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(12A12), phenylalanine (12A21), or arginine (1B2530 and 1NC9) (16, 26). Tryptophan substitution did not improve all HAADs that were tested (table S8); thus, increased potency requires a precise geometry.

Passive immunization and/or gene therapy to deliver HIV antibodies is increasingly being considered an option for prevention of HIV infection. To reduce the concentrations and numbers of antibodies required for protection to realistic and affordable levels, highly potent and broadly neutralizing antibodies are the reagents of choice for passive delivery. Although it is difficult to compare the potencies and breadth of antibodies characterized by using different virus panels, the natural form of NIH45-46 exhibits superior potency to VRC01 when compared against a panel of 82 tier 2 and 3 viruses representing all known HIV clades (16). A new set of HIV antibodies, the PGT antibodies that recognize the gp120 V3 loop and associated carbohydrates, exhibited median IC_{50} values up to 10-fold lower than VRC01 (18) but are less potent and broad than NIH45-46^{G54W} (Fig. 4B and table S9). The successful improvement of the potency and breadth of NIH45-46, already one of the most potent and broad of the currently available bNAbs (16), suggests that NIH45-46^{G54W} should be among a small number of characterized HIV antibodies considered for clinical testing. Because escape from even the best bNAbs is possible, optimal results may be achieved by using NIH45-46^{G54W} in combination with one or more other bNAbs.

Our results also have important implications for vaccine development; that is, the findings that contacts to the gp120 inner domain by NIH45-46 and increased interactions with the bridging sheet by NIH45-46^{G54W} improve the breadth and potency of a HAAD antibody suggest that immunogens used in vaccine strategies should include native inner domain/bridging sheet residues. Although this sort of immunogen may elicit some nonneutralizing CD4bs antibodies, raising the most potent and broad CD4bs antibodies will require contact with regions within the gp120 inner domain and bridging sheet in addition to the initial site of CD4 attachment on the gp120 outer domain.

References and Notes

1. A. S. Fauci *et al.*, *Science* **321**, 530 (2008).
2. M. J. McElrath, B. F. Haynes, *Immunity* **33**, 542 (2010).
3. G. B. Karlsson Hedestam *et al.*, *Nat. Rev. Microbiol.* **6**, 143 (2008).
4. A. J. McMichael, P. Borrow, G. D. Tomaras, N. Goonetilleke, B. F. Haynes, *Nat. Rev. Immunol.* **10**, 11 (2010).
5. S. Zolla-Pazner, T. Cardozo, *Nat. Rev. Immunol.* **10**, 527 (2010).
6. L. M. Walker *et al.*, *Science* **326**, 285 (2009); 10.1126/science.1178746.
7. J. F. Scheid *et al.*, *Nature* **458**, 636 (2009).
8. X. Wu *et al.*, *Science* **329**, 856 (2010); 10.1126/science.1187659.
9. L. Stamatatos, L. Morris, D. R. Burton, J. R. Mascola, *Nat. Med.* **15**, 866 (2009).
10. E. S. Gray *et al.*, *J. Virol.* **85**, 4828 (2011).
11. I. Mikell *et al.*, *PLoS Pathog.* **7**, e1001251 (2011).
12. A. Trkola *et al.*, *Nat. Med.* **11**, 615 (2005).

13. J. R. Mascola *et al.*, *Nat. Med.* **6**, 207 (2000).
14. A. J. Hessel *et al.*, *PLoS Pathog.* **5**, e1000433 (2009).
15. A. J. Hessel *et al.*, *Nature* **449**, 101 (2007).
16. J. F. Scheid *et al.*, *Science* **333**, 1633 (2011); 10.1126/science.1207227.
17. J. F. Scheid *et al.*, *J. Immunol. Methods* **343**, 65 (2009).
18. L. M. Walker *et al.*, *Nature* **477**, 466 (2011).
19. D. Corti *et al.*, *PLoS ONE* **5**, e8805 (2010).
20. M. Bonsignori *et al.*, *J. Virol.* **85**, 9998 (2011).
21. Q. J. Sattentau, R. A. Weiss, *Cell* **52**, 631 (1988).
22. M. Thali *et al.*, *J. Virol.* **67**, 3978 (1993).
23. B. Chen *et al.*, *Nature* **433**, 834 (2005).
24. J. M. Decker *et al.*, *J. Exp. Med.* **201**, 1407 (2005).
25. T. Zhou *et al.*, *Science* **329**, 811 (2010); 10.1126/science.1192819.
26. X. Wu *et al.*, *Science* **333**, 1593 (2011); 10.1126/science.1207532.
27. Materials and methods are available as supporting material on Science Online.
28. G. Nabel, P. Kwong, J. Mascola, *Philos. Trans. R. Soc. London Ser. B* **366**, 2759 (2011).
29. P. D. Kwong *et al.*, *Nature* **393**, 648 (1998).
30. N. Madani *et al.*, *Structure* **16**, 1689 (2008).
31. Taken from a crystal structure of Fab 21c plus CD4 domains 1 and 2 bound to the ZM135M.PL10a gp120 core at 2.45 Å (to be submitted to the PDB).
32. Geometric mean IC_{50} calculations were done without excluding resistant strains by entering values of 50 μ g/ml for strains with IC_{50} values >50 μ g/ml.
33. Y. Li *et al.*, *J. Virol.* **85**, 8954 (2011).

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Supporting Online Material

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Materials and Methods
Figs. S1 to S8
Tables S1 to S9
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The Intraepithelial T Cell Response to NKG2D-Ligands Links Lymphoid Stress Surveillance to Atopy

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Epithelial cells respond to physicochemical damage with up-regulation of major histocompatibility complex–like ligands that can activate the cytolytic potential of neighboring intraepithelial T cells by binding the activating receptor, NKG2D. The systemic implications of this lymphoid stress-surveillance response, however, are unknown. We found that antigens encountered at the same time as cutaneous epithelial stress induced strong primary and secondary systemic, T helper 2 (T_H2)–associated atopic responses in mice. These responses required NKG2D-dependent communication between dysregulated epithelial cells and tissue-associated lymphoid cells. These data are germane to uncertainty over the afferent induction of T_H2 responses and provide a molecular framework for considering atopy as an important component of the response to tissue damage and carcinogenesis.

A conserved feature of T lymphocytes is their subdivision into two main types. One, composed of $CD4^+$ and $CD8^+$ $\alpha\beta$ T cells specific for complexes of peptides and

major histocompatibility complex (MHC) molecules, has been termed “conventional” and is largely responsible for clonal, pathogen-specific memory responses that are the hallmark of adaptive immunity. The second T cell type, composed of cells expressing the $\gamma\delta$ T cell receptor (TCR), is not generally specific for peptide-MHC complexes and may instead recognize cell surface microbial and/or self-encoded moieties, some of which are up-regulated through cellular dysregulation. Although $\gamma\delta$ T cells exhibit some properties characteristic of adaptive immunity,

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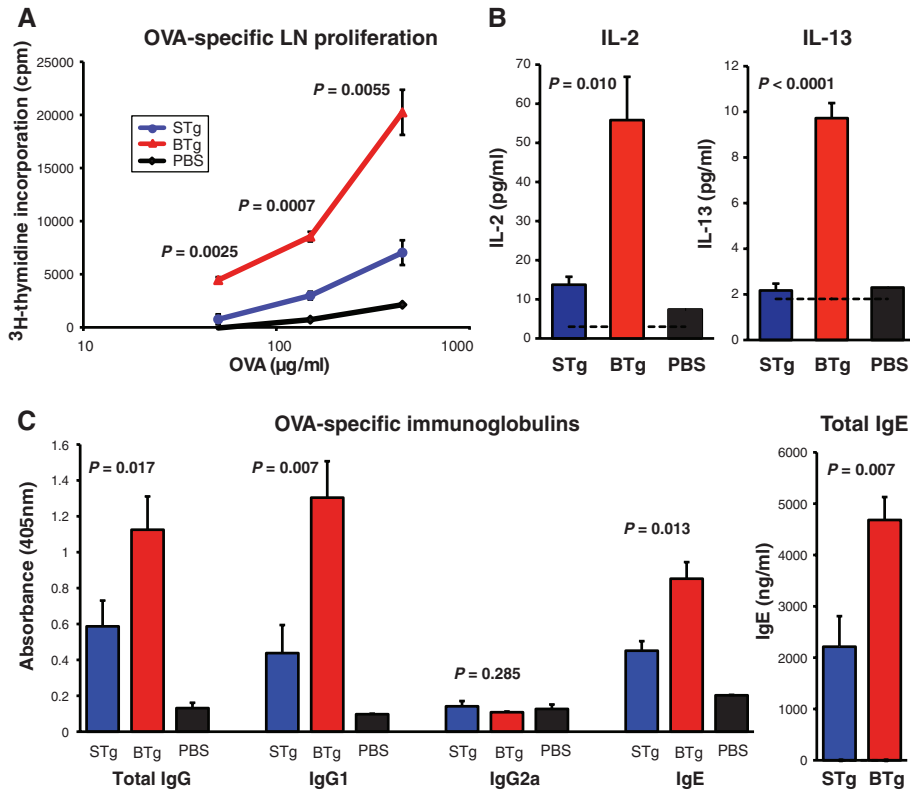


Fig. 1. Acute epidermal Rae-1 expression induces systemic immune effects. (A to C) Adjuvant-free ova was administered in phosphate-buffered saline (PBS) in epicutaneous patches to shaved areas of (red) BTg mice and (blue) STg mice dox-treated for 7 days. Ten days later, measurements were made of (A) skin draining LN ova-specific lymphocyte proliferation by means of [³H] thymidine incorporation; (B) of ova-dependent production of IL-2 and IL-13 by cells from draining LN, as measured with Luminex assay; and (C) of levels of serum ova-specific Ig and of total IgE. Black bars and lines show data for mice exposed to patches containing only PBS. Data expressed as mean ± 1 SEM; n = 6 to 8 mice per group, with experiment repeated on four independent occasions with comparable results.

a predominant function of these “unconventional” T cells is thought to be lymphoid stress-surveillance because they do not require major clonal expansion, because their functional potentials are pre-programmed during development, and because some may be activated in vivo independently of the TCR, by cytokines or by ligands for activating natural killer (NK) receptors, such as NKG2D (1). Such cells are major components of large but enigmatic tissue-resident T cell compartments, which include mouse dendritic epidermal T cells (DETCs) and human or mouse intraepithelial lymphocytes (IELs), among which CD4⁺CD8⁺αβ T cells may also contribute to rapid lymphoid stress-surveillance.

Because NKG2D ligands such as Rae-1 (mouse) and MHC class 1 chain-related protein A (MICA) (human) are activated by DNA damage (2) and are frequently expressed by tumor cells, such T cell responses—which have cytolytic potential—have been associated with local tumor surveillance. Indeed, DETC-deficient mice show increased susceptibility to chemical carcinogenesis (3, 4). However, whether lymphoid stress-surveillance describes a purely local response or whether it affects the systemic immune compartment remains a key question. To investigate this, transgenic Rae-1 expression was induced as described (4) specifically in keratinocytes by a doxycycline (dox)-dependent, bitransgenic (BTg) molecular switch (fig. S1A). This mode of Rae-1 induction avoids pleiotropic effects of applying agents that induce a stressed state within the epidermis. Rae-1 induction on otherwise normal epithelium promoted rapid morphological rearrangements of DETCs and of epidermal Langerhans cells (LCs), which are a tissue-associated dendritic

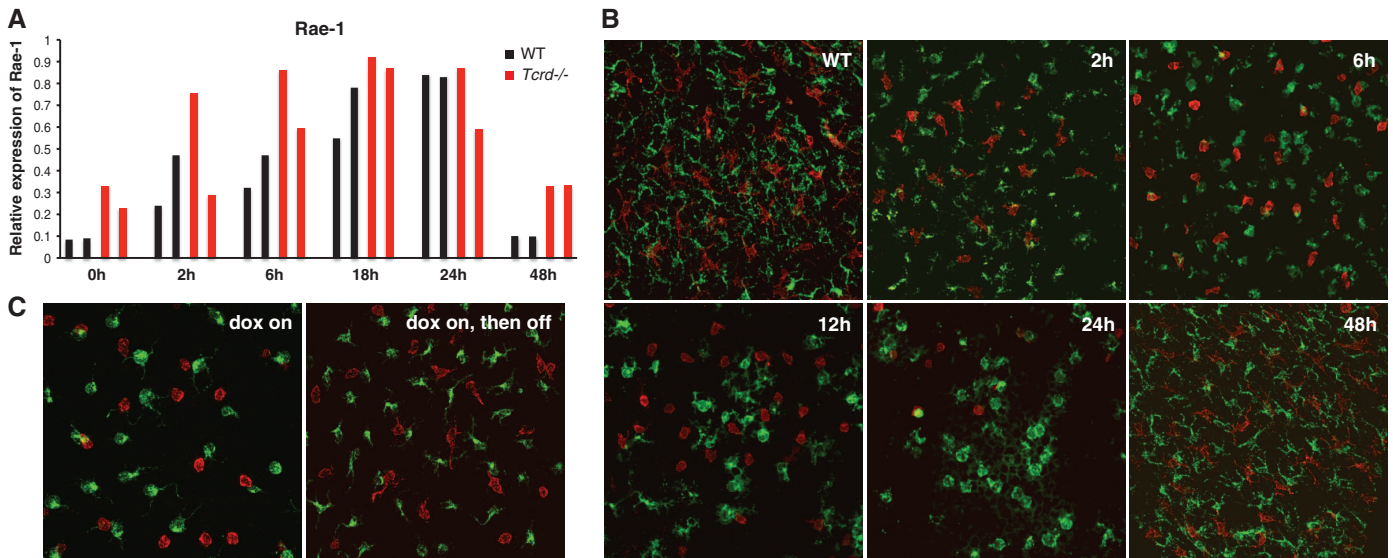


Fig. 2. Epidermal Rae-1 expression and local immune activation characterize the response to mild tissue abrasion. (A) Expression of endogenous Rae-1 mRNA in (black) WT and (red) *Tcrd*^{-/-} epidermis after tissue abrasion by tape-stripping. Quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed on isolated epidermis; each bar represents an individual mouse; data are expressed relative to the control gene cyclophilin. Six WT and four *Tcrd*^{-/-} mice

were analyzed per time-point in three separate experiments. (B) Representative confocal images show freshly isolated epidermal sheets from WT mice at various timepoints after tape-stripping: (red) DETCs and (green) LCs. (C) Confocal images show (red) DETCs and (green) LCs after 5 days on dox (left) and 3 days after withdrawal of dox (right). Original magnification was ×63 for (B) and (C). Micrographs are representative of analysis of 4 to 10 mice per condition.

cell (DC) subset lacking NKG2D (figs. S1B and S2) (4). Such changes were not seen in single transgenic (STg) mice that cannot induce dox-dependent Rae-1 up-regulation. While subject to acute Rae-1 up-regulation, patches containing highly purified ovalbumin (ova, a nominal nonself, non-microbe-associated antigen) or vehicle control were placed onto the skin of mice that had been gently shaved.

As in previous studies (5, 6) this provoked low but reproducible T helper 2 (T_H2) immune responses in STg mice, which were characterized by low levels of interleukin 4 (IL-4) and IL-13 and IL-13-dependent induction of ova-specific and generalized immunoglobulin G1 (IgG1) and IgE (5). In contrast, BTg mice showed significant increases in responsiveness, as judged by ova-specific proliferation of lymph node (LN) and splenic T cells (Fig. 1A and fig. S3); ova-dependent

LN IL-2 and IL-13 production (Fig. 1B); ova-specific IgG, IgG1, and IgE; and total IgE, which is customarily and substantially up-regulated in the context of antigen-specific IgE responses (Fig. 1C). Thus, localized epithelial up-regulation of a single self stress-antigen (Rae-1) can powerfully enhance the response to coincidentally encountered antigen. This “adjuvant-effect” amplified but did not dysregulate the immune response because there was no atypical induction of IgG2a.

The implication of these data is that lymphoid stress-surveillance of tissue damage may be a substantial driver of T_H2 responses developing during cutaneous antigen exposure. To investigate this, mild epidermal abrasion was induced in wild-type (WT) mice by means of tape-stripping, which transiently removes the outer dead cell layer—the stratum corneum—while leaving un-

derlying keratinocytes largely intact. The epidermis returns to normal within 48 hours (fig. S4). Such treatment mimics shaving and depilatory treatments in humans. Tape-stripping is known to activate LCs (6, 7), but whether it also induces a lymphoid stress-surveillance response is unknown. Rae-1 RNA was up-regulated in the epidermis within 2 hours, peaking at 18 to 24 hours, and returning toward baseline by 48 hours, which is consistent with morphological tissue repair (fig. S5). Rae-1 up-regulation was comparable in WT and TCR δ -deficient (*Tcrd*^{-/-}) mice, as expected for an event upstream of DETC activation (Fig. 2A). Consistent with the consequences of Rae-1 up-regulation seen in BTg mice, tape-stripping was followed by coincident rounding of DETCs and LCs and an aggregate loss of DETCs from the suprabasal and basal layers of the epidermis (Fig. 2B). The cellular changes

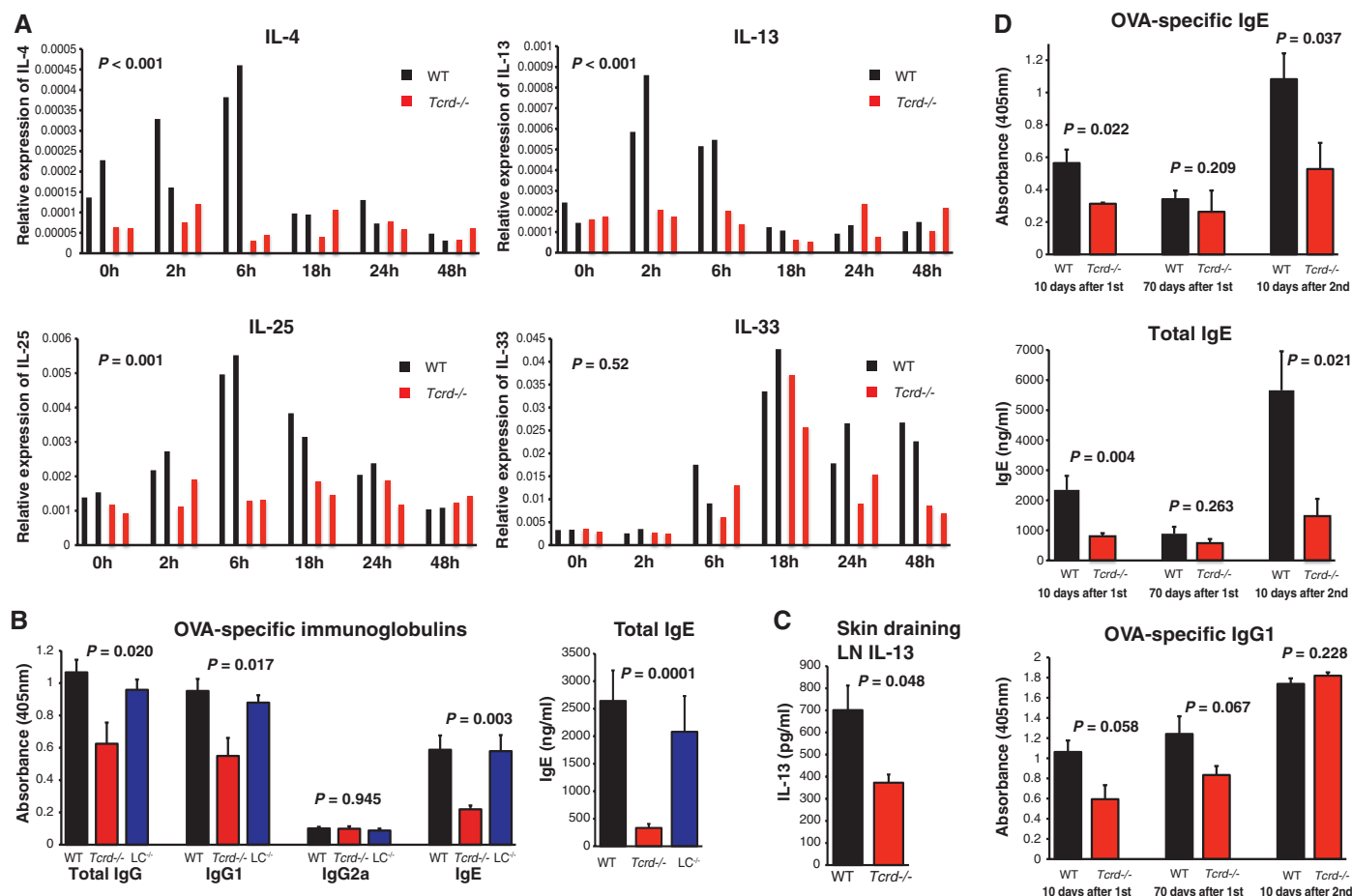


Fig. 3. Local stress-surveillance responses to mild abrasion are linked to $\gamma\delta$ T cell-dependent initiation of Type-2 responses. (A) Quantitative RT-PCR analysis of mRNA expression for the indicated cytokines over a 48-hour period after tape-stripping in (black) WT and (red) *Tcrd*^{-/-} epidermis. Data are expressed relative to the control gene cyclophilin; *P* values refer throughout the figure to statistically significant differences between WT and *Tcrd*^{-/-} mice. Two representative mice of each genotype are shown per timepoint. The entire experiment was repeated three times for WT mice ($n = 6$ mice per timepoint) and two times for *Tcrd*^{-/-} mice ($n = 4$ mice per timepoint). (B and C) After shaving, ova was applied as in Fig. 1 to dorsal skin of (black) WT, (red) *Tcrd*^{-/-}, or (blue) LC-deficient (LC^{-/-},

Langerin-DTA mice) mice. The Ig response (B) was assayed 10 days after primary immunization and IL-13 secretion from skin draining LN cells (C) after further 72 hours in vitro restimulation with ova, all performed by means of enzyme-linked immunosorbent assay (ELISA). Data are expressed as mean $\pm$ 1 SEM, and $n = 6$ to 8 mice per group. Experiments (B) and (C) were repeated independently six times for *Tcrd*^{-/-} and WT mice and twice for LC^{-/-} mice, with comparable results. (D) *Tcrd*^{-/-} (red) and WT (black) mice were rested for 70 days after primary epicutaneous antigen exposure; re-bled; reexposed to an ova-containing patch as described; and re-bled 10 days later. Ig responses were measured with ELISA. Data expressed as mean $\pm$ 1 SEM, and $n = 8$ mice per group.

in tape-stripped and BTg mice were rapidly reversed as Rae-1 expression declined (Fig. 2, B and C). In sum, mild cutaneous abrasion provokes the initial events of lymphoid stress-surveillance.

To define abrasion-induced lymphoid stress-surveillance, we analyzed local gene expression changes in sets of age- and gender-matched WT and *Tcrd*^{-/-} mice. As reported, after epithelial stress (8, 9) several cytokine mRNAs commonly attributed to epithelial cells were up-regulated, including IL-1, IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) (Fig. 3A and fig. S6). mRNAs for some T cell- and DC-associated interleukins—notably, IL-4, IL-13, interferon- γ (IFN γ), and IL-10—were also up-regulated. Such up-regulation of Rae-1 and T_H2 cytokines was likewise observed after careful shaving of mice (fig. S7).

The response to tape-stripping was radically different in *Tcrd*^{-/-} mice: Whereas IL-10 and TSLP up-regulation were unaffected, IL-4, IL-13, and

IL-25 RNAs were not induced, and those of IL-33 and IL-1 β less durable (Fig. 3A and fig. S6). Collectively, these data establish that $\gamma\delta$ T cells are required for the normal local induction of specific cytokines commonly associated with T_H2 responses to antigens encountered at epithelial surfaces. This conclusion was in part supported by newly generated lines of BTg mice with dox-inducible expression of either Rae-1 or H60c, another NKG2D ligand (10). When TCR $\gamma\delta$ ⁺ DETCs, LCs, and keratinocytes were purified from post-induction epidermis, rapid increases in IL-13 mRNA were specifically shown by DETCs (fig. S8), attesting to the rapid functional activation of the local T cell compartment in vivo. Moreover, because NKG2D ligands are in this case only transiently up-regulated on suprabasal keratinocytes in the absence of any other perturbation, local IL-13 up-regulation can be attributed to intrinsic lymphoid stress-surveillance, rather than

to obligate disruption of the epidermal barrier, as is often invoked (7, 11).

To determine whether such responses of $\gamma\delta$ T cells translated to effects on systemic immunity, shaved mice were exposed to ova-containing patches, as described. As before, there was substantial induction of ova-specific IgG, IgG1, and IgE, and of total IgE. However, whereas such responses were enhanced in BTg mice, they were significantly reduced in *Tcrd*^{-/-} mice that cannot mount epidermal lymphoid stress-surveillance (Fig. 3B). Again, there was no atypical induction of IgG2a.

Consistent with this, skin-draining LN cells from *Tcrd*^{-/-} mice produced significantly less ova-dependent IL-13 than did WT mice (Fig. 3C). Parallel treatment of other strains showed, as predicted, that $\alpha\beta$ T cells were required for IgG and IgE specific for antigen encountered during mild cutaneous abrasion, whereas less predictably congenital LC deletion had negligible impact (Fig. 3B and fig. S9). As more experiments were undertaken, it became clear that the dependence of ova-specific IgG and IgG1 on $\gamma\delta$ T cells was less than the dependence on $\alpha\beta$ T cells and did not always reach statistical significance (Fig. 3D and fig. S10); conversely, $\gamma\delta$ T cells were invariably important for IgE induced in this manner. Moreover, the characteristic induction of nonspecific IgE was fully ablated in *Tcrd*^{-/-} mice and in *Tcrb*^{-/-}*Tcrd*^{-/-} double-knockout mice (fig. S9). That $\gamma\delta$ T cells are a driving force for such IgE production was evident in the finding that *Tcrb*^{-/-} mice carry abnormally high levels of non-specific IgE, as was previously reported for *Tcrd*^{-/-} mice (12). Thus, the $\gamma\delta$ T cell compartment has a profound effect on the production of antigen-specific and total IgE after antigen exposure at mildly stressed epithelium.

T_H2 in comparison with T_H1 cell differentiation is often regarded as de facto irreversible because of the positive feedback loop established by GATA3 expression (13). Hence, the impact of stress-surveillance on primary T_H2-IgE responses may have long-term implications for T_H2-immunity. To examine this, WT and *Tcrd*^{-/-} mice were exposed to ova as before and then reexposed 70 days after initial challenge. Again, $\gamma\delta$ T cells were required for WT levels of ova-specific IgE induction (Fig. 3D). After 70 days, ova-specific and total IgE levels in WT mice had returned to normal. Although they were substantially boosted in mice reexposed to antigen at day 70 and reexamined 10 days later, this secondary response was greatly impaired in *Tcrd*^{-/-} mice (Fig. 3D). In contrast, ova-specific IgG1 had remained elevated 70 days after challenge and was boosted comparably in WT and *Tcrd*^{-/-} mice, again segregating the impact of lymphoid stress-surveillance on IgE and IgG1, respectively. In sum, the magnitude of antigen-specific IgE responses to antigen repetitively encountered at the skin is regulated by $\gamma\delta$ T cells.

To test whether this dependence of IgE up-regulation on $\gamma\delta$ T cells reflects its origin in

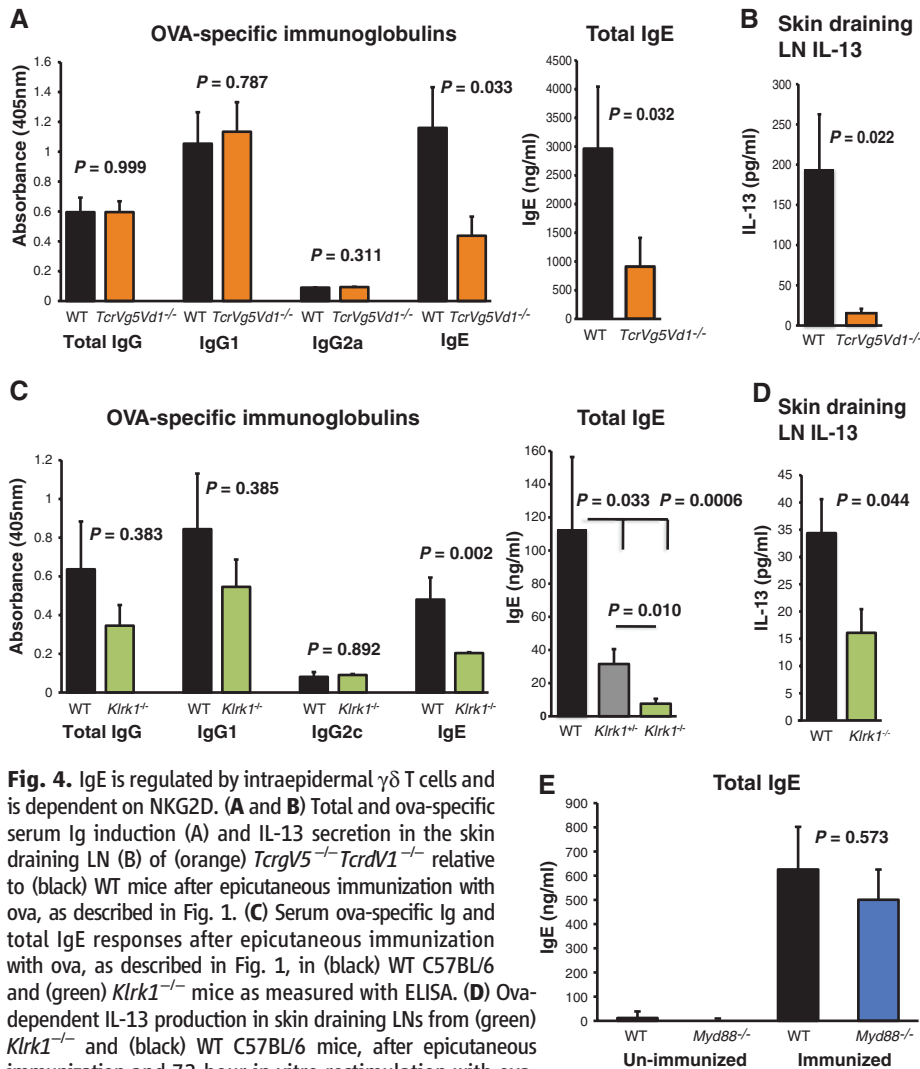


Fig. 4. IgE is regulated by intraepidermal $\gamma\delta$ T cells and is dependent on NKG2D. (A and B) Total and ova-specific serum Ig induction (A) and IL-13 secretion in the skin draining LN (B) of (orange) *TcrVg5*^{-/-}*TcrVd1*^{-/-} relative to (black) WT mice after epicutaneous immunization with ova, as described in Fig. 1. (C) Serum ova-specific Ig and total IgE responses after epicutaneous immunization with ova, as described in Fig. 1, in (black) WT C57BL/6 and (green) *Klrk1*^{-/-} mice as measured with ELISA. (D) Ova-dependent IL-13 production in skin draining LNs from (green) *Klrk1*^{-/-} and (black) WT C57BL/6 mice, after epicutaneous immunization and 72-hour in vitro restimulation with ova, determined with ELISA. Data are expressed as mean $\pm$ 1 SEM, and n = 6 to 10 mice per group. All experiments [(A) to (D)] were repeated twice with comparable results. (E) Total serum IgE response in (black) WT C57BL/6 and (blue) *Myd88*^{-/-} mice 10 days after primary epicutaneous immunization, as described in Fig. 1, or in unimmunized mice. Data are expressed as mean $\pm$ 1 SEM, and n = 4 unimmunized mice and n = 7 immunized mice per group.

lymphoid stress-surveillance, the studies were repeated in mice deficient in TCRV δ 1V δ 5 that lack only the normal DETC repertoire (4). Such mice showed substantially impaired ova-specific and total IgE; consistent with which, almost no ova-dependent IL-13 production could be evoked from skin-draining LN cells (Fig. 4, A and B). In these studies, there was no substantial impact on IgG1. Moreover, germane to the greater impact of local T cells on IgE versus IgG1, total IgE was already greatly up-regulated in WT mice (but not *Tcrd*^{−/−} mice) by day 7, whereas IgG1 up-regulation was not seen until day 10 (fig. S10). Consistent with IgE induction being substantially a product of lymphoid stress-surveillance, its up-regulation was significantly impaired in mice lacking the NKG2D receptor that mediates DETC responses to local Rae-1 up-regulation. Again, there was less impairment of IgG and IgG1 responses. There was an intermediate impairment of IgE responses in mice heterozygous for the gene that encodes NKG2D (*Klrk1*) (Fig. 4C). IL-13 production from skin-draining LN was also greatly reduced in *Klrk1*^{−/−} mice (Fig. 4D). Complementing these deficiencies in IgE production in NKG2D-deficient mice, IgE was readily detected in WT mice in squamous cell carcinomas that overexpress Rae-1 (fig. S11) (3, 4).

Although epithelial dysregulation has previously been linked to atopy—as in the genetic association of atopic dermatitis with mutations in the epidermal structural protein, filaggrin (14)—this has most often been attributed to the effects of microbes gaining access to local DCs, such as LCs, by way of barrier disruption (7, 11). However, shaving and ova-patching of WT mice and mice lacking MyD88, which transduces signals from bacteria-responsive Toll-like receptors (TLRs), induced comparable levels of Rae-1, IL-25, IL-13, and IgE (Fig. 4E and fig. S12), which is consistent with the proposal that these responses are initiated by epithelial dysregulation per se.

Given the critical role of IL-13 in epicutaneously induced T_H2 responses, and its strong association with clinical allergies, it is noteworthy that in comparison to conventionally polarized TCR $\alpha\beta$ ⁺ T_H2 cells, DETC activated in vitro or in vivo express an excess of IL-13 mRNA rela-

tive to IL-4 mRNA (fig. S13). Moreover, such cells' regulation of stress-dependent epithelial expression of IL-25, IL-33, and IL-1 seems also germane to the afferent promotion of T_H2 responses. Given that NKG2D-expressing mouse and human systemic $\gamma\delta$ cells and NKT cells also co-produce IL-13 and IFN γ (15, 16), and have been locally implicated in human allergic disease (17, 18), there may be multiple cellular mechanisms for stress-initiated T_H2 responses.

Although the same factors regulate B cell class-switching from IgM to IgE or to IgG1 (mouse) or IgG4 (human), an alternative, recently elucidated pathway favoring IgE class-switching (19) might accommodate the preferential induction of IgE by NKG2D-dependent stress-surveillance shown here and the germinal center-independent induction of IgE in $\alpha\beta$ T cell-deficient and germ-free mice (12, 20). In parallel, increasing tissue damage would expose DCs to IL-1 cytokines and/or to microbes, promoting conventional CD4⁺ $\alpha\beta$ T cell help for antigen-specific IgG1 responses. This can explain prior implications of TLRs in atopy (11, 21); the $\gamma\delta$ T cell-independence of IgG responses in multiply shaved and tape-stripped mice repeatedly exposed to antigen (22); and the reduced induction of antigen-specific IgG in shaved and ova-patched *Myd88*^{−/−} mice (fig. S14).

The humoral component of stress-surveillance may limit tissue damage by targeting foreign moieties, such as toxins, that are root causes of tissue dysregulation. The IgE effector response could promote toxin expulsion and (by reducing blood pressure) limit their systemic dissemination, the collateral cost being the IgE response to benign antigens co-encountered with tissue disruption (23). This linkage of lymphoid stress-surveillance and atopy evokes a long-standing implication in allergy of tumor-responsive NKT cells (24) and a large epidemiologic meta-analysis and a large Swedish cohort study, both asserting an inverse relationship, albeit weak, between IgE production and carcinogenesis (25). It also raises the possibility that increasing frequencies of atopic allergy might result from increased exposure to environmental toxins, rather than simply a paucity of environmental microbes.

References and Notes

1. A. C. Hayday, *Immunity* **31**, 184 (2009).
2. S. Gasser, S. Orsulic, E. J. Brown, D. H. Raulet, *Nature* **436**, 1186 (2005).
3. M. Girardi *et al.*, *Science* **294**, 605 (2001).
4. J. Strid *et al.*, *Nat. Immunol.* **9**, 146 (2008).
5. C. A. Herrick, L. Xu, A. N. McKenzie, R. E. Tigelaar, K. Bottomly, *J. Immunol.* **170**, 2488 (2003).
6. J. Strid, J. Hourihane, I. Kimber, R. Callard, S. Strobel, *Eur. J. Immunol.* **34**, 2100 (2004).
7. T. Nishijima *et al.*, *J. Invest. Dermatol.* **109**, 175 (1997).
8. L. C. Wood, S. M. Jackson, P. M. Elias, C. Grunfeld, K. R. Feingold, *J. Clin. Invest.* **90**, 482 (1992).
9. M. K. Oyoshi, R. P. Larson, S. F. Ziegler, R. S. Geha, *J. Allergy Clin. Immunol.* **126**, 976, 984, e1 (2010).
10. A. Takada *et al.*, *J. Immunol.* **180**, 1678 (2008).
11. T. A. Le *et al.*, *Int. Arch. Allergy Immunol.* **151**, 31 (2011).
12. L. Wen *et al.*, *Nature* **369**, 654 (1994).
13. W. Ouyang *et al.*, *Immunity* **12**, 27 (2000).
14. C. N. Palmer *et al.*, *Nat. Genet.* **38**, 441 (2006).
15. D. Vermijlen *et al.*, *J. Immunol.* **178**, 4304 (2007).
16. A. Bendelac, P. B. Savage, L. Teyton, *Annu. Rev. Immunol.* **25**, 297 (2007).
17. W. H. Wu *et al.*, *J. Allergy Clin. Immunol.* **126**, 290, 299, e1 (2010).
18. C. Iwamura, T. Nakayama, *Curr. Opin. Immunol.* **22**, 807 (2010).
19. T. Zhang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3040 (2010).
20. K. D. McCoy *et al.*, *Immunity* **24**, 329 (2006).
21. H. Hammad *et al.*, *Nat. Med.* **15**, 410 (2009).
22. A. L. Woodward *et al.*, *J. Allergy Clin. Immunol.* **107**, 359 (2001).
23. M. Profet, *Q. Rev. Biol.* **66**, 23 (1991).
24. M. Lisbonne *et al.*, *J. Immunol.* **171**, 1637 (2003).
25. M. Van Hemelrijc *et al.*, *Cancer Causes Control* **21**, 1657 (2010).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/334/6060/1293/DC1
Materials and Methods
Figs. S1 to S14
References (26–34)

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Supporting Online Material for

**The Intraepithelial T Cell Response to NKG2D-Ligands Links
Lymphoid Stress Surveillance to Atopy**

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This PDF file includes:

Materials and Methods

Figs. S1 to S14

References (26–34)

Materials and Methods

Mice. Mice expressing the reverse tetracycline-responsive transactivator domain under control of the epidermis-specific involucrin promoter were intercrossed with mice bearing Rae-1 or H60c transgenes under control of a tetracycline-dependent response element. Expression of Rae-1 or H60c was induced in offspring bi-transgenic (BTg) for the reverse tetracycline-responsive transactivator domain and tetracycline-dependent response element by administration of 3 mg of doxycyclin (Lillico) per gram of solid food (4). FVB/N LC-deficient (Langerin-DTA) mice were generated by using a transgene encoding the human Langerin promoter to drive expression of diphtheria toxin, as previously described (26). *Tcrd*^{-/-} (27), *Tcrb*^{-/-} (28), *Tcrq-V5*^{-/-} (29) and *Tcrd-V1*^{-/-} (30) mice were backcrossed onto the FVB/N background for >10 generations. *Tcrq-V5*^{-/-}*Tcrd-V1*^{-/-} and *Tcrdb*^{-/-} mice were generated by intercrossing the respective mutants. FVB/N mice (Jackson Laboratories) were bred for use as controls. *Klrk1*^{-/-} mice were generated as previously described (31) and *Klrk1*^{+/-} and C57BL/6 wild type mice used as controls. *Myd88*^{-/-} mice were generated as described (32) and C57BL/6 wild type mice used as controls. Mice were maintained under specific pathogen-free conditions and food and water provided *ad libitum*. Mice were used at ≥6 weeks of age. All studies complied with institutional guidelines and the UK Home Office for Laboratory Animal Care regulations.

Epicutaneous immunization. The upper backs of mice were closely shaved with surgical blades and the mice rested for 24h. A single patch (Johnson & Johnson) containing 100 µg endotoxin-free OVA (extracted from chicken eggs as described in (33)) in PBS, or PBS alone for control mice, was then fixed to the mouse back using Mastisol liquid adhesive. Patches detached naturally, usually within 4 days. Primary immune responses following epicutaneous immunizations were assayed on day 10 after application of the patch. For assay of memory responses, a second patch was applied similarly after re-shaving 70 days after the primary patch and secondary immune responses determined 10 days later.

Tape-stripping. The stratum corneum was removed from both sides of the earlobe by application and removal of cellophane tape (Scotch™) five to eight times. Skin was collected for examination at various time-points after tape-stripping.

Microscopy. Epidermal sheets were prepared from mouse ears that had been split into dorsal and ventral sides and floated dermal side down for 2h at 37°C in 20 mM EDTA. Epidermal sheets were gently lifted from the dermis, washed in PBS, and fixed in cold acetone for 20 min at -20°C. After washing in PBS, epidermal sheets were incubated for 1h at room temperature with 2% (wt/vol) BSA in PBS and were stained overnight at 4°C with antibodies specific for TCRγδ (GL3; BioLegend), MHCII (I-A/I-E; 2G9; BD PharMingen), Rae-1 (186107; R&D Systems) or isotype controls. Sheets were washed thoroughly in PBS and bound antibodies were detected for 90 min at 37°C with goat anti-hamster 546 and goat anti-rat 555 or FITC (all from Invitrogen) in PBS containing 1% (wt/vol) BSA. After extensive washing, epidermal sheets were mounted onto slides with antifade mounting medium (Vector). For microscopic analysis of

epidermal Rae-1 induction and skin histology, whole ear or back skin was embedded in OCT medium (Sakura Finetek) and snap-frozen in liquid nitrogen and t-Butanol (Sigma). 6 μm cross-sections were prepared using a Leica JUNG CM1800 cryostat and stored at -80°C . When defrosted, slides were air-dried for ~ 15 min, the sections encircled with an ImmEdge pen (Vector) and left to dry. Sections were then fixed in acetone for 5 min at -20°C and subsequently rehydrated for 7 min in 0.02M PBS containing 0.15M NaCl. Following blocking with 0.02M PBS containing 3% BSA and 0.15M NaCl for 1h at room temperature, sections were incubated with monoclonal antibodies against Rae-1 (186107) for 1h at room temperature. Sections were washed three times for 5 min in 0.02M PBS containing 0.15M NaCl and antibody binding was visualized by incubating the sections with goat anti-rat 555 (Invitrogen) for 1h at room temperature. Sections were washed three times for 5 min in 0.02M PBS containing 0.15M NaCl and mounted with a cover slip using anti-fade mounting medium with DAPI (Vector). Analysis of whole epidermal sheets and sections was performed using a Leica TCS SP2+AOBS confocal laser scanning microscope with digital processing using LCS v2.02 (Leica). Skin sections were additionally stained for hematoxylin and eosin and histology examined using a Nikon Microphot-sa microscope (Nikon) with Openlab (Improvision) software.

T cell proliferation and cytokine production. Draining axillary LN and spleen cell suspensions were obtained by mechanical disaggregation, and 2×10^5 cells cultured in 96-well flat-bottom plates (Nunc) in a total volume of 200 μl RPMI 1640 medium supplemented with 10% FCS, 50 μM 2-ME and 5 $\mu\text{g}/\text{ml}$ gentamycin. OVA (Grade V, Sigma) was added at concentrations ranging from 5 to 450 $\mu\text{g}/\text{ml}$. Control responses to an irrelevant antigen (BSA) or concanavalin A at 1 $\mu\text{g}/\text{ml}$ were also determined. Cultures were incubated at 37°C for 90h and pulsed with 1 μCi [^3H]thymidine (Amersham Pharmacia) for the last 16h. Cells were harvested and thymidine incorporation determined by liquid scintillation counting on a MicroBeta (Wallac).

Supernatants were assayed initially using a Luminex multiplex kit (Millipore) according to manufacturer's specifications, and samples analysed on a Luminex100 machine (cytokine data in Fig. 1 assayed using Luminex). Cytokines were analysed in duplicate from all supernatants (2×10^5 cells) and standard curves for each cytokine were generated using the reference cytokine concentrations supplied by the manufacturer. Raw data (mean fluorescent intensity) were then analysed using Star-Station software to obtain concentration values. The detection limit of the Luminex assay was 1.8 pg/ml . IL-13 cytokine analysis was additionally routinely performed using a DuoSet ELISA Development System (R&D Systems) according to manufacturer's instructions (IL-13 data in Fig. 3 and 4 assayed using DuoSet ELISA with supernatants collected from 5×10^6 (Fig. 3) or 2×10^6 (Fig. 4) cells). The detection limit of the IL-13 ELISA was 15 pg/ml .

Antibody responses. At the end of each experiment, mice were bled by cardiac puncture and sera prepared for specific antibody determinations. For IgG, IgG1 and IgG2a antibodies, 96-well Maxisorb plates (Nunc) were coated with OVA protein at 250 $\mu\text{g}/\text{ml}$ in carbonate-bicarbonate buffer at 4°C overnight. The plates were then blocked with 1% goat (total IgG) or rat (IgG1 and IgG2a) serum for 1h at 37°C . Following washing, appropriately diluted sera (100 μl in PBS) were added and the plates incubated at 37°C for 90 min. After washing, alkaline phosphatase-conjugated polyclonal goat anti-

mouse IgG Fc (Sigma), rat monoclonal anti-mouse IgG1 or IgG2a (also detects IgG2c in C57BL/6 mice) (both PharMingen) were added for 1h at 37°C. The alkaline phosphatase substrate pNPP (Sigma) was then added following further washing and absorbance measured at 405 nm. Antigen-specific and total IgE was measured by an IgE capture method. Sera to be tested and IgE standard were added to Maxisorb microtiter plate wells coated with 1 µg/ml rat monoclonal anti-mouse IgE (PharMingen) and blocked with 3% milk (Marvel) (for specific IgE) or 1% rat serum (for total IgE). Biotinylated OVA at a concentration of 100 µg/ml or biotinylated rat monoclonal anti-mouse IgE (PharMingen) at 1 µg/ml were then added and incubated for 2h at 37°C. After washing, alkaline phosphatase-streptavidin (PharMingen) was added for 1h followed by pNPP substrate.

Quantitative real-time PCR. Ears or back skin were collected and epidermis was isolated by floating skin dermal side down on 0.5M NH₄SCN in PBS for 40 min at 37°C. Epidermis and dermis was then separated using a dissection microscope and stored in RNA-later. The tissue was later lysed by a Qiagen TissueLyser II (Retsch) and total RNA isolated using RNeasy kit (Qiagen). Purified RNA was treated with RNase-free DNase (Promega) and reverse-transcribed with SuperScript II RT polymerase (Invitrogen) before amplification with primer pairs described below. Real-time quantitative PCR was performed on duplicate cDNA samples using SYBR Green Master Mix (Applied Biosystems) with forward and reverse primers at a final concentration of 300 nM in a reaction volume of 20 µL. Gene-specific PCR products were continuously measured by the 7900HT Fast Real-Time PCR System (Applied Biosystems) during 40 cycles at 60°C. Following primers were used: Rae-1: F TGGACACTCACAAGACCAATG, R CCCAGGTGGCACTAGGAGT, IL-1a: F TTGGTTAAATGACCTGCAACA, R GAGCGCTCACGAACAGTTG, IL1-β: F TTGACGGACCCCAAAAGAT, R GAAGCTGGATGCTCTCATCTG, IL-4: F CATCGGCATTTTGAACGAG, R CGAGCTCACTCTCTGTGGTG, IL-10: F GCCCAGAAATCAAGGAGCATT, R TGCTCCACTGCCTTGCTCTTA, IL-13: F GCTTATTGAGGAGCTGAGCAACA, R GCCAGGTCCACACTCCATA, IL-25: F TGGAGCTCTGCATCTGTGTC, R GATTCAAGTCCCTGTCCAATC, IL-33: F CACATTGAGCATCCAAGGAA, R AACAGATTGGTCATTGTATGTACTCAG, TSLP: F TCGAGGACTGTGAGAGCAAG, R TGTTTTGTGGGGAGTGAA. Products of these PCR amplifications were normalized to products of amplification of K5: F CCTGCAGAAGGCCAAGCA, R TGGTGTTTCATGAGCTCCTGGTA, cyclophilin: F CAAATGCTGGACCAAACACAA, R CCATCCAGCCATTCAGTCTTG, and GAPDH: F ACTCCACTCACGGCAAATTCA, R GCCTCACCCCATTTGATGTT. Data were evaluated relative to these reference genes using the 2^{-ΔΔCT} method.

Flow cytometry and cell sorting. Epidermis of BTg and STg mice was collected as above and dissociated into a single-cell suspension as previously described (4). Cell suspensions were blocked for 15 min on ice with 2.5 µg antibody to FcR (anti-FcR; CD16-CD32) in PBS containing 2% (vol/vol) FCS. The cell suspensions were then labeled with anti-MHCII and anti-CD45 antibodies (BD PharMingen) on ice, and sorted for DETC (CD45 single-positives), LC (CD45 and MHCII double-positives) and keratinocytes (double-negatives) on a MoFlo cell sorter (Beckman Coulter). Electronic gates were set on live cells with a combination of forward- and side-scatter properties and

7-amino- actinomycin D (Calbiochem) exclusion. The sorted cells were immediately lysed and the RNA extracted for qRT-PCR analysis using the Qiagen RNAeasy Mini Kit (Qiagen).

DETC culture. Epidermal cell suspensions were prepared from shaved body-wall skin by trypsinization as described (4) and stimulated with 1ug/ml anti-TCR δ antibody (GL3; BioLegend) for 48h. The cells were then removed from stimulation and cultured for a further 12 days in complete RPMI supplemented with IL-2. The cultures were analyzed by FACS for purity, and then stimulated at 200,000 cells/well with varying concentrations of Rae1-Fc fusion protein, Fc fusion control (both R&D Systems) and/or anti-TCR δ antibody (GL3). After 48h stimulation, supernatants were collected and analyzed by ELISA and the cells lysed and RNA extracted for qRT-PCR analysis.

Statistical evaluation. The statistical significance of differences between most experimental groups was determined with a two-tailed Student's t-test for unpaired data. For analysis of differences between *Klrk1*^{-/-}, *Klrk1*^{+/-} and WT C57BL/6 mice a nonparametric Mann Whitney test was performed. For analysis of differences between the WT and *Trcd*^{-/-} RT-PCR data, groups were compared using ANCOVA with the response modeled as polynomial functions of time. For each PCR product the significance of differences between groups was estimated using the smallest p-value for the differences between the polynomial coefficients. Calculations were performed in R (34). For all statistical evaluation, differences were considered significant at the p<0.05 level.

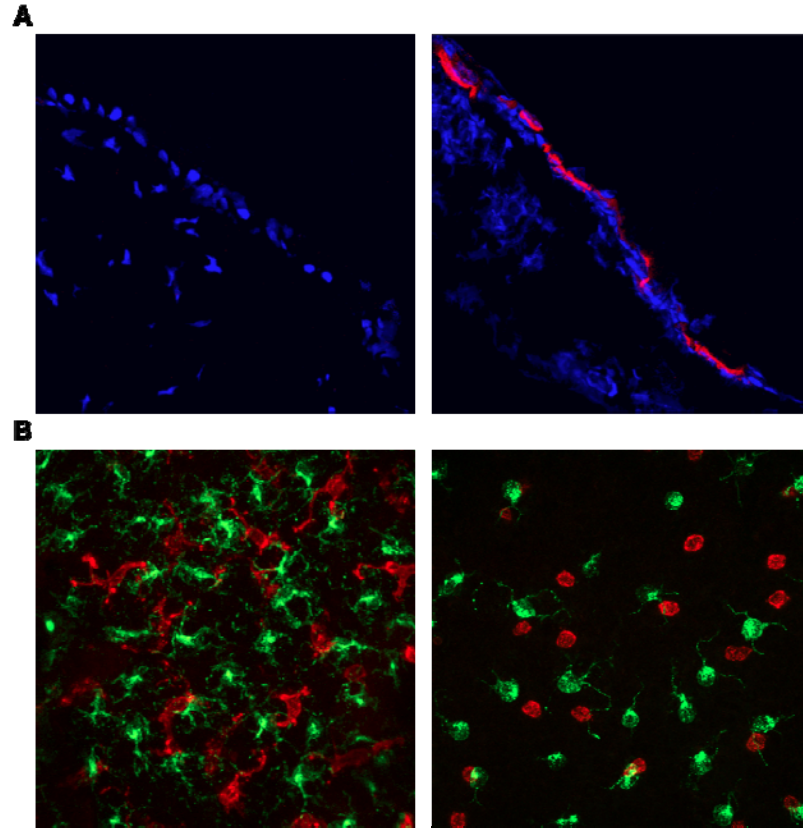


Fig. S1. Acute epidermal Rae-1 expression causes local immune activation

(A) Photomicrographs of transgenic Rae-1 expression [red] in dorsal epidermis of STg (control) mice [left] and BTg mice [right], both after doxycycline (dox) treatment and co-stained for nuclei using DAPI (blue). (B) TCRγδ⁺ DETC (red) and MHCII⁺ LC (green) in freshly isolated whole epidermal sheets of STg control mice [left] and BTg mice [right], both treated with dox for 5 days. Original magnification x63. Micrographs are representative of analysis of ≥ 10 mice per condition.

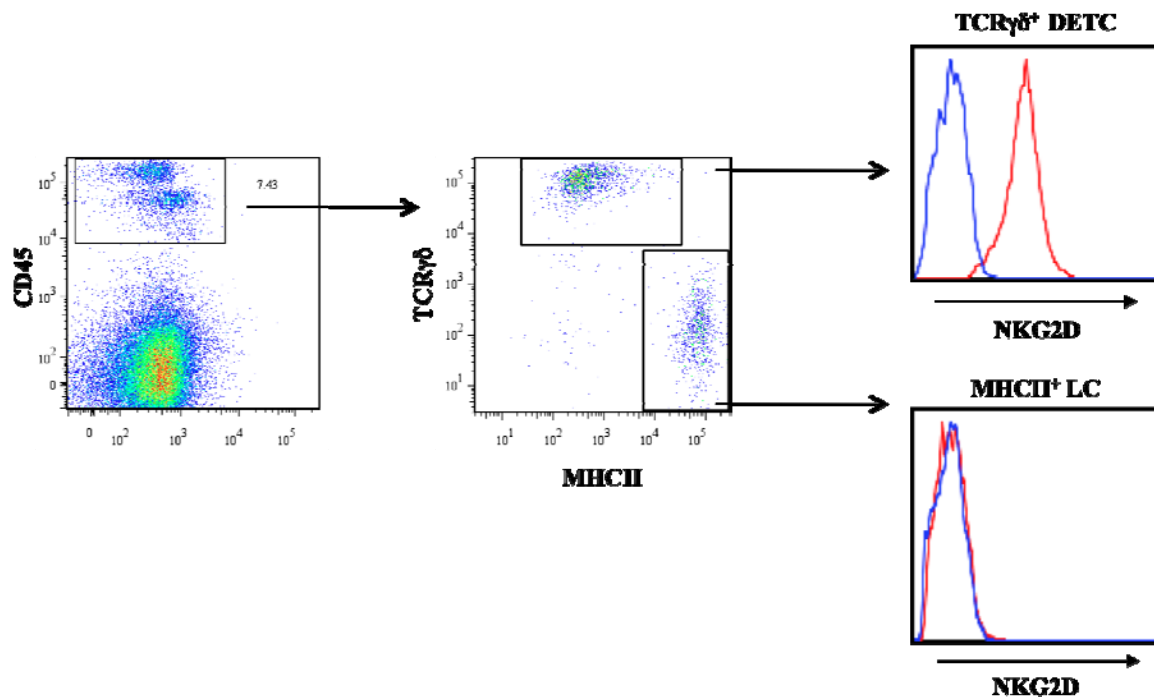


Fig. S2. DETC are the only NKG2D expressing cells in the epidermis

Whole epidermal cell-suspension were prepared and co-stained for CD45, TCRγδ, MHCII and NKG2D. Electronic gates were set on live cells with a combination of forward- and side-scatter properties and 7-amino- actinomycin D exclusion. Further gating on CD45⁺ cells shows that only TCRγδ⁺ DETC express NKG2D.

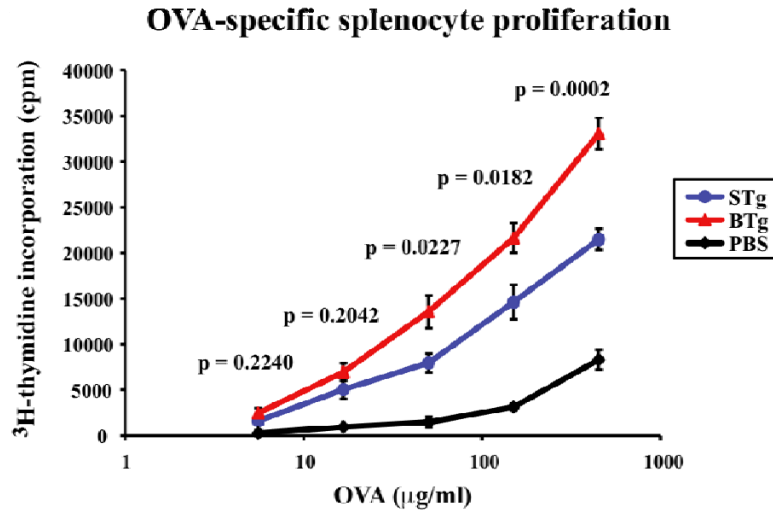


Fig. S3. Acute epidermal Rae-1 expression induces systemic immune effects with enhanced splenic T cell proliferation

Adjuvant-free ova was administered in PBS in epicutaneous patches to shaved areas of BTg mice (red) and STg mice (blue) dox-treated for 7 days, and 10 days later, measurements were made of ova-specific lymphocyte proliferation from spleen by [^3H] thymidine incorporation. Black line shows data for mice exposed to patches containing only PBS. Data expressed as mean $\pm$ 1 SEM; n = 6-8 mice/group with experiment repeated on 4 independent occasions with comparable results.

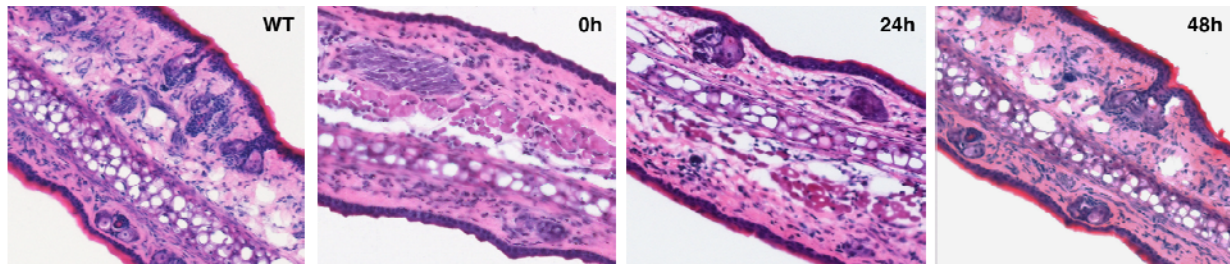


Fig. S4. Mild tissue abrasion removes the stratum corneum but leaves the epidermis and dermis intact

(A) H&E staining of WT mouse ears before [left panel] and immediately after tape-stripping (0h) and at 24h and 48h thereafter. The stratum corneum (deep pink) is transiently removed, causing mild damage to the epidermis but not to the dermis, akin to resting atopic dermatitis skin in humans. The epidermis is fully repaired by 48h. Cross-sections of frozen ear skin was investigated after staining and images are representative of analysis of ≥ 5 mice per condition. Original magnification x20.

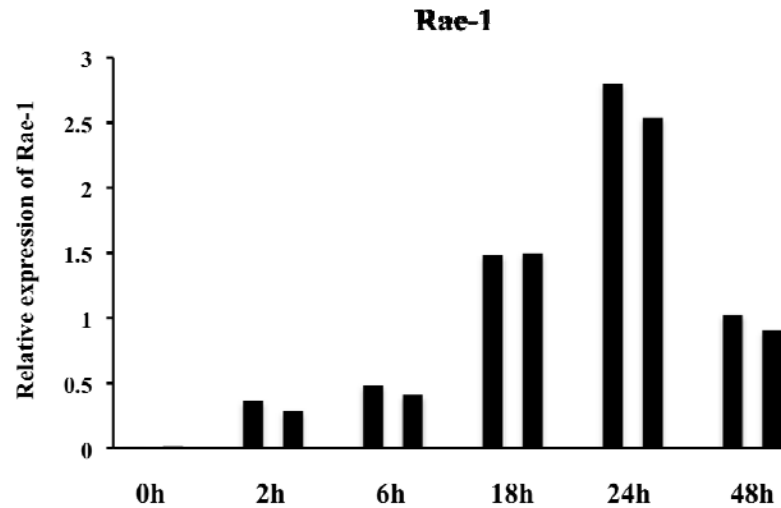


Fig. S5. Epidermal Rae-1 expression characterize the response to mild tissue abrasion

Expression of endogenous Rae-1 mRNA in WT epidermis at various time-points following tape-stripping. qRT-PCR was performed on isolated epidermis; each bar represents an individual mouse; data expressed relative to the control gene cyclophilin. 6 WT mice were analysed per time-point in 3 separate experiments.

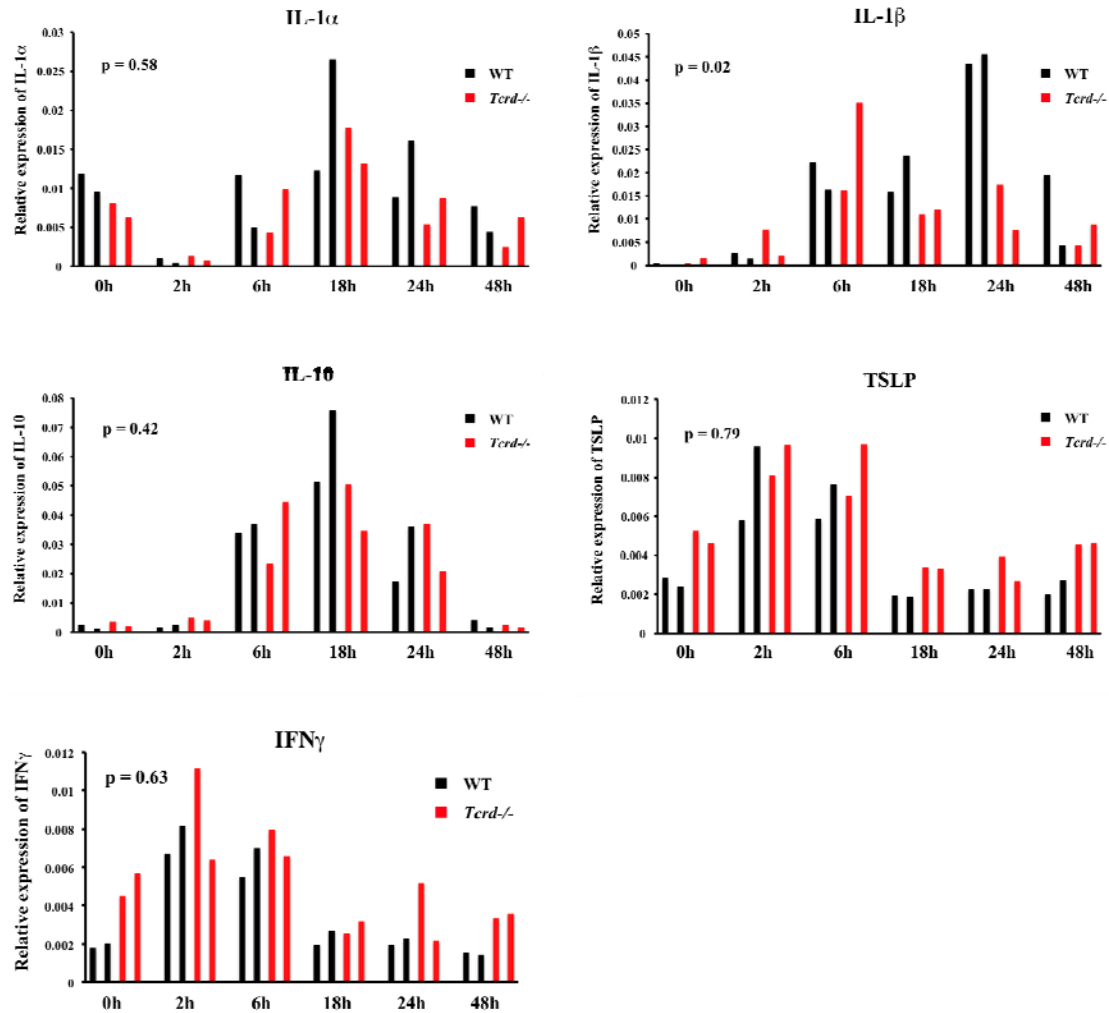


Fig. S6. Local stress-surveillance responses to mild abrasion induce a variety of cytokines in the skin

qRT-PCR analysis of mRNA expression for the indicated cytokines over a 48h period following tape-stripping in WT (black) and *Tcrd*^{-/-} epidermis (red). Data is expressed relative to the control gene cyclophilin; p-values refer to statistically significant differences between WT and *Tcrd*^{-/-} mice as a function of time. 2 mice of each genotype are shown per condition. The entire experiment was repeated 3 times for WT (n=6 per time-point) and 2 times for *Tcrd*^{-/-} (n=4 per time-point).

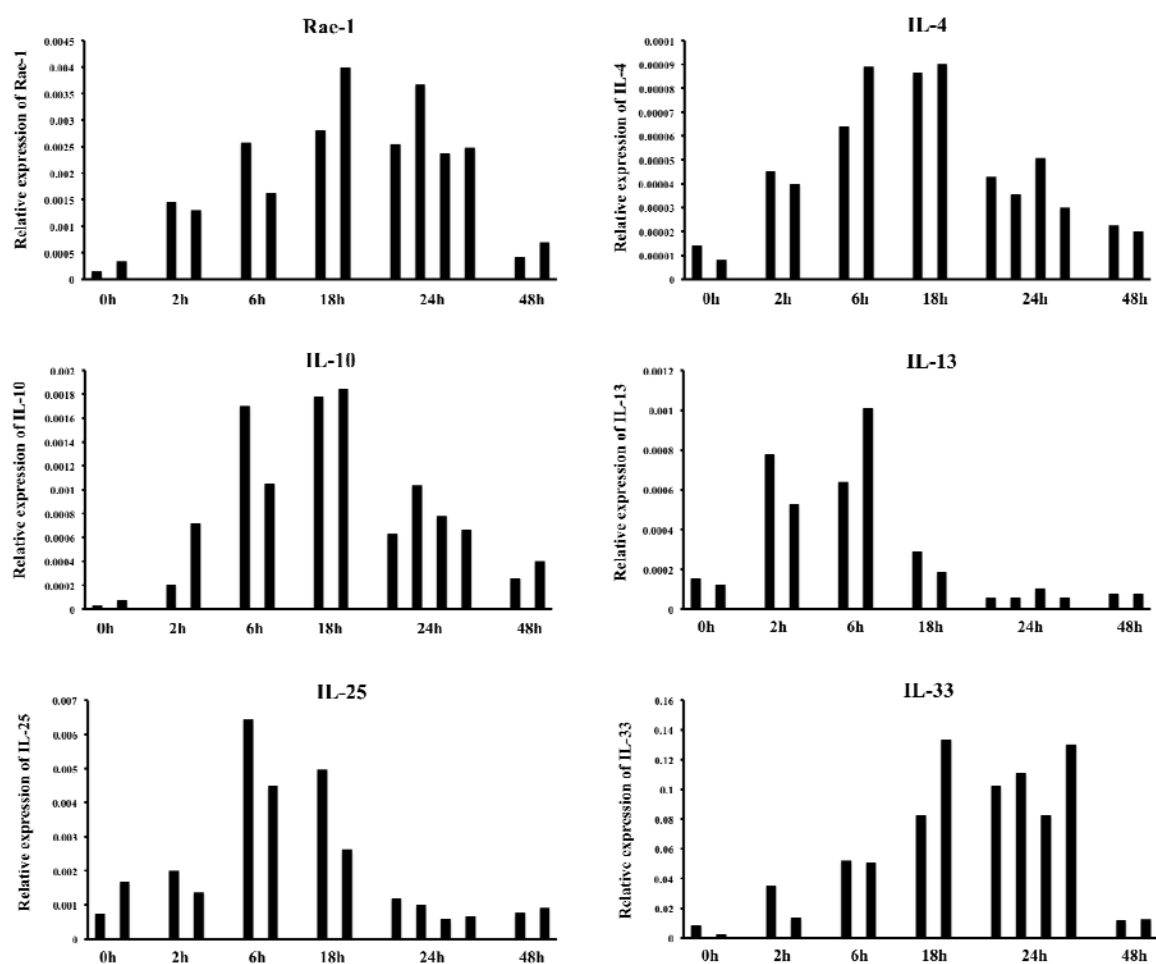


Fig. S7. Skin expression of Rae-1 and Th2 cytokines following shaving

The upper backs of WT mice were closely shaved and skin collected at various time-points after shaving. qRT-PCR was run on RNA extracted from isolated epidermis with each bar representing an individual mouse. Results are expressed relative to a reference gene, cyclophilin.

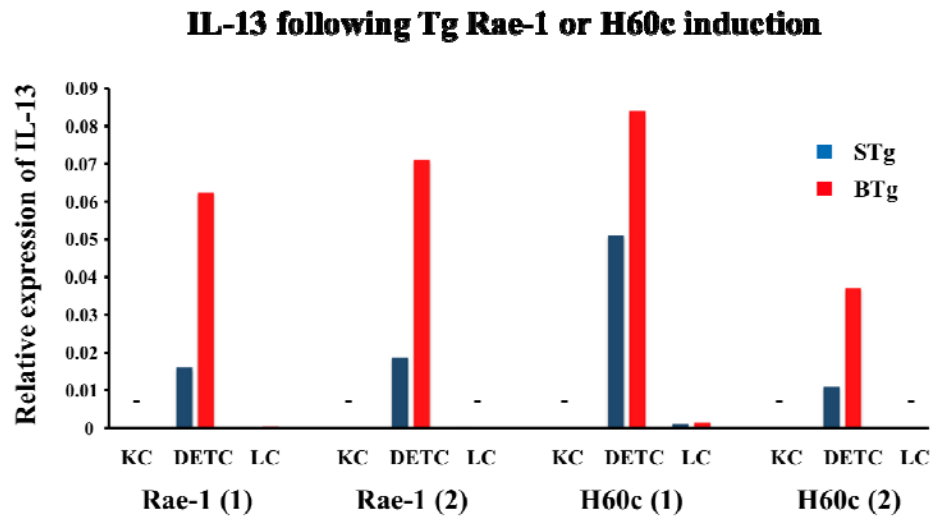


Fig. S8. Epidermal stress-surveillance is linked to DETC-induced Type-2 responses
 LC, DETC and keratinocytes (KC) were FACS sorted from isolated epidermis of BTg Rae-1 and H60c inducible mice (red) or STg control mice (blue) 72h after commencing dox treatment. Each bar represents mRNA pooled from 2 mice with two independent experiments shown for each strain. Results are expressed relative to the reference gene cyclophilin ((-) indicates sample below detection).

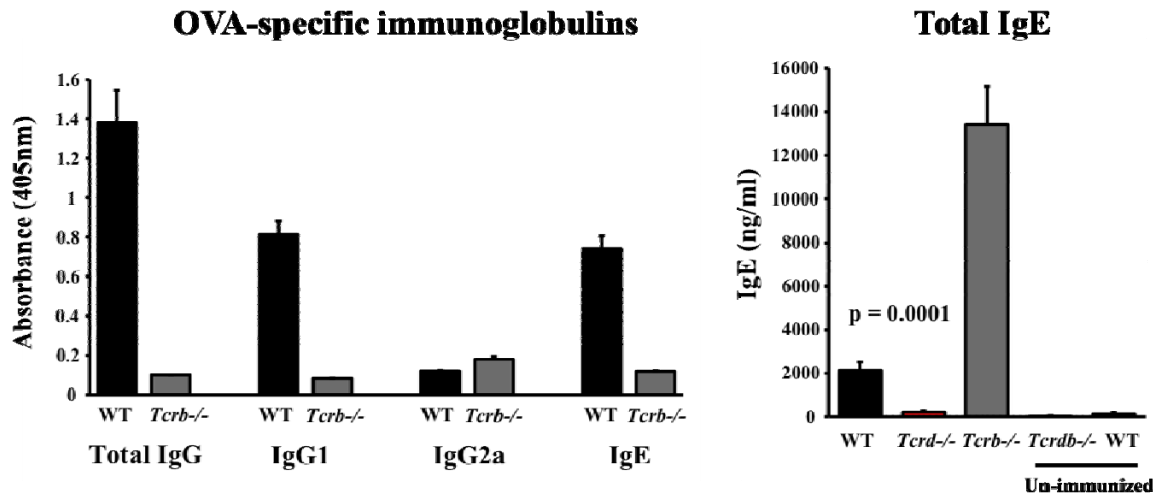


Fig. S9. Induction of ova-specific immunoglobulins is dependent on $\alpha\beta$ T cells but total non-specific IgE is not

Following shaving, ova was applied to dorsal skin of WT (black), *Tcrd*^{-/-} (red) or *Tcrb*^{-/-} (grey) mice. The Ig response was assayed 10 days after primary immunization by ELISA. Total IgE levels were also assayed by ELISA and compared to un-immunized WT and *Tcrd*^{-/-}*Tcrb*^{-/-} double-knockout mice. Data expressed as mean $\pm$ 1 SEM and n=6-8 mice/group. Experiments were repeated independently six times for *Tcrd*^{-/-} and WT and twice for *Tcrb*^{-/-} mice with comparable results.

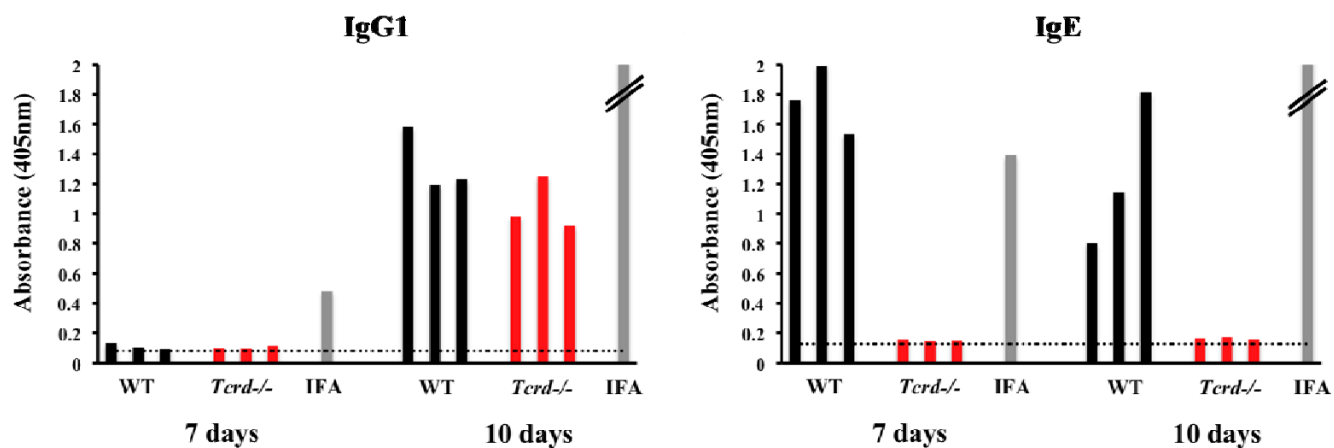


Fig. S10. IgE is rapidly induced and regulated by $\gamma\delta$ T cells

Serum ova-specific IgG1 and total IgE in WT (black) and *Tcrd*^{-/-} (red) mice 7 and 10 days after epicutaneous immunization. Controls include serum Ig measured 7 and 10 days post s.c. immunization of WT mice with ova in incomplete Freund's adjuvant (IFA).

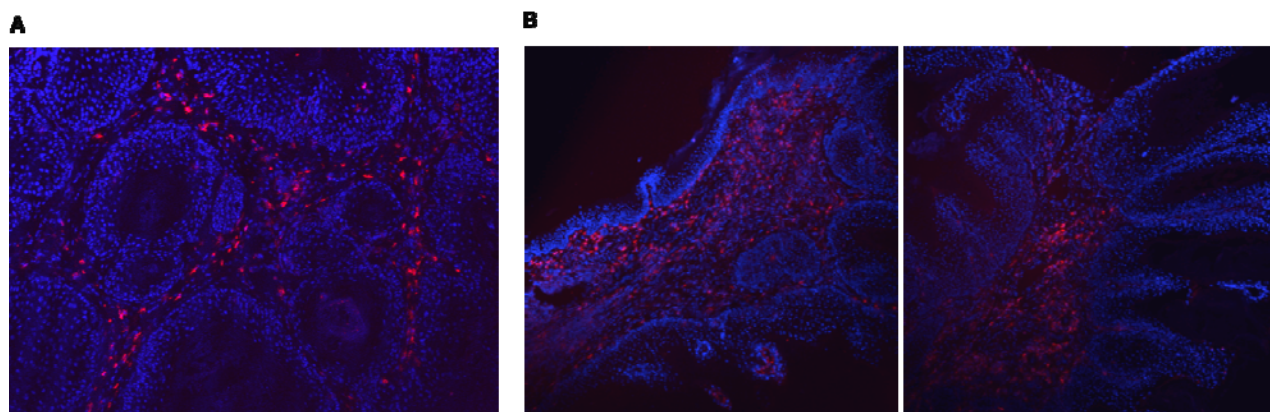


Fig. S11. IgE is readily-detected in chemically-induced skin tumours

Confocal images showing cross-sections of skin tumours, from WT mice subjected to low-dose two-stage chemical carcinogenesis, stained against IgE (red) and cell nuclei (blue). Both skin papillomas (A) and carcinomas (B) show high levels of IgE staining. Micrographs are representative of analysis of several separate papillomas and carcinomas from 4 individual mice. Original magnification x20.

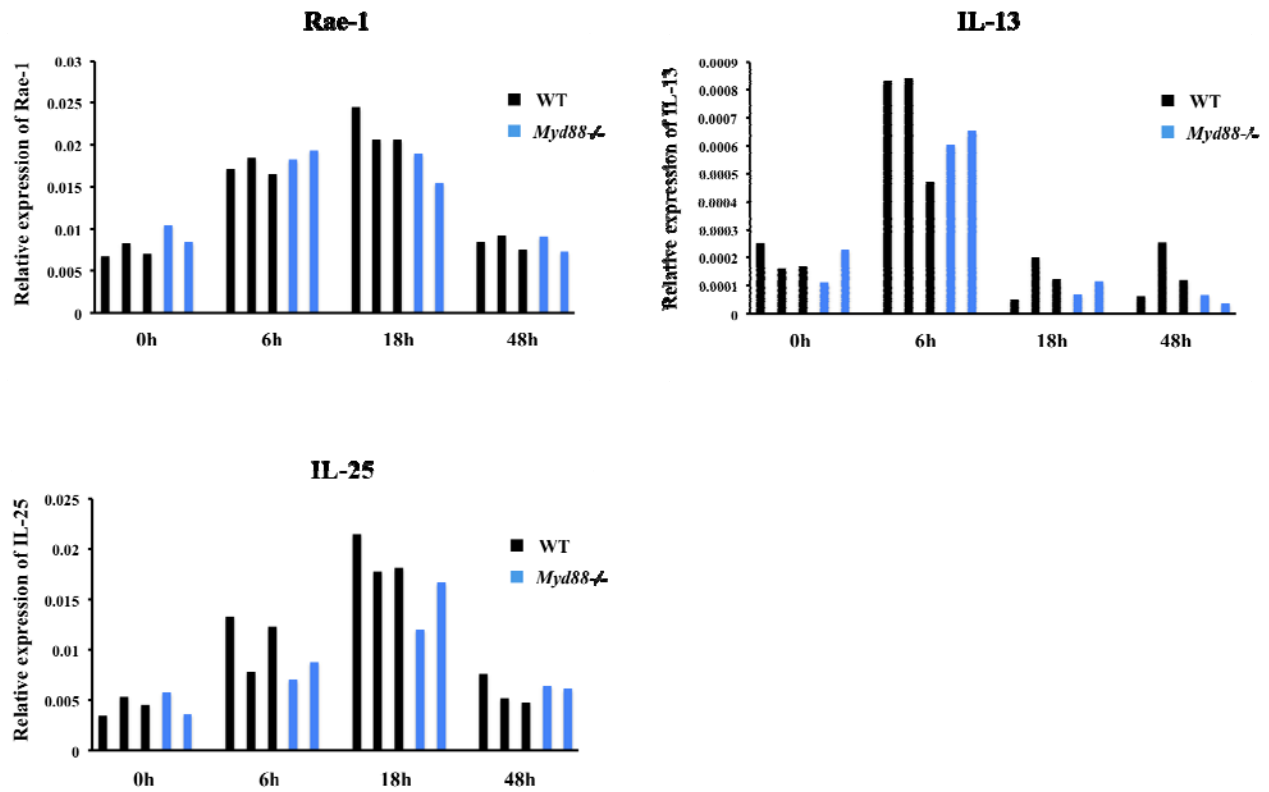


Fig. S12. Mice lacking MyD88 induce comparable levels of Rae-1, IL-13 and IL-25 in the epidermis following mild epicutaneous stress

qRT-PCR analysis of mRNA expression for Rae-1 and the indicated cytokines over a 48h period following tape-stripping in WT C57BL/6 (black) and *Myd88*^{-/-} epidermis (blue). Data expressed relative to the control gene cyclophilin; each bar represents an individual mouse.

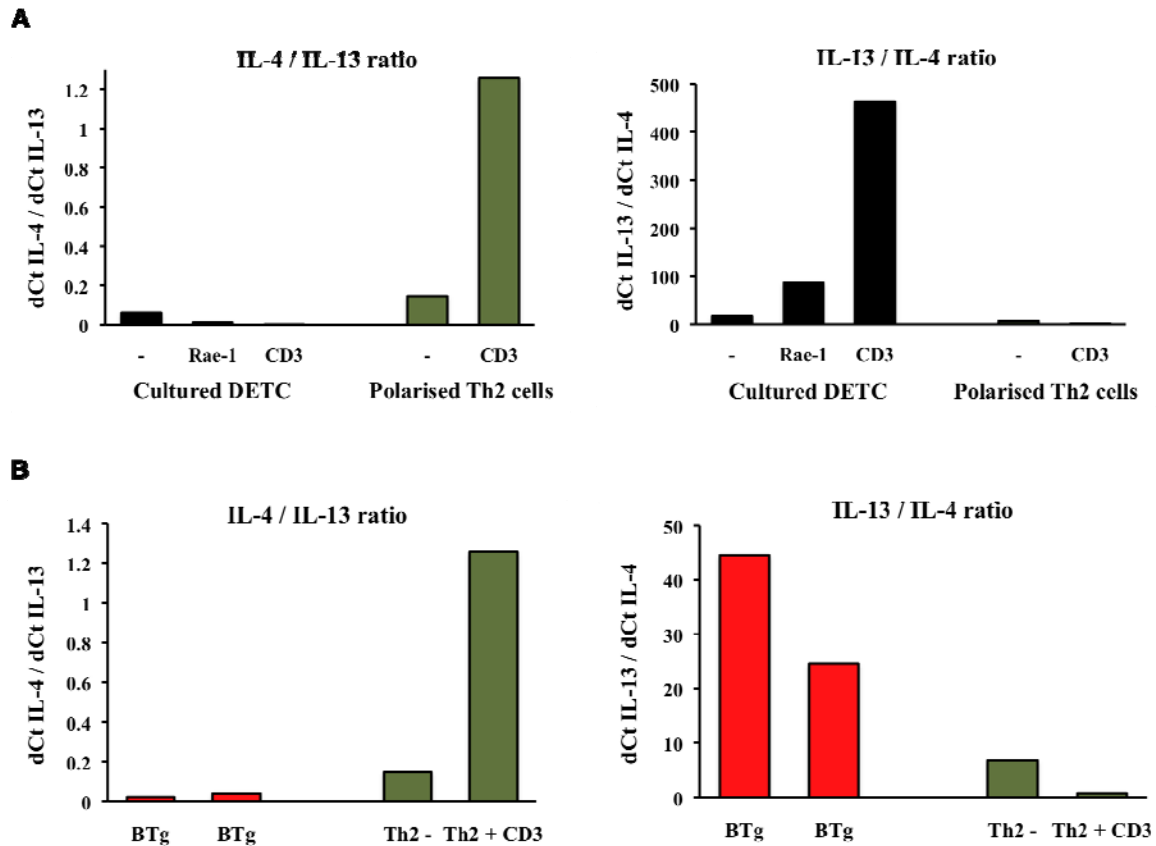


Fig. S13. DETC are distinguished from conventional Th2 cells by more overt IL-13 than IL-4 production

(A) RNA from cultured DETC lines (black), that were stimulated with Rae-1, CD3 or left unstimulated for 48h, were compared with conventionally polarized $\alpha\beta$ Th2 cells (green), stimulated with CD3 or left unstimulated (-), in their capacity to produce IL-13 and IL-4. qRT-PCR analysis of IL-13 and IL-4 mRNA is expressed relative to the reference gene cyclophilin and data is here shown as the ratio of IL-4 to IL-13 and *vice versa*. (B) qRT-PCR analysis as in (A) of IL-13 and IL-4 mRNA expressed by DETC isolated from Rae-1 inducible BTg mice given dox for 5 days. Expression of BTg DETC (red) IL-4 and IL-13 is analysed relative to cyclophilin and is here expressed as the cytokine ratio and compared to similarly analysed conventional Th2 cells (green). DETC from STg control mice do not express IL-4 or IL-13.

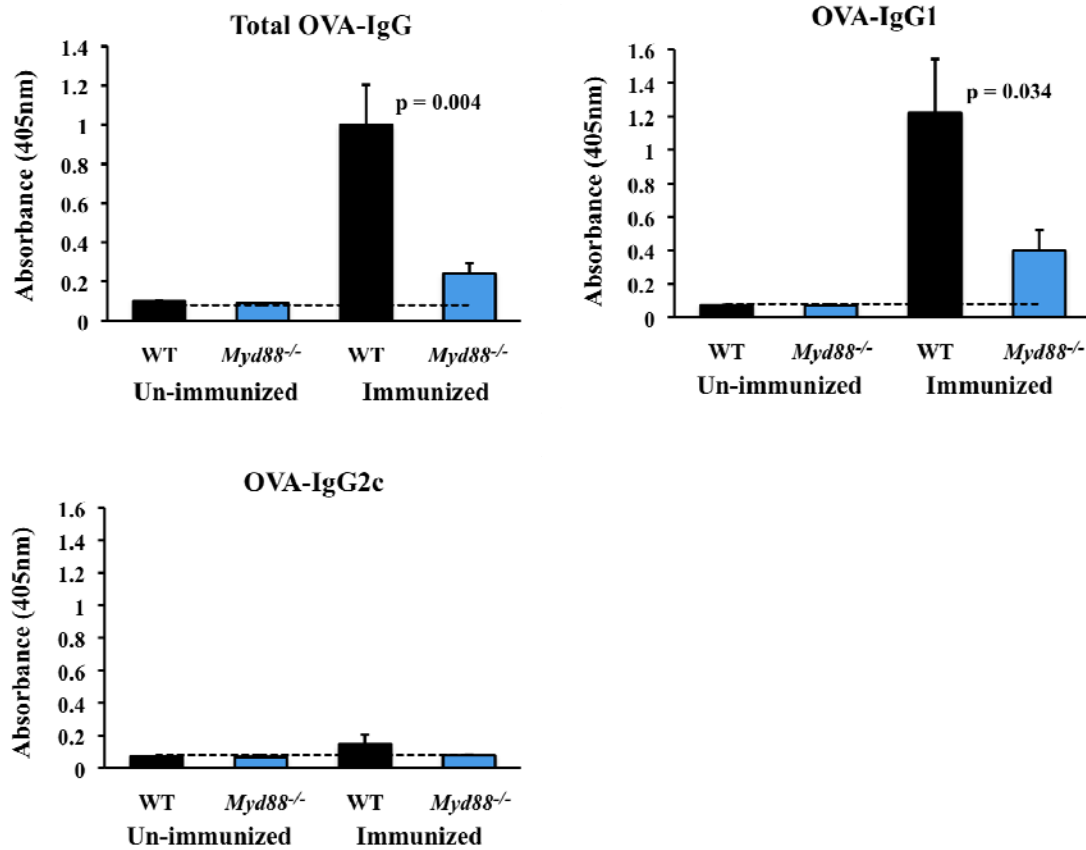


Fig. S14. Contribution of MyD88 to antigen-specific IgG responses following epicutaneous stress-immunization

WT C57BL/6 (black) and *Myd88*^{-/-} (blue) mice were epicutaneously immunized with ova by applying a patch to shaved back skin. Ten days after such primary immunization, induced serum levels of ova-specific total IgG, IgG1 and IgG2c were assayed by ELISA and compared to un-immunized mice. Data is expressed as mean $\pm$ 1 SEM; n=4 for un-immunized mice and n=7 for immunized mice.

References and Notes

1. A. C. Hayday, $\gamma\delta$ T cells and the lymphoid stress-surveillance response. *Immunity* **31**, 184 (2009). [doi:10.1016/j.immuni.2009.08.006](https://doi.org/10.1016/j.immuni.2009.08.006) [Medline](#)
2. S. Gasser, S. Orsulic, E. J. Brown, D. H. Raulet, The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature* **436**, 1186 (2005). [doi:10.1038/nature03884](https://doi.org/10.1038/nature03884) [Medline](#)
3. M. Girardi *et al.*, Regulation of cutaneous malignancy by $\gamma\delta$ T cells. *Science* **294**, 605 (2001). [doi:10.1126/science.1063916](https://doi.org/10.1126/science.1063916) [Medline](#)
4. J. Strid *et al.*, Acute upregulation of an NKG2D ligand promotes rapid reorganization of a local immune compartment with pleiotropic effects on carcinogenesis. *Nat. Immunol.* **9**, 146 (2008). [doi:10.1038/ni1556](https://doi.org/10.1038/ni1556) [Medline](#)
5. C. A. Herrick, L. Xu, A. N. McKenzie, R. E. Tigelaar, K. Bottomly, IL-13 is necessary, not simply sufficient, for epicutaneously induced Th2 responses to soluble protein antigen. *J. Immunol.* **170**, 2488 (2003). [Medline](#)
6. J. Strid, J. Hourihane, I. Kimber, R. Callard, S. Strobel, Disruption of the stratum corneum allows potent epicutaneous immunization with protein antigens resulting in a dominant systemic Th2 response. *Eur. J. Immunol.* **34**, 2100 (2004). [doi:10.1002/eji.200425196](https://doi.org/10.1002/eji.200425196) [Medline](#)
7. T. Nishijima *et al.*, Altered permeability and disordered cutaneous immunoregulatory function in mice with acute barrier disruption. *J. Invest. Dermatol.* **109**, 175 (1997). [doi:10.1111/1523-1747.ep12319282](https://doi.org/10.1111/1523-1747.ep12319282) [Medline](#)
8. L. C. Wood, S. M. Jackson, P. M. Elias, C. Grunfeld, K. R. Feingold, Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J. Clin. Invest.* **90**, 482 (1992). [doi:10.1172/JCI115884](https://doi.org/10.1172/JCI115884) [Medline](#)
9. M. K. Oyoshi, R. P. Larson, S. F. Ziegler, R. S. Geha, Mechanical injury polarizes skin dendritic cells to elicit a T(H)2 response by inducing cutaneous thymic stromal lymphopoietin expression. *J. Allergy Clin. Immunol.* **126**, 976, 984, e1 (2010). [doi:10.1016/j.jaci.2010.08.041](https://doi.org/10.1016/j.jaci.2010.08.041) [Medline](#)
10. A. Takada *et al.*, Two novel NKG2D ligands of the mouse H60 family with differential expression patterns and binding affinities to NKG2D. *J. Immunol.* **180**, 1678 (2008). [Medline](#)
11. T. A. Le *et al.*, Flagellin induces the expression of thymic stromal lymphopoietin in human keratinocytes via Toll-like receptor 5. *Int. Arch. Allergy Immunol.* **151**, 31 (2011). [doi:10.1159/000318679](https://doi.org/10.1159/000318679)
12. L. Wen *et al.*, Immunoglobulin synthesis and generalized autoimmunity in mice congenitally deficient in $\alpha\beta^+$ T cells. *Nature* **369**, 654 (1994). [doi:10.1038/369654a0](https://doi.org/10.1038/369654a0) [Medline](#)
13. W. Ouyang *et al.*, Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity* **12**, 27 (2000). [doi:10.1016/S1074-7613\(00\)80156-9](https://doi.org/10.1016/S1074-7613(00)80156-9) [Medline](#)

14. C. N. Palmer *et al.*, Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat. Genet.* **38**, 441 (2006). [doi:10.1038/ng1767](https://doi.org/10.1038/ng1767) [Medline](#)
15. D. Vemijlen *et al.*, *J. Immunol.* **178**, 4304 (2007). [Medline](#)
16. A. Bendelac, P. B. Savage, L. Teyton, The biology of NKT cells. *Annu. Rev. Immunol.* **25**, 297 (2007). [doi:10.1146/annurev.immunol.25.022106.141711](https://doi.org/10.1146/annurev.immunol.25.022106.141711) [Medline](#)
17. W. H. Wu *et al.*, Thymic stromal lymphopoietin-activated invariant natural killer T cells trigger an innate allergic immune response in atopic dermatitis. *J. Allergy Clin. Immunol.* **126**, 290, 299, e1 (2010). [doi:10.1016/j.jaci.2010.05.024](https://doi.org/10.1016/j.jaci.2010.05.024) [Medline](#)
18. C. Iwamura, T. Nakayama, Role of NKT cells in allergic asthma. *Curr. Opin. Immunol.* **22**, 807 (2010). [doi:10.1016/j.coi.2010.10.008](https://doi.org/10.1016/j.coi.2010.10.008) [Medline](#)
19. T. Zhang *et al.*, Downstream class switching leads to IgE antibody production by B lymphocytes lacking IgM switch regions. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3040 (2010). [doi:10.1073/pnas.0915072107](https://doi.org/10.1073/pnas.0915072107) [Medline](#)
20. K. D. McCoy *et al.*, Natural IgE production in the absence of MHC Class II cognate help. *Immunity* **24**, 329 (2006). [doi:10.1016/j.immuni.2006.01.013](https://doi.org/10.1016/j.immuni.2006.01.013) [Medline](#)
21. H. Hammad *et al.*, House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat. Med.* **15**, 410 (2009). [doi:10.1038/nm.1946](https://doi.org/10.1038/nm.1946) [Medline](#)
22. A. L. Woodward *et al.*, An obligate role for T-cell receptor $\alpha\beta^+$ T cells but not T-cell receptor $\gamma\delta^+$ T cells, B cells, or CD40/CD40L interactions in a mouse model of atopic dermatitis. *J. Allergy Clin. Immunol.* **107**, 359 (2001). [doi:10.1067/mai.2001.112695](https://doi.org/10.1067/mai.2001.112695) [Medline](#)
23. M. Profet, The function of allergy: Immunological defense against toxins. *Q. Rev. Biol.* **66**, 23 (1991). [doi:10.1086/417049](https://doi.org/10.1086/417049) [Medline](#)
24. M. Lisbonne *et al.*, Cutting edge: Invariant V alpha 14 NKT cells are required for allergen-induced airway inflammation and hyperreactivity in an experimental asthma model. *J. Immunol.* **171**, 1637 (2003). [Medline](#)
25. M. Van Hemelrijc *et al.*, *Cancer Causes Control* **21**, 1657 (2010). [Medline](#)
[doi:10.1007/s10552-010-9594-6](https://doi.org/10.1007/s10552-010-9594-6)
26. D. H. Kaplan, M. C. Jenison, S. Saeland, W. D. Shlomchik, M. J. Shlomchik, Epidermal langerhans cell-deficient mice develop enhanced contact hypersensitivity. *Immunity* **23**, 611 (2005). [doi:10.1016/j.immuni.2005.10.008](https://doi.org/10.1016/j.immuni.2005.10.008) [Medline](#)
27. S. Itohara *et al.*, T cell receptor delta gene mutant mice: Independent generation of alpha beta T cells and programmed rearrangements of gamma delta TCR genes. *Cell* **72**, 337 (1993). [doi:10.1016/0092-8674\(93\)90112-4](https://doi.org/10.1016/0092-8674(93)90112-4) [Medline](#)
28. P. Mombaerts, A. R. Clarke, M. L. Hooper, S. Tonegawa, Creation of a large genomic deletion at the T-cell antigen receptor beta-subunit locus in mouse embryonic stem cells by gene targeting. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3084 (1991). [doi:10.1073/pnas.88.8.3084](https://doi.org/10.1073/pnas.88.8.3084) [Medline](#)

29. C. A. Mallick-Wood *et al.*, Conservation of T cell receptor conformation in epidermal gammadelta cells with disrupted primary V γ gene usage. *Science* **279**, 1729 (1998). [doi:10.1126/science.279.5357.1729](https://doi.org/10.1126/science.279.5357.1729) [Medline](#)
30. H. Hara *et al.*, Development of dendritic epidermal T cells with a skewed diversity of gamma delta TCRs in V δ 1-deficient mice. *J. Immunol.* **165**, 3695 (2000). [Medline](#)
31. B. Zafirova *et al.*, Altered NK cell development and enhanced NK cell-mediated resistance to mouse cytomegalovirus in NKG2D-deficient mice. *Immunity* **31**, 270 (2009). [doi:10.1016/j.immuni.2009.06.017](https://doi.org/10.1016/j.immuni.2009.06.017) [Medline](#)
32. O. Adachi *et al.*, Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* **9**, 143 (1998). [doi:10.1016/S1074-7613\(00\)80596-8](https://doi.org/10.1016/S1074-7613(00)80596-8) [Medline](#)
33. D. L. Gibbons *et al.*, Suppression of airway inflammation by a natural acute infection of the intestinal epithelium. *Mucosal Immunol.* **2**, 144 (2009). [doi:10.1038/mi.2008.83](https://doi.org/10.1038/mi.2008.83) [Medline](#)
34. R Development Core Team, *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria, 2011).

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unized mice per group.