Abrogation of allergic reactions by a bispecific antibody fragment linking IgE to CD300a

Ido Bachelet, MSc,* Ariel Munitz, MSc,* and Francesca Levi-Schaffer, PhD

Jerusalem, Israel

Background: Initiated and regulated by mast cells, allergic responses are balanced through an intricate network of positive and negative signals. We have recently shown that the inhibitory receptor CD300a is expressed on human mast cells and modulates a large array of their functions.

Objective: We sought to evaluate CD300a as a negative regulator of allergic inflammation *in vivo* by means of a bispecific antibody linking CD300a with IgE.

Methods: Bispecific antibody fragments were generated by chemical conjugation of Fab′ fragments of anti-human IgE and CD300a ($IE1^H$) and anti-mouse IgE and CD300a ($IE1^M$). IgE-sensitized human mast cells were activated simultaneously with anti-IgE and $IE1^H$. Phosphorylation of signaling proteins and calcium influx were evaluated by using fluorescence-activated cell sorting. Degranulation was assessed on the basis of tryptase and IL-4 release. $IE1^M$ was administered simultaneously with allergen challenge in 2 murine models of allergic disease. Passive cutaneous anaphylaxis was assessed by means of dye exudation. Experimental airway inflammation was assessed on the basis of tryptase and cytokine content, eosinophilic infiltration, and lung histology (hematoxylin and eosin and periodic acid–Schiff stain).

Results: $IE1^H$ potently inhibited IgE-mediated mast cell degranulation in a dose-dependent manner by inhibiting the signaling events induced by FcεRI. $IE1^M$ completely abolished dye exudation in passive cutaneous anaphylaxis. $IE1^M$ abrogated allergic airway inflammation.

Conclusion: Our results demonstrate that specific targeting of CD300a on mast cells is a potent strategy for inhibiting allergic reactions.

Clinical implications: This work demonstrates a potent approach for the therapy of allergic diseases. (J Allergy Clin Immunol 2006;117:1314-20.)

Key words: *Allergy, CD300a, mast cells, bispecific antibody*

Abbreviations used

BALF: Bronchoalveolar lavage fluid
DNP: Dinitrophenol
ERK: Externally regulated kinase
FACS: Fluorescence-activated cell sorting
$IE1^H$: Fab′ fragments of anti-human IgE and CD300a
$IE1^M$: Fab′ fragments of anti-mouse IgE and CD300a
LAT: Linker for activation of T cells
MAPK: Mitogen-activated protein kinase
OVA: Ovalbumin
PCA: Passive cutaneous anaphylaxis
RBL: Rat basophilic leukemia
SHIP: Src homology 2–containing inositol phosphatase
SHP-1: Src homology 2–containing phosphatase

The past decades have witnessed the ongoing increase of allergic diseases, such as asthma, allergic rhinitis, and atopic dermatitis.[1] The central role of mast cells in the initiation and, more recently, in the perpetuation of the allergic process has been established throughout this time.[2] Allergen-induced cross-linking of the high-affinity IgE receptor (FcεRI) on mast cells triggers them to degranulate and release a vast array of both preformed and *de novo* synthesized mediators. The mast cells can then resynthesize and release these mediators during the late phase of the allergic reaction, when inflammatory cells and notably the eosinophils infiltrate the tissue.[3]

Immune effector cell function is stringently regulated by an intricate network of positive and negative signals. A critical part of these signals is transduced by immune inhibitory receptors, such as leukocyte Ig-like receptors, gp49B1, paired Ig-like receptor B, FcγRIIB, and sialic acid–binding lectins,[4] that are differentially distributed throughout the immune system. The functional cores of immune inhibitory receptors are specialized modules in their intracellular domain termed *immunoreceptor tyrosine-based inhibitory motifs*.[5] Coaggregation of inhibitory receptors with a kinase-associated activating receptor induces rapid immunoreceptor tyrosine-based inhibitory motif tyrosine phosphorylation and transforms the modules into high-affinity docking sites for SH2-containing protein phosphatases and inositol phosphatases, such as Src homology 2–containing phosphatase (SHP) 1 and 2 and Src homology 2–containing inositol phosphatase (SHIP).[6] These phosphatases act in combination to degrade phosphoinositide messengers and shut off activating phosphorylation events, thus terminating the activation

From the Department of Pharmacology, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem.

*These authors contributed equally to this work.

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Reprint requests: Francesca Levi-Schaffer, PhD, Department of Pharmacology, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120 Israel. E-mail: fls@cc.huji.ac.il.

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cascade at an early stage and inhibiting mediator release, maturation, adhesion, chemotaxis, and survival. We have recently shown that human mast cells express CD300a/IRp60, an inhibitory receptor belonging to the CMRF-35 gene cluster originally described on natural killer cells.[7,8] Cross-linking of CD300a induces the deployment of SHP-1 and SHIP to elicit strong negative signals on mast cell degranulation and survival.

We have decided to adopt a bifunctional protein-based approach to discern whether CD300a can be a potential target in treating allergic reactions. For this, we have designed and generated a bispecific antibody fragment that links CD300a with IgE. This construct completely abrogated IgE-induced signaling and activation in human and murine mast cells *in vitro*. Moreover, a construct targeting murine IgE and CD300a abolished the allergic-inflammatory response in 3 different animal models. Altogether, our data demonstrate that CD300a is a novel target for antiallergic therapy and that this approach provides a tool for the treatment of allergy and mast cell–associated disorders.

METHODS

Bispecific antibody design and construction

Bispecific antibody fragments were generated as previously described,[9,10] with slight modifications. The anti-human construct (Fab′ fragments of anti-human IgE and CD300a [$IE1^H$]) was generated by mean of agarose-immobilized pepsin digestion (Pierce, Rockford, Ill) of mouse anti-human IgE (clone 4C3; Serotec, Raleigh, NC) and mouse anti-human CD300a (clone P192, produced as described[8]), according to the manufacturer's instructions. The resulting $F(ab')_2$ fragments were reduced to Fab′ by means of 18 hours of incubation in reduction buffer (2 mmol/L cysteamine, 100 mmol/L Na_2HPO_4, 1 mmol/L EDTA, and 10 mmol/L sodium arsenite, pH 6.8). The anti-CD300a–derived Fab′ was thiol activated by 5 mmol/L 5,5′-dithiobis(2-nitrobenzoic acid) for 5 hours, and both Fab′ species were reconjugated for 18 hours in 100 mmol/L Na_2HPO_4/1 mmol/L EDTA, pH 6.8. All reagents used in conjugation were purchased from Sigma (Rehovot, Israel), except for cysteamine and sodium arsenite (Merck, Darmstadt, Germany), and were of analytic grade. The product of each stage was filtered and purified by means of centrifugal gel filtration, and the entire process was sampled to be monitored by means of SDS-PAGE and spectrophotometry. Fluorescence-activated cell sorting (FACS) analysis confirmed that the intermediate and final products retained their binding ability. The anti-mouse construct (Fab′ fragments of anti-human IgE and CD300a [$IE1^M$]) was generated similarly to $IE1^H$ but with rat anti-mouse IgE (clone LO-ME-3; Serotec) and rat anti-mouse CD300a (clone NKRL1-172224.111; R&D Systems, Minneapolis, Minn) selected from 4 clones recognizing murine CD300a. Control antibodies were generated by passing nonspecific mouse or rat IgG1 molecules through the same process (isotype control constructs) to rule out an unspecific inhibitory effect (eg, caused by residual coupling reagents).

Mast cell purification, culture, and mediator assays

Human cord blood mast cells and murine bone marrow mast cells were purified and activated as previously described.[7] Briefly, cells were sensitized with 5 μg/mL human IgE (Serotec) for 5 days. Cells were incubated with $IE1^H$ (0.01, 0.1, 1, and 10 μg/mL) for 30 minutes, washed, and activated with 5 μg/mL anti-IgE (Serotec) in Tyrode's gelatin buffer for 30 minutes at 37°C. Supernatants were removed for mediator measurement. Tryptase was measured as previously described.[7] IL-4 was measured with a Duoset ELISA (Diaclone, Besancon, France). Rat basophilic leukemia cells were kindly provided by Dr Ehud Razin (The Hebrew University of Jerusalem, Israel), and rat anti-human IgE (clone LO-hE-17) was purchased from Biosource (Camarillo, Calif). Written consent was obtained from cord blood donors. All procedures and experiments were approved by the Hadassah Hospital Helsinki Committee for Human Experimentation and the Institutional Ethical Committee for Animal Experimentation.

Measurement of signaling molecule phosphorylation by means of FACS

Sensitized mast cells were incubated with $IE1^H$ or isotype construct as previously described. The cells were then activated with 5 μg/mL anti-IgE and fixed at several time points (see text) in ice-cold 2.2% buffered formaldehyde. The cells were permeabilized and blocked in Hank's solution containing 0.1% wt/vol saponin, 10 mmol/L HEPES, 5% wt/vol BSA, and 5% vol/vol human serum and stained with polyclonal rabbit anti-phospho-Syk, phospho-LAT (phospho-linker for activation of T cells), phospho-ERK (phospho-externally regulated kinase; Cell Signaling, Beverly, Mass), or monoclonal mouse anti phospho-p38 mitogen-activated protein kinase (MAPK; BD Biosciences)–specific antibodies, followed by secondary Cy5-conjugated anti-rabbit or anti-mouse antibodies (Jackson Immunolaboratories, West Grove, Pa), respectively. Cells were washed between stages with cold Hank's solution containing 0.1% wt/vol saponin and 10 mmol/L HEPES.

Measurement of calcium mobilization by means of FACS

Sensitized mast cells were loaded with 5 μmol/L Calcium Green-1AM (Molecular Probes [Invitrogen], Carlsbad, Calif) at 37°C for 1 hour in loading buffer (RPMI, 2% FCS, and 10 mmol/L HEPES). After extensive washing in loading buffer, the cells were transferred to Tyrode's buffer containing 10 mmol/L $CaCl_2$ warmed to 37°C. Cytometry was initiated, and 40 seconds later, 5 μg/mL anti-IgE simultaneously with $IE1^H$ or isotype control construct was added, and flow was resumed for and additional 3 minutes. Arbitrary fluorescence units were calculated as fluorescence fold increase over the preactivated state level.

Animal models

For all experiments, 6- to 8-week-old female BALB/c mice (Harlan Laboratories, Jerusalem, Israel) were used. All experimental procedures were approved by the Institutional Ethical Committee for Animal Experimentation.

Passive cutaneous anaphylaxis. Passive cutaneous anaphylaxis (PCA) was performed as previously described.[11] Briefly, shaved mice were primed by means of intradermal injection on the dorsal side (30 μL) with either saline or 25 μg/mL dinitrophenol (DNP)-specific IgE (clone SPE7, Sigma) together with $IE1^M$ or isotype control construct (3 μg). After 2 hours, the mice were challenged intravenously with 1.5 mg of DNP with 1% Evan's blue in saline and killed 30 minutes later. The resulting dye spots were evaluated postmortem visually.

Experimental asthma. Experimental asthma was performed as previously described.[12] Briefly, mice were immunized with ovalbumin (OVA; Sigma) intraperitoneally on days 0 and 14. $IE1^M$ or isotype control construct (5 μg) was instilled 30 minutes before allergen challenge intranasally on days 24 and 27. The mice were killed on day 28 by means of isoflurane inhalation, and bronchoalveolar lavage was performed by washing 3 times with 0.8 mL of Tyrode's buffer.

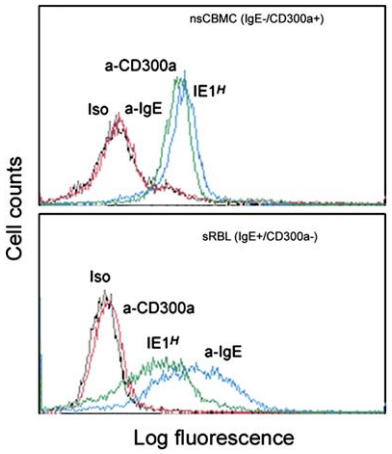

FIG 1. Recognition of IgE/CD300a by IE1^H. RBL cells sensitized with rat anti-human IgE antibodies followed by human IgE antibodies *(sRBL)* were used as IgE$^+$/CD300a$^-$ cells. Nonsensitized cord blood mast cells *(nsCBMC)* were used as IgE$^-$/CD300a$^+$ cells. FACS analysis was performed with IE1^H and compared with monoclonal anti-CD300a and anti-IgE antibodies as controls (representative of n = 3).

Bronchoalveolar lavage fluid (BALF) was analyzed for differential cell counts, as previously described,[13] and mediator content (see below). Lungs were fixed in 2.2% buffered formaldehyde and sectioned (7 μm), and slides were stained with hematoxylin and eosin or periodic acid–Schiff to evaluate inflammation, goblet cell numbers, and mucus production. The sections were examined by 2 blinded observers and graded using a standard scoring method (0 = normal, 1 = mild, 2 = intermediate, and 3 = severe). IL-4, IL-5, IL-13, and eotaxin-2 levels were measured with commercial ELISA kits (R&D systems). Tryptase content was measured by using an enzymatic-chromogenic assay.[7]

Data analysis

Mediators were measured in triplicate and from at least 3 different sets of experiments or cord blood batches. Three independent sets of experiments were performed for each animal model, with at least 4 animals in each treatment group. Data are presented as the means ± SD and were analyzed by means of ANOVA, followed by the paired Student *t* test, assuming equal variances.

RESULTS

Design and construction of IE1^H

Mouse mAbs were ''cut and pasted'' by using a chemical procedure to generate the bispecific construct, termed IE1^H. RBL cells primed with rat anti-human IgE antibodies were incubated with human IgE to yield an IgE$^+$/CD300a$^-$ control to confirm that the final product is capable of bivalent recognition. Unsensitized mast cells were used as an IgE$^-$/CD300a$^+$ control. Both cell types were recognized by IE1^H ($P < .001$, Fig 1), thus demonstrating the specificity of the designed antibody. Importantly, this result demonstrates that IE1^H does not interfere with IgE binding to FcεRI, which is critical for the construct's proper function. Furthermore, IE1^H selectively bound IgE-sensitized mast cells (see Fig E1, *A*, in the Online Repository at www.jacionline.org).

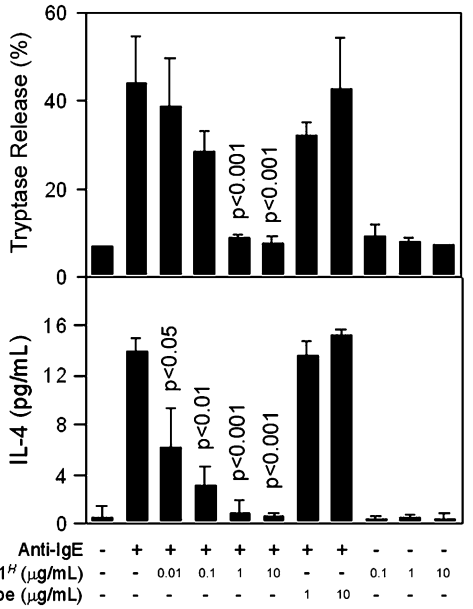

FIG 2. IE1^H inhibits IgE-induced human mast cell degranulation. IgE-sensitized mast cells were incubated with IE1^H or isotype control construct for 30 minutes and activated with anti-IgE antibodies for 30 minutes. Cell supernatants were analyzed for IL-4 (duoset ELISA) and tryptase (enzymatic-chromogenic assay; n = 3).

Inhibition of mast cell degranulation by IE1^H

Next we aimed to test the capability of IE1^H to inhibit mast cell activation *in vitro*. IgE-sensitized mast cells were activated by addition of anti-human IgE after incubation with IE1^H or isotype control construct. As shown, IE1^H inhibited, in a concentration-dependent fashion, the release of both tryptase and IL-4 (Fig 2). Importantly, a concentration as low as 1 μg/mL was sufficient to inhibit mast cell activation by 94% ($P < .001$). Furthermore, by using 10 μg/mL, no mediator release was detectable ($P < .001$). Mast cells were incubated with IE1^H in the absence of anti-IgE to rule out the possibility that IE1^H will induce coaggregation of FcεRI, hence initiating an activating cascade. As shown, IE1^H did not elicit mast cell activation in any concentration tested. In addition, the bispecific isotype control antibody did not inhibit mast cell activation.

Termination of activating signaling events by IE1^H

Inhibition of mast cell function through inhibitory receptors involves suppression of discrete biologic cascades that follow FcεRI coaggregation. These include activation of Lyn, Syk, linker for activation of T cells (LAT), Fyn, and phosphatidylinositol-3′-kinase, followed by a cytosolic calcium influx and activation of MAPKs.[14]

Mast cells were activated and assessed for Syk and p38 MAPK phosphorylation with FACS analysis to examine the effect of IE1^H on these events. The rapid phosphorylation of Syk and LAT observed after IgE-dependent activation was abolished by IE1^H at 1 μg/mL ($P < .02$ and

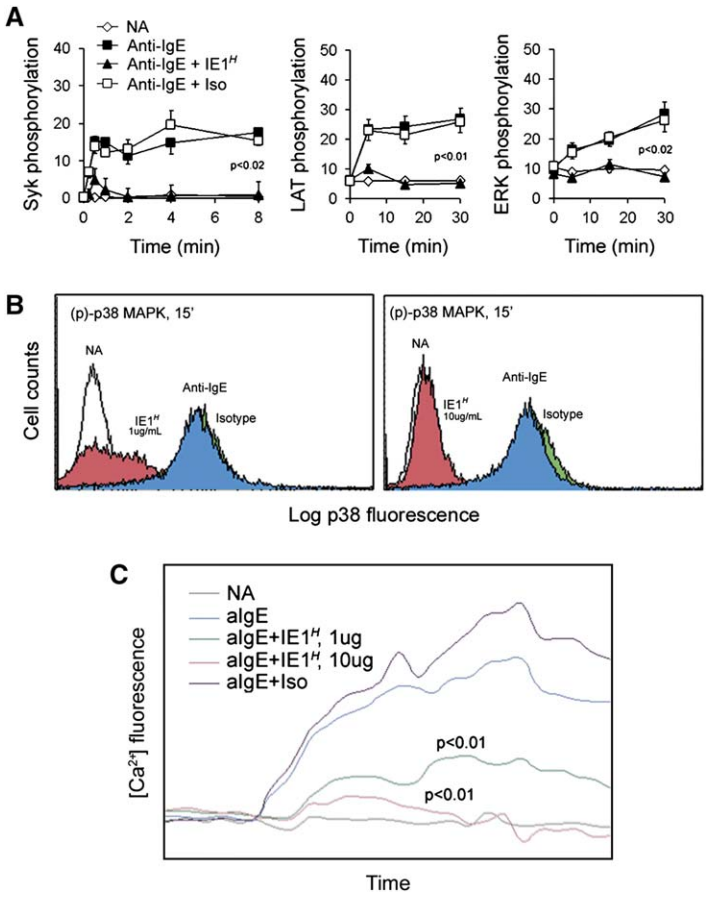

FIG 3. IE1^H inhibits IgE-induced signaling in human mast cells. IgE-sensitized mast cells were incubated with IE1^H or isotype construct and activated with anti-IgE. Cells were fixed and analyzed by means of FACS (**A** and **B**; n = 3). Calcium green-1AM–loaded cord blood mononuclear cells were activated during flow cytometry by anti-IgE. Calcium fluorescence was measured in the FL-1 channel and expressed as mean fluorescence intensity fold increase (**C**; n = 3).

$P < .01$, respectively) and 10 μg/mL (both $P < .001$, data not shown; Fig 3, *A*). Furthermore, the phosphorylation of externally regulated kinase (ERK) and p38 MAPK was similarly inhibited at 1 μg/mL (both $P < .02$) and 10 μg/mL ($P < .01$, data not shown; Fig 3, *B*). The increase in Ca^{2+} induced by anti-IgE was partially suppressed at 1 μg/mL (67%) and was completely abrogated at 10 μg/mL IE1^H (both $P < .01$; Fig 3, *C*). Notably, the bispecific isotype control did not induce an inhibitory effect in any of the aforementioned assays.

IE1^M abolishes allergic reactions *in vivo*

Finally, we examined whether selective targeting of CD300a on mast cells using a bispecific antibody can inhibit an allergic response *in vivo*. We generated a bispecific antibody recognizing the murine IgE and CD300a (accession no. BAC80268),[15] termed IE1^M. The specific anti-CD300a clone used for IE1^M construction was selected from 4 clones on the basis recognition analysis (data not shown). Similarly to IE1^H, IE1^M selectively recognized mast cells (see Fig E1, *B*, in the Online Repository at www.jacionline.org). The capacity of IE1^M to inhibit murine mast cell activation was first evaluated *in vitro* on murine mast cells and found to display inhibitory effects comparable with those of IE1^H (data not shown).

IE1^M was examined in 2 different models of allergic responses: PCA and experimental asthma. Injection of IE1^M before allergen challenge completely abrogated dye leakage resulting from selective mast cell inhibition (Fig 4). IE1^M did not inhibit IgE-independent cutaneous anaphylaxis, demonstrating its IgE dependence (see Fig E1, *C*, in the Online Repository at www.jacionline.org).

We next aimed to examine the inhibitory effect of IE1^M in experimental asthma while focusing on the inflammatory components, which in fact induce asthma symptoms and exacerbate them. We found that eosinophil levels in the BALF of saline-treated mice were nearly undetectable (0.97% ± 0.12%), whereas in the OVA-treated mice they were dramatically increased (55.14% ± 8.87%). Instillation of IE1^M before allergen challenge completely abolished this process (3.17% ± 1.05%, $P < .001$; Fig 5, *A*). The isotype control construct did not have any effect, and hence the isotype-treated mice displayed eosinophil levels comparable with those of OVA-challenged mice (51.64%

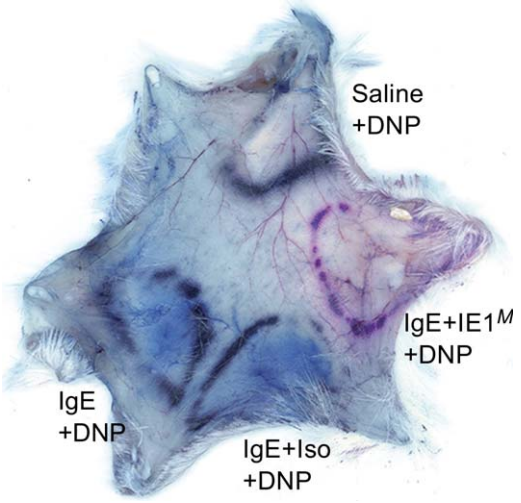

FIG 4. IE1^M inhibits PCA. Mice were sensitized dorsally by means of intradermal injection with DNP-specific IgE. IE1^M or isotype control construct was injected simultaneously, followed 2 hours later by systemic challenge (intravenous injection) with DNP in Evan's Blue dye solution. Mice were killed 20 to 30 minutes later and skinned, and dye spot diameters were assessed visually (representative of n = 6). *Iso,* Isotype.

± 4.4%). The inflammatory state in asthma is also reflected in the profile of T_H2 cytokines and mast cell mediators in the BALF. Therefore we measured the levels of tryptase, eotaxin-2, IL-4, IL-5, and IL-13 induced by allergen challenge. As shown in Fig 5, *B*, IE1^M reduced these mediators to the levels observed in saline-treated mice (tryptase, $P < .001$; eotaxin-2, $P < .01$; IL-4, $P < .001$; IL-5, $P < .001$; and IL-13, $P < .05$).

Subsequently, lung histologic analyses were performed to evaluate additional inflammatory parameters. As expected, OVA challenge induced a robust peribronchial and perivascular eosinophilic inflammation with epithelial damage (2.25 ± 0.29 inflammatory score, see Methods). In contrast, mice instilled with IE1^M displayed a normal phenotype (0.25 ± 0.35; perivascular: $P < .05$, peribronchial: $P < .01$) comparable with saline treatment (0.33 ± 0.26), without evident eosinophil aggregates or tissue damage. Importantly, lungs from mice treated with the isotype control construct (1.5 ± 0.91) displayed evident signs of inflammation (Fig 5, *C* and *D*).

Goblet cell content in the bronchi epithelium was also evaluated. In the saline-treated mice goblet cells were barely detectable (0.5% ± 1.19%), whereas the bronchi of OVA-challenged mice contained high numbers of mucus-containing goblet cells (62.5% ± 24.37%), with a prominent mucus secretion to the bronchial lumen. However, bronchi of IE1^M-treated mice were completely devoid of periodic acid–Schiff–positive cells (0.62% ± 1.65%, $P < .01$), in contrast to the bronchi of control-treated mice (45.71% ± 23.82%; Fig 5, *E* and *F*). To conclude, in all the examined aspects, IE1^M treatment before allergen challenge totally inhibited the development of allergic airway inflammation.

DISCUSSION

Bispecific antibodies represent a novel strategy to manipulate immune responses. This approach has been demonstrated in various settings, including cancer,[16] malaria,[17] and recently also allergy.[18] As mentioned, we have recently shown that CD300a potently inhibits critical effector functions of mast cells, namely activation and survival.[7] Taking into account the established role of mast cells in the initiation of the allergic response, we hypothesized that coaggregation of CD300a with FcεRI with a bispecific antibody will selectively inhibit mast cell functions *in vivo*.

An abundance of data exists regarding the expression and function of inhibitory receptors on immune effector cells,[6] and they have been indicated as modulators of allergic reactions.[19] Because these receptors are natural candidate targets for immune modulation, it is imperative to define their potential as such, beyond the scientific issue of characterizing their mechanism of action. Surprisingly, very few attempts were made to demonstrate targeted triggering of inhibitory receptors as an approach to suppress allergic reactions.[20] In this study we show that CD300a is a potent inhibitor of allergic reactions and that it can be selectively triggered to elicit its inhibitory function as a consequence of the targeting agent. It is noteworthy that CD300a is expressed both in human subjects and mice, unlike various inhibitory receptors with yet unidentified homologues in human subjects or vice versa.

Several factors dictated the bispecific antibody design. A natural candidate molecule providing cell specificity is FcεRI. Moreover, we have observed that the inhibitory effect elicited by CD300a was much enhanced when CD300a was coupled to FcεRI (data not shown), although it was not a required event. Yet we chose to link CD300a to IgE because it is likely that in atopic or allergic individuals mast cells are heavily occupied by IgE. Because of its high affinity to FcεRI, IgE might serve as a carrier that efficiently directs the bispecific antibody to FcεRI$^+$ cells, providing an additional advantage.

The requirement for simultaneous recognition of IgE and CD300a is aimed to restrict the antibody's targeting and function preferentially to cells coexpressing both antigens, principally mast cells and basophils, and to lower the probability for a nonselective immunosuppressive effect. Although in mice FcεRI is expressed almost exclusively on mast cells and basophils, the human receptor distribution is much wider.[14] Still, other human cells that coexpress FcεRI and CD300a, especially antigen-presenting cells, would make logical targets when aiming to inhibit allergic reactions.

The bispecific antibody, termed IE1^H, potently inhibited FcεRI-mediated mast cell degranulation *in vitro* by terminating the signaling cascades responsible for cell activation. Our previous observations demonstrate that CD300a acts by recruitment of SHP-1, SHIP, or both.[7] The robust inhibitory effect observed by CD300a could be explained by this potent use of effector phosphatases, which act on upstream signaling molecules involved

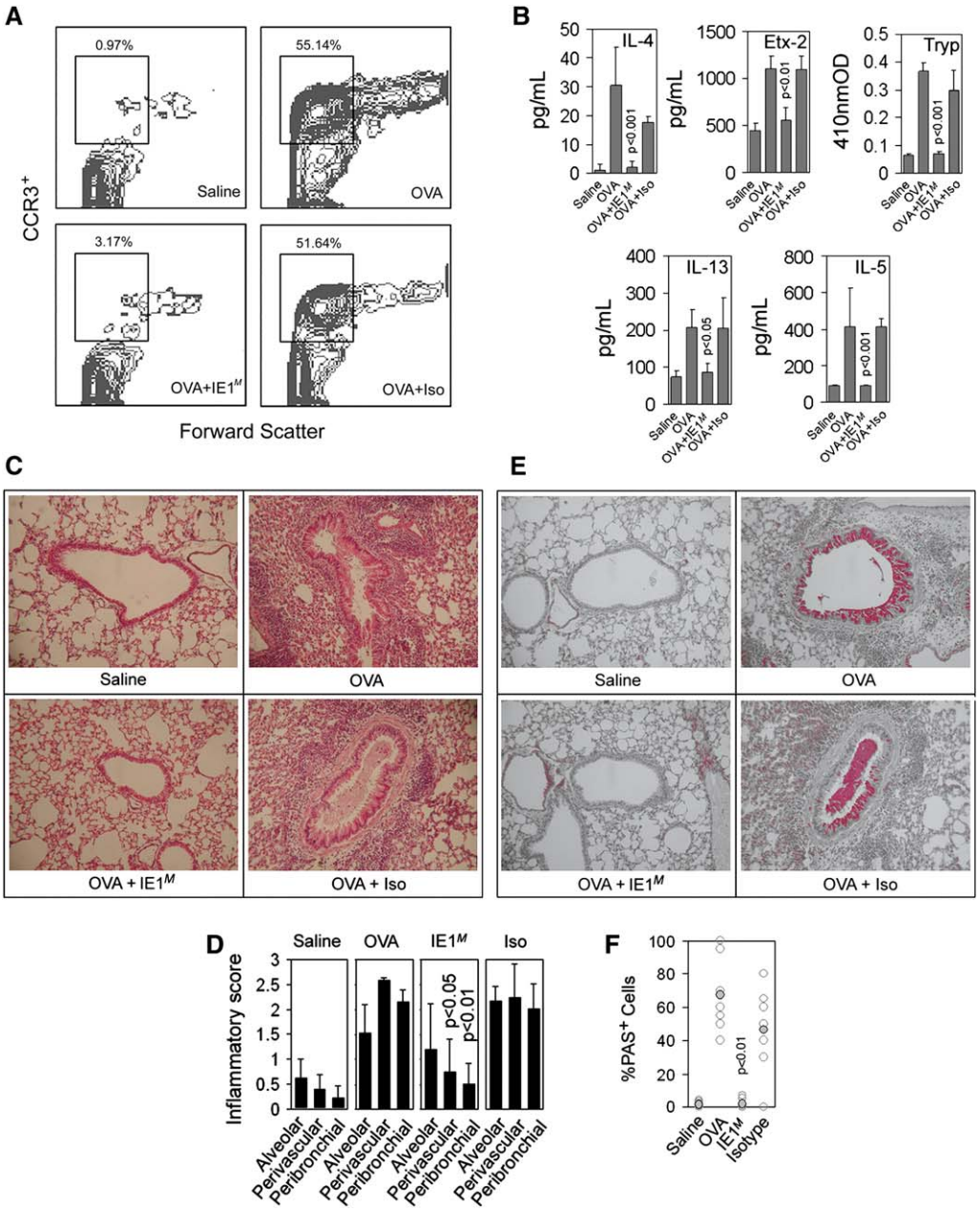

FIG 5. IE1^M inhibits airway inflammation in experimental asthma. OVA-immunized mice were instilled intranasally with IE1^M or with isotype construct before each allergen challenge. BALF was analyzed for mediator content **(A)**. Cells were analyzed by means of FACS **(B)**. Lungs were analyzed with hematoxylin and eosin **(C)** and scores were assigned (**D**). Lungs were analyzed with periodic acid–Schiff (**E**) and stained cells were quantified (**F**; n = 3 with 4-6 mice per group). *Etx-2*, Eotaxin-2; *Tryp*, tryptase; *Iso*, isotype.

in the activation process. Intriguingly, the inhibitory effect might result in part from interference with aggregation of lipid raft–associated receptors, adding to the overall potency of IE1^H. Selective targeted lipid raft interference could provide a different strategy achieving the same goal.

Inhibitory receptors also interfere with priming, survival, and proliferation signaling mediated by cytokines.[21] Indeed, IE1^H successfully inhibited IL-4/IL-13–induced phosphorylation of signal transducer and activator of transcription 6 in human mast cells (data not shown). Thus the inhibitory effect of IE1^H is not necessarily confined to FcεRI signaling. Taken together, these results demonstrated that targeting CD300a is a potent approach to inhibit the progression of allergic and possibly other T_H2-mediated diseases.

In vivo, IE1^M was tested in 2 models: PCA and experimental asthma. The antibody successfully inhibited the allergic response in PCA, a well-described model of

mast cell–induced hypersensitivity reactions.[11] IE1^M also abolished the inflammatory phenotype in experimental asthma, a model in which the central role of mast cells is still controversial.[22] In fact, our findings cannot rule out the intriguing possibility that IE1^M abrogated asthma by inhibiting basophils. This prospect is reinforced by recent findings demonstrating that basophils are capable of initiating allergic responses independently of mast cells or T lymphocytes.[23] We are currently investigating this hypothesis.

In summary, we have demonstrated that linking IgE with CD300a using a bispecific antibody fragment leads to potent inhibition of allergic reactions. The antibody inhibited *in vitro* mediator release and completely blocked allergic responses in murine models of PCA and experimental asthma. Our findings establish the role of CD300a as a critical modulator of the allergic setting and indicate this molecule as a novel target for allergy therapy. Furthermore, we stress the potential of this approach in combating unrestrained immune responses, such as autoimmunity and chronic inflammation. It is likely that such approaches will form the backbone concepts of immunotherapy in the near future.

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