Antigen-Conjugated Human IgE Induces Antigen-Specific T Cell Tolerance in a Humanized Mouse Model

Günther Baravalle, Alexandra M. Greer, Taylor N. LaFlam and Jeoung-Sook Shin

*J Immunol* 2014; 192:3280-3288; Prepublished online 7 March 2014; doi: 10.4049/jimmunol.1301751
http://www.jimmunol.org/content/192/7/3280

Supplementary Material
http://www.jimmunol.org/content/suppl/2014/02/28/jimmunol.1301751.DCSupplemental

References
This article cites 37 articles, 23 of which you can access for free at:
http://www.jimmunol.org/content/192/7/3280.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Antigen-Conjugated Human IgE Induces Antigen-Specific T Cell Tolerance in a Humanized Mouse Model

Günther Baravalle,* Alexandra M. Greer,* Taylor N. LaFlam, † and Jeoung-Sook Shin*

Dendritic cells (DCs) play an important role in immune homeostasis through their ability to present Ags at steady state and mediate T cell tolerance. This characteristic renders DCs an attractive therapeutic target for the induction of tolerance against auto-antigens or allergens. Accordingly, Ag-conjugated DC–specific Abs have been proposed to be an excellent vehicle to deliver Ags to DCs for presentation and tolerance induction. However, this approach requires laborious reagent generation procedures and entails unpredictable side effects resulting from Ab-induced crosslinking of DC surface molecules. In this study, we examined whether IgE, a high-affinity, non–cross-linking natural ligand of FcεRI, could be used to target Ags to DCs and to induce Ag-specific T cell tolerance. We found that Ag-conjugated human IgE Fc domain (Fce) effectively delivered Ags to DCs and enhanced Ag presentation by 1000- to 2500-fold in human FcεRIα-transgenic mice. Importantly, this presentation resulted in a systemic deletion of Ag-specific T cells and prevented these mice from developing delayed-type hypersensitivity, which is critically dependent on Ag-specific T cell immunity. Thus, targeting FcεRI on DCs via Ag-Fcε fusion protein may serve an alternative method to induce Ag-specific T cell tolerance in humans. The Journal of Immunology, 2014, 192: 3280–3288.

Because Abs are bivalent, their binding to cells can cross-link cell surface molecules. Surface molecule cross-linking often triggers stimulatory signaling in cells, the outcome of which varies depending on cell type (16–19). Importantly, clinically, development of human Abs is challenging, and it requires laborious manufacturing procedures, including the initial generation of mAbs in vivo, followed by extensive modifications of the Abs in vitro (20). Thus, there is a need for an alternative method to target DCs and for an animal model to better gauge its targeting efficacy in humans.

Targeting the high-affinity IgE receptor FcεRI with Ag-conjugated IgE could be a promising alternative method. Whereas FcεRI is expressed only by mast cells and basophils in steady state mice, it is additionally expressed by DCs and monocytes in humans (21). The FcεRIα-chain, a member of the Ig superfamily, binds IgE Fc domain at a 1:1 ratio of receptor to IgE with unusually high affinity (Kd = ~10−10 M) (22). When IgE/FcεRI complexes are cross-linked by multivalent Ags, a strong inflammatory signal is transmitted to cells, resulting in degranulation of mast cells and basophils and Ag presentation and cytokine production in DCs and monocytes (21). When bound by IgE alone, however, FcεRI does not transmit signaling in general; however, certain IgE clones, namely cytokinergic IgE, appear to induce signaling through IgE Fv domain (23).

In this study, we examined whether the human IgE Fc domain could be used to induce Ag-specific tolerance in vivo by using the previously described hFcεRIα-transgenic (Tg) mice (24), which express human FcεRIα under the control of its own promoter. We demonstrate that Ag-Fce fusion proteins strongly bind both human DCs and hFcεRIα-Tg mouse DCs, that such Ags are presented to Ag-specific T cells with a 1000–2500 fold greater efficiency than unlinked Ag are, and that this presentation leads to Ag-specific T cell deletion and tolerance.

Materials and Methods

Mice and cell lines

hFcεRIα transgenic mice (24), SMARTA mice (25), Foxp3<sup>GFP</sup> mice (26), and U937 cells transfected with hFcεRI (27) have been described previously. The human IgE secreting myeloma cell line U266 was purchased.
Human blood

Healthy human blood was obtained through an ongoing study under the UCSF Institutional Review Board–approved protocol 10-02596 with informed consent.

Abs

Abs to human CD3 (HIT3a), CD19 (HIB19), CD56 (MEM188), CD14 (HCD14), HLA-DR (L243), BDCA1 (L161), CD123 (6F6), hFcRhi (CRA-1), IgE (MHE-18), murine CD49b (Dx5), CD11b (M1/70), GR1 (RB6-8C5), Thy1.1 (H1S551), TCRVα2 (B20.1), TRCVPβ5 (MR-9-4), CD45.1 (A20), CD11c (N418), MHCII (TIB120), and isotype control Ab. Cells were gated as described in (\textit{V}3) and antibodies were purchased from BioLegend or eBioscience. Anti-CD131 (JOR50) was purchased from BD Biosciences. Anti-CD11c (M5/144A) and anti-CD19 (4D9) were purchased from Miltenyi Biotec. Anti-His (Penta) was purchased from Qiagen. HRP-conjugated Abs were purchased from Bio source.

Flow cytometry

Human PBMCs were isolated from whole blood via density gradient centrifugation using Ficoll Plaque Plus (GE Healthcare) (28). Murine blood was collected via cardiac puncture and PBMCs were isolated. Cells were labeled with various cell surface Abs and acquired on a FACS LSRII system (BD Biosciences). Only live (propidium iodide–negative [BioLegend]), singlet cells were used for all data analysis. Murine cells were gated as follows: conventional DC (cDC), CD11c+MHCII+; plasmacytoid DC (PDC), BDCA1+ DCs, PDCs, basophils, and monocytes, but not BDCA3+ DCs, B cells, T cells, or NK cells. PBMCs were stained as described in (\textit{A}) and gated by flow cytometry as shown in Supplemental Fig. 1. Anti-hFcRhi Ab staining is shown black, and isotype control Ab staining is shown gray. Data are representative of three independent experiments with blood collected from multiple donors.
incubated with 10 μg/ml anti-CD3 and 20 μg/ml anti-CD28 Ab (BD Biosciences).

**In vivo Ag presentation assay**

Indicated mice were injected i.v. with 0.5–1 × 10^6 CTV or CFSE-labeled Ag-specific CD4^+ or CD8^+ T cells. One day later, mice were injected i.v. with the indicated amounts of fusion proteins, OVA peptide (323–339; China peptides), or OVA protein (Fisher Scientific). Three, seven, or twelve days later, a single-cell suspension was prepared from spleen, lymph nodes, or blood, and stained for flow cytometry using fluorophore-labeled Abs directed against CD4, CD8, TCRβ85, and Thy1.1, and analyzed by flow cytometry. GFP^+CD4^+Thy1.1^+TCRβ85^+ cells were gated and cell numbers were determined.

**In vivo Treg induction assay**

Mice were injected i.v. with 0.5–1 × 10^6 CTV-labeled CD4^+GFP^+ T cells sorted from Foxp3^+OT-II mice expressing the congenic marker Thy1.1. One day later, mice were injected i.v. with OVAp-Fce. Three, seven, or twelve days later, CD4^+ T cells were isolated from whole spleen by using anti-CD4 Ab magnetic beads (Stemcell Technologies), stained using fluorophore-labeled Abs directed against CD4, CD8, TCRβ85, and Thy1.1, and analyzed by flow cytometry. GFP^+CD4^+Thy1.1^+TCRβ85^+ cells were gated and cell numbers were determined.

**T cell restimulation assay and delayed-type hypersensitivity assay**

Naive CD4^+ T cells (0.5–1 × 10^6) from OT-II mice were injected into hFceRIα-Tg mice or Tg^- control mice. After 24 h, 0.5 μg OVAp-Fce fusion proteins or PBS were injected. Seven days later, mice were injected s.c. with 30 μg OVA peptide (323–339) mixed with IFA (Sigma). An additional 7 d later, mice were examined for the development of OV A-specific T cell immunity by performing the T cell restimulation assay and delayed-type hypersensitivity (DTH) assay. For the T cell restimulation assay, 2 × 10^5 splenocytes were isolated and incubated with or without 20 μg/ml OVA peptide (323–339) in a 96-well plate for 4 d. [3H]Thymidine (1 Ci) was added to each well for the last 15 h of incubation. [3H]Thymidine incorporated into cells was determined using Microbeta counter (1450 MicroBeta Trilux; PerkinElmer). For DTH assay, mice were injected onto the right footpads with 3 mg OV A peptide (323–339; OV Ap) mixed with IFA and onto the left footpads with PBS. Seven days later, mice were injected s.c. with 2 × 10^5 CTV-labeled CD4+GFP^+ cells. Three, seven, or twelve days later, mouse footpads were removed, and the extent of inflammatory response was determined.

**Results**

**FceRI is expressed on BDCA1^+ DCs, PDCs, monocytes, and basophils in human PBMCs**

To determine which cells express FceRI in human blood, we performed flow cytometry on human PBMCs using an anti-FceRI Ab and a panel of cell type-specific Abs. We found that FceRI was expressed on a small fraction of PBMCs and that this fraction was composed of more than one cell population, as indicated by heterogeneous labeling by FceRI and CD14 Abs (Fig. 1A, upper panel). Further analysis revealed that these FceRI-expressing cells consisted of basophils, plasmacytoid DCs (PDCs), BDCA1^+ DCs, and monocytes (Fig. 1A, lower panel). To confirm that these cell types indeed expressed FceRI, we gated them independently of FceRI expression using cell type-specific markers (Supplemental Fig. 1) and subsequently examined FceRI expression. We found that BDCA1^+ DCs, PDCs, and basophils all expressed FceRI homogeneously, whereas monocytes appeared to express it heterogeneously (Fig. 1B). We also examined BDCA3^+ DCs, T cells, B cells, and NK cells by gating them using specific markers (Supplemental Fig. 1). None of these cells expressed FceRI at appreciable levels (Fig. 1B). Thus, FceRI is specifically expressed in BDCA1^+ DCs, PDCs, basophils, and a subpopulation of monocytes in human PBMCs.

**hFceRIα-Tg mice have a similar pattern of hFceRI expression compared with humans**

To determine whether hFceRIα-Tg mice express hFceRI in a similar fashion to humans, we performed flow cytometry of blood cells from hFceRIα-Tg mice using the same anti-human FceRI Ab. We found...
that basophils, monocytes, and PDCs expressed hFcRI; cDCs also expressed it, although levels were significantly lower than for other cell types (Fig. 2A). No FcεRI was found on lymphocytes, including B cells (Fig. 2A). A similar expression profile of hFcεRI was found in the spleen (Fig. 2B). Thus, hFcεRIα-Tg mice express hFcεRI in cell types similar to humans, albeit with slightly different expression levels.

Generation of OVAp-Fce recombinant protein

Having determined that hFcεRIα-Tg mice express hFcεRI similarly to humans, we decided to use these mice to examine whether IgE could be exploited to target Ags to DCs for presentation in vivo. For this study, we generated an OVAp-Fce fusion protein. First, a cDNA was cloned encoding a V<sub>ε</sub> signal sequence linked sequentially to OVA peptide (323–339; OVAp), human IgE Ce2-Cε4 domains (Fce), and 6XHis tag (Fig. 3A). In this study, the signal peptide was included to let the recombinant protein be secreted into the cell culture media. OVA peptide (323–339) was included as a model Ag, the presentation of which can be readily determined by using OT-II transgenic mice, which express OVA-specific TCRs. The 6XHis tag was added to ease purification of recombinant protein via affinity chromatography. This cDNA construct was transfected into 293T cell lines cultured in protein-free media. Media was subsequently examined for the presence of the recombinant protein by using nonreducing SDS-PAGE followed by Western blot analysis using an anti-hIgE Ab and an anti-His Ab. A single protein band was detected at the m.w. of OVAp-Fce dimers (Fig. 3D, 3E, “Supernatant”), which was expected because IgE heavy chains dimerize via intermolecular disulfide bonds (Fig. 3B) (29). To confirm dimerization, we ran the same sample on a reducing SDS-PAGE. As predicted, the reduced protein band appeared at about half the m.w. of the nonreduced protein band (Fig. 3F). We enriched and purified the recombinant protein by collecting media and running it through a Ni-NTA column. We found that the anti-IgE and anti-His reactive band was eluted from the column at high purity (Figs. 3C–3E, “Eluates”).

Next, we determined whether OVAp-Fce specifically binds to hFcεRI. OVAp-Fce was incubated with U937 cells that were either untransfected or transfected with hFcεRI (27); flow cytometry was performed using an anti-hIgE Ab that specifically reacts with hIgE Fc domain. We found that anti-hIgE Ab stained hFcεRI-transfected U937 cells but not untransfected cells (Fig. 3G), indicating that OVAp-Fce specifically binds to FceRI. We also determined whether OVAp-Fce cross-links FceRI. OVAp-Fce was incubated with hFcεRIα-Tg mouse bone marrow–derived mast cells, and degranulation was quantified by hexosaminidase release assay (30). No hexosaminidase was released upon incubation with the construct (Supplemental Fig. 2), indicating that OVAp-Fce does not cross-link FceRI.

OVAp-Fce binds to human DCs in vitro and to DCs of hFcεRIα-Tg mice in vivo

Next, we examined whether OVAp-Fce binds to human DCs. OVAp-Fce was incubated with human PBMCs, and its binding to cells was determined by flow cytometry with an anti-His Ab to avoid unintentional signal from endogenous IgE bound to FceRI. We found that anti-His Ab bound to all FceRI-expressing cells, including BDCA1<sup>+</sup> DCs, PDCs, basophils, and monocytes, but not to FceRI-nonexpressing cells such as BDCA3<sup>+</sup> DCs (Fig. 4A). This finding indicates that OVAp-Fce specifically binds to human DCs and other FceRI-expressing cells.

Next, we examined whether OVAp-Fce binds to DCs of hFcεRIα-Tg mice in vivo. OVAp-Fce was injected i.v. into hFcεRIα-Tg mice. One hour after injection, we isolated blood cells and performed flow cytometry using anti-His Ab and cell type specific Abs. We found that anti-His Ab bound to cDCs and other hFcεRI-expressing cells such as PDCs, but not to FceRI-negative cells such as B cells (Fig. 4B, upper panel). Like in blood, anti-His Ab stained cDCs and other FceRI-expressing cells, but not B cells in the spleen (Fig. 4C). To confirm that OVAp-Fce binding to DCs is mediated by hFcεRI, we performed the same experiment using Tg-negative control mice and found no anti-His Ab binding in any cell types (Fig. 4B, lower panel). Thus, OVAp-Fce binds to DCs in hFcεRIα-Tg mice in an hFcεRI-dependent manner.

Ag targeting to hFcεRI enhances Ag presentation in hFcεRIα-Tg mice by 1000–2500 fold in vivo

To determine whether Ag targeted to FceRI through our Ag-Fce fusion protein is presented in vivo, we injected OVAp-Fce into hFcεRIα-Tg mice that had been adoptively transferred with a mixture of CTV-labeled OT-II and CFSE-labeled SMARTA mouse CD4<sup>+</sup> T cells. We included SMARTA T cells, which are specific
for an irrelevant Ag, to determine whether T cell proliferation is Ag-specific or whether it is the result of nonspecific T cell activation. Proliferation of OT-II and SMARTA CD4+ T cells was examined 3 d after OVAp-Fc injection by determining the dilution of CFSE and CTV by flow cytometry. As expected, dilution of CTV was readily detected while dilution of CFSE was not observed (Fig. 5A). This finding indicates that Ag targeted to FcRI through Ag-Fc fusion protein is presented to Ag-specific T cells in hFcRRIα-Tg mice, resulting in the restricted proliferation of only Ag-specific cells.

Next, we examined the degree to which targeting Ags to FcRI enhances Ag presentation. For this study, CTV-labeled OT-II CD4+ T cells were transferred into hFcRRIα-Tg and Tg− control mice. One day later, mice were injected i.v. with 0.05–0.5 μg OVAp-Fc. Spleens were harvested 3 d later, and the dilution of CTV was examined by flow cytometry. We found that OTII CD4+ T cells proliferated in hFcRRIα-Tg mice, but not in control mice (Fig. 5B). To determine whether the lack of OT-II T cell proliferation in control mice is due to a general defect in Ag processing or presentation of these mice, we injected hFcRRIα-Tg mice and non-Tg control mice with OVA Ags at high doses, such as 10 μg OVA peptide (323–339) or 50 μg OVA protein. We found that OT-II CD4+ T cells proliferated in control mice and hFcRRIα-Tg mice to a similar degree (Fig. 5C, 5D), indicating that Ag processing and presentation are not significantly different between Tg+ and Tg− mice. Thus, it is the targeting of OVA to FcRI that dramatically enhances OTII T cell proliferation.

To quantify the increase in Ag presentation efficiency when Ags were targeted to FcRI via IgE, we compared the degree of OT-II T cell proliferation in hFcRRIα-Tg mice injected with OVAp-Fc

**FIGURE 5.** Ag targeting to FcRI enhances Ag presentation in hFcRRIα-Tg mice by 1000–2500 fold. (A) OVA presentation in hFcRRIα-Tg mice injected with OVAp-Fc. hFcRRIα-Tg mice were adoptively transferred with CTV (Cell Trace Violet)-labeled CD45.1+CD4+ OT-II T cells and CFSE-labeled CD45.1+CD4+ SMARTA T cells at equal numbers 1 d before i.v. injection with 0.2 μg OVAp-Fc. Three days after injection, spleens were harvested and cells were stained using Abs to CD45.1 and CD4 and analyzed by flow cytometry. CD45.1+CD4+ cells were gated, and the levels of CFSE and CTV were examined. (B) hFcRI-independent presentation of OVA in hFcRRIα-Tg mice injected with OVAp-Fc. hFcRRIα-Tg mice and non-Tg control mice were adoptively transferred with CTV-labeled CD45.1+CD4+ OT-II T cells 1 d before they were injected i.v. with OVAp-Fc at indicated dosages. Three days after injection, spleens were harvested and cells were stained, and CD45.1+CD4+ TCRVα2+ cells were analyzed by flow cytometry. Data from hFcRRIα-Tg mice are shown in black and data from non-Tg control mice are shown in gray. (C and D) hFcRI-independent presentation of OVA, hFcRRIα-Tg mice and non-Tg control mice were treated and analyzed as described in (B), except that they were injected with 10 μg OVA peptide (323–339) (C) or 50 μg OVA protein (D) instead of OVAp-Fc. (E and F) Comparison of OVA presentation in hFcRRIα-Tg mice with or without OVA targeting to hFcRI. (E) Data from Tg mice injected with 0.1 μg OVAp-Fc were overlaid with data from Tg mice injected with 10 μg OVA peptide. (F) Data from Tg mice injected with 0.05 μg OVAp-Fc were overlaid with data from Tg mice injected with 50 μg OVA protein. All data shown in (A)–(E) are representative of three independent experiments.

**FIGURE 6.** FcRI-targeted Ags are presented specifically by cDCs in hFcRRIα-Tg mice. (A and B) hFcRRIα-Tg mice were injected i.v. with 0.2 μg OVAp-Fc. Twenty-four hours later, spleens were harvested. cDCs (CD11c+), PDCs (mPDCA1+), basophils (Dx5+CD131+), monocytes (CD11b+GR1med/low), and B cells (CD19+) were isolated by FACS, mixed with CTV-labeled CD45.1+CD4+ OT-II T cells at a 1:5 ratio, and cultured at 37°C. As a negative control, CTV-labeled CD45.1+CD4+ OT-II T cells were cultured alone. Three days later, cells were harvested, stained using Abs to CD45.1, CD4, and TCRVα2, and analyzed by flow cytometry. CD45.1+CD4+ TCRVα2+ cells were gated and the percentage of CTV-dilution was determined. Shown in (A) are data from one representative experiment. Shown in (B) are data from three independent experiments including mean ± SEM. (C) hFcRRIα-Tg mice were injected i.v. with 1 μg OVAp-Fc. Twelve hours later, spleens were harvested and cells were isolated by FACS as described in (A) and (B), mixed with OT-II CD4+ T cells at a 1:10 ratio, and cultured at 37°C for 4 d. [3H]Thymidine was added for the last 15 h of the culture. As a positive and negative control, OT-II CD4+ T cells were cultured in the presence or absence of anti-CD3/anti-CD28 Ab. Experiments were performed twice as quadruplicates and data show one representative experiment including mean ± SEM. ****p < 0.005.
versus OVA protein or peptide (323–339). We found that 0.1 μg OVA peptide (323–339) induced OT-II T cell proliferation to a degree similar to 10 μg of OVA peptide (323–339; Fig. 5E), and that 0.05 μg of OVA-Fc elicited an OT-II T cell proliferation equivalent to that of 50 μg OVA protein (Fig. 5F). These findings indicate that Ag presentation increases by 1000–2500 fold when Ag is targeted to FcRI in hFcRlna-Tg mice.

It is noteworthy that targeting Ags to FcRI did not appear to enhance Ag presentation to CD8+ T cells. We generated OVA (257–264)-Fcy and injected it into hFcRlna-Tg and Tgα control mice that had received OT-I CD8+ T cells expressing OVA (257–264)-specific TCR. We found that OT-I CD8+ T cells proliferated in Tgα and Tgα mice to similar degrees (Supplemental Fig. 3), indicating that DC targeting of Ags via FcRI only enhances Ag presentation to CD4+ T cells in this mouse model.

*Figure 7.* Ag targeting to FcRI results in Ag-specific CD4+ T cell deletion in hFcRlna-Tg mice.

(A and B) Rapid deletion of OT-2 CD4+ T cells in hFcRlna-Tg mice injected with OVA-Fce. hFcRlna-Tg mice were adoptively transferred with CTV-labeled CD45.1+ OT-II CD4+ T cells 1 d before they were injected i.v. with 0.2 μg OVA-Fce. Three, seven, and twelve days after injection, spleens, inguinal lymph nodes, and blood were harvested. Cells from each tissue were stained and analyzed by flow cytometry for the frequency and CTV dilution of CD45.1+ cells observed on day 7, and they completely disappeared by day 12 (Fig. 7A). When we quantitated the frequency of CD45.1+ cells in the spleen, lymph node, and blood, we found that these cells were disappearing over time from all these organs, indicating systemic deletion (Fig. 7B). To determine whether FcRlna-Tg mice have an intrinsic defect in expanding Ag-specific T cells, we examined OT-II CD4+ T cell expansion in these mice after injection of OVA-Fce together with the immunostimulatory agent LPS. We found that OT-II CD4+ T cells proliferated substantially and continuously for the entire 12 d of examination in spleen, LN, and blood (Fig. 7C, 7D). This finding indicates that FcRlna-Tg mice are capable of expanding Ag-specific T cells when Ag is targeted to FcRI in the presence of inflammatory stimuli, but delete them when Ag is targeted to FcRI in the absence of such stimuli.

We also examined whether targeting Ags to FcRI induces Ag-specific regulatory T cells (Tregs) in hFcRlna-Tg mice. Mice were adoptively transferred with GFP+ CD4+ T cells that were isolated from Foxp3GFP OT-II mice by FACS (Fig. 8A) and labeled with CTV. OVA-Fce was injected i.v. the next day, and GFP expres-
sion by transferred OT-II CD4+ T cells was monitored by flow cytometry. Very few GFP+OT-II CD4+ cells were found at any days of examination following injection with OVAp-Fcε at high or low doses (Fig. 8B, 8C). Thus, Ag-targeting to hFcεRI hardly induces Tregs.

Ag-Fcε fusion protein inhibits development of Ag-specific T cell immunity in FcεRIa-Tg mice

Lastly, we investigated whether targeting Ags to FcεRI could inhibit development of Ag-specific T cell immunity and induce T cell tolerance in vivo. For this study, we examined whether OVAp-Fcε could prevent hFcεRIα-Tg mice from developing delayed-type hypersensitivity against OVA. hFcεRIα-Tg mice adoptively transferred with OT-II CD4+ T cells were injected i.v. with either OVAp-Fcε or PBS. Seven days later, mice were immunized with OVA peptide mixed with IFA. An additional 7 d later, OVA-specific T cell immunity was determined using either in vitro restimulation assay or DTH assay. For the in vitro restimulation assay, splenocytes were isolated from mice, incubated with OVA peptide, and examined for proliferation using [3H]thymidine. We found that cells from OVAp-Fcε–injected mice incorporated [3H]thymidine at significantly lower levels than cells from PBS-injected mice did. This finding indicates that OVAp-Fcε inhibits the development of OVA-specific T cell immunity in hFcεRIα-Tg mice (Fig. 9B). To verify that this inhibitory effect was mediated by hFcεRI, the same experiment was performed using control mice that do not express hFcεRI. Splenocytes from OVAp-Fcε–injected control mice incorporated [3H]thymidine as much as those from PBS-injected hFcεRIα-Tg mice did (Fig. 9B). Thus, OVAp-Fcε inhibits development of OVA-specific T cell immunity only when hFcεRI is expressed.

For DTH assay, mice were injected into the left footpads with OVA peptide mixed with IFA, and into the right footpads with IFA alone. Twenty-four hours after injection, the thickness of each footpad was measured. The thickness of the left footpad was subtracted from that of the right footpad to determine swelling induced by OVA rechallenge (Fig. 8A). We found that both PBS and OVAp-Fcε–injected mice showed appreciable swelling, but the degree was markedly less in OVAp-Fcε–injected mice, suggesting that OVAp-Fcε injection significantly inhibited mice from...
developing DTH (Fig. 8C). Importantly, OVAp-Fce failed to inhibit Tg− control mice from developing DTH (Fig. 8C) indicating that the inhibitory effect of OVAp-Fce in DTH development requires hFceRII expression. Taken together, these findings demonstrate that Ag targeting to FceRI inhibits the development of Ag-specific T cell immunity and induces T cell tolerance.

Discussion

In this study, we have found that Ag-Fce fusion proteins can be exploited to target Ags to DCs and induce Ag-specific T cell tolerance. We found that Ag-Fce bound to human DCs in vitro and to DCs of hFceRII-Tg mice in vivo, and that injection of hFceRII-Tg mice with Ag-Fce resulted in Ag presentation by DCs and subsequent systemic deletion of Ag-specific T cells. Importantly, injection of Ag-Fce into hFceRII-Tg mice significantly inhibited these mice from developing DTH.

Previous studies have indicated that the efficiency of Ag presentation can be increased by 100–1000 fold when Ags are conjugated with DC-specific Abs such as anti-DEC-205, DNGR1, DCIR, Langerin, Treml4, or DC-SIGN (9, 14, 31–33). We found in hFceRII-Tg mice that Ags conjugated with human Fce were presented by DCs at 1000–2500 fold higher efficiency than unconjugated Ags were. We predict that the efficiency of targeting of this construct to human DCs will be even higher than in hFceRII-Tg mice, because human DCs express much higher levels of FceRII than hFceRII-Tg mouse DCs do. Thus, the efficiency of Ag presentation in humans may increase markedly when Ags are conjugated with Fce than when it is conjugated with DC-specific Ab. In addition, generation of Ag-Fce fusion protein is more straightforward, as described in this study, and perhaps more cost-effective than the generation of Ag-DC-specific Ab fusion protein. A single cDNA cloning and transfection will generate Ag-Fce; there is no need to immunize animals, generate and select hybridomas, and humanize selected Abs.

The mechanism by which Fce-linked Ag is presented to CD4+ T cells remains to be determined. It has been known that IgE/FceRI cross-linking using multivalent Ags results in the entry of Ag/IgE/FceRII complexes to endocytic compartments in DCs and subsequent processing and presentation of the Ags to CD4+ T cells (34, 35). However, whether non–cross-linking Ag could follow the same pathway of DC entry has never been examined. Our finding that Fce-linked Ag, which binds to FceRI on DCs without cross-linking it, is presented to CD4+ T cells strongly suggests that cross-linking is not required for IgE/FceRII entry to DC endosomal compartments. Some other work in our laboratory indicates significant amounts of IgE and FceRI localized in endolysosomal compartments in BDC1+ DCs freshly isolated from human blood and DCs from hFceRII-Tg mice (unpublished observations). Because DCs do not make IgE themselves, the presence of IgE and FceRI in the endolysosomal compartment implies that FceRI traffics through endocytic pathway in DCs at steady state and that IgE enters DCs by binding to FceRI at cell surface. Ag-Fce is likely to follow the same pathway of entry.

Ag targeting to DCs in the absence of DC maturation stimuli has been proposed to induce T cell unresponsiveness, deletion, or conversion to Tregs (9, 36–38). Accordingly, we found that targeting Ags to FceRI via Ag-Fce fusion protein results in T cell deletion in hFceRII-Tg mice. Ag-Fce binding to FceRI is unlikely to activate DCs, mast cells, or basophils because it does not cross-link FceRI. Consistently, no degranulation was observed when mast cells were incubated with Ag-Fce. Thus, Ag presentation in these mice is likely to have been mediated by steady state immature DCs, which may then facilitate Ag-specific T cell deletion.

Indeed, when the DC maturation stimulus LPS was cojected with OVAp-Fce into hFceRII-Tg mice, OVAp-specific T cells expanded and persisted over 12 d after injection, supporting the important contribution of DC activation status to T cell fate determination. Few Ag-specific Tregs were observed in our study, although others have found robust Treg induction when Ag was targeted to DEC-205 or Langerin (14, 37). Possibly, injection of OVAp-Fce into the peripheral tissue sites rather than blood induced Treg cells, because these cells appear to be better generated by peripheral tissue migratory DCs (14).

DTH response is commonly examined to assess T cell-mediated immunity in vivo. A lack of DTH response to a recall Ag is regarded as an evidence of deficiency in primary T cell immunity. We found that OVAp-Fce injection i.v. markedly inhibited hFceRII-Tg mice from developing OVA-specific DTH and that this inhibitory effect was completely dependent on the expression of hFceRII in these mice. This finding strongly suggests that human FceRI-targeted Ag is capable of undermining primary T cell immunity and developing Ag-specific tolerance in vivo. We intend to test in the near future whether injection of hFceRII-Tg mice with a self-antigen–or allergen-linked Fce makes these mice resistant to autoimmune or allergic diseases.

In conclusion, this study proposes a novel approach to target Ags to human DCs for the induction of Ag-specific tolerance. Further studies on its efficacy, mechanism, and clinical safety may lead to the development of new therapeutics.

Acknowledgments

We thank Dr. Amy Putnam for blood drawing, Dr. Jean-Pierre Kinet for the hFceRII-Tg mice and U937 cells, Drs. Dirk Baumjohann and Laurence Cheng for helping with mice and cDNA construct, and Dr. Bibi Chatterjee for critical reading of the manuscript.

Disclosures

The authors have no financial conflicts of interest.

References

histocompatibility complex class I products and peripheral CD8+ T cell tolerance. J. Exp. Med. 196: 1627–1638.