GSDMB and a proxy SNP [rs12603332] for rs4065275 in *ORMDL3* [$R^2 = 0.98$], which has been shown to regulate IL-2 production in T cells).[8] For *CDHR3*, we tested a proxy SNP (rs10258293) for rs6967330 ($R^2 = 0.85$). In addition to its association with asthma risk, this variant in the *CDHR3* locus also confers increased risk of susceptibility to viral respiratory tract infections, a leading cause of wheeze in early life.[9] We hypothesized that the *GSDMB/ORMDL3* risk variants would be associated with the 2 wheeze trajectories with the largest percentages of patients with current asthma in early adolescence (persistent wheeze and midchildhood-onset wheeze), and that the *CDHR3* risk variant would confer increased risk of trajectories with higher rates of wheeze in early life (transient and persistent wheeze). Using an additive genetic model, we observed an increased odds (OR, 2.22 per risk allele; 95% CI, 1.04-4.77) of persistent wheeze for the proxy *ORMDL3* variant rs12603332 (C allele) but no clear association for transient or midchildhood-onset wheeze phenotypes. The *GSDMB* and *CDHR3* polymorphisms were not associated with any wheeze patterns.

The persistent wheeze trajectory observed in our cohort was strongly associated with parental history of asthma, nonwhite race/ethnicity, bronchiolitis in early life, and a genetic polymorphism in a known asthma susceptibility locus *(ORMDL3)*. The midchildhood-onset wheeze trajectory showed associations with atopic phenotypes (eczema in infancy and total serum IgE levels in adolescence). For the transient wheeze phenotype, the strongest measure of association was for bronchiolitis in infancy; however, it is important to note that bronchiolitis was associated with all of the more adverse wheeze trajectories (midchildhood, persistent, and transient) compared with never/infrequent wheeze.

In conclusion, using data from infancy to adolescence, our analysis of longitudinal wheeze phenotypes allowed us to visualize 4 longitudinal wheeze trajectories, including one not previously observed for Project Viva in a past trajectory analysis up to age 9 years. The trajectories observed had some common early-life predictors (maternal asthma and bronchiolitis), as well as unique predictors, such as an *ORMDL3* genetic variant (for persistent wheeze in white children) and atopic phenotypes (for midchildhood wheeze onset).

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Disruptive anti-IgE inhibitors prevent mast cell–dependent early airway response in viable atopic lung tissue

To the Editor

IgE plays a central role in the pathophysiology of allergic asthma. The therapeutic anti-IgE antibody omalizumab has been shown to inhibit allergic asthma symptoms by neutralizing free serum IgE and thereby preventing early and late phases of IgE-mediated allergic reactions after 12 to 16 weeks of treatment. Unfortunately, use of omalizumab is restricted to the treatment of moderate-to-severe asthma, ineffective to approximately one third of patients, and associated with high costs. Thus there is an urgent need for the development of new and more efficient therapeutic options to treat severe types of asthma. Here we report that the disruptive anti-IgE designed ankyrin repeat protein (DARPin, a registered trademark of Molecular Partners AG, Switzerland) bi53_79 (hereafter referred to as E002) might significantly improve treatment efficacy in a therapeutic setting in which allergic effector cells in the lung are sensitized with allergen-specific IgE.

In patients with IgE-mediated allergic asthma, mast cells are loaded with allergen-specific IgE, which is bound to the cell surface through the high-affinity IgE receptor (FcεRI).[1] Inhalation of allergens might lead to rapid activation of these airway mast cells through allergen-dependent cross-linking of FcεRI-bound IgE, followed by degranulation and release of mediators, including histamine. Histamine binds to the H1 receptor on airway smooth muscle cells and induces an allergen-specific immediate airway constriction defined as the early airway response (EAR).

The previously described disruptive anti-IgE DARPin E2_79 (hereafter referred to as E001) not only neutralizes free serum IgE with high specificity but further actively dissociates IgE-FcεRI complexes on human primary allergic effector cells.[2] Compared with classical antibody formats, DARPins represent alternative

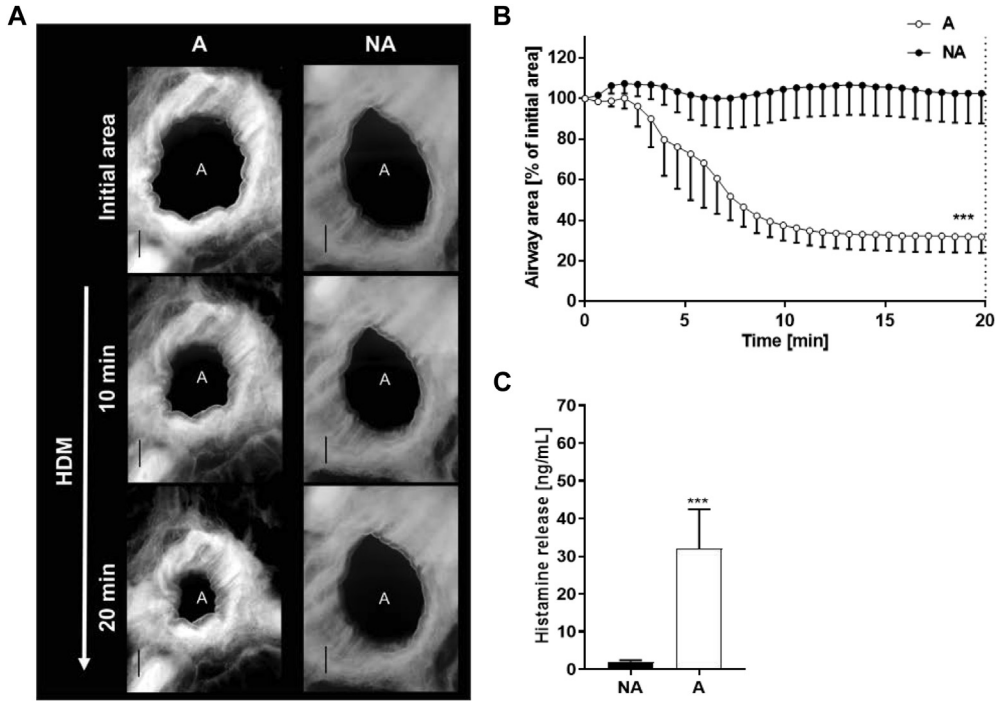

FIG 1. A, EAR in a sensitized human PCLS. PCLS were incubated overnight with 1% plasma of a donor with HDM allergy or plasma from a nonallergic donor. Bronchoconstriction provoked with HDM was monitored by using videomicroscopy. Reduction of the airway area was calculated as a percentage of the initial airway area. Representative microscopic images of the EAR in passively sensitized PCLS versus appropriate controls. Images of cross-sectioned airways before and 10 and 20 minutes after allergen (HDM) challenge. **B,** HDM provoked a reduction in airway area by 68% ± 10% in PCLS incubated with allergic plasma. PCLS incubated with plasma of a nonallergic donor showed no bronchoconstriction. **C,** HDM challenge of passively sensitized PCLS or PCLS treated with plasma of a nonallergic donor led to an average histamine release of 32 ± 10 and 2 ± 0 ng/mL, respectively. *Scale bar* = 200 μm. PCLS in duplicates, n = 3. ***$P < .001$, *t* test using the Mann-Whitney method. Data are expressed as means ± SEMs. *A,* 1% Allergic plasma; *NA,* 1% nonallergic plasma.

binding scaffolds. Their small molecular size (approximately 12 kDa) and rigid repetitive ankyrin structure lacking disulfide bridges allows highly efficient bacterial expression. E001 specifically targets human IgE, blocks its binding to FcεRI, and disrupts preformed IgE-FcεRI complexes through a mechanism termed facilitated dissociation in which subsite attachment points within IgE become partially exposed and accessible for E001 binding before full dissociation from FcεRI. In a humanized FcεRI transgenic mouse model, both E001 and its improved biparatopic variant, E002, inhibited allergic skin reactions with high potency after stimulation of passively sensitized cutaneous mast cells *in vivo*.[3]

In this study we sought to assess the efficacy of E002 to prevent mast cell activation in atopic precision-cut lung slices (PCLS), which represent a well-established technique with a high predictive value for the *in vivo* situation. PCLS contain all cells of the lower respiratory tract, such as epithelial cells, smooth muscle cells, fibroblasts, macrophages, and mast cells. Lung tissue remains viable and maintains functionality for days. Previous studies demonstrated the efficacy of bronchoconstrictors in a multispecies PCLS study and reported that histamine induces airway constriction in lung tissue of human subjects and nonhuman primates, whereas serotonin leads to airway constriction only in rodents.[4] Because this pathomechanism is critical for EAR, we functionally compared E002 to the therapeutic anti-IgE antibody omalizumab in human PCLS cultures *ex vivo*.

Allergen-induced airway constriction is mast cell dependent and occurs within the first 10 minutes on allergen exposure. This airway smooth muscle contraction is mainly induced by mediators, such as histamine, leukotrienes, and prostanoids, released from activated mast cells, which reach maximal concentrations a few minutes after allergen challenge.[5] Initially, we investigated allergen-induced airway constriction in human lung tissue *ex vivo*. Human PCLS that were passively sensitized with plasma of donors with house dust mite (HDM) allergy showed extensive bronchoconstriction after allergen challenge with HDM extract (Fig 1, *A* and *C*). Maximal reduction of the initial airway area (68% ± 10%) was achieved 20 minutes after allergen challenge (Fig 1, *A*). Airway constriction was significantly greater in PCLS that were sensitized with plasma of allergic donors when compared with PCLS that were sensitized with plasma from nonallergic subjects.

We further assessed whether the amount of released histamine correlates with the observed bronchoconstriction. Indeed, tissue culture supernatants of PCLS sensitized with plasma of allergic donors contained significantly greater histamine levels (32 ± 10 ng/mL) compared with control sensitized PCLS after allergen challenge (2 ± 0 ng/mL; Fig 1, *C*). Similar to the findings in bronchoalveolar lavage fluid of human asthmatic patients, we detected greater histamine quantities in supernatants of sensitized and allergen-provoked PCLS compared with those of nonsensitized tissue control subjects.

Omalizumab neutralizes free IgE with high potency, but it is not very efficient in accelerating the dissociation of preformed IgE-FcεRI complexes.[3] In an experimental setting in which omalizumab was added to the plasma of an allergic donor before it was

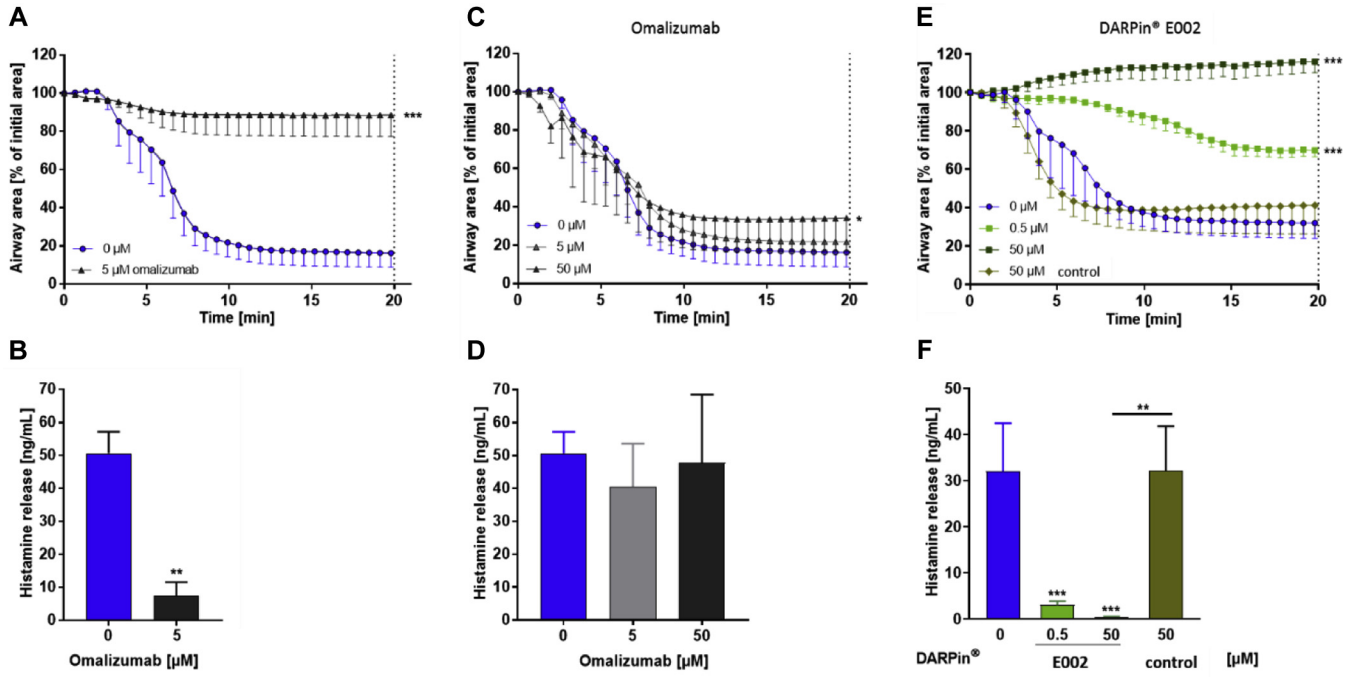

FIG 2. A, EAR is abolished with a preventive approach of omalizumab in sensitized human peripheral airways. One percent allergic plasma was pretreated with or without omalizumab (5 μmol/L) before adding to the PCLS. Bronchoconstriction provoked with HDM was monitored by using videomicroscopy. Reduction of the airway area was calculated as a percentage of the initial airway area. HDM provoked a reduction of the airway area by 84% ± 9% in PCLS incubated with allergic plasma. PCLS incubated with allergic plasma pretreated with omalizumab (5 μmol/L) showed only a maximal airway constriction of 12% ± 9%. **B,** HDM treatment of passively sensitized PCLS treated with or without omalizumab led to an average histamine release of 7 ± 5 and 51 ± 6 ng/mL, respectively. PCLS in duplicates, n = 3. $**P < .004$ and $***P < .001$, *t* test using the Mann-Whitney method. Data are expressed as means ± SEMs. **C,** Omalizumab does not prevent the EAR in a therapeutic setting. Passively sensitized PCLS were treated with or without omalizumab (5 or 50 μmol/L) 2 hours before allergen challenge. Bronchoconstriction provoked with HDM was monitored by using videomicroscopy. Reduction of the airway area was calculated as a percentage of the initial airway area. Passively sensitized PCLS untreated or treated with 5 or 50 μmol/L omalizumab showed maximal airway constriction of 84% ± 9%, 78% ± 8%, and 66% ± 17%, respectively. **D,** Passively sensitized PCLS untreated or treated with 5 or 50 μmol/L omalizumab led to an average histamine release of 51 ± 6, 40 ± 13, and 48 ± 21 ng/mL, respectively. PCLS in duplicates, n = 3. $*P < .02$, *t* test using the Mann-Whitney method. Data are expressed as means ± SEMs. **E,** E002 prevents EAR in a therapeutic setting. Passively sensitized PCLS were treated with or without E002 (0.5 or 50 μmol/L) 2 hours before the allergen challenge. Bronchoconstriction provoked with HDM was monitored by using videomicroscopy. Reduction of the airway area was calculated as a percentage of the initial airway area. Passively sensitized PCLS untreated or treated with 0.5 or 50 μmol/L E002 showed maximal airway constriction of 68% ± 10%, 30% ± 3%, and 0% ± 5%, respectively. Passively sensitized PCLS treated with control DARPin showed maximal airway constriction of 62% ± 10%. **F,** Passively sensitized PCLS untreated or treated with 0.5 or 50 μmol/L E002 led to an average histamine release of 32 ± 10, 3 ± 1, and 0.5 ± 0 ng/mL, respectively. Passively sensitized PCLS treated with control DARPin led to an average histamine release of 32 ± 10 ng/mL. PCLS in duplicates, n = 3. $**P < .004$ and $***P < .001$, *t* test using the Mann-Whitney method. Data are expressed as means ± SEMs.

incubated with PCLS, omalizumab efficiently inhibited airway constriction and significantly reduced histamine release from 51 ± 6 to 7 ± 5 ng/mL (Fig 2, *A* and *B*). Thus preventive treatment with omalizumab during passive PCLS sensitization completely abolished allergen-induced bronchoconstriction and histamine release.

These findings confirm the efficacy of omalizumab in neutralizing free IgE and thus in preventing IgE binding and sensitization of airway mast cells.[1] However, when we treated presensitized human PCLS with 50 μmol/L omalizumab for 2 hours before HDM challenge, we observed strong bronchoconstriction with a reduction of initial airway area to 66% ± 17% and prominent histamine release (48 ± 21 ng/mL; Fig 2, *C* and *D*). Thus when omalizumab was added after passive sensitization of PCLS with plasma of an allergic donor, it showed no inhibition of the EAR, confirming previous reports that omalizumab is a poor disruptor of pre-existing IgE-FcεRI complexes. In contrast, when we used the therapeutic setting, in which human PCLS were first sensitized with human plasma from an allergic donor and subsequently had E002 added, we observed a marked reduction of allergen-induced histamine release and bronchoconstriction (Fig 2, *E* and *F*). Allergen-dependent bronchoconstriction in passively sensitized human PCLS was significantly decreased at 0.5 μmol/L E002 and was completely abolished at 50 μmol/L. The total amount of 32 ± 10 ng/mL histamine in control DARPin-treated allergen-challenged human PCLS was reduced to 3 ± 1 ng/mL with 0.5 μmol/L E002. With increasing concentrations, the measured histamine levels further decreased to 2 ± 1

ng/mL at 5 μmol/L E002 and 0.5 ± 0 ng/mL at 50 μmol/L E002. Differences in airway response were significant between the treatment groups of E002 and control DARPin.

These data are in line with the previously reported disruptive activity of E002, and the results confirm a previous study reporting that intradermal injections of E002 six hours before antigen challenge are effective at inhibiting allergic skin reaction in passively sensitized humanized FcεRI transgenic mice.[3] In the same study it was described that omalizumab was only effective to inhibit the allergic skin reaction in mice when used at high concentrations. Indeed, omalizumab also accelerates the dissociation of receptor-bound IgE from isolated primary human blood basophils, although only at high concentrations or extended incubation periods. In contrast, E002 is 10,000-fold more efficient than omalizumab in dissociating IgE complexes.[3] Thus our data confirm dissociation of preformed IgE-FcεRI complexes considering that a 2-hour treatment at a relatively low concentration of 0.5 μmol/L E002 was sufficient to significantly inhibit the EAR in presensitized PCLS. Although E002 features lower IgE-binding affinity compared with omalizumab,[3] its main advantage is the efficacy with which it actively disrupts preformed IgE-FcεRI complexes through a facilitated dissociation mechanism.[2]

Currently, omalizumab is the only anti-IgE therapy approved to treat moderate-to-severe allergic asthma. It is well established that omalizumab efficiently decreases the concentration of free serum IgE and thereby decreases FcεRI expression on circulating basophils.[1] Moreover, there is evidence that omalizumab reduces IgE in inflamed tissues, such as the airway mucosa.[6] Previous studies have reported that patients with mild-to-moderate persistent asthma treated with omalizumab for 16 weeks had significant reduction in numbers of IgE-positive cells, as well as cells expressing FcεRI in the bronchial submucosa.[6] However, despite a significant decrease in FcεRI expression on mast cells within the tissue, it is suggested that the time course for this decrease is very slow. For instance, a significant reduction in mast cell FcεRI expression in skin biopsy specimens from patients with moderately severe allergic rhinitis was only observed after 10 weeks of omalizumab treatment.[7] These findings might be explained by either poor tissue penetrance of omalizumab or the remarkably slow dissociation rate of IgE from FcεRI on mast cells on omalizumab treatment.[8] This latter observation is crucial because the half-life of cell-bound IgE is in the range of weeks and therefore provides persistent sensitization of mast cells.[8] Because of its smaller molecular size, E002 might feature better tissue penetrance than antibodies and might therefore show an advantage to dissociate preformed IgE-FcεRI complexes at local sites of inflammation. From this point of view, combined therapy of omalizumab with E002 might represent an interesting alternative to improve treatment efficacy.

In summary, we provide evidence that disruptive IgE inhibitors, such as E002, might have a therapeutic advantage over conventional anti-IgE compounds that primarily aim at neutralizing free serum IgE. Our data indicate that active desensitization of mast cells in human lung tissue using E002 is remarkably rapid and efficient. Active disruption of IgE-FcεRI complexes on the cell surfaces of allergic effector cells represents a novel and interesting way to interfere with critical atopic pathways. Based on these characteristics, E002 might be a suitable candidate for the treatment of allergic asthma.

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METHODS

Source of lung material

The study included human lung tissue from 6 donors. Tissue was obtained from patients with cancer who underwent surgery because of lung tumors. Use of human lung tissue for research was approved by the ethics committee of the Hannover Medical School, Hannover, Germany. Experiments complied with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects. The privacy rights of human subjects were observed, and written consent was obtained from all patients. Personal data were not recorded to protect anonymity.

Preparation of PCLS

Preparation of PCLS was performed, as recently published in detail.[E1] Briefly, only lung lobes were used and filled up inside with medium and agarose solution. Soon after the agarose had solidified, lung tissue was sliced into equal parts to generate approximately 250-μm-thin PCLS using a Krumdieck tissue slicer (Alabama Research and Development, Munford, Ala). PCLS were then incubated under cell-culture conditions (37°C, 5% CO_2, and 100% air humidity) for 2 hours. PCLS were washed and used for further experiments. Tissue was passively sensitized in human plasma of allergic donors (HDM-IgE > 100 kU/L, specific IgE class 6; PlasmaLab International, Everett, Wash) overnight. For negative reference, PCLS were also incubated in human plasma of nonallergic donors. Treatment with inhibitor was performed after sensitization. Airway constriction was done as previously published.[E2] Single PCLS were watched by using stereomicroscopy. HDM extract was added, and airway constriction was watched for 20 minutes. Airway area before addition of allergen was set at 100%. Bronchoconstriction was expressed as the percentage decrease in airway area in comparison with initial airway area. Histamine release was analyzed by means of ELISA (Demeditec Diagnostics GmbH, Kiel, Germany). Viability was checked as published previously.[E1]

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