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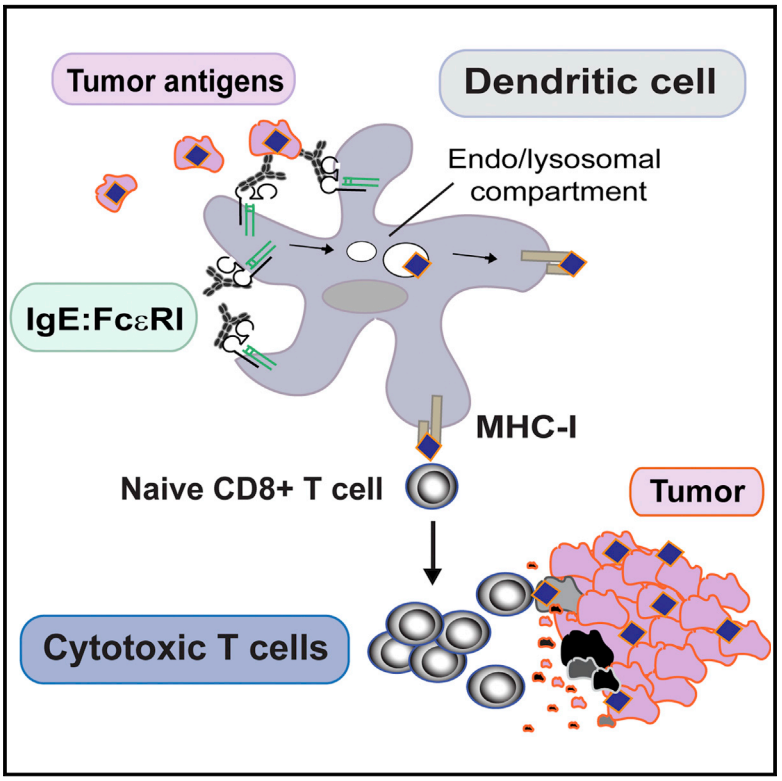
Report

IgE/FcεRI-Mediated Antigen Cross-Presentation by Dendritic Cells Enhances Anti-Tumor Immune Responses

Graphical Abstract



Highlights

- The high-affinity IgE receptor FcεRI facilitates antigen cross-presentation by DCs
- IgE and FcεRI efficiently prime CTLs in response to free low-dose soluble antigen
- MyD88 or IL-12 induction is not required for cross-presentation via IgE
- IgE-mediated cross-presentation by DCs improves anti-tumor responses in vivo

Authors

Barbara Platzer, Kutlu G. Elpek, ..., Shannon J. Turley, Edda Fiebiger

Correspondence

edda.fiebiger@childrens.harvard.edu

In Brief

Platzer et al. demonstrate a mechanism of cross-presentation executed by dendritic cells via IgE and the high-affinity IgE receptor FcεRI. IgE/FcεRI-mediated cross-presentation efficiently induces cytotoxic T cell responses, which are crucial for anti-tumor responses. This pathway provides a mechanistic explanation for epidemiologic data that show an inverse correlation between IgE-mediated allergies and cancer.

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IgE/FcεRI-Mediated Antigen Cross-Presentation by Dendritic Cells Enhances Anti-Tumor Immune Responses

Barbara Platzer,[1] Kutlu G. Elpek,[2] Viviana Cremasco,[2] Kristi Baker,[3] Madeleine M. Stout,[1] Cornelia Schultz,[1] Eleonora Dehlink,[1,5] Kai-Ting C. Shade,[4] Robert M. Anthony,[4] Richard S. Blumberg,[3] Shannon J. Turley,[2,6] and Edda Fiebiger[1,*]

[1]Division of Gastroenterology and Nutrition, Boston Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston, MA 02115, USA
[2]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02115, USA
[3]Division of Gastroenterology, Brigham and Women's Hospital and Department of Medicine, Harvard Medical School, Boston, MA 02115, USA
[4]Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Allergy, and Immunology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02129, USA
[5]Present address: Department of Pediatrics and Adolescent Medicine, Division of Pediatric Pulmonology, Allergology and Endocrinology, Medical University of Vienna, 1090 Vienna, Austria
[6]Present address: Department of Cancer Immunology, Genentech, One DNA Way, South San Francisco, CA 94080, USA
*Correspondence: edda.fiebiger@childrens.harvard.edu
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SUMMARY

Epidemiologic studies discovered an inverse association between immunoglobulin E (IgE)-mediated allergies and cancer, implying tumor-protective properties of IgE. However, the underlying immunologic mechanisms remain poorly understood. Antigen cross-presentation by dendritic cells (DCs) is of key importance for anti-tumor immunity because it induces the generation of cytotoxic $CD8^+$ T lymphocytes (CTLs) with specificity for tumor antigens. We demonstrate that DCs use IgE and FcεRI, the high-affinity IgE receptor, for cross-presentation and priming of CTLs in response to free soluble antigen at low doses. Importantly, IgE/FcεRI-mediated cross-presentation is a distinct receptor-mediated pathway because it does not require MyD88 signals or IL-12 induction in DCs. Using passive immunization with tumor antigen-specific IgE and DC-based vaccination experiments, we demonstrate that IgE-mediated cross-presentation significantly improves anti-tumor immunity and induces memory responses in vivo. Our findings suggest a cellular mechanism for the tumor-protective features of IgE and expand the known physiological functions of this immunoglobulin.

INTRODUCTION

It is well established that immunoglobulin E (IgE) plays a key role in allergies and mounts protective immune responses against helminthes (Galli and Tsai, 2012). Additionally, evidence for a role of IgE in tumor immunity has accumulated over the last decade. Epidemiological studies discovered an inverse correlation between elevated serum IgE levels (as seen in allergic patients) and the risk of developing childhood leukemia, pancreatic cancer, brain cancers, and ovarian cancer, indicating a possible function of IgE in anti-tumor immunity (Jensen-Jarolim et al., 2008; Josephs et al., 2013). Furthermore, IgE with specificity for tumor-associated antigens was found in humans (Fu et al., 2008; Staff et al., 2012), and the existence of tumor-protective features of IgE was supported by studies that employed murine models (Daniels-Wells et al., 2013; Daniels et al., 2012; Fu et al., 2008; Karagiannis et al., 2012; Nigro et al., 2009; Staff et al., 2012). However, the underlying cellular and molecular mechanisms remain largely unknown.

Tumor eradication depends heavily on the host's ability to successfully induce cytotoxic T cell (CTL) responses. Dendritic cells (DCs) contribute to tumor defense via major histocompatibility complex (MHC) class I-restricted cross-presentation, a pathway that efficiently generates CTLs in response to exogenous antigens such as those derived from tumors (Joffre et al., 2012; Mende and Engleman, 2005). Importantly, the low amount of circulating tumor antigens is considered a limiting factor for efficient responses via cross-presentation in vivo. For increased efficiency in monitoring the antigenic environment, DCs can use endocytic receptors that facilitate antigen uptake. Targeting of such receptors (e.g., DEC205) is currently being tested in cancer immunotherapy trials (Chatterjee et al., 2012; Dhodapkar et al., 2014; Tacken et al., 2007).

Fc gamma receptors (FcγRs) were among the first receptors identified to sample antigens for cross-presentation (reviewed in Platzer et al., 2014b). FcγRs allow DCs to detect antigen in the form of immunoglobulin G immune complexes (IgG-ICs), but not in its soluble free form. They belong to the immune recognition receptor family, with their ligand-binding α chains

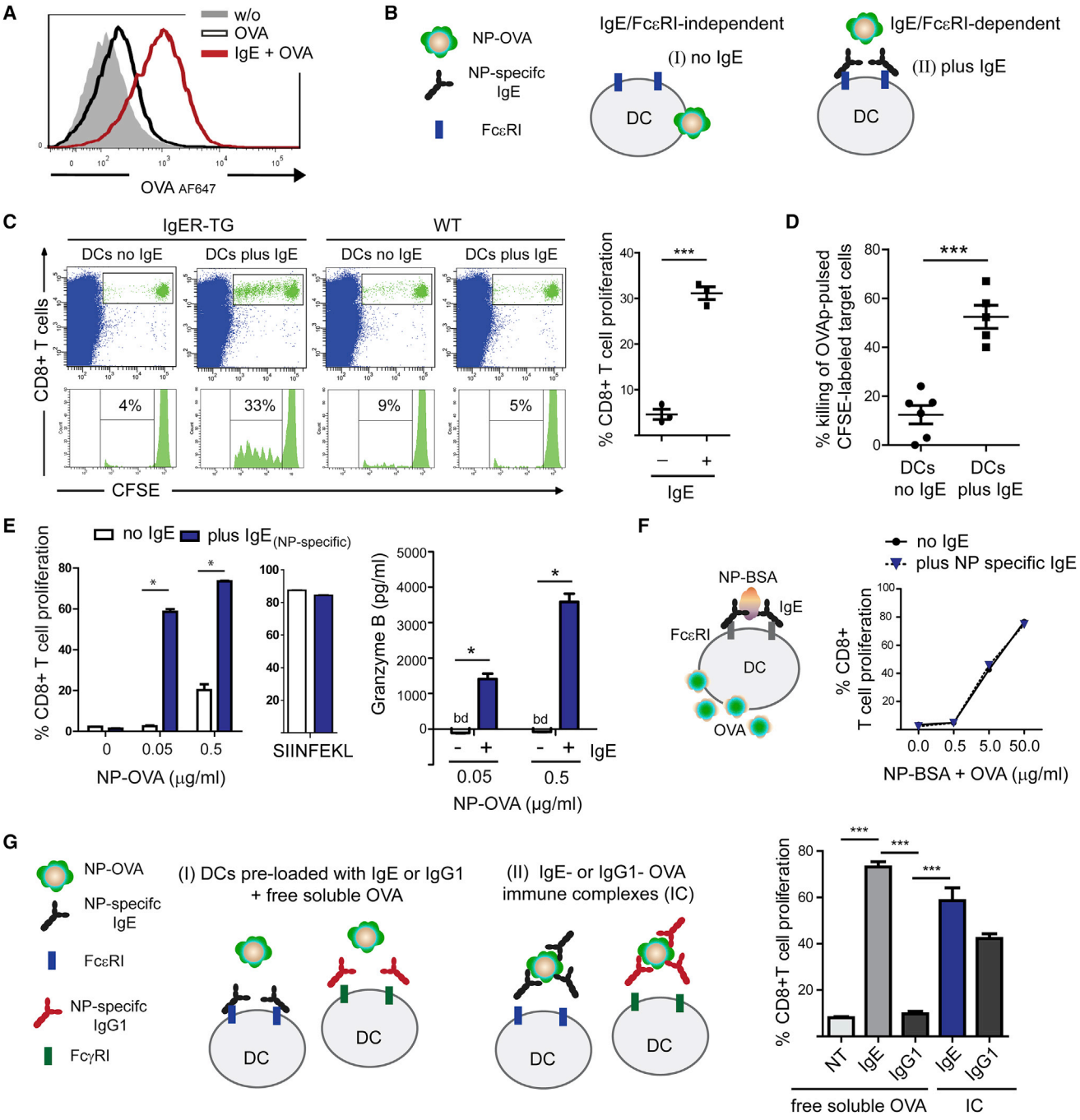

Figure 1. IgE/FcεRI-Mediated Antigen Uptake Allows for Cross-Priming of CTLs in Response to Free Soluble Antigen at Low Dose

(A) Binding and uptake of fluorescently labeled OVA (OVA^{AF647}) by DCs that were pre-loaded with OVA-specific IgE (histogram: red line). Representative histogram overlay. See also Figure S1.

(B–G) $CD8^+$ T cell priming via IgE.

(B) Schematic overview of IgE/FcεRI-independent (no IgE) and IgE/FcεRI-dependent (plus IgE) antigen sampling.

(C) In vivo T cell proliferation assay. Splenic DCs from IgE_R-TG and WT mice (without or with IgE) were pulsed in vitro with NP-OVA (0.05 μg/ml) and injected into WT recipients. Prior to DC injection, recipients received CFSE-labeled $CD8^+$ OT-I T cells. Representative FACS plots and quantification (mean ± SEM of 3 independent experiments, ≥2 mice per experiment).

(D) In vivo killing assay; dots represent individual mice (n = 2, mean ± SEM).

(E) In vitro T cell proliferation assay. Triplicates ± SEM of a representative experiment (n ≥ 5). Granzyme B production was determined by ELISA. bd, below the detection limit. Triplicates ± SEM, representative experiment (n = 3).

(legend continued on next page)

containing immunoglobulin-like domains and their FcRγ-chain dimer containing ITAM signaling modules. Interestingly, the high-affinity IgE receptor FcεRI has close structural similarities to FcγRs (Kinet, 1999). Therefore, we hypothesized that FcεRI might also contribute to cross-presentation.

Human, but not mouse, DCs constitutively express a trimeric isoform of FcεRI. Trimeric FcεRI contains the IgE-binding α chain and the common FcRγ-chain dimer, but lacks the FcεRI-β chain, which is a component of the tetrameric isoform as expressed on mast cells and basophils in humans and mice. Comparably to tetrameric FcεRI, trimeric FcεRI serves to coat the cell surface with monomeric IgE, creating the DC-bound IgE pool in humans (Galli and Tsai, 2012; Platzer et al., 2011). Since the DC-specific pool is absent in mice, we used animals that were humanized for their FcεRI expression on DCs (IgE_R-TG animals; Platzer et al., 2014a). Using this approach, we described an IgE/FcεRI-mediated cross-presentation pathway that allows for the generation of CTLs. We further demonstrated the contribution of this pathway to anti-tumor immune responses in vivo.

RESULTS

IgE/FcεRI-Mediated Cross-Presentation Efficiently Induces Proliferation of CTLs in Response to Low Doses of Free Soluble Antigen

We hypothesized that IgE/FcεRI-mediated uptake of tumor antigens by DCs and consequent cross-presentation allow IgE to contribute to cancer immunosurveillance. An examination of DCs from IgE_R-TG mice that were loaded with ovalbumin (OVA)-specific IgE and incubated with fluorescently labeled OVA showed that IgE-bearing DCs were far superior in capturing soluble free antigen compared with DCs lacking IgE (Figure 1A). Using the uptake of fluorescently labeled transferrin as a control, we showed that crosslinking of IgE/FcεRI does not change the overall endocytic capacity (Figure S1A). By following the intracellular route of IgE/FcεRI in a human cell line commonly used as model for antigen trafficking (Zwart et al., 2005), we found that the crosslinked receptor slowly entered $Rab5^+$ early endosomes, where it remained detectable over a prolonged period of time (~45 min) and then appeared in $Rab7^+$ and $LAMP1^+$ late endo-/lysosomal vesicles (Figures S1B and S1C). This slow receptor trafficking pattern suggests that IgE/FcεRI can indeed target antigens to compartments that favor MHC class I-specific presentation by protecting antigenic epitopes against rapid degradation in the more acidic environment of late endo-/lysosomes (Kreer et al., 2011). Using human DCs and human IgE with specificity for OVA, we next confirmed that IgE/FcεRI-mediated uptake shuttles free antigen into $Rab14^+$ endosomes (Figure S1D), which have been described as cross-presentation vesicles (Weimershaus et al., 2012). These data suggested that antigen that enters DCs via IgE/FcεRI is targeted for cross-presentation.

Cross-presentation of antigens by DCs primes naive $CD8^+$ T cells to proliferate and differentiate into CTLs. Thus, we first assessed whether IgE/FcεRI-mediated antigen cross-presentation promotes $CD8^+$ T cell proliferation by comparing IgE/FcεRI-dependent and -independent antigen uptake (schematic in Figure 1B). Splenic DCs were pre-loaded with hapten-specific IgE and pulsed with haptenized OVA ex vivo before being injected into recipient mice that had received carboxyfluorescein succinimidyl ester (CFSE)-labeled naive $CD8^+$ OT-IT cells that express an OVA-specific MHC class I-restricted T cell receptor. Only DCs that could use IgE/FcεRI-mediated antigen uptake induced significant T cell proliferation in response to low-dose free antigen (Figure 1C). We next tested the priming of functional CTLs through IgE/FcεRI-mediated cross-presentation by conducting an in vivo killing assay. In mice that had received DCs that used IgE/FcεRI for antigen uptake, an average killing rate of 52.4% ± 4.7% was observed, whereas the rate was only 12.4% ± 3.7% when DCs were restricted to the use of IgE-independent antigen sampling (Figure 1D). Then, we analyzed the consequences of IgE/FcεRI-dependent antigen uptake by performing in vitro antigen presentation experiments in the continuous presence of free antigen. This experimental setting, which mimics physiological antigen exposure over a prolonged time period, confirmed that IgE/FcεRI-mediated uptake significantly lowers the antigenic threshold for the induction of $CD8^+$ T cell proliferation (Figure 1E). Also, granzyme B, a component of secreted lytic granules, was generated via IgE/FcεRI-mediated cross-priming in DC/T cell co-cultures (Figure 1E). Importantly, these results demonstrate that prolonged antigen sampling via other uptake pathways may not functionally compensate for IgE/FcεRI-mediated cross-presentation if limiting amounts of free antigen are available.

We previously showed that IgE/FcεRI-crosslinking induces an intracellular signaling cascade involving SYK and ERK1/2 phosphorylation in DCs (Platzer et al., 2014a). To address whether such signals enhance the cross-presentation ability by themselves, we loaded DCs with hapten-specific IgE and incubated them with non-haptenized OVA, which restricted the antigen uptake for cross-presentation to the use of IgE-independent mechanisms. Simultaneously, we crosslinked FcεRI using haptenized bovine serum albumin (NP-BSA), an irrelevant antigen for OT-I T cells. We found that IgE/FcεRI crosslinking by NP-BSA did not alter the dose range required for antigen-specific T cell proliferation induced by IgE-independent antigen uptake. This set of experiments demonstrated that activation signals downstream of IgE/FcεRI that are not antigen specific do not increase the cross-presentation capacity of DCs (Figure 1F).

In a side-by-side comparison, we investigated whether cross-presentation of free low-dose antigen is a specific feature of IgE

(F) Signaling induced by NP-BSA through IgE/FcεRI crosslinking does not augment cross-presentation resulting from IgE-independent antigen uptake. DCs were loaded with NP-specific IgE (+) or not (−) prior to simultaneous stimulation with NP-BSA (50 μg/ml) and the indicated amounts of non-haptenized OVA. Representative experiment (n = 2).

(G) Comparison of IgE- and IgG-mediated antigen cross-presentation after incubation of DCs with free soluble antigen or immune complexes (ICs). Schematic overview: (I) DCs were pre-incubated with monomeric IgE or IgG1, washed, and treated with free soluble antigen; (II) IgE or IgG1 was pre-incubated with antigen to form ICs and then added to DCs. NT, not treated with antigen. Triplicates of a representative experiment (n = 2).

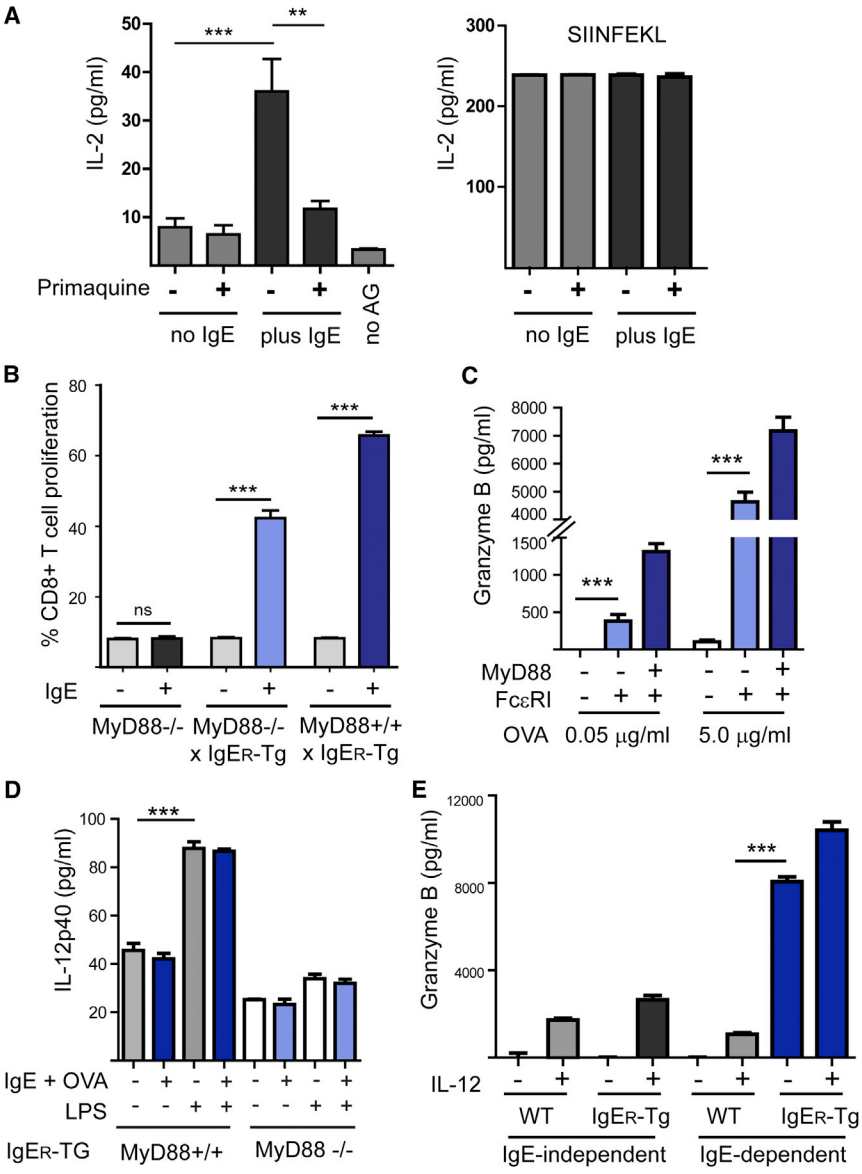

Figure 2. Cellular Mechanisms of IgE/FcεRI-Mediated Cross-Presentation

(A) IgE/FcεRI-mediated cross-presentation depends on endosomal trafficking. DCs pre-loaded with IgE or not were incubated with antigen in the presence of primaquine. OVA peptide (SIINFEKL) was used as control. Triplicates ± SEM of a representative experiment (n = 2).

(B–E) The IgE/FcεRI-mediated antigen cross-presentation pathway operates independently of MyD88 and IL-12.

(B and C) DCs from MyD88$^{-/-}$ mice expressing FcεRI (MyD88$^{-/-}$ × IgE$_R$-TG) induce efficient in vitro T cell proliferation and granzyme B production after IgE-dependent antigen uptake. See also Figure S2.

(D) Antigen-specific IgE/FcεRI crosslinking does not induce IL-12 in DCs. Splenic DCs were stimulated as indicated.

(E) Effects of exogenously added IL-12 on the cross-priming capacity of DCs.

(B–E) DCs were incubated with 0.05 μg/ml OVA. Biological triplicates ± SEM of representative experiments (n = 3).

or, alternatively, can be equally mediated by IgG. Importantly, IgE was unique in enabling DCs to cross-present free soluble antigen (Figure 1G). However, soluble antigen ICs were cross-presented with comparable efficiency irrespective of whether they had been generated with antigen-specific IgG or IgE (Figure 1G).

In summary, this data set demonstrates the existence of an IgE-mediated cross-presentation pathway in DCs.

Cellular Activation Requirements of IgE/FcεRI-Mediated Cross-Presentation

Cross-presentation via endocytic receptors is inhibited by primaquine, which blocks recycling endosomes and trafficking from early endosomes to the plasma membrane (Burgdorf et al., 2008). As expected from the FcεRI trafficking pattern in DCs (Figure S1), we found that IgE/FcεRI-mediated cross-presentation was susceptible to primaquine inhibition (Figure 2A), indicating that IgE/FcεRI routes soluble antigen into cross-presentation compartments similar to those targeted by the mannose receptor. Notably, cross-presentation facilitated by the mannose receptor depends strictly on Toll-like receptor (TLR) activation and MyD88 signals (Burgdorf et al., 2008). To further define the requirements for IgE/FcεRI-mediated cross-presentation, we compared DCs from MyD88$^{-/-}$, IgE$_R$-TG, and IgE$_R$-TG animals that were deficient for MyD88 (MyD88$^{-/-}$ × IgE$_R$-TG). We confirmed that the absence of MyD88 did not change FcεRI expression by the DCs (Figure S2A). As previously reported (Burgdorf et al., 2008), DCs from MyD88$^{-/-}$ or MyD88$^{-/-}$ × IgE$_R$-TG mice did not cross-prime CD8$^+$ T cells with soluble high-dose antigen in the absence or presence of lipopolysaccharide (LPS), but LPS increased the cross-priming capacity of MyD88$^{+/+}$ DCs (Figure S2B). Importantly, the IgE/FcεRI-mediated cross-presentation pathway remained active in the absence of MyD88, although a modest decrease in T cell proliferation and granzyme B production was detected (Figures 2B and 2C), implying that TLR signals further enhance the efficiency of IgE/FcεRI-mediated cross-presentation but are not strictly required. In line with the autonomy of the IgE/FcεRI-mediated cross-presentation pathway from MyD88 activation, we found that the pathway can operate independently of interleukin-12 (IL-12), as its induction was not detected after IgE/FcεRI-mediated antigen uptake in DCs (Figure 2D). Comparable to what was observed for TLR signals, addition of exogenous IL-12 increased granzyme B production in DC/T cell co-cultures (Figure 2E). Importantly, IgE/FcεRI-dependent cross-presentation in the absence of IL-12 still

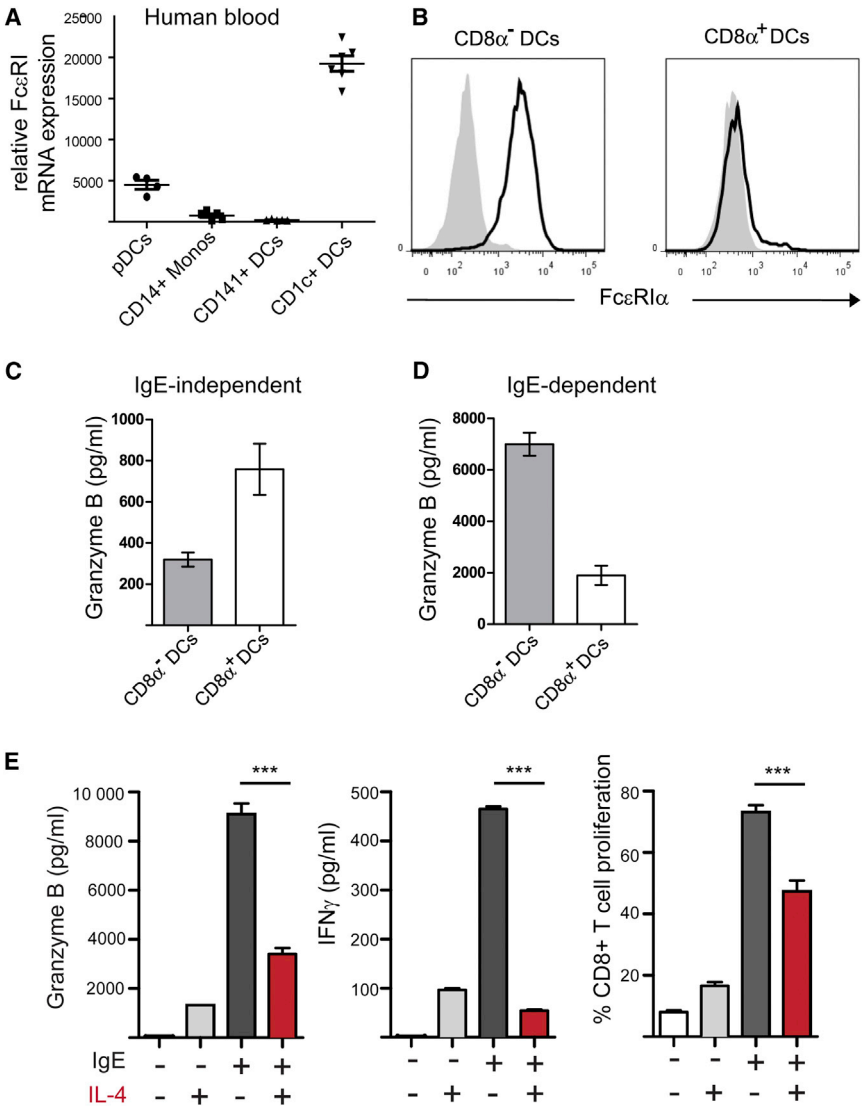

Figure 3. IgE/FcεRI-Mediated Cross-Presentation Is Executed by CD8α⁻ DCs and Is Blunted by IL-4

(A) Evaluation of FcεRI expression by human DC subsets. mRNA expression values were extracted from publically available microarray data (NCBI GEO data repository GSE35459).

(B) FcεRI expression on mouse CD8α$^+$ and CD8α$^-$ DCs from IgE$_R$-humanized mice. Histogram overlays of FcεRI (black lines) and isotype control (gray filled).

(C and D) FACS-sorted CD8α$^-$ and CD8α$^+$ splenic DCs were compared for their ability to induce granzyme B producing OT-I T cells following IgE/FcεRI-independent and -dependent antigen uptake. Data are shown as the mean ± SEM of triplicates of a representative experiment (n = 3).

(E) In vitro CTL generation in the presence of IL-4. Triplicates ± SEM of a representative experiment (n = 2). See also Figure S3.

induced more granzyme B than did IgE/FcεRI-independent cross-presentation with IL-12 (Figure 2E). In summary, this set of experiments delineates a cross-presentation pathway that significantly differs from the pathways described so far for free soluble antigens.

IgE/FcεRI-Mediated Antigen Uptake Endows the CD8α$^-$ DC Subpopulation with Efficient Cross-Presentation Capability

DC subsets differ substantially in their ability to cross-present antigens depending on the nature and form of the antigen, and the tissue environment. For example, overall, murine CD8α$^+$ DCs are considered to be the most potent cross-presenters for free soluble antigen, but cross-presentation of ICs is predominantly executed by CD8α$^-$ DCs (Bachem et al., 2010; den Haan et al., 2000; Joffre et al., 2012). Differences in the cross-presentation capacities of human DCs are more controversial (Segura et al., 2013). A potent way to regulate the cross-presentation ability of a DC subset is to regulate the expression of antigen uptake receptors that target the cross-presentation pathway. Thus, we defined FcεRI expression levels in different human DC populations (Figure 3A) by using publically available microarray data (Haniffa et al., 2013). Human CD1c$^+$ DCs, which are defined as functional homologs of murine CD8α$^-$ DCs, showed the highest transcript levels of FcεRI. FcεRI is further expressed in pDCs and, albeit at a rather low level, in CD14$^+$ monocytes. No transcripts were detected in human CD141$^+$ DCs, which are the homologs of murine CD8α$^+$ DCs (Figure 3A). Notably, this human DC subset expression pattern of FcεRI was accurately phenocopied in IgE$_R$-TG mice with high levels of surface receptor expression on CD8α$^-$ DCs (Figure 3B). Next, using fluorescence-activated cell sorting (FACS), we sorted DC subsets from the spleens of IgE$_R$-TG animals based on the expression of CD8α. In line with published literature, we found that CD8α$^+$ DCs were more efficient at priming CTLs with soluble free antigen than CD8α$^-$ DCs (Figure 3C). However, IgE/FcεRI-mediated cross-presentation was executed with the highest efficiency by CD8α$^-$ DCs (Figure 3D). This set of experiments confirms that expression of an antigen uptake receptor can expand the cross-presentation capacities of a DC subset tremendously.

IgE/FcεRI-Mediated Cross-Presentation Is Inhibited by IL-4

In allergy, where high levels of IgE are present and could promote cross-presentation, CD4$^+$ effector T cells that produce Th2-type cytokines such as IL-4 and IL-13 are predominantly found, but CD8$^+$ CTLs are not. In line with this absence of obvious CTL responses, we found that IL-4 significantly inhibited CTL generation via the IgE/FcεRI-mediated cross-presentation pathway

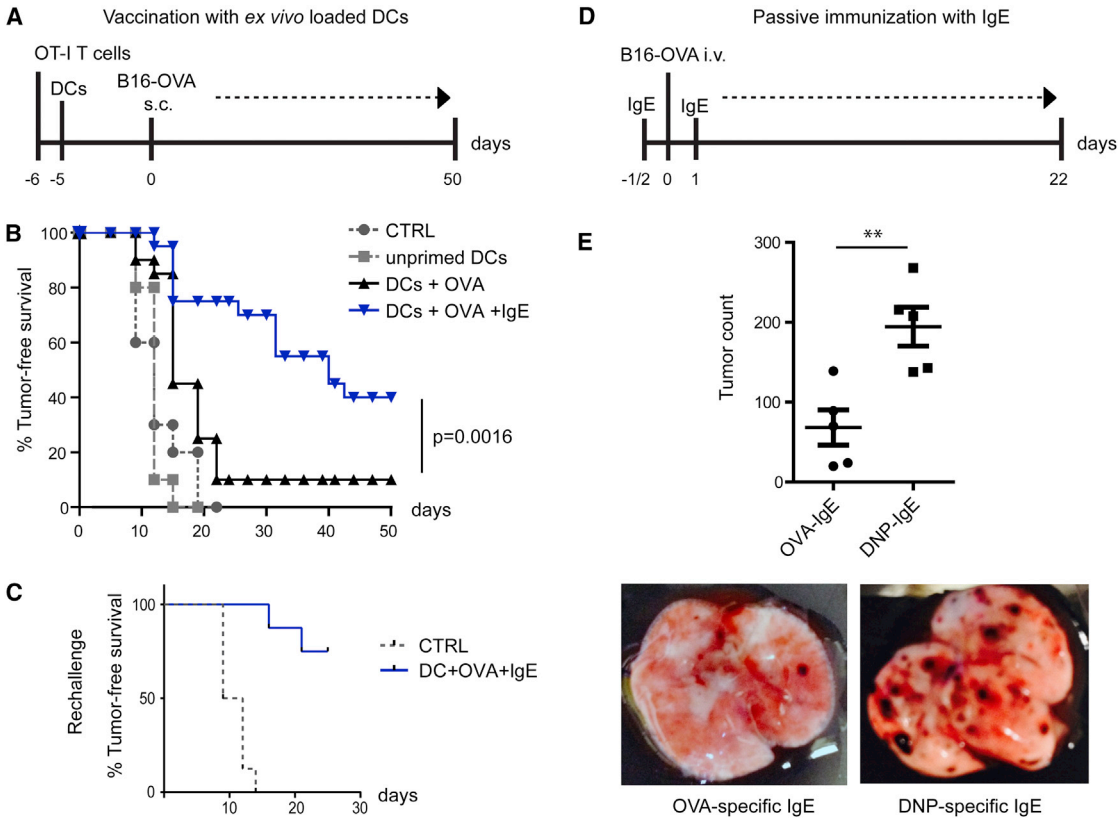

Figure 4. IgE/FcεRI-Mediated Cross-Presentation Promotes Anti-Tumor Immunity

(A) Outline of the tumor-protection experiment.

(B) Vaccination with DCs loaded with tumor-specific antigen via IgE/FcεRI increases tumor-free survival. Control (CTRL) mice did not receive DCs. Unprimed DCs were loaded with IgE but not incubated with antigen. Pooled data from two experiments; 20 mice per group, CTRL and unprimed, n = 10 mice. Tumor cells were injected subcutaneously (s.c.).

(C) Tumor-free mice from the experiments shown in (B) were re-challenged with OVA-expressing B16 tumor cells and monitored for tumor growth.

(D and E) Tumor-specific IgE mediates tumor protection in vivo.

(D) Overview of the tumor experiment after passive immunization with IgE. Mice in which expression of FcεRI was restricted to DCs were treated with OVA-specific IgE or DNP-specific IgE, and OVA-expressing B16 cells were injected i.v.

(E) Tumor count in lungs. Quantification (symbols are representative of n = 5 mice per group, mean ± SEM, **p < 0.01) and representative images are shown.

(Figures 3E and S3). This set of data indicates that during Th2-type inflammation, IL-4 prevents an overshooting CTL response induced by IgE, and explains why allergen-specific $CD8^+$ T cells are highly uncommon.

IgE/FcεRI-Mediated Cross-Presentation Affects the Efficiency of Anti-Tumor Responses In Vivo

Thus far, we have shown that IgE/FcεRI-mediated antigen uptake can be extremely efficient in priming CTL responses. Since induction of tumor-specific CTLs is a major goal in cancer immunotherapy, we next performed a classical tumor vaccination experiment. Our approach involved ex vivo antigen loading of DCs because IgE/FcεRI crosslinking on mast cells and basophils can result in systemic activation of the allergic effector cascade, including increased IL-4 production, which we found to inhibit CTL priming (Figure 3E). DCs were isolated from IgE_R-TG mice, loaded with antigen-specific IgE, pulsed with soluble free OVA, and injected into recipient mice. Next, mice were injected subcutaneously with OVA-expressing B16 melanoma cells (Figure 4A). We found significant protection from tumor development, as evidenced by prolonged tumor-free survival, in animals that could use IgE/FcεRI-mediated cross-presentation compared with animals that could not (Figure 4B). Importantly, 40% of the mice did not display any signs of tumor until day 50. To test whether the tumor-free survivors had developed memory T cell responses, we re-injected the animals with B16 melanoma cells. Indeed, mice that had been vaccinated with DCs capable of IgE/FcεRI-mediated cross-presentation remained protected upon re-challenge (Figure 4C).

We next asked whether in vivo recognition of tumor-specific antigen via tumor-specific IgE conveys anti-tumor protection (Figures 4D and 4E). Thus, we used passive immunization with tumor-specific IgE (i.e., OVA-specific IgE) and tumor-irrelevant IgE (i.e., dinitrophenyl [DNP]-specific IgE). To avoid activation of mast cells and basophils, we used an IgE_R-TG strain in which the FcεRI expression was restricted to DCs (Platzer et al., 2014a). When we monitored the capacity of OVA-expressing B16 cells to metastasize to the lung after intravenous injection (Baker et al.,

2013), we found that the pulmonary tumor burden was significantly reduced in mice that received IgE with tumor specificity (Figure 4E).

In summary, these experiments demonstrate the contribution of tumor-specific IgE and the IgE/FcεRI-mediated cross-presentation pathway by DCs to anti-tumor defense in vivo. Furthermore, the experiments suggest that targeting of this pathway might be therapeutically useful.

DISCUSSION

A novel role of IgE beyond its protective functions against helminth pathogens and its detrimental effect in allergy has recently become the center of attention. IgE was found to confer immunity against insect venoms (Marichal et al., 2013; Palm et al., 2013). Our study provides evidence for an additional physiological function of IgE by demonstrating that DCs use IgE to direct exogenous soluble free antigen into the cross-presentation pathway through the high-affinity Fc receptor FcεRI. We demonstrate that IgE-mediated antigen cross-presentation greatly enhances the ability of DCs to prime CTL responses, which are known to be crucial for immuno-surveillance against cancer. This finding is remarkable because, to our knowledge, it is the first demonstration that IgE induces MHC class I-dependent $CD8^+$ T cell responses, and thus may provide a mechanistic explanation for epidemiological studies that describe an inverse correlation of high IgE levels and cancer.

At first glance, IgE/FcεRI-mediated cross-presentation resembles other known receptor-mediated cross-presentation pathways, but its cellular requirements are distinct. A main difference compared with the IgG-mediated pathway, which has been long appreciated for its efficiency in eliciting CTLs (Regnault et al., 1999; Schuurhuis et al., 2002), is the nature of the antigen. DCs use low-affinity FcγRs for uptake of IgG-ICs. Since the latter low-affinity receptors cannot serve to coat the cell surface with monomeric IgG, and the high-affinity IgG receptor CD64, which engages monomeric IgG at the cell surface, is not expressed on DCs (Guilliams et al., 2014), the IgG/FcγR pathway is not suited to sample free antigens and is restricted to IgG-ICs. In contrast, monomeric IgE binds to the DC surface via FcεRI (Kinet, 1999), which then allows DCs to sense soluble free antigens with high efficiency, establishing a unique quality of IgE/FcεRI-mediated cross-presentation.

Several other surface receptors, such as the mannose receptor and DC-SIGN, have been described as scaffolds that allow for entry into endosomal cross-presentation compartments (Schuette and Burgdorf, 2014). The soluble model antigen OVA, which we used here, was also demonstrated to be shuttled into the cross-presentation pathway by the mannose receptor, although this seems to require higher concentrations than does IgE/FcεRI-mediated cross-presentation. More importantly, mannose receptor-dependent cross-presentation requires the concomitant occurrence of danger signals such as TLR stimulation (Burgdorf et al., 2008). Our data demonstrate that the IgE-mediated pathway does not require such additional signals, as it was found to be functional in DCs from $MyD88^{-/-}$ mice. In fact, not even the induction of IL-12 by DCs appears to be a requirement for efficient generation of CTLs in vitro. The finding that IgE/FcεRI-mediated cross-presentation operates with free low-dose antigen in the absence of danger signals from TLR ligands or inflammatory cytokines implies that it is not biased toward antigens that contain microbial molecular patterns or an inflammatory setting. The independence from additional activation signals highlights the IgE/FcεRI-mediated cross-presentation pathway as an ideal candidate for tumor antigen surveillance at steady state. Interestingly, the efficiency of the IgE/FcεRI-mediated cross-presentation pathway was counterbalanced by its susceptibility to downmodulation by the Th2-type cytokine IL-4. The latter finding is important because it explains why IgE-mediated induction of CTLs is not an immunological characteristic of allergic responses, and adds to the literature that describes Th2 responses as disadvantageous during tumor defense (Palucka and Banchereau, 2012; Sasaki et al., 2008).

Despite major research efforts, DC-based vaccination strategies aimed at inducing durable and efficient T cell responses against cancer antigens have been only moderately successful (Tacken et al., 2007). IgE-mediated antigen presentation may be particularly attractive for therapeutic purposes because of its ability to efficiently induce CTL responses via cross-presentation and simultaneously induce antigen-specific $CD4^+$ T helper cells (Platzer et al., 2014a). Concomitant induction of $CD8^+$ and $CD4^+$ T cell responses to tumor antigens was shown to improve anti-tumor immunity because synergy between these two T cell types promotes the killing of cancer cells (Tomita et al., 2013). Importantly, we also previously showed that IgE-mediated antigen uptake by DCs does not induce allergic Th2-type immune responses by itself (Platzer et al., 2014a), which could counteract efficient CTL responses. Thus, given that human $CD1c^+$ DCs constitutively express high levels of FcεRI, IgE-mediated targeting of tumor antigens directly to this specific DC subset might be a promising strategy to improve anti-tumor immunotherapy.

In summary, our study identifies an IgE-dependent cross-presentation pathway that is mediated by FcεRI on DCs. The most outstanding properties of this cross-presentation pathway are its high sensitivity for recognizing soluble free antigens, its autonomy from DC activation signals, and its efficiency in inducing tumor protection.

EXPERIMENTAL PROCEDURES

Mice

Wild-type, IgE_R-transgenic, IgE_R × murine FcεRIα$^{-/-}$, $MyD88^{-/-}$, and IgE_R × $MyD88^{-/-}$ animals were bred under specific-pathogen-free conditions at Boston Children's Hospital (Boston, MA). OT-I TCR transgenic animals and some WT recipient mice were purchased from The Jackson Laboratory. All animal studies were approved by the animal care and use committees of Boston Children's Hospital, Brigham and Women's Hospital, or the Dana-Farber Cancer Institute.

Measurement of Cytokine Production, Flow Cytometry, Antibodies, and Reagents

Details regarding measurement of cytokine production, flow cytometry, antibodies, and reagents can be found in Supplemental Experimental Procedures.

Antigen Uptake via IgE, T Cell Proliferation, and In Vivo CTL Assays

All experiments were performed using previously described standard protocols (Baker et al., 2011; Platzer et al., 2014a; Sharma et al., 2009) and are fully described in Supplemental Experimental Procedures.

Tumor Models

For the vaccination experiment, untreated DCs and DCs pre-loaded with NP-specific IgE were pulsed with 0.05 μg/ml NP-OVA for 1 hr ex vivo. Then, 2×10^5 DCs were injected into recipient mice that had received OT-I cells 24 hr before. After 5 days, recipient mice were subcutaneously injected with 2×10^5 OVA-expressing B16 melanoma cells. Tumor growth was monitored, and mice that became moribund or had a tumor size $\geq$ 2000 mm^2 were sacrificed. For the lung metastasis model, IgE_R × murine FcεRIα$^{-/-}$ mice were injected intravenously with 2×10^5 OVA-expressing B16 melanoma cells as described previously (Falo et al., 1995). At 8 hr before and 24 hr after injection of the tumor cells, the mice received 10 μg of murine OVA-specific IgE (clone E-C1; Chondrex) or mouse anti-DNP-IgE (clone SPE-7; Sigma-Aldrich). Mice were sacrificed after 22 days and tumor nodules were counted independently by two investigators (one of whom was blinded).

Statistical Analysis

Data are presented as the mean ± SEM of three or more independent experiments unless stated otherwise. Statistical analysis was performed using PRISM software (GraphPad Software). Significance was assessed using unpaired two-tailed Student's t tests for all comparisons between two populations or one-way ANOVA tests for multiple comparisons with Bonferroni's or Tukey's tests.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.02.015.

AUTHOR CONTRIBUTIONS

B.P. and E.F. conceived and designed the experiments. B.P., K.B., C.S., M.M.S., and E.D. performed the trafficking and antigen presentation studies. B.P., K.G.E., V.C., and M.M.S. performed in vivo killing and tumor experiments. K.-T.C.S. and R.M.A. provided human IgE. S.J.T., R.S.B., and E.F. obtained funding and oversaw the study. B.P. and E.F. wrote the manuscript. All authors edited the manuscript.

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REFERENCES

Bachem, A., Güttler, S., Hartung, E., Ebstein, F., Schaefer, M., Tannert, A., Salama, A., Movassaghi, K., Opitz, C., Mages, H.W., et al. (2010). Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. J. Exp. Med. *207*, 1273–1281.

Baker, K., Qiao, S.W., Kuo, T.T., Aveson, V.G., Platzer, B., Andersen, J.T., Sandlie, I., Chen, Z., de Haar, C., Lencer, W.I., et al. (2011). Neonatal Fc receptor for IgG (FcRn) regulates cross-presentation of IgG immune complexes by CD8-CD11b+ dendritic cells. Proc. Natl. Acad. Sci. USA *108*, 9927–9932.

Baker, K., Rath, T., Flak, M.B., Arthur, J.C., Chen, Z., Glickman, J.N., Zlobec, I., Karamitopoulou, E., Stachler, M.D., Odze, R.D., et al. (2013). Neonatal Fc receptor expression in dendritic cells mediates protective immunity against colorectal cancer. Immunity *39*, 1095–1107.

Burgdorf, S., Schölz, C., Kautz, A., Tampé, R., and Kurts, C. (2008). Spatial and mechanistic separation of cross-presentation and endogenous antigen presentation. Nat. Immunol. *9*, 558–566.

Chatterjee, B., Smed-Sörensen, A., Cohn, L., Chalouni, C., Vandlen, R., Lee, B.C., Widger, J., Keler, T., Delamarre, L., and Mellman, I. (2012). Internalization and endosomal degradation of receptor-bound antigens regulate the efficiency of cross presentation by human dendritic cells. Blood *120*, 2011–2020.

Daniels, T.R., Leuchter, R.K., Quintero, R., Helguera, G., Rodríguez, J.A., Martínez-Maza, O., Schultes, B.C., Nicodemus, C.F., and Penichet, M.L. (2012). Targeting HER2/neu with a fully human IgE to harness the allergic reaction against cancer cells. Cancer Immunol. Immunother. *61*, 991–1003.

Daniels-Wells, T.R., Helguera, G., Leuchter, R.K., Quintero, R., Kozman, M., Rodríguez, J.A., Ortiz-Sánchez, E., Martínez-Maza, O., Schultes, B.C., Nicodemus, C.F., and Penichet, M.L. (2013). A novel IgE antibody targeting the prostate-specific antigen as a potential prostate cancer therapy. BMC Cancer *13*, 195.

den Haan, J.M., Lehar, S.M., and Bevan, M.J. (2000). CD8(+) but not CD8(−) dendritic cells cross-prime cytotoxic T cells in vivo. J. Exp. Med. *192*, 1685–1696.

Dhodapkar, M.V., Sznol, M., Zhao, B., Wang, D., Carvajal, R.D., Keohan, M.L., Chuang, E., Sanborn, R.E., Lutzky, J., Powderly, J., et al. (2014). Induction of antigen-specific immunity with a vaccine targeting NY-ESO-1 to the dendritic cell receptor DEC-205. Sci. Transl. Med. *6*, 232ra251.

Falo, L.D., Jr., Kovacsovics-Bankowski, M., Thompson, K., and Rock, K.L. (1995). Targeting antigen into the phagocytic pathway in vivo induces protective tumour immunity. Nat. Med. *1*, 649–653.

Fu, S.L., Pierre, J., Smith-Norowitz, T.A., Hagler, M., Bowne, W., Pincus, M.R., Mueller, C.M., Zenilman, M.E., and Bluth, M.H. (2008). Immunoglobulin E antibodies from pancreatic cancer patients mediate antibody-dependent cell-mediated cytotoxicity against pancreatic cancer cells. Clin. Exp. Immunol. *153*, 401–409.

Galli, S.J., and Tsai, M. (2012). IgE and mast cells in allergic disease. Nat. Med. *18*, 693–704.

Guilliams, M., Bruhns, P., Saeys, Y., Hammad, H., and Lambrecht, B.N. (2014). The function of Fcγ receptors in dendritic cells and macrophages. Nat. Rev. Immunol. *14*, 94–108.

Haniffa, M., Collin, M., and Ginhoux, F. (2013). Ontogeny and functional specialization of dendritic cells in human and mouse. Adv. Immunol. *120*, 1–49.

Jensen-Jarolim, E., Achatz, G., Turner, M.C., Karagiannis, S., Legrand, F., Capron, M., Penichet, M.L., Rodríguez, J.A., Siccardi, A.G., Vangelista, L., et al. (2008). AllergoOncology: the role of IgE-mediated allergy in cancer. Allergy *63*, 1255–1266.

Joffre, O.P., Segura, E., Savina, A., and Amigorena, S. (2012). Cross-presentation by dendritic cells. Nat. Rev. Immunol. *12*, 557–569.

Josephs, D.H., Spicer, J.F., Corrigan, C.J., Gould, H.J., and Karagiannis, S.N. (2013). Epidemiological associations of allergy, IgE and cancer. Clin. Exp. Allergy *43*, 1110–1123.

Karagiannis, S.N., Josephs, D.H., Karagiannis, P., Gilbert, A.E., Saul, L., Rudman, S.M., Dodev, T., Koers, A., Blower, P.J., Corrigan, C., et al. (2012). Recombinant IgE antibodies for passive immunotherapy of solid tumours: from concept towards clinical application. Cancer Immunol. Immunother. *61*, 1547–1564.

Kinet, J.P. (1999). The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. Annu. Rev. Immunol. *17*, 931–972.

Kreer, C., Rauen, J., Zehner, M., and Burgdorf, S. (2011). Cross-presentation: how to get there - or how to get the ER. Front. Immunol. *2*, 87.

Marichal, T., Starkl, P., Reber, L.L., Kalesnikoff, J., Oettgen, H.C., Tsai, M., Metz, M., and Galli, S.J. (2013). A beneficial role for immunoglobulin E in host defense against honeybee venom. Immunity *39*, 963–975.

Mende, I., and Engleman, E.G. (2005). Breaking tolerance to tumors with dendritic cell-based immunotherapy. Ann. N Y Acad. Sci. *1058*, 96–104.

Nigro, E.A., Brini, A.T., Soprana, E., Ambrosi, A., Dombrowicz, D., Siccardi, A.G., and Vangelista, L. (2009). Antitumor IgE adjuvanticity: key role of Fc epsilon RI. J. Immunol. *183*, 4530–4536.

Palm, N.W., Rosenstein, R.K., Yu, S., Schenten, D.D., Florsheim, E., and Medzhitov, R. (2013). Bee venom phospholipase A2 induces a primary type 2 response that is dependent on the receptor ST2 and confers protective immunity. Immunity *39*, 976–985.

Palucka, K., and Banchereau, J. (2012). Cancer immunotherapy via dendritic cells. Nat. Rev. Cancer *12*, 265–277.

Platzer, B., Ruiter, F., van der Mee, J., and Fiebiger, E. (2011). Soluble IgE receptors—elements of the IgE network. Immunol. Lett. *141*, 36–44.

Platzer, B., Baker, K., Vera, M.P., Singer, K., Panduro, M., Lexmond, W.S., Turner, D., Vargas, S.O., Kinet, J.P., Maurer, D., et al. (2014a). Dendritic cell-bound IgE functions to restrain allergic inflammation at mucosal sites. Mucosal Immunol., Published online September 17, 2014. http://dx.doi.org/10.1038/mi.2014.85.

Platzer, B., Stout, M., and Fiebiger, E. (2014b). Antigen cross-presentation of immune complexes. Front. Immunol. *5*, 140.

Regnault, A., Lankar, D., Lacabanne, V., Rodriguez, A., Théry, C., Rescigno, M., Saito, T., Verbeek, S., Bonnerot, C., Ricciardi-Castagnoli, P., and Amigorena, S. (1999). Fcgamma receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. J. Exp. Med. *189*, 371–380.

Sasaki, K., Pardee, A.D., Okada, H., and Storkus, W.J. (2008). IL-4 inhibits VLA-4 expression on Tc1 cells resulting in poor tumor infiltration and reduced therapy benefit. Eur. J. Immunol. *38*, 2865–2873.

Schuette, V., and Burgdorf, S. (2014). The ins-and-outs of endosomal antigens for cross-presentation. Curr. Opin. Immunol. *26*, 63–68.

Schuurhuis, D.H., Ioan-Facsinay, A., Nagelkerken, B., van Schip, J.J., Sedlik, C., Melief, C.J., Verbeek, J.S., and Ossendorp, F. (2002). Antigen-antibody immune complexes empower dendritic cells to efficiently prime specific CD8+ CTL responses in vivo. J. Immunol. *168*, 2240–2246.

Segura, E., Durand, M., and Amigorena, S. (2013). Similar antigen cross-presentation capacity and phagocytic functions in all freshly isolated human lymphoid organ-resident dendritic cells. J. Exp. Med. *210*, 1035–1047.

Sharma, R.K., Elpek, K.G., Yolcu, E.S., Schabowsky, R.H., Zhao, H., Bandura-Morgan, L., and Shirwan, H. (2009). Costimulation as a platform for the development of vaccines: a peptide-based vaccine containing a novel form of 4-1BB ligand eradicates established tumors. Cancer Res. *69*, 4319–4326.

Staff, C., Magnusson, C.G., Hojjat-Farsangi, M., Mosolits, S., Liljefors, M., Frödin, J.E., Wahrén, B., Mellstedt, H., and Ullenhag, G.J. (2012). Induction of IgM, IgA and IgE antibodies in colorectal cancer patients vaccinated with a recombinant CEA protein. J. Clin. Immunol. *32*, 855–865.

Tacken, P.J., de Vries, I.J., Torensma, R., and Figdor, C.G. (2007). Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting. Nat. Rev. Immunol. *7*, 790–802.

Tomita, Y., Yuno, A., Tsukamoto, H., Senju, S., Kuroda, Y., Hirayama, M., Irie, A., Kawahara, K., Yatsuda, J., Hamada, A., et al. (2013). Identification of promiscuous KIF20A long peptides bearing both CD4+ and CD8+ T-cell epitopes: KIF20A-specific CD4+ T-cell immunity in patients with malignant tumor. Clin. Cancer Res. *19*, 4508–4520.

Weimershaus, M., Maschalidi, S., Sepulveda, F., Manoury, B., van Endert, P., and Saveanu, L. (2012). Conventional dendritic cells require IRAP-Rab14 endosomes for efficient cross-presentation. J. Immunol. *188*, 1840–1846.

Zwart, W., Griekspoor, A., Kuijl, C., Marsman, M., van Rheenen, J., Janssen, H., Calafat, J., van Ham, M., Janssen, L., van Lith, M., et al. (2005). Spatial separation of HLA-DM/HLA-DR interactions within MIIC and phagosome-induced immune escape. Immunity *22*, 221–233.