Epithelial damage and tissue γδ T cells promote a unique tumor-protective IgE response

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IgE is an ancient and conserved immunoglobulin isotype with potent immunological function. Nevertheless, the regulation of IgE responses remains an enigma, and evidence of a role for IgE in host defense is limited. Here we report that topical exposure to a common environmental DNA-damaging xenobiotic initiated stress surveillance by γδ TCR+ intraepithelial lymphocytes that resulted in class switching to IgE in B cells and the accumulation of autoreactive IgE. High-throughput antibody sequencing revealed that γδ T cells shaped the IgE repertoire by supporting specific variable-diversity-joining (VDJ) rearrangements with unique characteristics of the complementarity-determining region CDR3. This endogenous IgE response, via the IgE receptor FcεRI, provided protection against epithelial carcinogenesis, and expression of the gene encoding FcεRI in human squamous-cell carcinoma correlated with good disease prognosis. These data indicate a joint role for immunosurveillance by T cells and by B cells in epithelial tissues and suggest that IgE is part of the host defense against epithelial damage and tumor development.

IgE is an ancient and highly conserved immunoglobulin isotype found in all mammals1. It is thought that IgE has evolved to provide protection against infection by macroparasites, such as helminths. However, although the abundance of IgE is elevated in both mice and humans with helminth infection, IgE is not critical for protective immunity against helminths, and much of the IgE raised is not parasite-specific2. An alternative hypothesis suggests that IgE is important for immune responses to environmental toxins such as venoms3, and indeed, published data indicate that IgE can provide protection against bee venom and limit snake-venom toxicity4,5. Furthermore, aberrant IgE responses that cause allergies are frequently directed against environmental irritants and non-replicating agents. A role for IgE in defending the host against immediate danger would be consistent with the very rapid mobilization of its effector functions. Therefore, a broader paradigm proposes that IgE represents an arm of early immunological host defense against xenobiotics or large parasites that threaten tissue integrity6. However, what drives these IgE responses in vivo, what they recognize and whether they have a wider role in immunological defense remain unknown.

IgE responses occur most frequently in epithelial tissues such as the skin, lungs and gut, which contain resident IgE-binding cells. These barrier surfaces are continuously exposed to challenges from environmental xenobiotics, and a prominent feature of their response to external insults is the induction of type 2 immunity7 and IgE8,9. In mouse skin epithelia, a resident γδTCR+ population of intraepithelial lymphocytes (IELs) controls tissue homeostasis via the induction of IL-1310, a type 2 cytokine, and also promotes IgE after challenge with protein allergens on stressed tissue11. These IELs can directly sense epithelial-cell dysregulation via stress-sensing receptors, such as NKG2D, and this pathway has a key role in promoting the IgE response12,13. The ability of IELs to detect epithelial-cell stress and initiate a restorative response has been called ‘lymphoid stress surveillance’ and has an important role in the early detection of stressed and pre-malignant cells14. Indeed, tissue-specific IELs regulate epithelial tissue integrity and are key to protection of the host against carcinogenesis15,16.

Many toxins and xenobiotics are also carcinogens. Hence, in defending against environmental xenobiotics, IgE might simultaneously confer protection against transformation and against cancer in exposed tissues. Here we report the development of a potent and dominant IgE response following topical exposure to the common environmental xenobiotic and carcinogen DMBA (7,12-dimethylbenz[a]anthracene). This endogenous IgE response was autoreactive and was required for protection against cutaneous carcinogenesis. Furthermore, we found that tissue-resident γδTCR+ IELs had a unique role in initiating and regulating IgE production, driving an early, innate-like response, which directed a subsequent adaptive response supported by IL-4-producing CD4+ γδ T cells. Deep sequencing of IgE and IgG1 from germinal-center (GC) B cells and plasma cells (PCs) indicated that γδTCR+ IELs shaped a distinct IgE repertoire that was dependent on the presence of DNA damage in epithelial cells. Thus, lymphoid stress surveillance promoted a unique IgE response that was part of an early host-defense mechanism that provides protection against cancer.

Results
Carcinogen-induced epithelial-cell damage triggers IgE responses. DNA-damaging xenobiotics such as DMBA are

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commonly found in the environment and have been linked to carcinogenesis\(^1\). We found that a single topical application of DMBA to the back skin of wild-type mice induced an IgE response in serum within 4 d (Fig. 1a). Exposure to DMBA was associated with upregulation of the NKG2D ligand Rae-1 on skin epithelial cells (Supplementary Fig. 1a), indicative of epithelial-cell stress. The number of epithelial cells containing breaks in double-stranded DNA (dsDNA), assessed by staining for the phosphorylated histone H2A variant H2AX (γH2AX), peaked between 3 d and 7 d after exposure to DMBA (Supplementary Fig. 1b,c). Epidermal Langerhans cells (LCs) must metabolize DMBA to the more mutagenic compound DMBA-trans,3,4-dihydridiol to cause substantial damage to DNA. Langerin-DTA mice, which do not have LCs (and have transgenic expression of the gene encoding human Langerin, modified to drive expression of diphtheria toxin subunit A (DTA)), showed less epithelial-cell DNA damage following a single dose of DMBA than did their non-transgenic littermates (control mice) (Supplementary Fig. 1d) and failed to show increased serum IgE, in contrast to the control mice (Fig. 1b). However, Langerin-DTA mice generated IgE in response to other topical challenges\(^2\) and, in contrast to the control mice (Fig. 1b), failed to show increased serum IgE, induced IgE (Supplementary Fig. 1f,g). Once-weekly exposure to damaging skin challenges, such as ultraviolet (UV) irradiation, also showed less epithelial-cell DNA damage (Supplementary Fig. 1d,e). Other DNA-modified to drive expression of diphtheria toxin subunit A (DTA)), have transgenic expression of the gene encoding human Langerin, expressed IgE (Supplementary Fig. 1d) and lacked skin mast cells, showed susceptibility to tumor development similar to that of their Cpa3\(^{3-5}\) mice, which have sequence encoding Cre recombine inserted into the locus encoding mast-cell carboxypeptidase (Cpa3) and lack skin mast cells, showed susceptibility to tumor development similar to that of their Cpa3\(^{3-5}\) littermates following DMBA carcinogenesis (Supplementary Fig. 3b), which suggested that most probably, FcεRI\(^+\) basophils mediated the protection against carcinogenesis. Splenic CD45\(^{+}\)Kit CD41\(^+\) FcεRI\(^+\) basophils from Igγ\(^{−/−}\) mice produced IL-4 and IL-6 and deranged equivalent to their wild-type counterparts after stimulation with the phorbol ester PMA and ionomycin; however, the skin-tumor cytokine environment was significantly altered in Igγ\(^{−/−}\) mice relative to that in wild-type mice (Supplementary Fig. 3c–e).

To investigate whether similar IgE responses occur in humans, we analyzed newly incised skin SCC samples from 12 patients. FcεRI\(^+\) cells were present in all tumor and peri-lesional skin samples, varying in frequency from around 1% to over 40% of the tumor infiltrating CD45\(^+\) leukocytes (Fig. 3c). The FcεRI\(^+\) cells were present mainly in the peritumoral infiltrate at the interface between the stroma and the neoplastic keratinocytes, with some entering the tumor and/or hair follicle (average, 11.7 \(\pm\) 2.9 cells per mm\(^3\) (mean \(\pm\) s.e.m.)) (Supplementary Fig. 4). FcεRI\(^+\) cells accumulated more in the skin than in blood samples from the same donor; however, tumor tissue contained fewer FcεRI\(^+\) cells than did histopathologically ‘healthy’ peri-lesional skin from the same donor (Fig. 3c). In an additional cohort of 56 patients with SCC, gene-expression analysis revealed that expression of FCER1A mRNA was higher in unaffected skin areas than in developed tumors, with the lowest expression of FCER1A mRNA detected in high-risk and metastatic SCC (Fig. 3d). A significant linear trend was observed between the expression of FCER1A mRNA and the risk of more-advanced SCC (Fig. 3d). Together these data indicated that IgE, via FcεRI, provided protection against DMBA-induced SCC and that the presence of fewer FcεRI-expressing leukocytes in human SCC correlated with the risk of more-severe disease.

Carcinogen-induced humoral immunity depends on αβ T cell-derived IL-4. Conventional and unconventional modes of inducing class switching to IgE have been described\(^4\). We first explored whether induction of tumor-protective IgE was dependent on αβ T cells. Mice deficient in the β-chain of the T cell antigen receptor (TCR) (Terb\(^{−/−}\) mice), which lack αβ T cells, had no enlargement of skin-draining LN, no GCs and no IgG1\(^+\) PCs following two topographical applications of DMBA to the ear skin, in contrast to their wild-type counterparts (Fig. 4a). The response was restored by the transfer of polyclonal CD4\(^+\) T cells into Terb\(^{−/−}\) mice, but not by the transfer of OT-II CD4\(^+\) T cells, which have transgenic expression of a major histocompatibility complex class II–restricted (ovalbumin-specific) TCR (Supplementary Fig. 5a); this indicated that it was dependent on a normal TCR repertoire. Furthermore, injection of blocking antibody to the B cell–stimulatory molecule CD40L partially inhibited LN enlargement and repressed GC formation and the generation of IgE\(^+\) and IgG1\(^+\) PCs in wild-type mice.

Topical exposure to a carcinogen induces class switching in local B cells. To investigate the origin of the DMBA-induced IgE response, we assessed B cells in the skin-draining lymph nodes (LN) during acute exposure to DMBA (before malignancy). Two applications of DMBA, 3 d apart, on the ear skin induced the enlargement of skin-draining LNs plus the formation of GCs and class switching of GC B cells (Fig. 2a), as well as an increased number of CD138\(^+\) PCs, which had class switched mainly to IgE and IgG1, with little or no induction of IgG2a (Fig. 2b). There was no induction of GCs or class-switched PCs in the spleen (data not shown), which suggested that the response was localized to the skin-draining LNs. Weekly exposure to DMBA on the back skin for 5 weeks also induced GCs and IgE\(^+\) and IgG1\(^+\) PCs. However, under these conditions, the GC structures and the IgG1\(^+\) PCs diminished from week 3, which resulted in a dominant IgE response (Fig. 2c). By week 5 after exposure, nearly 80% of all CD138\(^+\) PCs in the LNs had switched to IgE (Fig. 2c). Therefore, the exposure to DMBA promoted a local type 2 B cell response with dominant and prevailing production of IgE.

Carcinogen-induced IgE provides protection against carcinogenesis. To explore whether the IgE response induced by DMBA was important for host defense, we assessed skin-tumor formation in mice deficient in the gene encoding the heavy chain of IgE (Igh7\(^−/−\), called ‘Igh7\(^−/−\) here), which thus lack IgE. Following once-weekly exposure to DMBA, Igh7\(^−/−\) mice developed tumors sooner and more rapidly than did their wild-type counterparts, as well as significantly more and larger tumors than those of their wild-type counterparts (Fig. 3a). The IgG1 response in the DMBA-treated Igh7\(^−/−\) mice was identical to that of their wild-type counterparts (Supplementary Fig. 2a). In addition, the number of FcεRI\(^+\) effector cells in their tissue was similar to that of wild-type mice (Supplementary Fig. 3a). mice deficient in the gene encoding FcεRI (FcεRI\(^−/−\) mice) also had enhanced susceptibility to DMBA carcinogenesis relative to that of wild-type mice (Fig. 3b), despite the finding of similar concentrations of IgE and of IgG1 in serum from these mice (Supplementary Fig. 2b); this suggested that the protective effect of IgE was mediated by signaling through FcεRI. CD45\(^{+}\) Kit FcεRI\(^+\) basophils represented the main IgE\(^+\) cell population in the tumors (Fig. 1d). Cpa3\(^{3-5}\) mice, which have sequence encoding CRE recombine inserted into the locus encoding mast-cell carboxypeptidase (Cpa3) and lack skin mast cells, showed susceptibility to tumor development similar to that of their Cpa3\(^{3-5}\) littermates following DMBA carcinogenesis (Supplementary Fig. 3b), which suggested that most probably, FcεRI\(^+\) basophils mediated the protection against carcinogenesis. Splenic CD45\(^{+}\) Kit CD41\(^+\) FcεRI\(^+\) basophils from Igh7\(^−/−\) mice produced IL-4 and IL-6 and deranged equivalent to their wild-type counterparts after stimulation with the phorbol ester PMA and ionomycin; however, the skin-tumor cytokine environment was significantly altered in Igh7\(^−/−\) mice relative to that in wild-type mice (Supplementary Fig. 3c–e).

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Fig. 1 | Carcinogen-induced epithelial-cell damage triggers a rapid local and systemic IgE response. a–c, ELISA of IgE in serum from blood obtained from wild-type FVB mice (n=10) (a) or from Langerin-DTA mice (n=5) and their non-transgenic littermates (control mice) (NLC) (n=4) (b) before treatment (Pre-bleed) and 4 d after treatment with a single topical dose of 200 nmol DMBA or vehicle control (Acetone) on shaved back skin (a,b) and from wild-type FVB mice (n=9 per group) at various times (horizontal axis) before and after topical exposure to 200 nmol DMBA once weekly (c).

d, Flow cytometry (left) of IgE-bearing cells in naive skin, skin 7 d after exposure to DMBA and DMBA-induced skin tumors (left margin), and quantification of those cells from such mice (right; cells in tumor presented as frequency among CD45+ and IgE+ and IgG1+ and IgM+ cells). Numbers adjacent to outlined areas (left half) indicate percent CD45 and n and IgG1 and IgM positive cells of total live cells (Supplementary Fig. 5b). Similar defects in class switching to IgE+ and IgG1+ PCs were seen Il4−/− mice, which cannot produce IL-4, although Il4−/− mice showed an enlargement of LNs similar to that of wild-type mice and had some GC B cells (Fig. 4a). Il4−/− mice had some class switching to IgG2a, which was never observed in wild-type or in Tcrb−/− mice (data not shown). Chimeras generated by the transfer of wild-type bone marrow into Il4−/− host mice (WT→Il4−/− chimeras) underwent induction of IgE+ and IgG1+ PCs similar to the induction of such cells in WT→WT chimeras following topical treatment with DMBA, whereas Il4−/→WT chimeras underwent significantly less induction of IgE+ and IgG1+ PCs than did WT→Il4−/− chimeras (P<0.0001 and P<0.01, respec-
**Fig. 2 | Topical exposure to a carcinogens induces local B cell class switching, which results in a dominant IgE response.**

**a.** Flow cytometry (middle) of GC gated B cells from the skin-draining LNs of wild-type FVB mice left untreated (Naive) or exposed topically to DMBA on the dorsal side of the ears twice, 3 d apart, and analyzed 7 d after the final exposure (DMBA), assessing class switching via intracellular immunoglobulin staining, and quantification of total LN cells (far left) and GC B cells (defined as B220⁺CD95⁺GL7⁺ LN cells throughout) (far right) in such mice (n = 9 (Naive) or n = 11 (DMBA)). Numbers adjacent to outlined areas (middle) indicate percent GL7⁺CD95⁺ cells (top row); IgE⁺IgG1⁻ cells (left gate) and IgE⁻IgG1⁺ cells (right gate) (bottomleft); or IgG2a⁺IgG1⁻ cells (left gate) and IgG2a⁺IgG1⁺ cells (right gate) (bottom right). **b.** Flow cytometry (left) of PCs in the mice in a, with intracellular immunoglobulin staining for analysis of isotype switching, and quantification of IgG1⁺, IgE⁺ or IgG2a⁺ PCs (gated as FSC⁺CD95⁺CD138⁺ cells throughout) in such mice (right). Numbers in outlined areas (left) indicate percent CD138⁺FSChi cells (PCs) (top row) or as in a (bottom row). **c.** Flow cytometry (right) of axillary LN B cells from wild-type FVB mice exposed to DMBA once weekly on shaved back skin, assessed at weeks 2–5 after exposure (above plots), and frequency of GC B cells (among B220⁺ lymphocytes) and IgG1⁺ or IgE⁺ (key) class-switched PCs (among CD138⁺ PCs) in such mice (n = 3 per time point) analyzed weekly for 1–5 weeks (horizontal axis) beginning 7 d after the final exposure (left). Numbers in outlined areas (right), as in a (bottom left). Each symbol (a,b) represents an individual mouse; small horizontal lines indicate the mean (± s.e.m.). NS, not significant (P > 0.05); *P < 0.05, ***P < 0.001 and ****P < 0.0001 (two-tailed Student’s t-test for unpaired data). Data are from one experiment representative of five (a,b) or two (c) independent experiments with similar results (mean ± s.e.m. in c).
Induction of tumor-protective IgE requires γδ TCR+ IELs. Skin γδ TCR+ IELs are key for protective immunity to chemical carcinogenesis and are strong inducers of local type 2 immune responses\textsuperscript{11,13}. We found that following topical exposure of the ear skin to DMBA, Tcrd\textsuperscript{−/−} mice, which lack γδ T cells, had less LN hypertrophy than that of wild-type mice but had a normal number of GC B cells (Fig. 5a). Tcrd\textsuperscript{−/−} mice developed IgG1+ PCs similar to the development of such cells in wild-type mice but had significantly impaired IgE PC responses compared with those of wild-type mice (Fig. 5a).

The decrease in IgE was detected as early as day 4, a time at which the number of IgE+ PCs and serum IgE had increased in wild-type mice but remained undetectable in Tcrd\textsuperscript{−/−} mice (Fig. 5a,b). GCs were not yet enlarged in wild-type mice at day 4 (Fig. 5a), which suggested that the γδ T cell–dependent IgE response at this time might have been of extrafollicular origin. To explore this, we used CD19\textsuperscript{−/−}Bcl6\textsuperscript{0/0} mice, which have a B cell–specific defect in the transcription factor Bcl-6. CD19\textsuperscript{−/−}Bcl6\textsuperscript{0/0} mice did not develop a GC response following topical application of DMBA, despite LN enlargement, but the number of IgE+ and IgG1+ PCs in their LNs was equivalent to that in the LNs of their Bcl6–heterozygous littermates and wild-type mice (Supplementary Fig. 5d).

We next sought to determine whether the γδ T cells involved in the response were located in the skin itself or in a secondary lymphoid organ such as the draining-LNs. Skin-resident γδ TCR+ IELs are radiation resistant\textsuperscript{11}, and in Tcrd\textsuperscript{−/−}→WT bone-marrow
chimeras or WT→Tcrd−/− bone-marrow chimeras, γδ T cells were detected only in the skin or LNs, respectively (Fig. 5c). When these chimeras were treated topically with DMBA, IgE was significantly reduced only in WT→Tcrd−/− chimeras, in which γδ TCR+ IELs were missing from the skin, but γδ T cells were present in the LNs, compared with the IgE in WT→WT chimeras (Fig. 5d). The number of IgG1+ PCs was similar in Tcrd−/−→WT, WT→Tcrd−/−, WT→WT and Tcrd−/−→Tcrd−/− chimeras (Fig. 5d). The abundance of Vγ4+ and other ‘non-Vγ5’ γδ T cells in the skin of Tcrd−/−→WT and WT→Tcrd−/− chimeras was similar to that in the skin of WT→WT chimeras (Fig. 5c). In addition, following topical treatment with DMBA, Tcrδ-V5−/+/−TcrV1−/− mice, which lack only the canonical Vγ5Vδ1+ IELs, had fewer IgE+ PCs than did wild-type mice (Fig. 5e), which suggested that Vγ5+ IELs were most probably the IgE-promoting γδ T cell subset. These observations indicated that DMBA-induced IgE depended on γδTCR+ IELs in the tissue.

γδ T cells promote a distinct IgE repertoire in response to a carcinogen. To understand the nature of the humoral response produced during epithelial-cell damage, and the role of γδ T cell immunosurveillance, we sorted B220+CD95+GL7+ GC B cells and FSC+CD95+CD138+ PCs from the skin-draining LNs of wild-type and Tcrd−/− mice 7 d after the second of two topical exposures to DMBA. Analysis of the IgG1 and IgE heavy-chain repertoires by high-throughput sequencing revealed that IgE-producing clones had the largest clonal expansion in wild-type mice (Supplementary Fig. 6a). As expected, the average size for IgG1+ clones was greater among PCs than among GC B cells (Fig. 6a). In contrast, IgE+ clones among PCs and GC B cells were equivalent and were larger than IgG1+ clones (Fig. 6a). Furthermore, Tcrd−/− mice had defective IgE+ clonal expansion in GC B cells compared with that of wild-type mice, while IgG1+ clonal expansion was similar (Fig. 6b). The Alakazam tool15, an analysis framework for the sequencing of adaptive immunoreceptor repertoires, indicated that the IgG+ PC

**Fig. 4 |** Carcinogen-induced humoral immunity depends on αβ T cell-derived IL-4. a. Flow-cytometry analysis of IgE and IgG1 in PCs from the skin-draining LNs of wild-type, Il4−/− and Tcrb−/− FVB mice (above plots) exposed topically to DMBA on the dorsal side of the ears twice, 3 d apart, and analyzed 7 d after the final exposure (bottom row), and total LN cells, GC B cells and IgG1+ or IgE+ PCs in naïve wild-type mice (n = 6) and such DMBA-treated wild-type FVB mice (n = 6), Il4−/− FVB mice (n = 5) and Tcrb−/− FVB mice (n = 5) (key) (top row). Numbers in outlined areas (bottom row) indicate IgG1+IgG1− PCs (top left) or IgE+IgG1+ PCs (bottom right). b. Quantification of GC B cells and IgG1+ or IgE+ PCs in the skin-draining LNs of Tcrb−/− mice reconstituted with wild-type CD4+ αβ T cells (WT CD4, n = 3 host mice) or Il4−/− CD4+ αβ T cells (Il4−/− CD4, n = 4 host mice) 1 d before topical exposure of host mice to DMBA as in a, assessed by flow cytometry. c. Tumor susceptibility of wild-type and Il4−/− mice (n = 9 per group) after DMBA-induced carcinogenesis (presented as in Fig. 3a,b). Each symbol (a,b) represents an individual mouse; small horizontal lines indicate the mean (± s.e.m.). *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (two-tailed Student’s t-test for unpaired data (a,b)), or P values (in plots), log-rank (Mantel-Cox) test (tumor latency) or linear regression (tumor incidence and area) (c). Data are representative of three independent experiments with similar results (a,b); mean ± s.e.m. (middle and right in c).
Fig. 5 | Induction of tumor-protective IgE requires γδ TCR+ IELs. a, Total LN cells, GC B cells and IgG1+ or IgE+ PCs in the skin draining LNs of wild-type and Tcrd−/− FVB mice (key) left untreated (Naive) or exposed topically to DMBA on the dorsal side of the ears twice, 3 d apart; analyzed by flow cytometry 4 or 7 d after the final exposure (horizontal axis): mice: n = 10 (wild-type naive), n = 8 (wild-type 4 d), n = 10 (wild-type 7 d), n = 5 (Tcrd−/− 4 d) or n = 10 (Tcrd−/− 7 d). b, ELISA of IgE in serum from wild-type and Tcrd−/− mice (key; n = 10 per group) topically exposed to DMBA on shaved back skin, with blood obtained before (Pre-bleed) and 4 d after exposure (horizontal axis). c, d, Quantification of γδ T cells in the LNs and Vγ5+ IELs, Vγ4+ cells and other ‘non-Vγ5’ γδ T cells in the skin (c) and IgG1+ or IgE+ PCs in the skin-draining LNs (d) of WT→WT and Tcrd−/−→Tcrd−/− (control) chimeras and Tcrd−/−→WT and WT→Tcrd−/− chimeras (n = 5 per group) after exposure to DMBA and analysis (by flow cytometry) at day 7 as in a, e. Quantification of IgE+ PCs in the skin-draining LNs of wild-type FVB mice (n = 6) and Tcrg-V5−/Tcrd-Vγ5− FVB mice (n = 8) exposed to DMBA and analyzed as in a. ND, not detected. Each symbol represents an individual mouse; small horizontal lines (a, c–e) indicate the mean (± s.e.m.). *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (one-way ANOVA multiple comparison (a–d) or two-tailed Student’s t-test for unpaired data (e)). Data are from one experiment representative of three (a, b) or two (c, d) independent experiments with similar results (mean ± s.e.m. in b).

IgG1+ and IgE+ PCs in the skin-draining LNs of wild-type mice in all but the most common clones (Fig. 6c). In contrast, the diversity of the IgG1+ PC repertoire in Tcrd−/− mice was similar to that of wild-type mice (Fig. 6c).

We also analyzed the physicochemical properties of the complementarity-determining CDRH3 regions by principal-component analysis (PCA) of Kidera factors, which are a set of independent factors that encapsulate information from 188 different biophysical characteristics of all 20 amino acids. We observed distinct differences between GC B cells and PCs, as well as between IgG1-switched clones and IgE-switched clones in wild-type mice, in the biophysical characteristics of CDRH3 protein (Fig. 6d), which indicated that the majority of IgE+ PC clones were of extrafollicular origin and had switched directly from IgM to IgE. That conclusion was supported by clonal analysis, which indicated that <30% of IgE+ PC clones were shared with IgG1+ PC clones and only around 30% of IgE+ PC clones were shared with IgE+ GC clones (Supplementary Fig. 6b,c). Furthermore, Kidera PCA showed that the properties of IgE and IgG1 in Tcrd−/− mice were different from those of IgE and IgG1 in wild-type mice (Fig. 6d), which suggested that immunosurveillance by γδ T cells had a role in selecting the physicochemical properties of the CDRH3 regions.

Selection events are also expected to shape the B cell repertoire in terms of the use of genes encoding the variable, diversity and joining (VDJ) regions of the immunoglobulin heavy chain. We found that only five main genes of the Igh-V family and five main genes of the Igh-D family were commonly used, but all four Igh-J genes were used, by both IgG1+ and IgE+ GC B cells and PCs (Supplementary Fig. 6d–f). The third-most-frequent D region–encoding gene in IgE+ and IgG1+ GC B cells and PCs could not be annotated by the Immunogenetics (IMGT) information system, most probably due to an Igh-D gene in the genome of the FVB mouse strain that is
Fig. 6 | γδ T cells promote a distinct IgE repertoire in response to carcinogen. **a**, b. High-throughput sequencing and heavy-chain-repertoire analysis of IgG1 and IgE in sorted GC B cells and PCs from the skin-draining LNs of wild-type mice (a) and Tcrd−/− mice (b) (n = 6 per group) (key) 7 d after the second of two topical exposures to DMBA, presented as clone size (average values (ave)) for IgG1+ or IgE+ GC B cells (GC) and PCs (key). c. Clonal diversity (average values) of the IgG1 and IgE PC repertoires in wild-type and Tcrd−/− mice as in a,b, presented as the general diversity index (qD) plotted against clonal frequency (q). P < 0.05 for IgE from q < 2. **d**, Kidera-factor PCA of IgG1+ or IgE+ GC B cells and PCs (key) in wild-type mice (left) and Tcrd−/− mice (right) as in a,b, e. Three-dimensional plot of the use (average values) of VDJ-encoding gene families (along axes) in IgE+ PCs in wild-type mice (top) or Tcrd−/− mice (bottom) as in a,b, V-encoding and D-encoding genes used < 1% in the repertoire have been removed for clarity. **f,g**, VD repertoire of IgE+ PCs in wild-type and Tcrd−/− mice as in a,b, presented as frequency of use of VD12 (f) or of other combinations of V regions (vertical axis) and D regions (horizontal axis) after exclusion of VD12 (g) among the total repertoire. **h,j**, Kidera-factor PCA (h), CDRH3 length (in amino acids (AAs)), frequency of aromatic amino acids (phenylalanine, histidine, tryptophan and tyrosine), small amino acids (alanine, cysteine, aspartic acid, glycine, asparagine, proline, serine, threonine and valine), aliphatic amino acids (alanine, isoleucine, leucine and valine) and basic amino acids (histidine, lysine and arginine), and isoelectric point (pI) (i), and frequency of mutations in the gene encoding CDRH3, relative to germline mutations (j), in V3Dx+ clones and other VD families in wild-type IgE+ PCs. Each symbol (a,b,i,j) represents an individual mouse; small horizontal lines indicate the mean (± s.e.m.). *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (two-tailed Student’s t-test for unpaired data (a,b,f,g) or one-way ANOVA multiple comparison (i,j)). Data are presented as mean ± s.e.m. in f,g.
absent from the IMGT genomic data, as the heavy-chain repertoire is highly variable among inbred strains. We annotated this newly identified gene as ‘Dx’. Three-dimensional analysis of VDJ use in IgE+ PCs indicated that V1D2 was the dominant selection (Fig. 6e), consistent with data showing that the immunoglobulin heavy-chain V1 family is most frequently used in mouse immunoglobulins and J-region usage was approximately equal among the four J-region family groups (Fig. 6e). The VDJ analysis also revealed that some VDJ combinations in wild-type mice, particularly the newly identified ‘V3Dx’ segment, were significantly less selected in IgE+ PCs from Tcrd−/− mice than in those from wild-type mice (Fig. 6f–g).

We next compared the physicochemical properties of CDRH3 in the wild-type V3Dx+ clones with those of V3D2+ clones, which used the same V region–encoding gene but a different D region–encoding gene, V14D2+ clones, which did not require γδ T cell selection. Kidera-factor PCA indicated that the V3Dx+ clones had unique physicochemical characteristics of their CDRH3 (Fig. 6h) and had a lower frequency of aromatic and small amino acids, but a higher

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**Fig. 7 | DMBA-induced IgE is autoantigenic and differs from TPA-induced IgE.**

a, Microscopy of the binding of autoreactive IgE (red) to HEp-2 cells in serum from DMBA-treated wild-type or Tcrd−/− mice and TPA-treated wild-type mice (above images), with serum diluted 1:25, 1:5 and 1:25, respectively, to match IgE titers; nuclei are in blue. Original magnification, ×63.
b, ELISA of anti-dsDNA IgE in serum from DMBA-treated wild-type mice (n=16) or Igh7−/− mice (n=6) and naive wild-type mice (n=5).
c, Frequency of keratinocytes positive for γH2AX (as a measure of dsDNA breaks) among CD45– skin epithelial cells from mice (n=8 per group) before (Naive) and 1 or 3 d (horizontal axis) after topical treatment with DMBA or TPA (key), assessed by flow cytometry.
d–g, High-throughput sequencing and heavy-chain repertoire analysis of IgE in sorted PCs from the skin-draining LNs of wild-type mice (n=6 per group) 7 d after topical exposure to DMBA or TPA. d, Average clonal diversity throughout the IgE+ PC repertoire (presented as in Fig. 6c; *P<0.05 throughout the repertoire). e–g, Kidera-factor PCA showing CDRH3 properties (e), three-dimensional plot of use of VDJ-encoding gene families (average values) in TPA-induced IgE+ PCs and use of the five most common V-region families as a proportion of the total repertoire and frequency of the V3Dx clones (g). Each symbol (b,c) represents an individual mouse; small horizontal lines (b) indicate the mean (±s.e.m.). *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 (one-way ANOVA multiple comparison (b,c) or two-tailed Student’s t-test for unpaired data (g)). Data presented as mean±s.e.m. in g.
frequency of aliphatic and basic amino acids, as well as higher isoelectric point than that of clones of the other VD combinations (Fig. 6i). These characteristics suggested that the antigen-binding site was hydrophobic, with slight positive charge in the loop at blood pH. In addition, the V3DX-expressing clones had shorter CDHR3 regions than those of the V1D2* or V3D2* clones (Fig. 6i), indicative of antigen selection[13,25], and carried fewer mutations (Fig. 6j), which indicated that they were more germline in nature. In sum, these data indicated that in response to DMBA, γ8TCR+ T cells shaped the IgE repertoire by supporting specific VDJ rearrangements, which resulted in unique characteristics of the CDHR3 antigen-binding site, suggestive of polyreactive autologous binding.

DMBA-induced IgE is autoantigenic and differs from TPA-induced IgE. We next explored whether the IgE responses induced by topical DMBA were autoreactive. We screened the binding of IgE from the serum of DMBA-treated wild-type mice to human HEp2 epithelial cells and observed several strong staining patterns (Fig. 7a and Supplementary Fig. 7a) indicative of both autoreactivity of IgE and cross-species reactivity of IgE. The most common staining patterns of IgE on HEp2 cells were ‘nuclear dots’, indicative of anti-nuclear specificity, and ‘cytoplasmic vesicles’ that resembled stress granules (Fig. 7a and Supplementary Fig. 7a). In addition, we detected IgE reactivity to dsDNA (Fig. 7b) and to damaged epithelial cells, frequently around hair follicles, in epidermal sheets isolated from Fcer1a−/− mice 24 h after exposure to DMBA (Supplementary Fig. 7c–g). We detected no autoactivity to HEp2 cells for IgE from the serum of DMBA-treated Tcra−/− mice or IgE from the serum of wild-type mice treated topically with the inflammatory chemotherapeutic agent cisplatin (Supplementary Fig. 7c), which did not elicit DNA damage in epithelial cells (Fig. 7c), but triggers general skin inflammation.

We next sorted FSc−CD95+CD138+ PCs from the skin-draining LNs of wild-type mice exposed topically to DMBA or TPA and compared their IgE heavy-chain repertoires by high-throughput sequencing. Alakazam analysis indicated that TPA-induced IgE+ PCs exhibited significantly less diversity throughout the repertoire than that of DMBA-induced IgE+ PCs (Fig. 7d). Kidera-factor PCA indicated more heterogeneity in the CDHR3 regions of TPA-induced IgE than in those of DMBA-induced IgE, with little overlap between the two responses (Fig. 7e). In parallel, use of VDJ-encoding gene families among TPA-induced IgE was dominated by V1 encoding genes, with significantly less selection for V3-encoding genes and V14-encoding genes than that among DMBA-induced IgE (Fig. 7f,g). Notably, TPA also did not generate the V3DX-expressing clones, which we had found to be promoted by γ6 T cells in response to DMBA-induced DNA damage (Fig. 7g). Together these results indicated that exposure of the skin to DMBA drove a unique, autoreactive IgE repertoire that was distinct from that induced in response to TPA, which induced local acute inflammation.

Discussion

Here we found that IgE was rapidly induced by cutaneous exposure to the carcinogen DMBA and accumulated in the skin and tumors on FcRε-expressing basophils. The IgE response required γ8TCR+ IELs, was autoreactive and was protective against epithelial carcinogenesis. FcRε-expressing cells also accumulated in human skin SCCs, and the level of FcRε expression correlated with disease severity. Together our data indicated that IgE contributed to early tissue immunosurveillance against environmental xenobiotics or toxins and had a role in protection against epithelial tumor development.

Polyaromatic hydrocarbons such as DMBA are ubiquitous and arise from many sources, including car emissions and tobacco smoke[19,33]. They are the main organic constituent in air pollution, and their levels correlate with the prevalence and severity of atopic dermatitis[23,24]. In mice, chronic exposure of the skin to air pollutants can induce atopic dermatitis–like features and IgE production. However, while gene expression in the skin depends on ligation of the aromatic hydrocarbon receptor by polyaromatic hydrocarbons, the increase in IgE does not[26]. Consistent with that, we found that the initiation of IgE induction after topical application of DMBA depended on epithelial DNA damage, not direct engagement of the aromatic hydrocarbon receptor. Host DNA can selectively induce IgE[26], which would suggest that dying cells have unique IgE-adjuvant activity. Furthermore, expression of autologous stress antigens, such as NKG2D ligands, on damaged skin epidermis promotes IgE production[5]. Topical application of TPA also induces the expression of NKG2D ligands[26], which might partly explain the induction of IgE by this agent. However, sequencing analysis revealed that different types of tissue damage initiated distinct IgE repertoires. In humans, self-reactive IgE has been found in patients with systemic lupus erythematosus[27,28], bullous pemphigoid[29], atopic dermatitis[30] or primary immune deficiencies[31]. Such data suggest that IgE responses are potently promoted by autologous cell stress and/or damage, which is induced by exposure of the skin to environmental xenobiotics.

IELs provide a first line of defense in epithelial tissues. A large proportion of IELs carry γ8TCRs and are autoreactive in nature, as they express receptors for tissue-specific stress molecules[26]. We found that following DMBA-induced epithelial injury, IELs rapidly elicited IgE in the parafollicular regions and subsequently triggered a conventional adaptive response that required polyclonal αβ TCR+ CD4+ T cells that produced IFN-γ. This mode of inducing IgE differs from the regulation of self-reactive ‘natural IgE’, which is also driven by γδ T cells but is independent of αβ T cells and major histocompatibility complex class II[32]. Deep sequencing of IgG1+ and IgE+ GC B cells and PCs demonstrated that γδ T cells shaped the repertoire and the nature of the antibodies produced. Thus, antibody deep sequencing revealed a previously unrecognized mode of B cell selection dictated by tissue-resident IELs.

γδ T cells and tissue-specific γδ IELs are key participants in host defense against cancer, which is considered to reflect innate properties such as cytotoxicity and cytokine production[11,13,27]. Here we found that they were able to provide a second line of host defense via the initiation of adaptive autoreactive IgE responses and FcεRI-expressing effector cells. Our findings that endogenous IgE was able to suppress tumor growth are supported by evidence that immunization with tumor-antigen-specific IgE can eradicate tumors and is more effective than immunization with IgG1[34,35]. How IgE inhibited tumor growth in our model remains to be determined; however, it required expression of FcεRI, and mast cells were not essential, which would suggest it might involve soluble factors and/or cytotoxicity mediated by basophils.

In humans, epithelial skin cancers are on the rise[38,39]. In addition to UV irradiation, environmental chemical carcinogens can also lead to the development of skin SCC, as indicated by the causal link, in chimney sweeps, between scrotal SCC and exposure to soot, which contains polyaromatic hydrocarbons[40]. Moreover, tobacco smokers, who are exposed to large amounts of polyaromatic benz[a]anthracene, have a 50% increased likelihood of developing skin SCC[41]. Studies of IgE and cancer risk in humans have shown a negative, albeit weak, correlation between serum IgE and overall cancer risk[42,43]. In addition, FCER1A is reported to be among the top 50 genes associated with positive survival in 39 human malignancies[44]. The presence of intratumoral γδ T cells is the most favorable prognostic indicator of survival in patients with cancer[44], while a large number of infiltrating PCs is also strongly associated with good clinical outcome[44]. Consistent with those observations, we found that FcεRI-expressing cells were abundant in human SCC, and there was an inverse correlation between expression of FCER1A and more-advanced disease. Of note, the use of omalizumab, a monoclonal antibody that blocks...
IgE has been associated with more-frequent development of cancer, particularly epithelial and solid-organ cancers. Together these findings suggest that the IgE-FcεRI axis is part of a tumor-protective immune response and provide support for the ‘toxin hypothesis’, which proposes that IgE is a host defense mechanism against non-infectious cell-damaging xenobiotics.

**Methods**

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41590-018-0161-8.

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tumor samples for Nanostring; K.B. and M.H. generated SCC NanoString data and K.G. analyzed it; M.B. assisted with data interpretation and manuscript preparation; D.D.-W. assisted with sequencing analysis and interpretation; and J.S. performed and analyzed some experiments, directed the study and wrote the manuscript.

**Competing interests**
The authors declare no competing interests.

**Additional information**
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Methods

Mice. Genetically altered mice were generated as previously described. Tcrd−/− mice, Tcrd−/+ mice, Il4−/− mice, Langerin-DTA (LC-deficient) mice and Treg V9−/− Tcrd−/− mice were on the FVB/N background after >10 backcrosses. Igh-7−/− mice, K6−/− mice, and Cpa1−/− mice were on the BALB/c background after more than ten backcrosses; and OT-II Tg mice, Tcrd−/+ mice, CD19-Cre mice and B6+/+ mice were on the C57BL/6 background. Strain-matched wild-type control mice were purchased from Charles River. Mice were bred and maintained in individually ventilated cages under specific pathogen-free conditions. Age-matched female mice were used for all experiments at ≥25 weeks of age and were selected at random from a large pool when allocated to experiments. All studies were approved by Imperial College AWEB (Animal Welfare and Ethical Review Body) and by the UK Home Office. Experiments involving cancer studies strictly adhered to the guidelines set out by the National Cancer Research Institute (NCRI) and in the document ‘Guidelines for the Welfare and Use of Animals in Cancer Research’. All studies using animals were conducted following the document Animal Research: Reporting In Vivo Experiments (ARRIVE).

Chemical cutaneous damage and carcinogenesis. Chemicals 7,12-dimethylbenz[a]anthracene (DMBA) and 12-0-tetradecanoylphorbol-13-acetate (TPA) were purchased from Sigma and were dissolved in acetone and 100% ethanol, respectively. Acute damage was induced by exposure of the dorsal sides of the ear skin to a single dose or repeated doses of 200 nM DMBA or 2.5 nM TPA, in 25 μl. In brief, 200 nM DMBA was carefully and slowly applied by pipette, in a 100-μl volume, to the entire shaved skin area. Mice were allowed to ‘rest’ for 1 week, and 100 nM DMBA was then applied weekly. Hair regrowth during the experiment was gently removed by clipping with trimmers. Mice were monitored for chemical cutaneous damage and cutaneous tumour incidence and monitored and killed at a narrower once weekly. Back skin and tumours were evaluated by visual inspection by an observer blinded to the experimental groups.

UV irradiation. The back skin of FVB mice was shaved using electrical clippers 3 d before UV irradiation. Mice were exposed to the UV light source in their cages placed under an UV lamp with a UV output of approximately 270–350 nm (predominantly UVB) (Philips TL12 lamps). The total dose of UVB radiation was monitored during each exposure using an IL-400 A UVB photometer (International Light). Mice were given 100 mJ/cm2 three times a week.

Tissue processing. Tissue was cut into small 1-mm2 pieces using a scalpel blade and incubated for 2 h in digestion buffer containing 25 μg/ml Liberase (Roche), 250 μg/ml DNase (Roche) and 1 × DNase buffer (1.21 Tris base, 0.5 g MgCl2 and 0.073 g CaCl2) at 37°C. Following digestion, tissue was transferred into C-tubes (Miltenyi Biotech) containing RPMI-1640 medium (Thermo Fisher) supplemented with 10% heat-inactivated fetal calf serum, 1% penicillin-streptomycin-glutamine (Thermo Fisher) and was physically disrupted using a Miltenyi cell dissociator. Cell suspensions were then filtered and cells were counted and measured with a caliper once weekly. Back skin and tumours were evaluated by visual inspection by an observer blinded to the experimental groups.

Flow cytometry. Cell suspensions were blocked for non-specific binding using antibody to FcγR (2.4G2, BD) and 2% normal rat serum (Sigma) before any staining protocols. For staining of cell-surface markers, cell suspensions were stained with fluorochrome-conjugated antibodies or the appropriate isotype-matched control antibody with the addition of a fixable, live/dead discrimination dye (Invitrogen) for 25 min and were subsequently washed. Intracellular staining was then carried out using an Intracellular Staining kit according to the manufacturer's instructions (ThermoFisher Scientific). Cells were fixed for 10 min at 4°C with Perm buffer and stained for intracellular markers for 25 min. For intranuclear staining of γH2AX, cells were fixed and permeabilized in ice-cold 70% ethanol at −20 °C for 2 h, blocked with 2% normal mouse serum (Sigma), Fc- and block-2% FCS for 15 min, followed by 45 min of staining for γH2AX at room temperature. Stained cells were analyzed using a BD FACsVerse and a Fortessa X20 (BD Biosciences). Data analysis was performed using FlowJo 10 for Mac (TreeStar).

ELISA. For IgG1 and IgG2a antibodies, NUNC Immune Maxisorp 96-well plates (Thermo Scientific) were coated with 5 μg/ml goat anti-mouse IgG + L (1010-01, Southern Biotech) in borate-buffered saline at 37 °C for 3 h. After washing, plates were blocked with PBS containing 0.5% BSA for 1 h at room temperature and the appropriately diluted serum was added, followed by incubation overnight at 4°C. After washing, plates were incubated with alkaline phosphatase–conjugated polyclonal goat anti-mouse IgG1 (1070-04) or IgG2a (1080-04) (also detects IgG2c in C57BL/6 mice) (both from Southern Biotech) for 5 h at 4°C. Following further washing, the alkaline phosphatase substrate pNPP (Sigma) was added and absorbance was measured at 405 nm. Total IgE was measured by an IgE-capture method. Serum to be tested and the IgE standard were added to plate wells coated with anti-IgE (RI-28, PharMingen) and blocked with 1% rat serum. Biotinylated rat monoclonal antibody to mouse IgE (R35-92, PharMingen) and added with 1% rat serum and 3% milk. Serum samples were screened at 1:25 dilution. 100 ng/ml biotinylated herring sperm dsDNA (Promega) was then added, followed by incubation overnight at 4°C. Total IgE, Tween, plates were incubated with alkaline phosphatase–conjugated streptavidin (PharMingen) for 1 h at 37°C. After washing, detection was carried out by addition of the alkaline-phosphatase substrate pNPP (Sigma) and absorbance was measured at 405 nm. Autoantibody levels are presented in arbitrary ELISA units.

H&E staining. Tumour specimens were fixed in 10% buffered formalin overnight, embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin (H&E). Tumour tissue sections were analyzed using a BX51 microscope (Olympus) and image analysis was performed using the ImageJ software package (National Institutes of Health, Bethesda, MD).

Immunofluorescent staining of skin samples. Skin specimens were fixed in 4% paraformaldehyde for 1–2 h at room temperature, dehydrated through a series of increasing concentrations of ethanol, and embedded in paraffin wax. 5 μm-thick sections were cut, mounted on poly-L-lysine-coated glass slides, allowed to air-dry overnight, stained at 4°C with Alexa Fluor 488-conjugated mouse anti-CD45, Alexa Fluor 568-conjugated goat anti-CD3, Alexa Fluor 594-conjugated goat anti-IgE and Alexa Fluor 647-conjugated goat anti-IL-4 (all Invitrogen) and counterstained with DAPI (Vector Laboratories) and were visualized with a Leica TCS confocal laser-scanning microscope (Leica).
were determined. Complementary DNA (cDNA) was synthesized from RNA with an iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's instructions. cDNA was diluted in nuclease-free double-deionized water for qRT-PCR. All primers were single-stranded DNA oligonucleotides (Sigma) that were intron spanning as verified by NCBI Primer-Blat tool. Real-time PCR products were detected with SYBR Green (Life) measured continuously with a Viia 7 Real-Time PCR system (Applied Biosystems).

Ct values for genes of interest were normalized against Ct values of the housekeeping gene encoding cyclophilin (Cyp). Amplicons were purified by gel extraction using a QIAquick PCR purification kit (Qiagen) and were deep sequenced by long-read 454 pyrosequencing on the Genome Sequencer FLX system (Roche). Raw sequencing data were presented in FASTA format and unproductive sequences were removed (data cleanup stages as described). Analysis was performed as previously described. In brief, sequences were assigned to individual samples according to their multiplex identifier. V(D)J gene assignment and V(D)J sequences were referenced using the Phyre High-Fidelity DNA Polymerase (New England Biologs) to ensure accuracy and robust performance for large PCR products. PCR was performed with a primer in the constant C region (5'-CTAGGGTCTAGGAGGCAGTGCC-3') or C region (5'-GGAGATACGTGCTCTGCAAGTT-3') in combination with a promiscuous Y region primer (binding all Y region heavy-chain genes) (5'-GAGGTCGGTGGTCATGTGGTG-3'). All primers were labeled at either end with multiplex identifiers for multiplexing. PCR thermal cycling was as follows: 98 °C, 30 s; 50 °C (98 °C, 30 s; 60 °C, 30 s; 72 °C, 35 s); and 72 °C, 10 min. Amplicons were purified by gel extraction using a QIAquick PCR purification kit (Qiagen) and were deep sequenced by long-read 454 pyrosequencing on the Genome Sequencer FLX system (Roche). Raw sequencing data were presented in FASTA format and unproductive sequences were removed (data cleanup stages as described).

### Immunoglobulin sequencing and analysis

Mice were treated twice with DMBA or TPA to the dorsal side of the ear skin, 3 d apart. 1 week after the final exposure, draining LNs were collected and GC B cells (B220+GL7+) and Ab-TGGACACTCACAAGACCAATG-3' and R (5'-GTTTGTGCACCTCATTGGG-3'). Sequences were amplified using primers with a 5'-CACATTGAGCATCCAAGGAA-3' and R (5'-AACAGTTGGCTATGTAGTACTCAG-3') and Cys, F (5'-CAAATGTCGGACAAACAGAA-3') and R (5'-CATCACCAGGCCTAGTCTG-3').

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**Human tissue.** For flow cytometry, freshly excised SCC tissue and peripheral blood were obtained from 12 patients during surgery at the Dermatology Department of University Hospital Southampton NHS Foundation Trust. Ethics approval was provided by the South Central Hampshire B NRES Committee (reference number 07/H0504/187). All participants recruited to the study provided informed consent in accordance with the Freedom from Research Ethics Service (EoSRES) REC 1. SCC tumors were graded by an experienced dermatopathologist as low risk or high risk based on the Scottish Intercollegiate Guidelines Network for cutaneous SCC. In addition, peri-lesional skin (histopathologically normal looking skin > 4 mm away from the tumor edge) and abdominal non-UV-exposed skin were also analyzed. Total RNA was extracted using a Qiagen RNeasy extraction kit and was directly hybridized using the NanoString PanCancer and PanCancer Immune expression panel and analyzed on an nCounter (NanoString).

**Statistical evaluation.** The statistical significance of difference between experimental groups was determined using two-tailed Student's t-test for unpacked data, Wilcoxon test for paired samples, one-way ANOVA or linear regression, where appropriate, with results deemed significant at P < 0.05. Asterisk significance correlates as follows: *P < 0.05; **P < 0.01; ***P < 0.001; and ****P < 0.0001. Statistics were analyzed with GraphPad Prism 6.00 for Mac (GraphPad).

**Data availability statement.** The data supporting the findings of this study is available from the corresponding author upon request. RNA sequencing data are available from the public repository on the National Center for Biotechnology Information's Sequence Read Archive in raw format (accession codes: BioProject, PRJNA417372; BioSample, SAMN07985450, SAMN07985451, SAMN07985452, SAMN07985453, SAMN07985454 and SAMN07985455).

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- Clearly defined error bars

State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

BD FACS Diva software version 8.0.1, Quant Studio RT-PCR version 1.3, ELISA Ascents software version 2.6.

Data analysis

All software used throughout this work is publicly available: FlowJoV8.8.7/10, ImageJ, Graph Pad Prism 7, Illustrator, Alakazam from the Change-O toolkit (http://clip.med.yale.edu/changeo/) and BRepertoire (http://mabra.biomed.kcl.ac.uk/BRepertoire/).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data supporting the findings of this study are available from the corresponding author upon request. RNA sequencing data is available from the public.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑ Life sciences
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**
No statistical method was used to predetermine sample size. Sample sizes were chosen based on previous experience with the experimental protocols. The antibody responses examined in this work were highly reproducible with little variability. Carcinogenesis experiments have a greater biological variation, however the protocols are well-known, and much previous experience guided us to increase our samples sizes to minimum 10 animals per experimental group to ensure statistical power in these longitudinal studies.

**Data exclusions**
No data points were excluded from data sets.

**Replication**
All the experimental data provided are based on biological replicates and are representative of independent experiments. All experiments were reliably reproduced.

**Randomization**
For animal work, mice of a particular genotype were randomly selected for experiment from a larger cohort - other than that, no randomization was used.
For human tissue, individual SSC tumours were graded by an experienced dermatopathologist as low risk or high risk based on the Scottish Intercollegiate Guidelines Network for cutaneous SCC, as outlined in the materials and methods.

**Blinding**
For reporting on tumour development following chemically induced carcinogenesis, back skin and tumours were evaluated by an observer blinded to the experimental groups. For most other experiments, the investigators were not blinded to group allocation.

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a                              | n/a     |
| ☑ Unique biological materials    | ☑ ChIP-seq |
| ☑ Antibodies                     | ☑ Flow cytometry |
| ☑ Eukaryotic cell lines          | ☑ MRI-based neuroimaging |
| ☑ Palaeontology                  |         |
| ☑ Animals and other organisms    |         |
| ☑ Human research participants    |         |

**Antibodies**

**Antibodies used**
For all flow cytometry staining, cell suspensions were incubated with 2% Rat Serum (Sigma) and Fc block (clone 93, BD Bioscience) for 30mins on ice, prior to staining. The following antibodies were then used: ebioscience: anti-CD45 (30-F11), B220 (RA3-6B2), CD95 (Jo2), GL7 (GL-7), IgE (2G3), IgG1 (M1-14012), TCRβ (H57-597), TCRγδ (eBioGL3), Vγ5 (536), CD117 (2B8), IL-4 (11B11); BD Biosciences: CD38 (90/CD38), CD138 (281-2), IgG2a (R19-15); Biologend: CD41 (MWRReg30), FcεRI (MAR-1), IL-6 (MPS-20F3), CD63 (NVG-2), Vy4 (UC3-10Ab), CD3 (145-2C11); Millipore; yH2AX (JBW301). All antibodies were used at a dilution of 1:100-1:200 as per respective manufacturers recommendation.
OVA-loaded tetramers were provided by the NIH tetramer facility - Emory University Vaccine Center 954 Gatewood Road Atlanta, GA 30329. Reagents are provided to qualified investigators at no cost.

**Validation**
All antibodies were validated by the manufacturers and by our own and colleagues’ labs where they are used extensively. All antibodies were titrated to determine optimal staining concentration.
Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Only mice were used in this study and groups were age- and sex-matched for all experiments. In general females mice at 7-9 weeks were used throughout. Tcrd-/-, Tcrb-/-, Il4-/-, Langerin-DTA (LC-deficient) and VgSvD/a-/- were on the FVB/N background after 10 backcrosses; Igh-7-/-, Fcer1a-/- and Cpa3Cre/+ were on the BALB/c background after 10 backcrosses; and OTII Tg, Tcrb-/-, CD19-Cre and Bcl6f/f were on the C57Bl/6 background. Genetic backgrounds of individual strains and controls are indicated where appropriate. Humoral responses were also examined in male animals and were comparable. All mouse studies were approved by Imperial College AWERB (Animal Welfare and Ethical Review Body) and by the UK Home Office. Experiments involving cancer studies strictly adhered to the guidelines set out by the National Cancer Research Institute (NCRI) and Workman et al. in ‘Guidelines for the Welfare and Use of Animals in Cancer Research’. All studies using mice were conducted following the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines.

Wild animals

n/a

Field-collected samples

n/a

Human research participants

Policy information about studies involving human research participants

Population characteristics

Two cohorts of human research participants were involved in this study:
A: freshly excised SSC tumour tissue and peri-lesional skin for flow cytometric analysis from 12 patients: All tumor tissue used in the study was confirmed SSCs with a diameter > 8mm. Non-lesional skin was histopathologically normal and > 6mm away from the tumor edge.
B: For SSC tumor gene expression, fixed frozen paraffin embedded skin samples from 56 patients obtained from the University of Dundee, Tayside NHS Trust and Greater Glasgow and Clyde NHS Trust were analyzed.

Recruitment

In A: freshly excised SCC tissue and peripheral blood were obtained from 12 patients during surgery at the Dermatology Department, University Hospital Southampton NHS Foundation Trust. Ethics were provided by the South Central Hampshire B NRES Committee (reference number 07/H0504/187). All participants recruited to the study provided informed consent for blood and tissue samples to be used for research purposes.
In B: The study was conducted according to the Declaration of Helsinki Principles and all patients donating samples to this study provided written, informed consent in accordance with ethical approval from the East of Scotland Research Ethics Service (EoSRES) REC 1. SSC tumors were graded by an experienced dermatopathologist as low risk or high risk based on the Scottish Intercollegiate Guidelines Network for cutaneous SCC. In addition, peri-lesional skin (histopathologically normal looking skin > 4 mm away from the tumor edge) and abdominal non-UV exposed skin were also analyzed.

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Tumour tissue, skin, skin-draining LNs, spleens and blood were collected and processed using standard methods as detailed in the Material and Methods section.

Instrument

BD FACSVerse and BD LSRFortessa X-20

Software

BD FACS DIVA software version 8.0.1 and FlowJo software, version 8.8.7 and version 10

Cell population abundance

For cell sorting: 10,000 to 200,000 cells were collected for each population. The purity of each population was over 95% and was determined by rerunning the sorted population on a FACS.
For FACS analysis: the population abundance varied in different tissues and in different analysis - the relevant cell abundance is indicated within the results section.

Gating strategy

Flow cytometric analysis focused around identifying activated B cells, mainly germinal center B cells (GC B cells) and antibody-secreting plasma cells (PCs). In all experiments, cells were first gated on singlets (FSC-H vs FSC-A) before setting a lymphocyte gate based on SSC-A vs FSC-A. Dead cells and T cells were excluded from the analysis by using a Live/Dead dye (Invitrogen) and pan CD3 antibody respectively. GC B cells were gated as B220+, CD95+ GL7+. PCs were identified as FScH, CD95+, CD138+. Additional intracellular staining was carried out to assess isotype class switching. Isotype control antibodies were used to
determine if the staining was specific. Fluorescence Minus One (FMO) controls were used to determine the gating boundaries.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.