CD300f associates with IL-4 receptor α and amplifies IL-4–induced immune cell responses

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IL-4 receptor (R) α, the common receptor chain for IL-4 and IL-13, is a critical component in IL-4– and IL-13–mediated signaling and subsequent effector functions such as those observed in type 2 inflammatory responses. Nonetheless, the existence of intrinsic pathways capable of amplifying IL-4Rα–induced responses remains unknown. In this study, we identified the myeloid-associated Ig receptor CD300f as an IL-4–induced molecule in macrophages. Subsequent analyses demonstrated that CD300f was colocalized and physically associated with IL-4Rα. Using *Cd300f*$^{-/-}$ cells and receptor cross-linking experiments, we established that CD300f amplified IL-4Rα–induced responses by augmenting IL-4/IL-13–induced signaling, mediator release, and priming. Consistently, IL-4– and aeroallergen-treated *Cd300f*$^{-/-}$ mice displayed decreased IgE production, chemokine expression, and inflammatory cell recruitment. Impaired responses in *Cd300f*$^{-/-}$ mice were not due to the inability to generate a proper Th2 response, because IL-4/IL-13 levels were markedly increased in allergen-challenged *Cd300f*$^{-/-}$ mice, a finding that is consistent with decreased cytokine consumption. Finally, CD300f expression was increased in monocytes and eosinophils obtained from allergic rhinitis patients. Collectively, our data highlight a previously unidentified role for CD300f in IL-4Rα–induced immune cell responses. These data provide new insights into the molecular mechanisms governing IL-4Rα–induced responses, and may provide new therapeutic tools to target IL-4 in allergy and asthma.

IL-4 receptor | eosinophil | macrophage | CD300f | inflammation

Interleukin (IL) 4 and IL-13 play pivotal roles in shaping the nature of type 2 immune responses. IL-4 is required for induction of IgE antibodies by B cells and the subsequent development of naïve CD4$^+$ T cells into Th2 cells (1). Furthermore, IL-4 and IL-13 can activate multiple cells of the myeloid lineage, including macrophages, dendritic cells, and eosinophils (2, 3). For example, IL-4/IL-13–activated myeloid cells display an alternatively activated phenotype, which is associated with the induction of a distinct genetic signature, including the expression of specific mediators and enzymes (4). Furthermore, IL-4 induces rapid eosinophil mediator release and priming (5). Thus, IL-4 and IL-13 are primary therapeutic targets in Th2 diseases such as allergy and asthma.

The majority of studies concerning IL-4 and/or IL-13 have focused either on defining the cellular source for these cytokines or on the respective expression and function of their receptor chains. These studies revealed that the biological functions of IL-4 largely overlap with those of IL-13 due to the utilization of shared signaling components such as IL-4Rα, IL-13Rα1, and STAT-6 (6). Importantly, signaling elicited by these receptor chains is regulated by various mechanisms. For example, differential expression of the common γ-chain and IL-13Rα1 chains in distinct cells renders them responsive to IL-4, IL-13, or both (7). Furthermore, biochemical studies have demonstrated that the IL-4Rα chain possesses an intrinsic immunoreceptor tyrosine-based inhibitory motif (ITIM), which can suppress IL-4 (and likely IL-13) signaling (8). In addition, stress-induced phosphoprotein 1 (STIP1) homology and U box-containing protein 1 (STUB1) interacts with IL-4Rα and targets it for degradation, thus terminating IL-4 or IL-13 signaling (9). It is unknown whether an additional receptor system exists that may act to amplify IL-4Rα signaling and subsequent IL-4/IL-13–induced responses.

CD300 family members consist of nine transmembrane glycoprotein receptors, which are expressed by a variety of immune cells including eosinophils, dendritic cells, macrophages, and B cells (10). The only CD300 family members that possess ITIMs in their intracellular domains are CD300f and CD300a, and are thus potentially capable of suppressing immune cell activation by recruitment of phosphatases (10). Importantly, despite its known inhibitory activities (11, 12), CD300f can also exert cellular activation and is required for phagocytosis of apoptotic cells via recruitment of p85α of the PI3K signaling pathway (13, 14). The finding that the genetic loci (human chromosome 17q22-25) of CD300 members are under strong positive evolutionary selection suggests potent immune regulatory roles for these molecules (15). Indeed, recent studies using *Cd300f*$^{-/-}$ mice revealed key roles for CD300f in governing the activation of inflammatory myeloid cells, mast cells, and eosinophils (11, 12, 16). However, the overall physiological function of CD300f is still largely unknown.

Significance

IL-4 receptor (R) α is a critical component in IL-4– and IL-13–mediated signaling and subsequent effector functions such as those observed in allergy. Thus, it is a primary therapeutic target in diseases such as atopic dermatitis and asthma. Despite extensive studies, it is unknown whether an additional receptor system exists that may act to amplify IL-4Rα signaling and subsequent IL-4/IL-13–induced responses. We now report that CD300f is physically associated with IL-4Rα and potently amplifies IL-4Rα–induced responses in vitro and in vivo. Our results establish CD300f as a previously unidentified IL-4Rα coreceptor. To the best of our knowledge, this is the first report of an additional receptor that serves to amplify the IL-4 signaling pathway.

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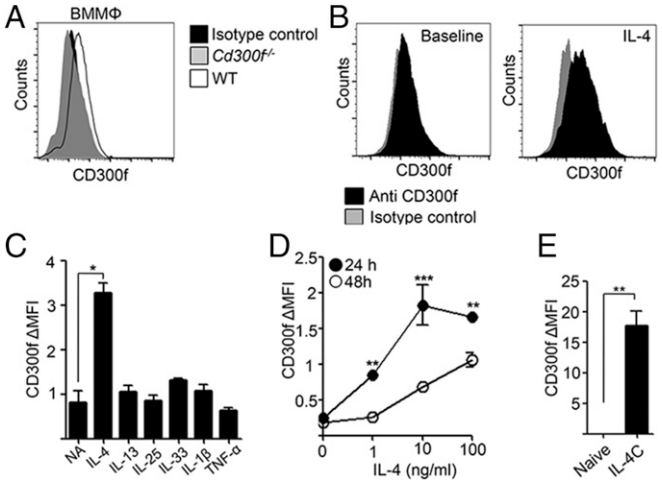

Fig. 1. IL-4 up-regulates the expression of CD300f in macrophages. (*A* and *B*) WT and *Cd300f*$^{-/-}$ bone marrow-derived macrophages (BMMΦs) were stained for CD300f expression at baseline (*A*) and following cytokine activation (10 ng/mL, 24 h; *A* and *B*). (*C* and *D*) Time kinetics and dose-dependent effects of IL-4 on CD300f expression. NA, nonactivated. (*E*) Expression of CD300f by naïve and IL-4–treated peritoneal macrophages (CD11b^{+}/F4/80^{+} cells) is shown. Data represent $n = 3$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; error bars are mean ± SEM.

In this study, we demonstrate that CD300f is an IL-4–induced molecule in macrophages that is physically associated with IL-4Rα. Our in vitro and in vivo analyses establish that CD300f amplifies IL-4/IL-13–induced immune cell responses, including aeroallergen-induced allergic airway inflammation. Collectively, these findings add fundamental knowledge regarding the complexity of IL-4R signaling, especially in myeloid cells, and may have substantial implications in designing new therapies for allergic diseases such as asthma.

Results

IL-4 Up-Regulates the Expression of CD300f in Macrophages. We have recently demonstrated that CD300f is differentially expressed in macrophages from various tissues. For example, colonic, adipose, and peritoneal cavity macrophages hardly express CD300f, whereas alveolar and splenic macrophages express high CD300f levels (*SI Appendix*, Fig. S1). These data suggested that CD300f expression might be dynamically regulated, possibly by cytokines. To assess this hypothesis, bone marrow (BM)-derived macrophages (17) were incubated with various cytokines including IL-33, IL-4, IL-13, IL-25, IL-1β, and TNF-α. First, we confirmed the low baseline expression of CD300f in BM-derived macrophages by staining wild-type (WT) and *Cd300f*$^{-/-}$ cells with anti-CD300f or isotype-matched control antibodies. Anti-CD300f stained *Cd300f*$^{-/-}$ cells displayed nearly identical fluorescence intensity levels as isotype control stained *Cd300f*$^{-/-}$ cells [Fig. 1*A*; mean fluorescence intensity (MFI) 0.45 and 0.53, respectively]. Out of the full panel of cytokines that were assessed, IL-4 specifically increased the expression of CD300f (Fig. 1 *B* and *C*; increased MFI from 1.3 at baseline to 4.29 after IL-4 treatment). Of note, IL-4 increased the expression of CD300f in BM-derived macrophages in a time- and concentration-dependent fashion (Fig. 1*D*). The ability of IL-4 to increase CD300f expression in macrophages was specific to CD300f, because CD300a expression was not increased (*SI Appendix*, Fig. S2). Next, we determined whether IL-4 was also capable of increasing the expression of CD300f in vivo. Consistently, i.p. administration of IL-4 markedly increased the expression of CD300f in peritoneal macrophages that hardly express CD300f under baseline conditions (Fig. 1*E* and *SI Appendix*, Fig. S1). Thus, CD300f is an IL-4–induced molecule in macrophages.

CD300f Is Physically Associated with IL-4Rα. Next, we examined the spatial distribution of CD300f in macrophages using confocal microscopy. CD300f was strongly codistributed, and associated with IL-4Rα both under baseline conditions and following IL-4 stimulation (Fig. 2*A*). Quantitation of colocalized pixels of CD300f and IL-4Rα revealed that 78.85 ± 13.49% of detected CD300f was colocalized with IL-4Rα and, vice versa, 51.55 ± 15.62% of IL-4Rα colocalized to the same region as CD300f (Fig. 2*B*). A similar colocalization pattern was demonstrated in BM-derived dendritic cells that highly express CD300f (*SI Appendix*, Fig. S3). Although IL-4 activation increased the expression of CD300f in macrophages, it did not increase the level of colocalization, which was observed under baseline conditions (Fig. 2 and *SI Appendix*, Fig. S3). Notably, and despite our efforts to precipitate CD300f or IL-4Rα from BM-derived macrophages, we were unable to pull down any of these proteins (even alone) with the currently available commercial antibodies. As an alternative approach, to definitely demonstrate the physical association between CD300f and IL-4Rα, we cotransfected HEK-293T cells with IL-4Rα– (Fig. 2*C*; transfected cells are marked as "+"; nontransfected cells are marked as "-") and c-Myc–tagged CD300f constructs or empty vectors. Subsequently, the cells were lysed, c-Myc–precipitated (IP: anti–c-Myc), and blotted with anti–IL-4Rα (IB: anti–IL-4Rα) or anti–c-Myc (IB: anti–c-Myc) as a surrogate tag for CD300f expression. Notably, c-Myc coprecipitated with IL-4Rα only in cells that were cotransfected with CD300f and IL-4Rα (Fig. 2*C*). Importantly, the expression of c-Myc and IL-4Rα was similar in total lysates of CD300f and empty vector transfected cells, respectively (Fig. 2*D*). These coimmunoprecipitation assays revealed that IL-4Rα was physically associated with CD300f (Fig. 2*C*), and raised the possibility that CD300f might act as an IL-4Rα coreceptor.

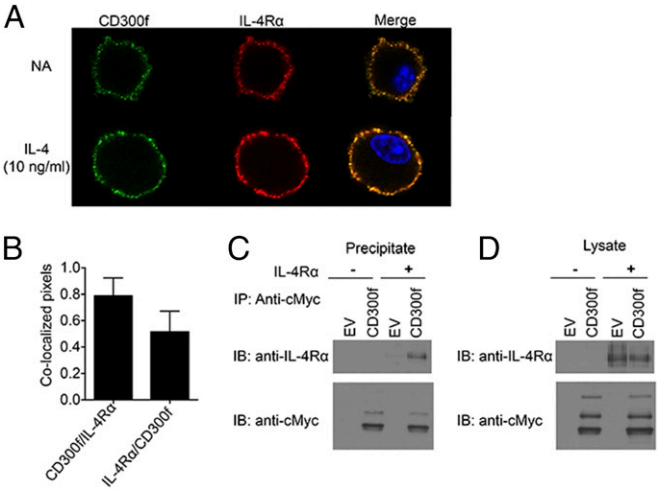

Fig. 2. CD300f physically associates with IL-4Rα. (*A*) Colocalization of CD300f and IL-4Rα on the surface of WT BM-derived macrophages at baseline and following IL-4 activation (10 ng/mL, 24 h) is shown. (*B*) The graph shows the percentage of pixels positive for CD300f colocalizing with pixels positive for IL-4Rα (*Left*) and the percentage of pixels positive for IL-4Rα colocalizing with pixels positive for CD300f (*Right*). (*C* and *D*) HEK-293T cells were cotransfected with IL-4Rα and Myc-tagged CD300f constructs (+ and - indicate IL-4Rα transfected and nontransfected cells, respectively). (*C*) CD300f was immunoprecipitated using an anti-Myc antibody and immunoblotted with anti–IL-4Rα. (*D*) Western blot analysis of IL-4Rα and c-Myc expression in HEK-293T cell lysates is shown. Data represent $n = 3$ (*A* and *B*) or $n = 2$ (*C*); error bars are mean ± SEM.

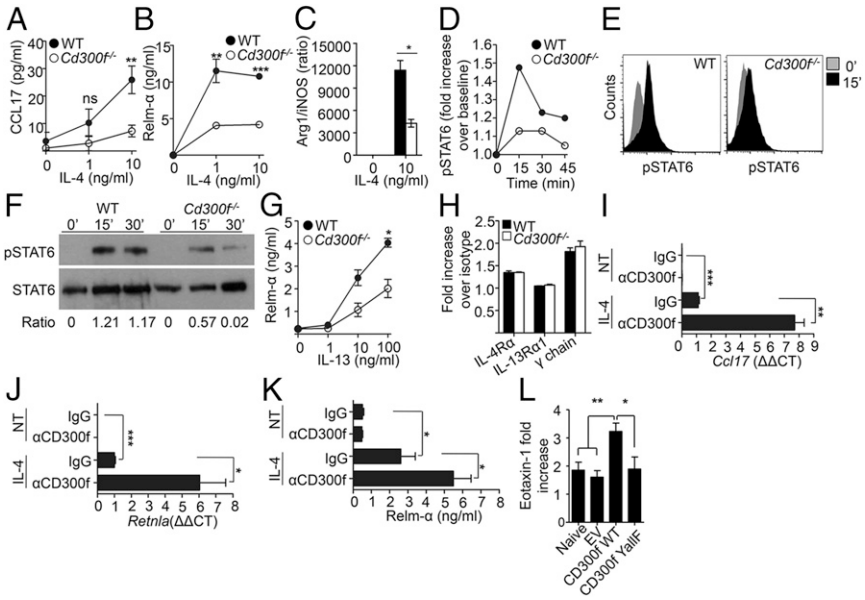

Fig. 3. CD300f amplifies IL-4–induced activation in macrophages. WT and *Cd300f*$^{-/-}$ BM-derived macrophages were activated with IL-4 (*A*–*F*) or IL-13 (*G*). Protein levels of CCL17 (*A*) and Relm-α (*B* and *G*) were assessed. Quantitative PCR analysis of arginase 1 (Arg1) and inducible nitric oxide synthase (iNOS) were done and plotted as an Arg1/iNOS ratio (*C*). STAT-6 phosphorylation (*D*–*F*) was determined by phosphoflow (*D* and *E*) and Western blot (*F*) and is presented as the ratio between phospho- and total STAT-6 (*F*, *Bottom*). A representative histogram overlay of phospho–STAT-6 is shown at time 0 (0′) and 15 min (15′) after IL-4 stimulation. Surface expression of IL-4 receptor chains was determined by flow cytometry (*H*). CD300f (αCD300f) or control IgG was cross-linked on WT BM-derived macrophages with or without IL-4 (10 ng/mL, 48 h). Thereafter, CCL17 and Relm-α levels were assessed (*I*–*K*). (*L*) NIH 3T3 cells were infected with empty vector (EV), WT CD300f (WT), or mutant CD300f, which lacks intracellular tyrosines (YallF). The transfected cells were activated with IL-4 (24 h), and CCL11 secretion was assessed and normalized to the nonactivated control. Data represent $n = 3$ (except for *F* and *L*, where $n = 2$); ns, nonsignificant; NT, nontreated; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; error bars are mean ± SEM.

CD300f Regulates IL-4–Induced Macrophage Activation. To define the role of CD300f in IL-4Rα–induced responses, BM-derived macrophages were obtained from WT and *Cd300f*$^{-/-}$ mice and stimulated with IL-4. IL-4–activated *Cd300f*$^{-/-}$ BM-derived macrophages displayed a significant impairment in their ability to respond to IL-4 and exhibited decreased Relm-α and CCL17 secretion (Fig. 3 *A* and *B*). Furthermore, the ratio between arginase 1 and inducible nitric oxide synthase, two hallmark enzymes that are modulated by IL-4 activity in macrophages, was attenuated in IL-4–activated *Cd300f*$^{-/-}$ cells (Fig. 3*C*). Indeed, IL-4–activated *Cd300f*$^{-/-}$ cells displayed reduced STAT-6 phosphorylation as determined by phosphoflow (Fig. 3*D* and representative overlay in Fig. 3*E*) and Western blot analyses (Fig. 3*F*). The activity of CD300f in IL-4–induced responses was specific, because *Cd300a*$^{-/-}$ cells displayed comparable levels of Relm-α secretion and phosphorylation of STAT-6 in response to IL-4 stimulation (*SI Appendix*, Fig. S4).

IL-13 activates macrophages via the type 2 IL-4R, which also uses the IL-4Rα chain. Thus, we hypothesized that CD300f would regulate IL-13–induced responses as well. Indeed, IL-13–induced Relm-α secretion was impaired in *Cd300f*$^{-/-}$ BM-derived macrophages (Fig. 3*G*). The inability to respond to IL-4 (or IL-13) was not due to decreased IL-4R chains, because *Cd300f*$^{-/-}$ BM-derived macrophages had comparable levels of IL-4R chains to WT cells (Fig. 3*H*).

As an additional approach, CD300f was activated on the surface of WT BM-derived macrophages using antibody-based receptor cross-linking. CD300f receptor activation in the absence of IL-4 had no activation effect in macrophages (Fig. 3 *I*–*K*). However, cross-linking of CD300f in the presence of IL-4 augmented the synthesis and secretion of CCL17 and Relm-α (Fig. 3 *I*–*K*). Furthermore, insertion of CD300f into NIH 3T3 cells resulted in enhanced secretion of CCL11 following IL-4 stimulation. Of note, insertion of a mutant form of CD300f that lacks all of the intracellular tyrosine residues of CD300f (CD300f YallF) impaired the ability of CD300f to augment IL-4–induced CCL11 secretion (Fig. 3*L*).

These data establish that CD300f amplifies IL-4–induced signaling and subsequent cellular responses in BM-derived macrophages.

Impaired IL-4–Induced Activation in *Cd300f*$^{-/-}$ Mast Cells, Eosinophils, and Dendritic Cells. We were interested to determine whether the role of CD300f in IL-4Rα–induced responses was macrophage-specific. To this end, WT and *Cd300f*$^{-/-}$ BM-derived mast cells were stimulated with IL-4, and IL-4–induced mediator release and STAT-6 phosphorylation were assessed. In contrast to macrophages, IL-4 did not increase the expression of CD300f in BM-derived mast cells (Fig. 1 and *SI Appendix*, Fig. S5). However, *Cd300f*$^{-/-}$ BM-derived mast cells displayed significantly decreased levels of IL-4–induced Relm-α secretion and showed attenuated phosphorylation of STAT-6 (Fig. 4 *A* and *B*). Similarly, *Cd300f*$^{-/-}$ BM-derived eosinophils (18) displayed significantly decreased levels of IL-4–induced Relm-α, CCL17, and CCL24 secretion (Fig. 4 *C*–*E*). Furthermore, IL-4–induced STAT-6 phosphorylation was decreased in *Cd300f*$^{-/-}$ eosinophils (Fig. 4*F*). In addition, CD300f was necessary for IL-4–induced eosinophil priming. WT eosinophils, which were pretreated with IL-4, displayed a 20–25% increase in their responsiveness to eotaxin-induced chemotaxis (Fig. 4*G*) (11). Consistently, *Cd300f*$^{-/-}$ eosinophils displayed increased chemotactic responses to eotaxin stimulation (Fig. 4*G*). However, IL-4 failed to prime the chemotactic responses of *Cd300f*$^{-/-}$ eosinophils toward eotaxin (Fig. 4*G*). *Cd300f*$^{-/-}$ eosinophils displayed decreased responsiveness to IL-13 as well (Fig. 4 *H* and *I*). Moreover, *Cd300f*$^{-/-}$ BM-derived dendritic cells exhibited decreased IL-4–induced Relm-α secretion (Fig. 4*J*) and STAT-6 phosphorylation as determined by phosphoflow (Fig. 4*K* and representative overlay in Fig. 4*L*) and Western blot analyses (Fig. 4*M*). The inability to respond to IL-4 was not due to decreased IL-4R chains, because *Cd300f*$^{-/-}$ BM-derived cells had comparable levels of IL-4Rα expression to WT cells (*SI Appendix*, Fig. S6).

CD300f Regulates IgE Production by IL-4–Activated B Cells. IL-4 plays a critical role in class switch recombination in B cells via the type 1 IL-4R. Because B cells express CD300f (*SI Appendix*, Fig. S7), we aimed to define whether CD300f regulates IL-4–induced B-cell responses as well. To this end, WT and *Cd300f*$^{-/-}$ splenic B cells were stimulated with IL-4 and anti-CD40. Thereafter, total cell counts and IgE secretion were determined. Stimulation of WT B cells with IL-4 and anti-CD40 resulted in an 11-fold increase in B-cell counts (day 9), whereas IL-4– plus anti-CD40–stimulated *Cd300f*$^{-/-}$ cell numbers increased only 2.7-fold (Fig. 4*N*). Subsequently, IL-4– plus anti-CD40–stimulated *Cd300f*$^{-/-}$ B cells displayed markedly decreased IgE secretion (Fig. 4*O*). Taken together, these data establish that CD300f regulates IL-4Rα–induced responses in numerous CD300f-expressing IL-4/IL-13–responsive immune cells, at least in vitro.

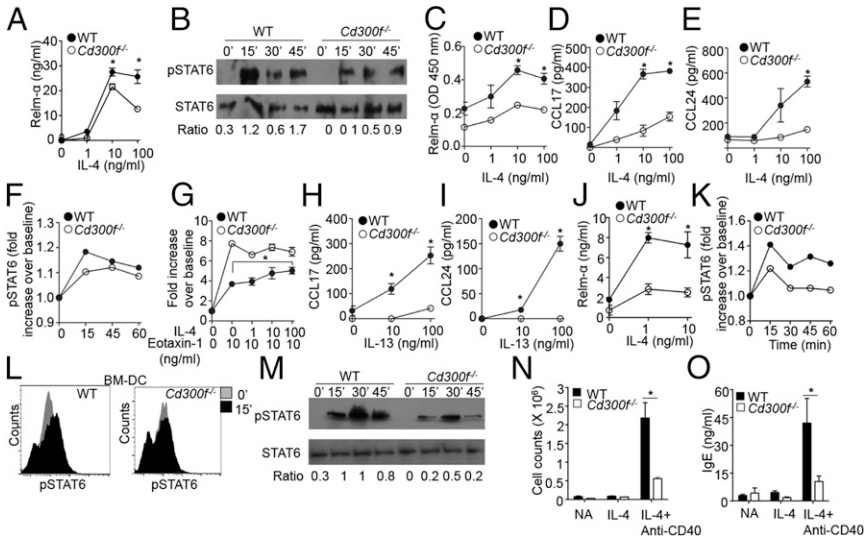

Fig. 4. CD300f amplifies IL-4–induced activation in mast cells, eosinophils, dendritic cells, and B cells. WT and *Cd300f*$^{-/-}$ BM-derived mast cells (*A* and *B*), eosinophils (*C–I*), or dendritic cells (DCs) (*J–M*) were activated with IL-4 (*A–G*) or IL-13 (*H* and *I*). Thereafter, Relm-α (*A*, *C*, and *J*), CCL17 (*D* and *H*), and CCL24 (*E* and *I*) secretion was determined. STAT-6 phosphorylation (*B*, *F*, and *K–M*) was determined by phosphoflow (*F*, *K*, and *L*) and Western blot (*B* and *M*) and is shown as the ratio between phospho- and total STAT-6 (*B* and *M*, *Bottom*). A representative histogram overlay of phospho–STAT-6 is shown at times 0 and 15 min after IL-4 stimulation. (*G*) WT and *Cd300f*$^{-/-}$ BM-derived eosinophils were primed with IL-4 and subjected to eotaxin-1–induced chemotaxis. Purified splenic B cells were left nonactivated or stimulated with IL-4 or IL-4 + anti-CD40. Thereafter, B-cell counts were performed (*N*) and IgE levels were determined (*O*). Data represent $n = 3$; $^{*}P < 0.05$; error bars are mean ± SEM.

CD300f Regulates IL-4–Induced Responses in Vivo. To further establish the role of CD300f in IL-4–induced cellular responses, a direct in vivo approach was used in which IL-4 was administered into the lungs of WT and *Cd300f*$^{-/-}$ mice. Thereafter, IL-4–induced mediator release and cellular recruitment were assessed. Indeed, IL-4–induced chemokine expression (Fig. 5 *A* and *B*) and subsequent cellular infiltration (e.g., neutrophils and eosinophils) were significantly reduced (4- and 6.5-fold, respectively) in *Cd300f*$^{-/-}$ mice (Fig. 5 *C–E*).

CD300f Regulates Aeroallergen-Induced Eosinophilic Inflammation. The role for CD300f in IL-4–induced inflammatory responses prompted us to determine whether CD300f governs the development of aeroallergen-driven allergic airway inflammation, where IL-4Rα has a cardinal role mediating IL-4 and IL-13 responses (6, 19). First, the expression of CD300f was determined in various lung cellular populations following aeroallergen challenge (i.e., *Aspergillus fumigatus*; Asp). Under baseline conditions, CD300f was differentially expressed in various lung cells [Fig. 6*B*, region (R)1–R6] with the exception of lymphocytes (Fig. 6*B*, R1). Interestingly, allergen challenge caused a significant increase in CD300f expression only in alveolar macrophages (defined as CD45^{+}/CD11c^{+}/Gr-1^{-}/CD11b$^{-/lo}$/Siglec-F^{+}; Fig. 6 *C* and *D*), eosinophils (defined as CD45^{+}/CD11c^{lo}/Gr-1^{lo}/CD11b^{+}/Siglec-F^{+}; Fig. 6 *E* and *F*), and mast cells (defined as CD45^{+}/c-kit^{+}/FcεR1^{+}; Fig. 6 *G* and *H*), whereas its expression in all other lung cell populations was unchanged (*SI Appendix*, Fig. S8).

Subsequently, WT and *Cd300f*$^{-/-}$ mice were challenged with Asp and allergic airway inflammation was assessed. Asp-induced serum IgE levels were significantly impaired (approximately twofold decrease) in *Cd300f*$^{-/-}$ mice in comparison with Asp-challenged WT mice (Fig. 6*I*). Whereas Asp-challenged WT mice displayed noticeable elevation of the classical IL-4/IL-13–associated chemokines CCL17 and CCL22 (Fig. 6 *J* and *K*), Asp-challenged *Cd300f*$^{-/-}$ mice exhibited decreased CCL17 levels and to a lesser extent CCL22 (Fig. 6 *J* and *K*). In agreement with these data, Asp-induced total and differential cell counts in the lungs were significantly lower in *Cd300f*$^{-/-}$ mice (Fig. 6 *L–P*). Decreased chemokine content and subsequent cellular infiltration in Asp-challenged *Cd300f*$^{-/-}$ mice were not due to the inability to generate a proper Th2 response, because bronchoalveolar lavage (BAL) fluid levels of IL-4 and IL-13 were actually increased in Asp-challenged *Cd300f*$^{-/-}$ mice in comparison with WT mice (Fig. 6 *Q* and *R*). Despite elevated IL-4 and IL-13 levels in the lungs of *Cd300f*$^{-/-}$ mice, mucus production and airway resistance were similar in Asp-challenged WT and *Cd300f*$^{-/-}$ mice (*SI Appendix*, Fig. S9), likely due to the lack of CD300f expression in nonimmune lung cells (*SI Appendix*, Fig. S9).

CD300f Is Up-Regulated in Monocytes and Eosinophils Obtained from Active Allergic Rhinitis Patients. Given that CD300f is up-regulated by macrophages and eosinophils in settings of allergic airway inflammation, we were interested to examine whether atopic allergic individuals would display increased CD300f expression. The expression of CD300f was increased in eosinophils (CD16^{-}/Siglec-8^{+}) and monocytes (CD14^{+}/CD16^{-}) obtained from allergic rhinitis patients (*SI Appendix*, Fig. S10).

Discussion

IL-4Rα is a critical receptor in type 2 immune settings, as it mediates the signaling of both IL-4 and IL-13 (1, 6). Therefore, this signaling axis has drawn considerable attention (20). Previous studies have largely focused either on the effector functions of IL-4/IL-13 or the relative expression and role of their receptor chains (i.e., type 1 and type 2 IL-4Rs) in various type 2 immune responses. However, endogenous mechanisms that regulate

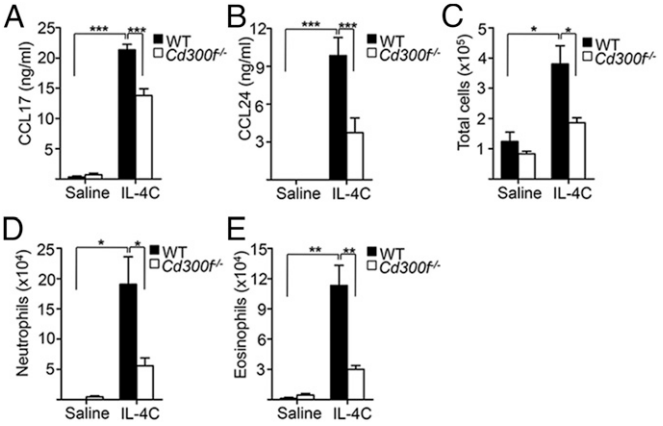

Fig. 5. CD300f amplifies IL-4–induced responses in vivo. IL-4 complex (IL-4C) was administered to WT and *Cd300f*$^{-/-}$ mice. CCL17 (*A*) and CCL24 (*B*) as well as total (*C*) and differential cell counts (*D* and *E*) in the bronchoalveolar lavage fluid are shown. Data are representative of two independent experiments, with six mice per experimental group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; error bars are mean ± SEM.

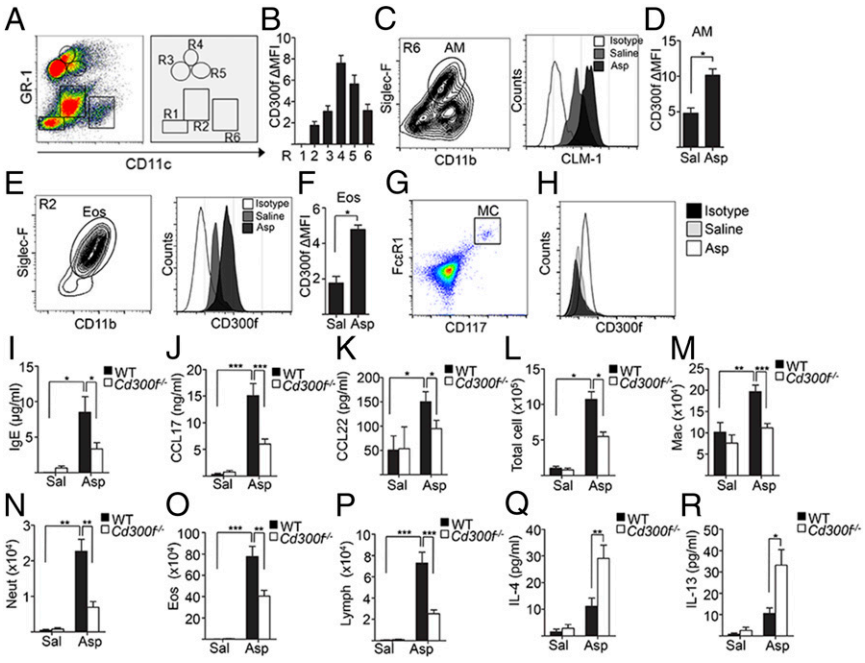

Fig. 6. CD300f amplifies aeroallergen-induced IL-4–mediated responses. Single-cell suspensions were obtained from the lungs of saline (Sal) or Asp-challenged WT mice, and baseline CD300f expression in the various cellular populations was assessed (*A* and *B*). Expression of CD300f by alveolar macrophages (AM; *C* and *D*), eosinophils (Eos; *E* and *F*), and mast cells (MC; *G* and *H*) is shown. WT and *Cd300f*$^{-/-}$ saline- or Asp-challenged mice were assessed for serum IgE levels (*I*), BAL CCL17 (*J*), and CCL22 (*K*) expression. Total (*L*) and differential BAL cell counts (*M*–*P*) as well as BAL IL-4 (*Q*) and IL-13 (*R*) content were determined. Data are representative of three independent experiments with more than eight mice per experimental group. Lymph, lymphocytes; Mac, macrophages; Neut, neutrophils. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; error bars are mean ± SEM.

IL-4Rα–induced effects are largely unknown. We now show that CD300f is physically associated with IL-4Rα. Using three independent techniques (*Cd300f*$^{-/-}$ mice and/or cells, cross-linking experiments, and CD300f transfection experiments), we demonstrate that CD300f amplifies IL-4Rα–induced signaling responses. To the best of our knowledge, this is the first report of another receptor that may serve as a coreceptor for the IL-4 signaling pathway.

Using a clinically relevant aeroallergen mouse model of asthma, we demonstrate that CD300f expression is increased following aeroallergen challenge in macrophages, mast cells, and eosinophils. These data are consistent with our finding that IL-4 increased the expression of CD300f in macrophages and that IL-33 induced the expression of CD300f in eosinophils (21). Of note, activation of macrophages with IL-13 did not increase the expression of CD300f. This result is likely explained by the fact that macrophages are significantly more sensitive to IL-4 than IL-13, due to the relative abundance of the type 1 IL-4R in comparison with the type 2 IL-4R (7). Despite this, CD300f amplified IL-13–induced responses in macrophages and eosinophils. Thus, CD300f likely regulates both type 1 and type 2 IL-4R–induced responses by interactions with IL-4Rα. Directly related, we have recently identified miR-511 as an alternatively activated macrophage-associated microRNA that is induced by IL-4 or IL-13 (22). Overexpression of miR-511 induced marked genetic alterations in macrophages and induced the expression of CD300f. It is thus tempting to speculate that IL-4/IL-13–induced miR-511 increases the expression of CD300f (likely via an indirect pathway).

We demonstrate that aeroallergen induction of IgE- and Th2-associated chemokines and subsequent recruitment of inflammatory cells were largely dependent on CD300f. Importantly, the relatively high expression level of IL-4 and IL-13 in the BAL fluid of Asp-challenged *CD300f*$^{-/-}$ mice suggests that the altered inflammatory response, which was observed in Asp-challenged *CD300f*$^{-/-}$ mice lungs, was not due to a general defect in the ability of *Cd300f*$^{-/-}$ mice to generate a proper Th2 response. These findings are consistent with previous reports demonstrating that antigen presentation by CD300f-expressing myeloid cells and consequent T-cell responses were comparable in WT and *Cd300f*$^{-/-}$ mice in a mouse model of experimental autoimmune encephalomyelitis (16). Decreased chemokine production and cellular infiltration in response to allergen challenge likely reflect the requirement of CD300f for allergen-induced IL-4 effector functions. We have recently shown that cellular recruitment in experimental asthma models is mainly dependent on IL-4 signaling via the type 1 IL-4R expressed by hematopoietic cells, whereas the clinical features of asthma [e.g., mucus production, airway hyper responsiveness (AHR), and fibrosis] are largely dependent on IL-4/IL-13 signaling via the type 2 IL-4R, which is predominantly expressed by structural cells such as epithelial and smooth muscle cells (23). Interestingly, allergen-challenged *Cd300f*$^{-/-}$ displayed no alteration in AHR or mucus production despite elevated IL-4/IL-13 levels. This is explained by the finding that lung epithelial cells and smooth muscle cells do not express CD300f. Hence, IL-4Rα signaling in these cells remains intact.

A recent study assessing the function of CD300f in a mast cell-dependent model of asthma suggested an inhibitory function for CD300f in allergic airway inflammation. To this end, *Cd300f*$^{-/-}$ mice displayed an exaggerated disease phenotype (12). The differences between our findings and this study may be due to numerous factors. CD300f is capable of acting as a coactivating receptor or an inhibitory receptor. Thus, CD300f can suppress IgE-induced mast cell activation but may still be required for IL-4–induced mast cell responses. Therefore, in murine models of allergic airway disease that are dependent on cross-linking of IgE on the surface of mast cells, CD300f will act as an inhibitory receptor. In contrast, in murine models that are IgE-independent and have a strong IL-4 signature [such as the aeroallergen model we used in our study (23, 24)], CD300f may function as an IL-4 coreceptor. In addition, the availability of CD300f ligands in the different models may also impact the outcome of CD300f signaling. Alternatively, optimal CD300f signaling is obtained by the generation of heterocomplexes with additional CD300 receptors (25). Thus, it is possible that differential expression of other CD300 family members may affect the observed phenotype. Hence, it is possible that each, or any combination, of these factors may be sufficient to alter the intracellular signaling events that are delivered by CD300f. Subsequently, conclusions from both studies may be correct, and the specific

circumstances surrounding a given model and protocol will need to be noted in future studies with the multiple *Cd300f* mouse models that are currently available (13, 16, 26).

Interestingly, whereas the binding of ceramide to CD300f promotes inhibitory signals in mast cells (12), phosphatidylserine (PS) induces a PI3K-mediated response upon CD300f ligation (27). These data suggest the possibility that ceramide could act as a ligand of CD300f when this molecule is not forming part of the IL-4R complex. Alternatively, the inclusion of CD300f in that complex could modify or mask the ceramide-binding site and promote a new docking surface for PS. Supporting this hypothesis, although the binding of PS to CD300f is most probably a calcium-dependent interaction with the polar region of this lipid, similar to the one described for human CD300a (28), the lack of the polar head in ceramide necessarily implicates a different docking surface. The association of a PS receptor, in this case CD300f (13, 27), with IL-4Rα as a means of amplifying type 2 immune response may have a biological basis, because cell injury/death plays a key role in the initiation of type 2 immune responses (29). Furthermore, many parasites and parasite-infected cells express high levels of PS (30, 31). Thus, CD300f ligands are readily available in settings where IL-4 is present, especially in vivo, whereas in vitro, dying cells in the culture may be a source for CD300f ligands.

In summary, we demonstrate a previously unidentified and unique requirement for CD300f in IL-4Rα–induced responses predominantly in immune cells. These data provide a new understanding into the signaling mechanisms required for IL-4Rα–induced responses and implicate CD300f as a necessary component of the IL-4/IL-13 signaling complex in multiple immune cells.

Materials and Methods

Complete methods can be found in *SI Appendix, Materials and Methods*.

Mice. Generation of *Cd300f*$^{-/-}$ and *Cd300a*$^{-/-}$ mice was previously described (11, 16). WT C57BL/6 mice were originally obtained from Harlan Laboratories and grown in-house. All mice (age-, weight-, and sex-matched) were used and housed under specific pathogen-free conditions according to protocols approved by the Tel Aviv University Institutional Animal Care Unit.

Bone Marrow-Derived Cell Cultures. Macrophage, dendritic cell, and eosinophil cultures were generated and stimulated with IL-4 as previously described (17, 18).

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