FIGURE 1

(a) RT-PCR of RNA from tail tissue biopsies, analyzing the induction of Rae-1 transgene expression in the skin of bi-transgenic mice (BiTg) and single-transgenic mice (SingleTg) after 120 h on doxycycline. Results are compared with amplification of β-actin in the same sample (below). (b) Confocal microscopy of an epidermal sheet isolated from the ear skin of a wild-type FVB mouse, showing highly dendritic MHC class II–positive Langerhans cells (green) in nonoverlapping association with TCRγδ+ DETCs (red). Original magnification, x63. (c) Flow cytometry of epidermal cells from body wall skin, stained with NKG2D-specific (red) or isotype control (blue) antibodies, for gated CD3+ TCR γδ+ DETCs or CD3− MHC class II–positive Langerhans cells (MHCII+ LC). Data are representative of 21 (a), 15 (b) or 24 (c) experiments.
FIGURE 2

(a) Confocal microscopy of epidermal sheets freshly isolated from mice treated with doxycycline for defined time periods. Arrows in top right image indicate juxtaposition of MHC class II–positive Langerhans cells (green) and TCR γδ+ DETCs (red). Original magnification, X63 (main images) with ×2.5 'zoom' (enlargement of boxed area). At least ten bi-transgenic and six single-transgenic mice were analyzed per condition. (b–d) Flow cytometry of epidermal cell suspensions from ear skin of mice after 120 h on doxycycline (n = 6 mice). Left (graphs): each dot represents an individual mouse; small horizontal lines indicate the mean. Right (histograms and dot plots): data from one representative mouse. (b) CD69 expression on CD3+ TCR γδ+ DETCs. (c) TCR expression on CD3+ TCR γδ+ DETCs. (d) CD86 expression on CD3- MHC class II–positive Langerhans cells. *, P = 0.002; **, P < 0.001. Data are representative of three (a) or four (b–d) experiments.
FIGURE 3

(a–f) Confocal microscopy of TCR<sup>Y8</sup> DETCs (red) and TCRαβ<sup>+</sup> cells (green) in epidermal sheets freshly isolated from mice treated with doxycycline for defined time periods. At least ten bi-transgenic and six single-transgenic mice were analyzed per condition; images of representative fields were obtained after analysis of whole epidermal sheets. (g,h) Confocal microscopy of the reversibility of changes induced by Rae-1 expression in the epidermis of bi-transgenic mice given doxycycline (dox) for 120 h, then taken off doxycycline for 72 h. (g) TCR<sup>Y8</sup> DETCs are red; MHC class II–positive Langerhans cells are green. (h) TCR<sup>Y8</sup> DETCs are red; TCRαβ<sup>+</sup> cells are green. Original magnification, ×63. Data are representative of four (a–f) or three (g,h) experiments.
FIGURE 4

a

b

WT spleen

BITg epidermis

Tcra-/- epidermis

TCR Vβ2

TCR Vβ3

TCR Vβ4

TCR Vβ6

TCR Vβ7

TCR Vβ10b

TCR Vβ14

C

WT spleen

Tcra-/- epidermis

BITg epidermis

TCRγδ DETC

CD3

CD1d tetramer

+ α-GalCer

+ α-GalCer

+ α-GalCer
(a,b) Flow cytometry of wild-type spleen cell suspensions and of epidermal cell suspensions prepared from the ears of Tcrd⁻/⁻ and of bi-transgenic mice after 120 h on doxycycline. Stringent isolation ensured no dermal contamination, and no TCR⁺ cells were found in single-transgenic or wild-type controls. Plots are of individual mice; at least ten bi-transgenic mice were analyzed per condition. (a) Plots gated on CD3⁺TCR⁺ epidermal cells. (b) TCRβ repertoire in bi-transgenic and Tcrd⁻/⁻ epidermis and wild-type (WT) spleen; plots gated on CD3⁺TCRβ⁺ cells. (c) Binding of α-galactosylceramide–CD1d tetramers to epidermal T cells; plots gated on CD3⁺TCRβ⁻ splenic or epidermal cells. Seven bi-transgenic, three wild-type and three Tcrd⁻/⁻ mice were analyzed. Numbers in quadrants or outlined areas indicate percent cells in each. Data are representative of four (a,b) or two (c) experiments.
(a) Flow cytometry of intraepidermal T cell populations. Top row, gating on all epidermal cells; stained with anti-TCR $\gamma^5$ (GL3) and anti-V $\gamma^5$ (536); bottom row, gating on TCR $\gamma^8^+$ cells; stained with anti-V $\gamma^5V\delta^1$ (17D1) and anti-V $\gamma^5$ (536). Numbers in quadrants indicate percent cells in each. (b) Tumor development in mice subjected to low-dose two-stage chemical carcinogenesis (200 nmol DMBA initiation; 10 nmol weekly TPA promotion). Statistical analysis, Table 1. Data are representative of three experiments (a) or four experiments with 10–14 mice in each (b; error bars, s.e.m.).
(a,b) Induction of tumor formation by a low-dose two-stage chemical carcinogenesis protocol (200 nmol DMBA initiation, 10 nmol weekly TPA promotion) in wild-type mice and in mice rendered deficient in Langerhans cells via diphtheria toxin transgene regulated by the Langerin promoter (Langerin-DTA): 5.67 ± 1.63 versus 1.00 ± 0.47 tumors per mouse at week 16, respectively (P < 0.005). (b) Wild-type and Langerhans cell–deficient mice from the low-dose experiment in a. (c) Tumor induction by a high-dose two-stage chemical carcinogenesis protocol (400 nmol DMBA; 40 nmol TPA weekly) in wild-type and Langerhans cell–deficient mice: 20.20 ± 0.59 versus 2.39 ± 0.59 tumors per mouse at week 16, respectively (P < 0.0000001). (d,e) Induction of tumor formation by the low-dose two-stage chemical carcinogenesis protocol in a for mice deficient in either all T cells (Tcrb−/−) or both T cells and Langerhans cells (Tcrb−/− Langerin-DTA; d) and for mice deficient in either all CD4+ T cells (Cd4−/−) or both CD4+ T cells and Langerhans cells (Cd4−/− Langerin-DTA; e): 9.20 ± 1.80 tumors per mouse for Tcrb−/− versus 0.36 ± 0.28 tumors per mouse for Tcrb−/− Langerhans cell–deficient (P < 0.0005); 6.14 ± 1.48 tumors per mouse for Cd4−/− versus 0.36 ± 0.20 tumors per mouse for Cd4−/− Langerhans cell–deficient (P < 0.001). Data are representative of three (a,b) or two (c–e) experiments with 10–15 mice (a), 10 mice (c,d) or 10–14 mice (e) in each.