OT-I splenocytes intravenously into recipient mice 24 h before immunization of LN dissection. We performed epicutaneous infection by scarification as previously described[9]. Every experiment was performed at least twice (and often multiple times). We used fingolimod (FTY-720, Sigma) diluted in PBS and injected it intraperitoneally (i.p.) at 1 µg per g of mouse body weight: for example, typically 25 µg/mouse i.p. daily for 7 d, beginning 2 d before the DNFB challenge.

Parabiotic surgery. We performed parabiosis surgery as previously described[8]. Briefly, we shaved the flank of sex- and age-matched mice 1 d before surgery. We then anesthetized them to full muscle relaxation with ketamine and xylazine (10 mg/g) i.p. After skin disinfection (betadine solution and 70% ethanol, 3×), we made two matching skin incisions from the olecranon to the knee joint of each mouse pair. We then bound the elbow and knee joints by a single 5-0 silk suture and closed the dorsal and ventral skins with staples and 5-0 silk suture. Betadine solution was used to cover the full length of the dorsal and ventral incision. We kept the mice on heating pads and continuously monitored them until recovery from anesthesia and surgery. We used 2.5 mg/g flunixin subcutaneously for analgesia every 12 h for 48 h after the operation. After an interval of the indicated weeks, parabiotic mice were surgically separated by a reversal of the above procedure for subsequent experiments.

Lymphadenectomy. Briefly, we shaved mice on the flank and belly 1 d before surgery. We anesthetized the mice to full muscle relaxation with ketamine and xylazine (10 mg/g) i.p. After skin disinfection (betadine solution and 70% ethanol, 3×), we performed a 1 cm skin incision on a longitudinal line 0.5 cm medial to the mammary gland. We everted the lateral skin to reveal the inguinal lymph node, which we then excised. We closed the incision with two surgical clips, disinfected with betadine 1%, kept the mice on heating pads and continuously monitored them until recovery. We used 2.5 mg/g flunixin subcutaneously for analgesia every 12 h for 24 h.

Viruses and infections. We obtained recombinant MVA from B. Moss (US National Institutes of Health) and grew them on DF-1 cells. Viral titers were determined using CV-1 cells and 2×10^6 p.f.u. of MVA used for epicutaneous infection by skin scarification, as previously described[8].

Preparation of cell suspensions. We harvested lymph nodes and spleen and passed them through a 70-mm nylon cell strainer to prepare cell suspensions, in which we lysed red blood cells with hypotonic solution (Gibco). For skin tissue, we removed hair, chopped skin into small fragments and incubated them in Hank's balanced salt solution (Gibco) supplemented with 1 mg/ml collagenase A (Pharmingen) and 40 mg/ml DNase I (Roche) at 37 °C for 30 min. After filtering through a 70-mm nylon cell strainer, we collected the cells and washed them thoroughly with cold PBS before staining.

Antibodies and flow cytometry. We obtained the following anti-mouse antibodies from BD Pharmingen: CD8a (53-6.7), CD4 (L3T4), CD45.1 (A20), CD44 (IM7), CD62L (MEL-14), CD69 (H1.2F3), CD103 (M290), CD122 (TM-Beta 1). We used all antibodies at a dilution of 1:200. We purchased fluorescence-conjugated anti-mouse CD127 (A7R34) from eBioscience. To examine E- or P-selectin ligand expression, we incubated cells with rmE-Selectin/Fc Chimera or rmP-Selectin/Fc Chimera (R&D Systems) in conjunction with APC-conjugated F(ab9)2 fragments of goat anti-human IgG F(c) antibody (cat. 109-136-098, Jackson ImmunoResearch). We analyzed data on a FACSCanto flow cytometer using FACSDiva software.

Isolation of gDNA and *TRB* high-throughput sequencing. We extracted DNA from 22–24 mg of skin (ear or de-boned tail skin), vagina, lung, gut or 6–12 mg of LN using Qiagen's DNeasy mini-columns according to the manufacturer's instruction and stored it at −20 °C. We then shipped it on dry ice to Adaptive Biotechnologies. All TCR-β characterization was performed by Adaptive Biotechnologies using the ImmunoSeq TCR-β 'survey level' mouse assay. To address amplification bias in the multiplex PCR, we divided a single C57Bl/6 mouse thymus into 12 sections and ran each thymus section independently through HTS. Taking CDR3 sequences found in only one sample (which produces a population made up almost exclusively of CDR3 sequences represented by a single T cell in the input material), we calculated the read coverage (compared to average) associated with each V and J gene segment and used these factors to normalize sequencing output. To estimate the number of T cells bearing each rearrangement, we began with the same population of CDR3 sequences as above and calculated the mean number of sequencing reads obtained per T cell of input in this population (after normalizing for amplification bias). Next we divided the total sequencing output of each of the 12 thymus samples by this mean coverage to estimate the number of T cells present in each of the 12 subsections. These data were used to model the relationship between the number of unique CDR3 sequences obtained and the total number of T cells sequenced. For the 12 thymus sections, these values were highly correlated (total T cells = 1.22 * unique CDR3 rearrangements; $r^2 = 0.98$). This relationship depends on the clonal structure of the T cell population but should only depart from our model in the case of highly clonal T cell populations. In each subsequent sample this relationship was used to estimate the total number of T cells assayed and thus the mean coverage per input T cell. Finally, the number of T cells bearing each unique CDR3 sequence was estimated as number of sequencing reads obtained/estimated mean coverage. OT-I TCR tracking was based on the OT-I Vβ5.2/Dβ2/Jβ2.6 sequence 5-TACTTCTGTGCCAGCT CTCGGGCCAATTATGAACAGTACTTCGGTCCCGGCA-CCAGGCT-3′

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(YFCASSRANYEQYFGPGTR). TCR-β characterization of human samples (n = 3 subjects) was performed using the Adaptive Biotechnologies ImmunoSeq human TCR-β assay, survey level. All raw data can be accessed at http://adaptivebiotech.com/papers/nmed69531a.

VDJ family lists corresponding to graphs in Figure 2b. The analyzed mouse TCR subfamilies are described here. They are portrayed in this order in **Figure 2b**, from left to right for both the *x*- and *y*-axis. TCR-BV1-0, -BV2-0, -BV3-0, -BV4-0, -BV5-0, -BV6-0, -BV7-0, -BV8-0, -BV9-0, -BV10-0, -BV12-1, -BV12-2, -BV12-3, -BV13-1, -BV13-2, -BV13-3, -BV14-0, -BV15-0, -BV16-0, -BV17-0, -BV19-0, -BV20-0, -BV21-0, -BV22-0, -BV23-0, -BV24-0, -BV26-0, -BV27-0, -BV28-0, -BV29-0, -BV30-0, -BV31-0. TCR-BJ1-1, -BJ1-2, -BJ1-3, -BJ1-4, -BJ1-5, -BJ1-6, -BJ1-7, -BJ2-1, -BJ2-2, -BJ2-3, -BJ2-4, -BJ2-5, -BJ2-7, -BJ1-5*1, -BJ1-5*2, -BJ1-5*3 (the last three use three different set of primers for the same J1-5 subtype). Human TCR subfamilies analyzed were TCR-BV01-01, BV02-01, BV04-01, -BV04-02, -BV04-03, -BV05-01, -BV05-03, -BV05-04, -BV05-05, -BV05-06, -BV05-08, -BV06-01, -BV06-04, -BV06-05, -BV06-06, -BV06-07, -BV06-08, -BV06-09, -BV07-02, -BV07-03, -BV07-04, -BV07-06, -BV07-07, -BV07-08, -BV07-09, -BV09-01, -BV10-01, -BV10-02, -BV10-03, -BV11-01, -BV11-02, -BV11-03, -BV12-01, -BV12-02, -BV12-05, -BV13-01, -BV14-01, -BV15-01, -BV16-01, -BV18-01, -BV19-01, -BV20-01, -BV21-01, -BV22-01, -BV23-01, -BV25-01, -BV27-01, -BV28-01, -BV29-01, -BV30-01 and TCR-BJ01-01, -BJ01-02, -BJ01-03, -BJ01-04, -BJ01-05, -BJ01-06, -BJ02-01, -BJ02-02, -BJ02-03, -BJ02-04, -BJ02-05, -BJ02-06, -BJ02-07. More detail is available on Adaptive's website at: http://adaptivebiotech.com/papers/nmed69531a.

Statistical analysis. We determined statistical significance in values between experimental groups using either two-way ANOVA wherever appropriate (followed by *post hoc* analysis using the Holm–Bonferroni method), or one-way ANOVA. The distribution and variance were normal and similar in all groups. We considered any $P > 0.05$ as not significant. In all mouse ear-swelling measurements and FACS experiments, we give values as mean of independent replicates ± s.d. Two-way ANOVA with Holm–Bonferroni *post hoc* analysis was used for **Figure 3b–g** and **Supplementary Figures 1d**, **3d**, **7d–f**,**h–i**,**n**, **8e–g** and **9b**. One-way ANOVA was used for **Figure 3h** and **Supplementary Figures 1b**, **5b**, **7g**,**i**,**k**,**m**,**o**, **8b–d**,**h** and **9c**,**d**. Each of these experiments was replicated at least once (i.e., performed twice), and typically replicated twice or more. Numbers of T cell clones cannot be assumed to follow a normal distribution, so we performed comparisons in **Supplementary Figure 6** by two-tailed Mann–Whitney *U* test.