CD48 Is Critically Involved in Allergic Eosinophilic Airway Inflammation

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Rationale: **Despite ongoing research, the molecular mechanisms controlling asthma are still elusive. CD48 is a glycosylphosphatidylinositol-anchored protein involved in lymphocyte adhesion, activation, and costimulation. Although CD48 is widely expressed on hematopoietic cells and commonly studied in the context of natural killer and cytotoxic T cell functions, its role in helper T cell type 2 settings has not been examined.**

Objectives: **To evaluate the expression and function of CD48, CD2, and 2B4 in a murine model of allergic eosinophilic airway inflammation.**

Methods: **Allergic eosinophilic airway inflammation was induced by ovalbumin (OVA)–alum sensitization and intranasal inoculation of OVA or, alternatively, by repeated intranasal inoculation of *Aspergillus fumigatus* antigen in wild-type, STAT (signal transducer and activator of transcription)-6–deficient, and IL-4/IL-13–deficient BALB/c mice. Gene profiling of whole lungs was performed, followed by Northern blot and flow cytometric analysis. Anti-CD48, -CD2, and -2B4 antibodies were administered before OVA challenge and cytokine expression and histology were assessed.**

Measurements and Main Results: **Microarray data analysis demonstrated upregulation of CD48 in the lungs of OVA-challenged mice. Allergen-induced CD48 expression was independent of STAT-6, IL-13, and IL-4. Neutralization of CD48 in allergen-challenged mice abrogated bronchoalveolar lavage fluid and lung inflammation. Neutralization of CD2 inhibited the inflammatory response to a lesser extent and neutralization of 2B4 had no effect.**

Conclusions: **Our results suggest that CD48 is critically involved in allergic eosinophilic airway inflammation. As such, CD48 may provide a new potential target for the suppression of asthma.**

Keywords: asthma; CD48; CD2; 2B4

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

CD48 is an activation molecule able to facilitate various cellular activities. Its role in asthma is unknown.

What This Study Adds to the Field

CD48 is upregulated in experimental asthma. Anti-CD48–based therapies may be useful for asthma and perhaps various allergic diseases.

Asthma is a chronic inflammatory disease of the airways characterized by airflow obstruction, bronchial hyperresponsiveness, and airway inflammation (1–3).

Studies of airway inflammation in the lungs of individuals with asthma have revealed the accumulation of a large number of inflammatory cells, increased mucus production and submucosal mucus gland hyperplasia/metaplasia, epithelial shedding, and smooth muscle cell hypertrophy (3, 4). Notably, chronic inflammation of the asthmatic lung leads to structural changes, which in turn exacerbate the hyperresponsiveness observed in this disease (4).

Experimentation in the study of asthma has provided a rationale for the development of multiple therapeutic agents that interfere with specific inflammatory pathways (5–8). However, development of the disease phenotype is likely related to the interplay of a large number of pathways. Genome searches have revealed that at least 19 genes contribute to asthma susceptibility and microarray studies of asthmatic tissue revealed the involvement of hundreds of genes (3). Moreover, microarray analysis has demonstrated increased expression of 291 genes commonly associated with murine disease pathogenesis rather than a particular mode of disease induction (7). Therefore, a central issue still under investigation is the identification of fundamental molecules/pathways that govern the processes underlying inflammation in asthma.

CD48 is a glycosylphosphatidylinositol-anchored protein belonging to the CD2 subfamily (9). It is expressed mainly on hematopoietic cells and exists in both membrane-associated and soluble forms (9, 10). It is a low-affinity ligand for CD2 and is implicated as an important costimulatory molecule in lymphocyte activation (11, 12). Interestingly, whereas CD2-deficient mice display normal T cell development and function, CD48-deficient mice exhibit significant defects in $CD4^+$ T cell activation (13, 14), indicating a broad immunologic role for CD48. Indeed, CD48 has been described as facilitating cell adhesion (15, 16), innate responses to bacterial infection (17–19), and graft rejection (20–23).

In addition, CD48 is a high-affinity ligand for 2B4 (24). CD48–2B4 interactions can modulate T cell, B cell, and natural killer (NK) cell functions and cross-talk (25–27). Studies with 2B4 gene–targeted mice demonstrated that 2B4–CD48 interactions are essential for expansion and activation of murine NK cells (26). The absence of functional 2B4–CD48 interactions impairs NK cell cytotoxic response and IFN-γ release on tumor target exposure (27). Furthermore, activated NK cells significantly increase the CD3-dependent proliferation of $CD8^+$ and $CD4^+$ T cells by a 2B4–CD48-dependent mechanism (25).

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The contribution of CD48 has not been explored in helper T cell type 2 (Th2) settings. Thus, the applicability of CD48-dependent stimulatory pathways that have been investigated in Th1 settings to allergy is not obvious.

In this study, we investigated the contribution of CD48 and its ligands to allergic eosinophilic airway inflammation. We report that CD48 is upregulated in two murine models of allergic eosinophilic airway inflammation (7). Furthermore, experiments with anti-CD48, anti-CD2, and anti-2B4 neutralizing monoclonal antibodies (mAbs) demonstrate that CD48 is critically involved in allergic eosinophilic airway inflammation.

METHODS

Reagents and Chemicals

All chemicals used in this study were purchased from Sigma (Rehovot, Israel) and were of the best available grade.

Antibodies

Fluorescein isothiocyanate (FITC)-conjugated anti-CCR3 (CC chemokine receptor-3) was obtained from R&D Systems (Minneapolis, MN). Anti-CD3–allophycocyanin (APC), anti-VLA (very late activation antigen)-4–phycoerythrin (PE) (clone DX5), anti-CD4–PE/cyanine 5 (Cy5), anti-rat IgG–PE, anti-rat IgG–FITC, streptavidin–PE, and streptavidin–Cy5 were all purchased from eBioscience (San Diego, CA). Anti-B220–APC, anti-CD2, and anti-CD48–PE were obtained from BioLegend (San Diego, CA). Anti-2B4 mAb (a kind gift from V. Kumar, University of Chicago, Chicago, IL) and anti-CD2 were conjugated to biotin according to a standard protocol (28).

Mice

All experiments involving animals and primary animal cells were approved by the institutional animal experimentation ethics committee. BALB/c mice were obtained from the National Cancer Institute (Frederick, MD) or Harlan Laboratories (Jerusalem, Israel) and housed under specific pathogen-free conditions. BALB/c mice deficient in signal transducer and activator of transcription (STAT)-6 or IL-4 receptor α chain were obtained from Jackson Laboratory (Bar Harbor, ME). BALB/c IL-13–deficient mice and mice deficient in both IL-4 and IL-13 were kindly provided by A. McKenzie (29). BALB/c mice carrying the tetracycline-inducible IL-13 transgene under the regulation of the Clara cell-10 (CC10) lung promoter have been previously described (30).

Allergen Sensitization and Challenge

Allergic eosinophilic airway inflammation was induced as described (7). In neutralization experiments, anti-CD48, anti-CD2 (BioLegend), anti-2B4, or appropriate isotype-matched controls (hamster IgG and rat IgG) were administered intraperitoneally on Day 23 (24 hours before allergen challenge) and on Days 24 and 27, 1 hour before allergen challenge (250 μg/mouse in 300 μl of saline). These concentrations were chosen because they had been shown to have a neutralizing effect *in vivo* (20, 23, 31). All neutralization studies have been conducted on 4–6 mice per experimental group for at least three times (i.e., total of 12–16 mice.

Microarray Hybridization and Analysis

Microarray hybridization was performed on total lung RNA by the Affymetrix Gene Chip Core facility at Cincinnati Children's Hospital Medical Center (Cincinnati, OH), as previously described (7). Data for each allergen challenge time point were normalized to the average of the saline-treated mice. *See* the online supplement for additional detail on the method.

Northern Blot Analysis

Northern blot analysis was performed as previously described (7). *See* the online supplement for additional detail on the method.

Flow Cytometry

Total bronchoalveolar lavage fluid (BALF) cells (2×10^5) of treated mice were incubated with the aforementioned antibodies in a final volume of 100 μl of Hanks' balanced salt solution supplemented with 0.1% bovine serum albumin and 0.02% sodium azide for 30 minutes on ice. Thereafter, differential cell populations were electronically gated and assessed for expression of CD48, CD2, or 2B4. *See* the online supplement for additional detail on the method.

Mediator Assessment

Cytokines were measured with kits purchased from the following sources: IL-5 (eBioscience), IL-4 and IL-13 (BioLegend), and eotaxin-2 and tumor necrosis factor (TNF)-α (R&D Systems). ELISA procedures were performed according to the manufacturers' instructions. Lower detection limits for the various assays were as follows: 7.8, 2, 16, 32, and 16 pg/ml, respectively.

Quantification of Lung Inflammation

Histological studies were performed as follows: the right upper lobe of saline or allergen-challenged lungs was fixed in 3.7% paraformaldehyde, embedded in paraffin, deparaffinized, and stained with hematoxylin and eosin or with periodic acid–Schiff reagent.

See the online supplement for additional detail on the calculation method.

Statistical Analysis

Statistical significance was calculated by parametric analysis (analysis of variance, followed by the Tukey-Kramer *post hoc* test or Student *t* test). Values were considered significant at $p < 0.05$ (32).

RESULTS

DNA Microarray Analysis Identifies CD48 as an Allergen-induced Gene in Allergic Eosinophilic Airway Inflammation

Quantitative microarray analysis revealed that CD48, but not CD2 or 2B4, mRNA expression was significantly increased in both the ovalbumin (OVA)- and *Aspergillus*-induced allergic eosinophilic airway inflammation models (Zimmermann and coworkers [7] and Figures 1A and 1B, respectively). Kinetic analysis

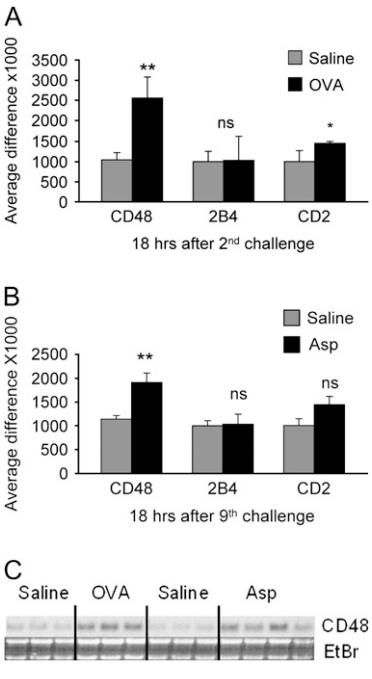


Figure 1. DNA microarray analysis identifies CD48 as an allergen-induced gene in allergic eosinophilic airway inflammation. Expression of CD48 in ovalbumin (OVA)–challenged mice (*A*) and *Aspergillus fumigatus* (Asp)–challenged mice (*B*) as measured by gene chip analysis is shown. The average difference for the hybridization signal after saline (*shaded columns*) and allergen (*solid columns*) challenge is depicted (n = 3 mice for *Aspergillus* control group, n = 4 mice for OVA control group, and n = 4 mice for OVA and *Aspergillus* experimental groups). *$p < 0.05$; **$p < 0.01$; ns = not significant. (*C*) The induction of CD48, 2B4, and CD2 in allergen-challenged mice as measured by Northern blot analysis. Total RNA was electrophoresed, transferred, and hybridized with a radiolabeled sequence-confirmed CD48 cDNA probe. The location of 18S RNA is shown. Each lane represents an extract from one separate mouse. EtBr = ethidium bromide.

revealed that CD48 mRNA was significantly upregulated 18 hours after the second OVA allergen challenge. In addition, CD48 was upregulated 18 hours after the ninth *Aspergillus* allergen challenge. Subsequently, these data were confirmed by Northern blot analysis (Figure 1C) and reverse transcription-polymerase chain reaction (data not shown). Although there was low basal expression of CD48 in the lungs of saline-treated mice, the level of CD48 mRNA was significantly upregulated after OVA and *Aspergillus* challenge.

CD48 Expression Is Independent of STAT-6, IL-4, and IL-13

Next, we aimed to establish whether signaling pathways that are key regulators of the allergic inflammatory response, such as STAT-6, IL-4, and IL-13, are involved in the upregulation of CD48. OVA- and *Aspergillus*-induced allergic eosinophilic airway inflammation protocols were performed with STAT-6, IL-13, and IL-4/IL-13 gene–targeted mice. Thereafter, total lung mRNA was extracted and subjected to Northern blot analysis (Figures 2A–2D). CD48 expression was found to be enhanced in the absence of each of these factors in both the OVA- and *Aspergillus*-induced models. Nevertheless, inducible IL-13 transgenic mice displayed elevated levels of CD48 starting after 6 days of IL-13 induction, indicating that IL-13 overexpression is sufficient for CD48 overexpression (Figure 2E).

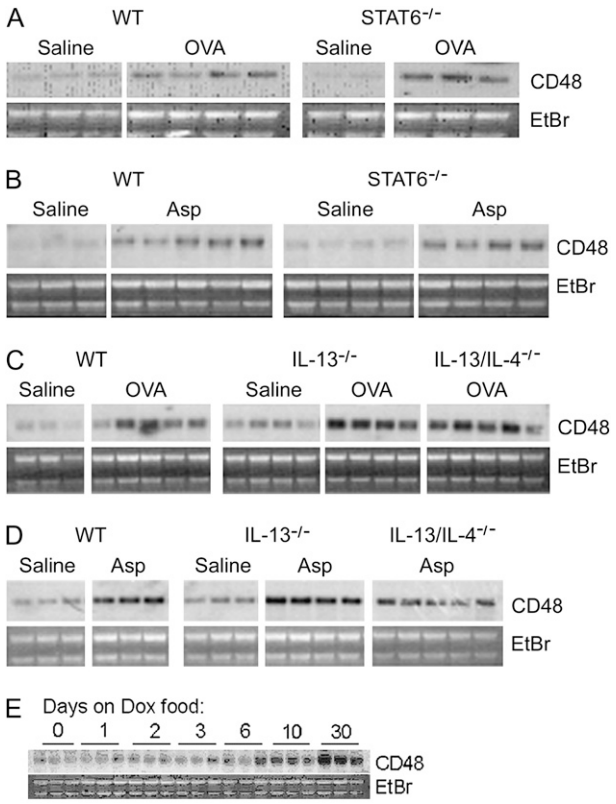


Figure 2. CD48 expression is independent of signal transducer and activator of transcription (STAT)-6, IL-4, and IL-13. RNA was extracted from the lungs of wild-type (WT) mice (*A–D*, *left*), STAT-6–deficient mice (*A* and *B*, *right*), and IL-13– and IL-4/IL-13–deficient mice (*C* and *D*, *middle* and *right*, respectively). Mice that express tetracycline-inducible IL-13 were fed doxycycline (Dox)–containing food for the indicated time periods (*E*). For all Northern blot assays, total RNA was electrophoresed, transferred, and hybridized with a radiolabeled sequence-confirmed CD48 cDNA probe. The location of 18S RNA is shown. Each lane represents an extract from one separate mouse. Asp = *Aspergillus*; OVA = ovalbumin.

Cellular Source of CD48, CD2, and 2B4 in Lungs

Subsequently, we determined the cellular source of CD48 and its ligands. Most of the cells in the lung and BALF expressed CD48; however, eosinophils expressed the highest levels of CD48 and were the main cellular source for its expression, comprising about 50–65% of $CD48^+$ cells in the lung (Figure 3). Interestingly, murine eosinophils did not express 2B4 and 2B4 expression was restricted to NK and natural killer T (NKT) cells. In addition, CD2 expression was limited to NKT, NK, and $CD4^+$ T cells (Figure 3).

Neutralization of CD48 Attenuates Eosinophilic Inflammation, and Th2 and Proinflammatory Cytokine Expression, in BALF

The demonstration that CD48 is upregulated in allergic eosinophilic airway inflammation raised the possibility that this type of inflammation is dependent on CD48 and its ligands. Consequently, we used specific neutralizing antibodies for CD48, CD2, and 2B4 to investigate their roles in this experimental regimen (Figures 4A–4F) (20–23). These antibodies were also analyzed by us to determine whether they deplete targeted cells *in vivo*. Administration of anti-CD48, anti-CD2, and anti-2B4 mAbs did not alter splenic and peripheral blood cellular composition or numbers (*see* Figure E1 in the online supplement), and did not have any effect on bone marrow eosinophils (data not shown).

Anti-CD48 mAb treatment before allergen challenge considerably reduced BALF inflammation. For example, eosinophilic inflammation was significantly decreased on CD48 pretreatment (about 85%). Interestingly, anti-CD2 mAb pretreatment inhibited BALF inflammation to a lesser extent and caused an approximately 45% reduction in BALF eosinophils. Pretreatment with anti-2B4 mAb did not alter eosinophilic inflammation (Figure 4A). In addition, OVA-challenged mice displayed increased IL-4, IL-5, IL-13, TNF-α, and eotaxin-2 levels (Figures 4B–4F). However, mice pretreated with anti-CD48 mAb showed a pronounced reduction of these cytokines (75–93% decrease). In contrast, mice treated with anti-CD2 or anti-2B4 mAb exhibited only an approximately 40–50% decrease in the BALF cytokine profile or had no effect, respectively. Notably, all of the aforementioned effects were specific because mice that were treated with control antibodies displayed cytokine levels equivalent to those of OVA-challenged mice. Importantly, the effect of CD48 treatment was observed for at least 48 hours after administration.

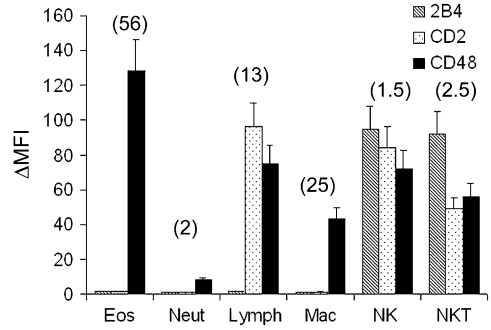


Figure 3. Cellular source of CD48, CD2, and 2B4 in the lungs of mice sensitized with ovalbumin (OVA)–alum and challenged with OVA. Eighteen hours after the last allergen challenge the lungs were harvested and expression of CD48, CD2, and 2B4 on various cell types was analyzed. Data are represented as the change in mean fluorescence intensity (ΔMFI) ± SD (n = 4 mice). Eos = eosinophils; Neut = neutrophils; Lymph = $CD4^+$ lymphocytes; Mac = macrophages; NK = NK cells; NKT = NKT cells. Values in parentheses indicate the percentage of the indicated cell type in bronchoalveolar lavage fluid (BALF).

Neutralization of CD48 Attenuates Lung Inflammation

These findings indicate that CD48 has a significant role in the allergen-induced inflammatory response. Accordingly, lung histology followed by quantitative analysis was performed to assess the effects of CD48 and its ligands on several parameters of lung inflammation (Figures 5A–5D). As shown, OVA-challenged mice displayed evident perivascular and peribronchial eosinophilic inflammation, epithelial damage, and airway muscle thickening. Anti-CD48–treated mice had a striking reduction in alveolar space, lung perivascular and peribronchial inflammation, and epithelial shedding (Figures 5A–5D). This effect was specific to CD48 treatment because anti-CD2 treatment induced a mild inhibitory effect only on the peribronchial inflammatory score, and anti-2B4 treatment seemed to enhance lung inflammation. Importantly, control antibodies did not alter these features.

Neutralization of CD48 Attenuates Goblet Cell Hyperplasia, Mucus Production, and Smooth Muscle Thickening in Lungs

One of the main features of allergic eosinophilic airway inflammation is mucus production and goblet cell hyperplasia. As assessed by periodic acid–Schiff reagent staining, allergen challenge increased goblet cell hyperplasia and mucus production. This effect was significantly reduced by anti-CD48 pretreatment (Figures 6A and 6B) but not by anti-2B4 treatment. Anti-CD2 treatment induced a negligible effect.

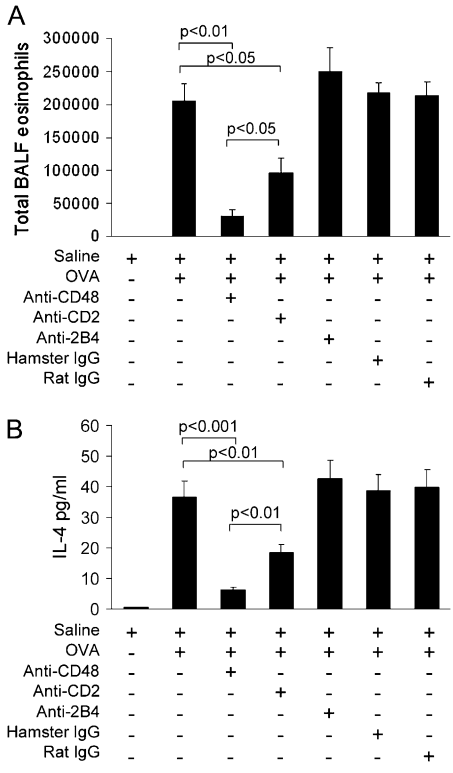
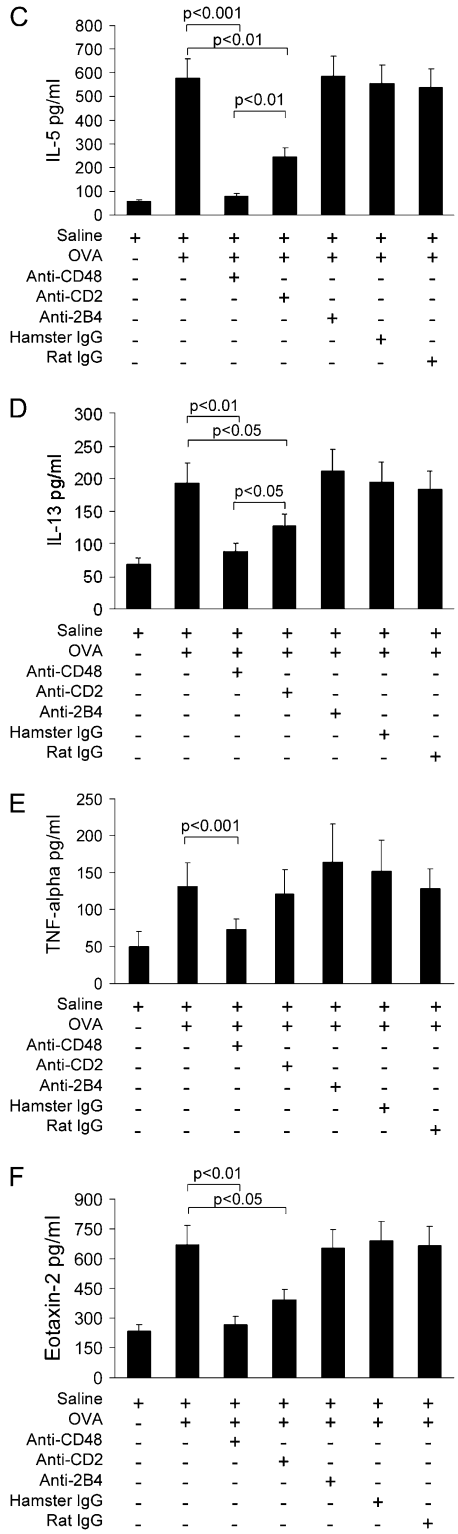


Figure 4. Neutralization of CD48 attenuates eosinophilic inflammation and helper T cell type 2 and proinflammatory cytokine expression in bronchoalveolar lavage fluid (BALF). Mice sensitized with ovalbumin (OVA)–alum were treated with anti-CD48, anti-CD2, or anti-2B4 monoclonal antibodies (mAbs) or control antibodies (rat IgG or hamster IgG) on Day 23 and on Days 24 and 27, 1 hour before allergen challenge (250 μg/mouse). Twenty-four hours after the last allergen challenge bronchoalveolar lavage was performed and BALF cells were stained for differential cell identification. $CCR3^+/VLA\text{-}4^+/CD3^-/SSC^{high}$ (high side scatter) cells were gated and considered eosinophils (*A*). IL-4, IL-5, IL-13, tumor necrosis factor (TNF)-α, and eotaxin-1 (*B–F*, respectively) in BALF were detected by ELISA according to the manufacturers' instructions. Data are presented as means ± SD of n = 3 experiments (4–6 mice per group per experiment). OVA = ovalbumin.



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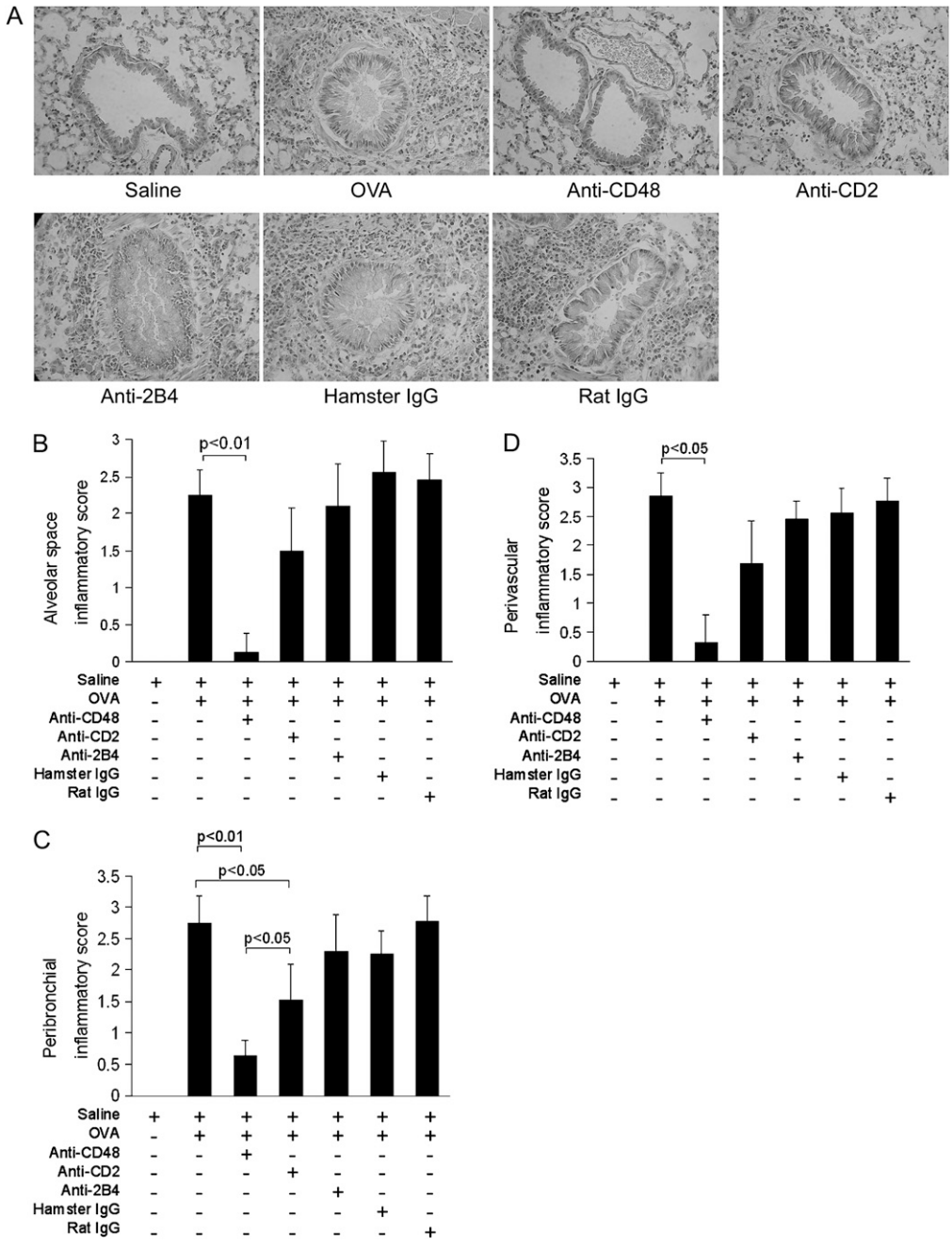

Figure 5. Neutralization of CD48 attenuates lung inflammation. Mice were sensitized, challenged, and treated as described in text. Lung tissue was fixed, paraffin embedded, and stained with hematoxylin and eosin (H&E) for assessment of inflammation. Representative photomicrographs (original magnification, ×40) of airway inflammation in the various treatment groups (*A*). Quantitative analysis of alveolar space (*B*), lung perivascular (*C*), and peribronchial (*D*) inflammation is presented. Data represent means ± SD of n = 3 experiments (4–6 mice per group per experiment). OVA = ovalbumin.

In addition, the thickness of the peribronchial smooth muscle layer was significantly greater in OVA-challenged mice than in saline-challenged mice. Anti-CD48 mAb–treated mice displayed significantly less smooth muscle thickening (Figure 6C). Anti-CD2–treated mice exhibited a minor reduction, and anti-2B4 and control antibodies had no effect.

DISCUSSION

A central issue in understanding the complexity of asthma is to define the molecular mechanisms that govern this disease process. Therefore, in the present study we focused on the contribution of CD48, CD2, and 2B4 to allergic eosinophilic airway inflammation. By using global transcript expression profiling (7), we found that CD48 is upregulated in two murine models of allergic eosinophilic airway inflammation and is an element of the "asthma genome signature" that was described by Zimmermann and coworkers (7). Although CD2 was found to be upregulated in the OVA-induced airway inflammation model, it did not appear significantly upregulated in the *Aspergillus* model. Thus, the only CD2 subfamily receptor that is likely involved in disease pathogenesis and not in the mode of induction is CD48. Therefore, our findings regarding CD48 were further validated by Northern blot and polymerase chain reaction.

Our data suggest that the expression of CD48 is regulated by a factor in the inflammatory milieu. Among the key molecules in the allergic inflammatory response are STAT-6, IL-4, and IL-13 (7, 33–36). These molecules govern several genes that are key regulators of allergic eosinophilic airway inflammation, such as trefoil factor-2 (TFF2), a disintegrin and metalloproteinase domain-8 (ADAM8), eotaxin-1, and arginase (7, 33, 34, 37). Mechanistic analysis of these pathways revealed that CD48 was

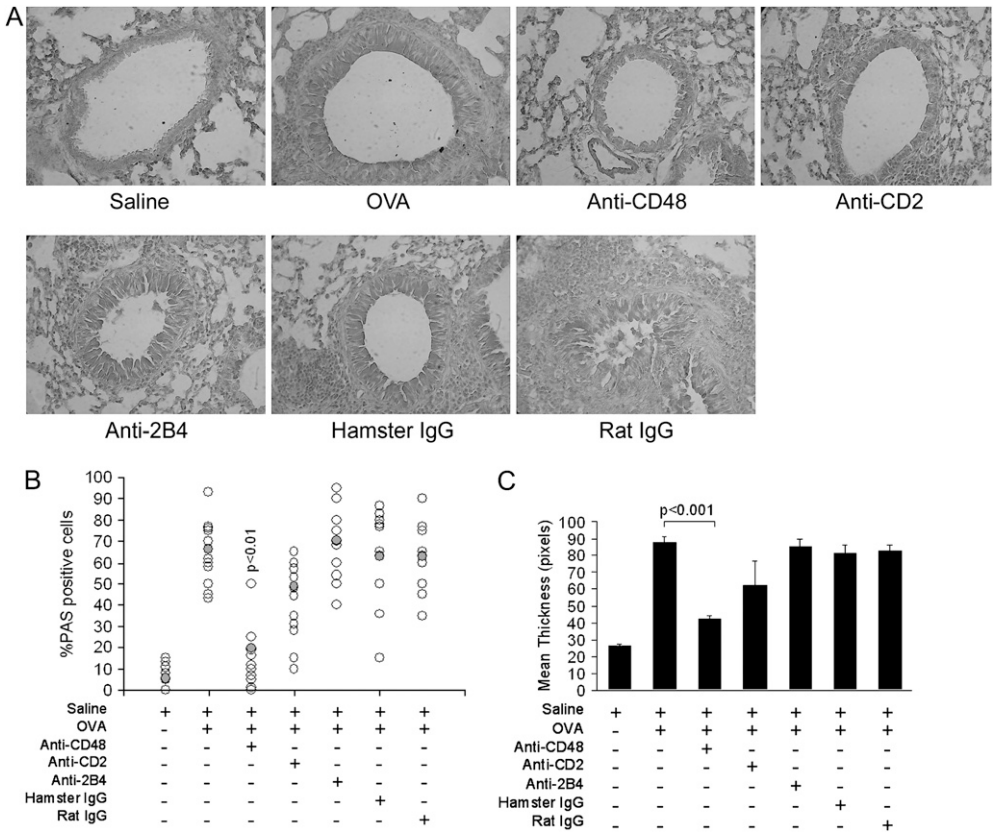

Figure 6. Neutralization of CD48 attenuates goblet cell hyperplasia, mucus production, and smooth muscle thickening in lungs. Mice were sensitized, challenged, and treated as described in text. Lung tissue was fixed, paraffin embedded, and stained with periodic acid–Schiff reagent (PAS) for assessment of goblet cell hyperplasia and mucus production. (A) Representative photomicrographs (original magnification, ×40) of airway PAS staining in the various treatment groups. (*B*) Quantitative analysis of PAS^+ cells in the bronchial epithelium. *Shaded circles* represent the mean peribronchial smooth muscle thickness in pixels of three midsized bronchioles per mouse. (*C*) Analysis of peribronchial smooth muscle thickening. Data represent means ± SD of n = 3 experiments (4–6 mice per group per experiment). OVA = ovalbumin.

upregulated in the absence of STAT-6, IL-4, and IL-13. Interestingly, CD48 expression was found to be spontaneously upregulated in inducible IL-13 transgenic mice. Thus, we can hypothesize that compensatory mechanisms (yet to be defined) present in the lungs of allergen-challenged mice can upregulate CD48 in the absence of a single pathway. We could not rule out the possibility that CD48 is upregulated in the lungs of OVA-challenged mice as a result of local inflammation and recruitment of CD48-expressing cells. Yet, upregulation of CD48 was unchanged in mice deficient in STAT-6, IL-4, IL-13, and eotaxin-1/IL-5, which fail to develop an eosinophil infiltrate (data not shown). Thus, on allergen challenge lung expression of CD48 remains unaltered (33). The finding that CD48 is upregulated in the absence of eosinophils, which constitute the major inflammatory component in both the OVA and *Aspergillus* experimental models, could be partially explained by the fact that compensatory mechanisms will take place such as the recruitment of other $CD48^+$ cells such as lymphocytes and neutrophils. Indeed, although STAT-6–deficient mice have attenuation of many features of experimental asthma (e.g., pulmonary eosinophilia), they are either only partially protected or not protected at all from other aspects of the disease that are less specific for allergy, such as lung neutrophilia (33). Alternatively, it is probable that diverse pathways upregulate CD48 at the single-cell level. Indeed, phytohemagglutinin, phorbol myristate acetate, IL-4, interferons, and Epstein-Barr virus infection all upregulate CD48 expression on various hematopoietic cells (16, 38). Relevant to this study, we have established that the expression of CD48 on both murine and human eosinophils is upregulated specifically by IL-3 (39). Thus, CD48 is upregulated at the single-cell level by at least two mediators that are expressed in the asthmatic milieu: IL-3 and IL-4.

The abundance of pathways that regulate CD48 expression *in vivo* highlights the importance of CD48. This led us to investigate the CD48–CD2–2B4 axis in allergic eosinophilic airway inflammation pathogenesis. To examine this, we administered anti-CD48, anti-CD2, and anti-2B4 neutralizing antibodies (20, 23, 31) before allergen challenge. Strikingly, neutralization of CD48 significantly reduced eosinophilic inflammation and cytokine expression (i.e., Th2 cytokines IL-5, IL-4, and IL-13; and proinflammatory cytokines TNF-α and eotaxin-2) in the BALF. Moreover, it abrogated lung inflammation (alveolar space, perivascular, and peribronchial), airway smooth muscle thickening, epithelial shedding, goblet cell hyperplasia, and mucus production. Neutralization of CD2 caused an approximately 40–50% reduction in these inflammatory parameters whereas anti-2B4–treated mice displayed no significant effect.

Several mechanisms could account for the antiinflammatory effects produced by blocking CD48. CD48-deficient mice show considerable defects in $CD4^+$ T cell activation (14). The inhibitory effect of anti-CD48 treatment in our settings is likely to be only partially dependent on T cell costimulation via CD2, because anti-CD2–treated mice displayed a mild reduction of the disease parameters in comparison with anti-CD48 mAb–treated mice. Supporting this finding is the observation that CD2-deficient mice do not display the same effects observed in CD48-deficient mice. This suggests a broader and more substantial role for CD48 in the immune system than has been previously recognized. Several lines of evidence support a broad immunologic role for CD48. CD48 can activate B cells and mast cells, and interact with heparan sulfate on epithelial cells (11, 17–19, 40). Furthermore, IL-18, which has been previously described as a Th1-inducing cytokine, was shown also to promote Th2 cytokine production and to promote IgE production. In addition, transgenic overexpression of pro–IL-18 by keratinocytes induces

the development of atopic dermatitis (41, 42). Intriguingly, IL-18 interacts with CD48 to induce its signaling cascades (43). Thus, IL-18 stimulation of CD48 may promote allergic responses.

NKT cells have been described as regulating Th2 immune responses (44). They have been attributed significant effector functions in allergic settings particularly by releasing IL-4 and IL-13 but not IFN-γ (44, 45). These cells express a variety of NK receptors, among them 2B4, and are able to bias systemic and local T cells to differentiate into Th2 cytokine–producing cells (46). Furthermore, IL-5–producing NK cells have been reported to promote allergic inflammation (47). Although the role and pathways regulating NKT cell functions in asthma are still to be determined, our study suggests that 2B4 does not play a significant role in their activation in allergic settings.

CD48 can induce signal transduction, as it binds Lck, Fyn, and G proteins (48, 49). Cross-linking of CD48 on purified tonsillar B cells significantly increased CD40-mediated activation (11), and cross-linking CD48 in combination with IL-4 and/or IL-10 is able to induce B cell aggregation, proliferation, and IgG secretion (11). Mast cells have been shown to bind the fimbrial adhesion molecule FimH through interactions with CD48, resulting in mast cell degranulation and phagocytosis (17–19). Although CD48 has not been studied on these cells in the context of allergic reactions, it may participate in mast cell functions in allergy as well. Moreover, we have shown that CD48 is an activation receptor on human eosinophils and triggers the release of eosinophil peroxidase and eosinophil-derived neurotoxin (39). Consequently, by blocking CD48 we may inhibit lymphocyte, eosinophil, and mast cell activation and cross-talk. Importantly, masking of CD48 on murine eosinophils (isolated from IL-5 transgenic mice) did not alter their ability to transmigrate through A549 epithelial cells in response to eotaxin-1 (data not shown). Thus, the significant reduction of BALF inflammation is probably not due to inhibition of cellular trafficking.

The involvement of CD48 was also recognized in experimental colitis (50). In the latter study, CD48 has been shown to inhibit IL-4 release, suggesting its role in Th2-related reactions (50). Nevertheless, this is the first study to demonstrate a significant role for CD48 in Th2-type settings and especially in allergic eosinophilic airway inflammation.

Although this study reveals a prominent role for CD48 in allergen-challenged mice, several limitations oblige us to be cautious concerning our conclusions regarding its role in human disease. In humans, CD58, which is upregulated on eosinophils from asthmatic donors (51), has a higher affinity than CD48 for CD2. In addition, our *in vivo* data suggest that 2B4 does not participate in murine pulmonary allergic inflammation. However, because of its limited expression on murine cells and its greater expression on human leukocytes, it may play a significant role in human asthma.

Nevertheless, our observations that CD48 is upregulated on human eosinophils from asthmatic donors (tissue and peripheral blood) (39), together with its abundant expression in allergen-challenged lungs and the relatively low effect of anti-CD2 treatment, reinforce our hypothesis that CD48 is critically involved in human asthma pathogenesis and is therefore a potential target for asthma therapy.

Conflict of Interest Statement: A.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. I.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. F.D.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.E.R. participated as a speaker financed by Merck, and received consulting fees and/or stock options from Ception Therapeutics ($37,000), GlaxoSmithKline ($5,000), and Medacorp ($10,000). He received $45,000 from Cambridge Antibody Technology in 2005 as a research contract/grant. F.L.-S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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