2. Han ED, MacFarlane RC, Mulligan AN, Scafidi J, Davis AE 3rd. Increased vascular permeability in C1 inhibitor-deficient mice mediated by the bradykinin type 2 receptor. *J Clin Invest*. 2002;109(8):1057-1063.
3. Qiu T, Chiuchiolo MJ, Whaley AS, et al. Gene therapy for C1 esterase inhibitor deficiency in a murine model of hereditary angioedema. *Allergy*. 2018. [Epub ahead of print]. https://doi.org/10.1111/all.13582
4. Santos RA, Simoes e Silva AC, Maric C, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci USA*. 2003;100(14):8258-8263.
5. Siltari A, Korpela R, Vapaatalo H. Bradykinin -induced vasodilatation: role of age, ACE1-inhibitory peptide, mas- and bradykinin receptors. *Peptides*. 2016;85:46-55.
6. Czaller I, Molnár K, Csuka D, Varga L, Farkas H. Successful outcome using C1-inhibitor concentrate in acute pancreatitis caused by hereditary angioedema. *Gastroenterol Nurs*. 2011;34(1):60-63.
7. Brickman CM, Tsokos GC, Balow JE, et al. Immunoregulatory disorders associated with hereditary angioedema. I. Clinical manifestations of autoimmune disease. *J Allergy Clin Immunol*. 1986;77(5):749-757.
8. Chen Q, Vera-Portocarrero LP, Ossipov MH, Vardanyan M, Lai J, Porreca F. Attenuation of persistent experimental pancreatitis pain by a bradykinin b2 receptor antagonist. *Pancreas*. 2010;39(8):1220-1225.
9. Hirata M, Hayashi I, Yoshimura K, et al. Blockade of bradykinin B(2) receptor suppresses acute pancreatitis induced by obstruction of the pancreaticobiliary duct in rats. *Br J Pharmacol*. 2002;135(1):29-36.

SUPPORTING INFORMATION

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Longitudinal trends of serum IgE and *IL5RA* expression throughout childhood are associated with asthma but not with persistent wheeze

To the Editor,

Wheezing episodes are common in young infants affecting every third child before the age of 3.[1,2] While wheezing episodes in early life are mostly triggered by viral lower respiratory tract infection, the development of asthma and airway hyperresponsiveness seems to be promoted by immunoglobulin E (IgE)–mediated lung inflammation.[3-5] Although the development of an asthma phenotype does not necessarily require an atopic status, early childhood asthma is mostly associated with atopy while nonatopic, intrinsic asthma is a rather rare condition.[3,4] Considering the critical role of IgE in the development of impaired lung function in early childhood, several studies tried to differentiate children with transient wheezing episodes from those with persistent wheeze based on early childhood serum IgE levels providing evidence that persistently wheezing children seem to have elevated IgE levels already very early in life.[6] Notably, although not every child with persistent or late-onset wheeze will be diagnosed with asthma, none of the earlier studies focusing on IgE, wheeze, and asthma distinguished between persistent wheeze and asthma (eg, [4,6]). Thus, one major aim of the present study was to evaluate the longitudinal association between serum IgE levels, wheeze, and asthma considering persistent wheeze and asthma as independent endotypes. In addition to serum IgE, we included interleukin-5 receptor-α (*IL5RA*) measured by qPCR from whole-blood RNA in our analyses as *IL5RA* has been closely linked to both IgE and asthma development.[7]

Data and samples from two population-based German birth cohorts, LINA[8] and LISA,[9] were used for this study. While the majority of birth cohorts dispose of only few blood sampling time points, the LINA study with annual clinical visits and blood sampling until the age of 8 offers the opportunity to study the timing of sensitization, wheezing symptoms, and asthma onset in a tightly time-resolved manner. The LISA study with available blood samples at ages 2, 6, 10, and 15 was used as a replication cohort. For the current analyses, only children with longitudinal blood samples (LINA age 1-8, n = 98; LISA age 2, 6, 10, and 15, n = 453) and available RNA (LINA age 1, 4, and 8; LISA age 15) were included (for the description of the study population, see Figure S1 and Table S1, Supporting Information Methods). Wheezing children without an asthma diagnosis were grouped according to their wheezing endotypes: transient (reported wheezing up to the age of 3 but not thereafter), late-onset (wheezing reported after age 3), and persistent wheeze (wheezing during the first 3 years of life and at least one time thereafter). These children were compared to apparently healthy controls and children diagnosed with asthma, respectively. The asthma outcome was defined according to the question asked at each follow-up (see Figure S1): "Has a physician diagnosed your child with asthma during the past 12 months?" Within the studied LINA and LISA subpopulations, 10.2% (10/98) and 10.8% (49/453) of the children were ever diagnosed with asthma, respectively.

As expected, in both cohorts the specific IgE concentrations (sIgE) (Figure 1A) and sensitization percentages (Figure S2) against aeroallergens (sx1/rx1) were significantly different between groups over time with highest levels in asthmatic children (one-way repeated-measurement ANOVA; Figure 1A). Compared to healthy controls, LINA children diagnosed with asthma later in life showed significantly higher sIgE levels already starting at age 4 (post hoc

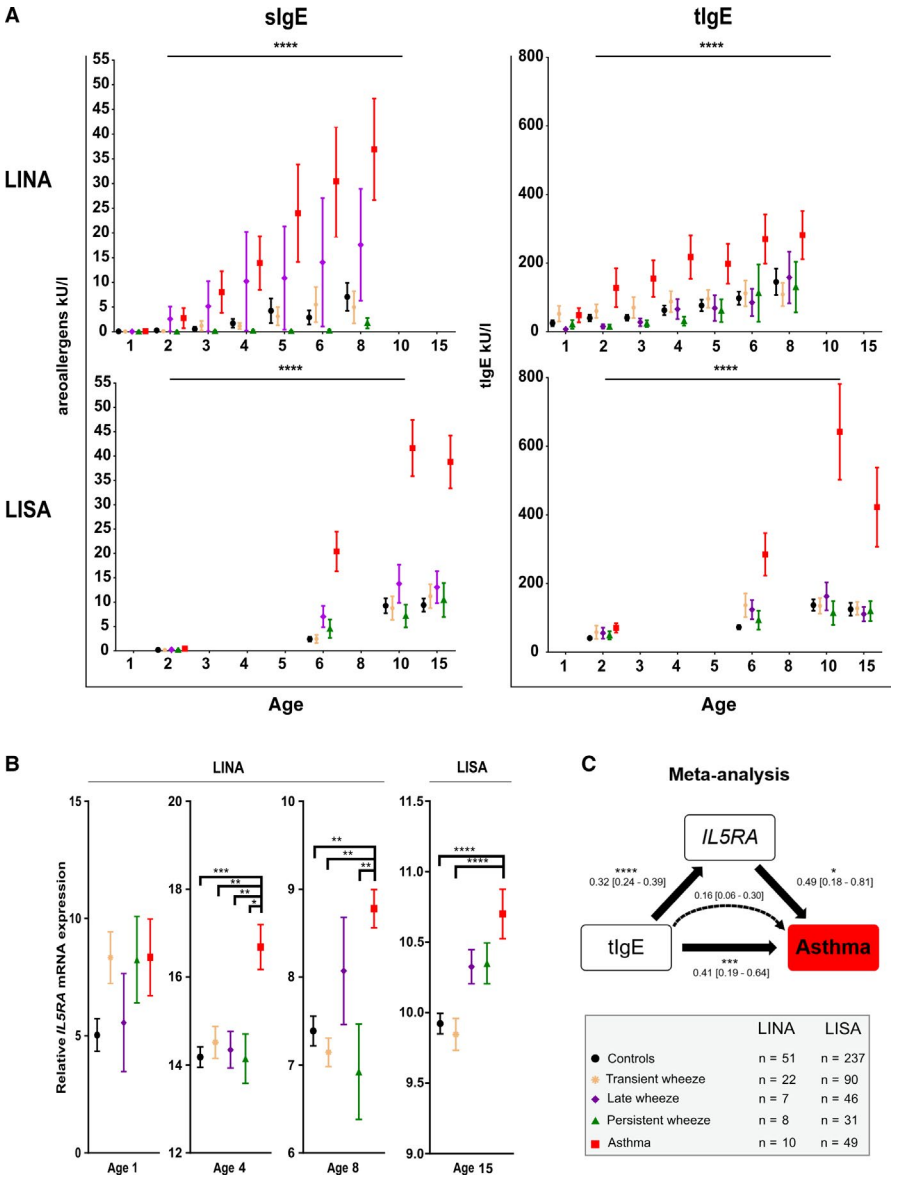

FIGURE 1 A, Longitudinal pattern of specific and total serum IgE levels in the LINA and LISA cohorts. Given are mean ± SEM. A repeated-measurement one-way ANOVA was applied to determine the difference between groups over time. B, Comparison of relative *IL5RA gene expression in asthma, different wheezing endotypes, and controls in the LINA and the LISA cohort, respectively. One-way ANOVA followed by Dunnett's post hoc test was applied to determine the difference between asthma and the other groups. C, Mediation meta-analysis for the relationship of tIgE, IL5RA expression, and asthma development for 8-y-old children in LINA and 15-y-old children in LISA together. Models were adjusted for gender, maternal history of atopy, mode of delivery, and prenatal tobacco smoke exposure. The effect sizes for each path of the mediation analysis are given as unstandardized b-values with lower and upper confidence intervals (P < 0.05 *; P < 0.01**; P < 0.001***; P < 0.0001****)*

TABLE 1 Dunnett's test (*P*-value) comparing specific IgE against aeroallergens (sx1/rx1) between asthmatic and nonasthmatic children of controls and wheezing endotypes in the LINA cohort

Compared phenotypes			Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 8
Asthma	vs	Controls	1.0	0.952	0.291	**0.028**	**0.0001**	**0.0001**	**0.0001**
		Persistent	1.0	0.978	0.525	0.095	**0.0006**	**0.0001**	**0.0001**
		Late	1.0	1.000	0.977	0.946	0.138	**0.044**	**0.011**
		Transient	1.0	0.958	0.460	**0.040**	**0.0002**	**0.0001**	**0.0001**

P-values < 0.05 are indicated in bold.

TABLE 2 Dunnett's test (*P*-value) comparing specific IgE against aeroallergens (sx1/rx1) between asthmatic and nonasthmatic children of controls and wheezing endotypes in the LISA cohort

Compared phenotypes			Age 2	Age 6	Age 10	Age 15
Asthma	vs	Controls	1.0	**0.0001**	**0.0001**	**0.0001**
		Persistent	1.0	**0.0009**	**0.0001**	**0.0001**
		Late	1.0	**0.0019**	**0.0001**	**0.0001**
		Transient	1.0	**0.0001**	**0.0001**	**0.0001**

P-values < 0.05 are indicated in bold.

TABLE 3 Dunnett's test (*P*-value) comparing total IgE between asthmatic and nonasthmatic children of controls and wheezing endotypes in the LINA cohort

Compared phenotypes			Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 8
Asthma	vs	Controls	0.969	0.204	0.062	**0.005**	**0.041**	**0.001**	**0.017**
		Persistent	0.983	0.253	0.150	**0.017**	0.123	0.058	0.074
		Late	0.936	0.292	0.196	0.090	0.182	**0.026**	0.218
		Transient	1.000	0.515	0.318	0.051	0.169	**0.011**	**0.004**

P-values < 0.05 are indicated in bold.

TABLE 4 Dunnett's test (*P*-value) comparing total IgE between asthmatic and nonasthmatic children of controls and wheezing endotypes in the LISA cohort

Compared phenotypes			Age 2	Age 6	Age 10	Age 15
Asthma	vs	Controls	0.917	**0.0001**	**0.0001**	**0.0001**
		Persistent	0.998	**0.015**	**0.0001**	**0.0001**
		Late	0.997	**0.024**	**0.0001**	**0.0001**
		Transient	0.993	**0.015**	**0.0001**	**0.0001**

P-values < 0.05 are indicated in bold.

TABLE 5 Longitudinal association of specific IgE (aeroallergens sx1/rx1), total IgE, and *IL5RA* expression of asthmatic children compared to healthy controls in the LINA and LISA cohorts (wheezing children were excluded from these analyses; for results of wheezing endotypes, see Figure S3). Given are ORs with upper and lower 95% confidence intervals and *P*-values from adjusted generalized estimating equations

	LINA			LISA		
	OR	CI	*P*-value	OR	CI	*P*-value
sIgE	1.13	1.03-1.22	0.006	1.46	1.33-1.59	<0.0001
tIgE	1.20	1.06-1.35	0.003	1.71	1.38-2.11	<0.0001
IL5RA	1.04	1.03-1.06	0.0001	—[b]		

[a]Adjusted for gender, maternal history of atopy, parental educational level, mode of delivery, and prenatal tobacco smoke exposure (cotinine level).
[b]RNA only available in the LISA cohort for age 15.

comparison by Dunnett's test; Table 1). From age 6 onward in both cohorts, asthmatic children also showed significantly higher sIgE concentrations compared to all wheezing endotypes including persistent wheeze (Tables 1/2). Similarly, total IgE (tIgE) was significantly different between groups over time (Figure 1A). However, differences in tIgE between wheezing endotypes and asthmatic children were less clear and consistent compared to sIgE (Tables 3/4).

Asthmatic children twice as often had high IgE concentrations (sIgE > 0.35 kU/L; tIgE > 100 kU/L) compared to healthy controls or any of the wheezing endotypes (Figure S2). Only in LINA, late-onset wheezers also showed high sIgE levels, which most likely was related to those children not yet diagnosed with asthma during our observation period until the age of 8. Noteworthy, the persistent wheezing group was rather more similar to the healthy controls than to the asthma group.

Similarly to IgE, *IL5RA* mRNA expression was significantly increased only in children with asthma compared to controls and all wheezing endotypes starting at age 4 (Figure 1B). As demonstrated by adjusted generalized estimating equations (adjGEEs), tIgE, sIgE, and *IL5RA* showed a significant longitudinal association with the development of asthma (Table 5) but not with persistent wheezing or any other of the wheezing endotypes in both cohorts (IgE: Figure S3; *IL5RA*: data not shown). Within the LINA study, an increased expression of *IL5RA* was observed already at the age of 4 in children diagnosed with asthma (Figure 1B). Subsequent mediation analyses revealed that *IL5RA* expression transmits the effect of tIgE on asthma, as shown by a significant direct (unstandardized b = 0.41, CI = 0.19-0.64) and indirect (unstandardized b = 0.16, CI = 0.06-0.30) effect in a meta-analysis based on LINA and LISA data (Figure 1C).

IL5RA is expressed on eosinophils, basophils, mast cells, and B cells. Unfortunately, we neither determined these cell types in the blood nor do we have protein data that might account for functional properties. Therefore, we were not able to identify the cellular source of this receptor in the context of asthma development within this study. A further limitation of this study is the parent-reported physician-diagnosed asthma, which might be less accurate as a diagnosis made in a clinical setting. In addition, there might be misdiagnosed asthma children within the persistent and late-onset wheezing subgroups. Together with the low numbers of patients in the different subgroups, these limitations might have reduced the power of this study.

Nevertheless, this study provides interesting new data of potential clinical relevance. For the first time, we show high resolved longitudinal IgE and *IL5RA* data in children with wheezing symptoms and asthma compared to healthy controls. Based on these data, a clear difference between persistent wheezing and asthma evolved, underlining the necessity to consider both endotypes as different entities. Results of the mediation analyses suggest that the development

of asthma requires both a high IgE level and an increase in *IL5RA* mRNA expression. Furthermore, there is some evidence from the LINA study that *IL5RA* activation, studied here as *IL5RA* mRNA expression, may occur already before the asthma phenotype is established (see Figure S4 showing *IL5RA* mRNA expression at the age of 4 in those 7 children diagnosed with asthma later in life). Although this result is based on a small case number and therefore has to be interpreted with caution, our data may encourage further studies in this direction. In early life, high IgE levels and concomitant increase in *IL5RA* mRNA expression might help to distinguish children developing asthma from those children with wheezing symptoms but who never will suffer from asthma.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Bloomberg GR. Recurrent wheezing illness in preschool-aged children: assessment and management in primary care practice. *Postgrad Med*. 2009;121(5):48-55.
2. Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson children's respiratory study: 1980 to present. *J Allergy Clin Immunol*. 2003;111(4):661-675.
3. Just J, Bourgoin-Heck M, Amat F. Clinical phenotypes in asthma during childhood. *Clin Exp Allergy*. 2017;47(7):848-855.
4. Peat JK, Toelle BG, Dermand J, van den Berg R, Britton WJ, Woolcock AJ. Serum IgE levels, atopy, and asthma in young adults: results from a longitudinal cohort study. *Allergy*. 1996;51(11):804-810.

5. Kusel MM, de Klerk NH, Kebadze T, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol*. 2007;119(5):1105-1110.
6. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med*. 1995;332(3):133-138.
7. Virkud YV, Kelly RS, Croteau-Chonka DC, et al. Novel eosinophilic gene expression networks associated with IgE in two distinct asthma populations. *Clin Exp Allergy*. 2018;48(12):1654-1664.
8. Trump S, Bieg M, Gu Z, et al. Prenatal maternal stress and wheeze in children: novel insights into epigenetic regulation. *Sci Rep*. 2016;6:28616.
9. Tischer C, Standl M, Lehmann I, Schaaf B, von Berg A, Heinrich J. Sleeping on animal fur is related to asthma outcomes in later childhood. *Eur Respir J*. 2015;46(1):107-114.

SUPPORTING INFORMATION

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Differential effects of mesenchymal stem cells on T cells isolated from childhood allergies and autoimmune diseases

To the Editor,

Mesenchymal stem cells (MSCs) are multipotent stromal cell precursors and are involved in immunoregulation. They can suppress the maturation, proliferation, or effector function of a wide range of innate and adaptive immune cells, including T cells.[1] Numerous studies have reported that MSCs can inhibit Th1 and Th17 cells and ameliorate Th1- or Th17-mediated autoimmune diseases.[2,3] However, the effects of MSCs on Th2 cells and Th2-mediated immune responses are not well understood. In addition, the therapeutic potential of MSCs in children with autoimmune diseases is rarely studied. In this study, we investigated the effect of MSCs on cultured Th2 cells and T cells isolated from children with allergic asthma and examined the therapeutic potential of MSCs in autoimmune disease by using blood samples from pediatric patients.

To study the effect of MSCs on the development of Th2 cells in vitro, CD4 T cells were cultured in Th2-polarizing conditions in the presence or absence of MSCs and assayed Th2 cell function after 5 days of culture. MSCs were isolated from choriodecidual tissues of human placenta (denoted as pcMSCs) as described in our previous study.[4] pcMSCs markedly suppressed the production of interleukin (IL)-4, IL-5, and IL-13 and proliferation of Th2 cells (Figure S1A-C). We further investigated the suppression of Th cells already differentiated to the Th2 phenotype by pcMSCs. We demonstrated that pcMSCs downregulated CD25, IL-4, IL-5, and IL-13 expression in Th2 cells (Figure S1D-F) and therefore suppressed the activation of Th2 cells.

Further, we examined whether MSCs exerted a similar effect in Th2-mediated asthma. We collected peripheral blood mononuclear cells (PBMCs) from children with allergic asthma. PBMCs were activated using anti-CD3 and anti-CD28 antibodies in the presence or absence of pcMSCs. We determined the activation, proliferation, and cytokine production of stimulated T cells. A representative flow cytometry analysis of PBMCs is shown in Figure 1A. The suppressive effect of pcMSCs on the activation and proliferation of both $CD4^+$ and $CD8^+$ T cells was dose-dependent (Figure 1B-E). Remarkably, the Th2-cytokine IL-5 was dramatically reduced in the conditioned supernatant of PBMCs by pcMSC coculture and the suppression was found even at low MSC-to-PBMC ratio (1:80) (Figure 1F). Th1 and Th17 cytokines (interferon (IFN)-γ and IL-17, respectively) were also downregulated by pcMSCs (Figure 1G and 1H). In addition, bone marrow–derived MSCs (bmMSCs) [5] also suppressed IL-5 production (Figure 1I). The direct effect of MSCs on $CD4^+$ T cells was defined by using purified $CD4^+$ T cells (Figure S2). Hence, MSCs suppressed the activation, proliferation, and cytokine (especially IL-5) production of T cells isolated from children with allergic asthma. Our results were consistent with the previous study,[6] which showed that bmMSCs suppress the proliferation of PBMCs isolated from adult allergic asthmatic patients and this effect was allergen-specific. However, we also observed a small number of patients whose T cells were not reactive under anti-CD3 and anti-CD28 antibody stimulation (Figure S3, MSC: PBMC = 0). Coculturing these nonreactive T cells with pcMSCs promoted their activation, proliferation, and cytokine production (Figure S3). Hence, apart from this exception, we inferred that MSCs could suppress the activation, proliferation, and cytokine (especially IL-5) production of T cells isolated from children with allergic asthma.

We then studied the therapeutic potential of MSCs in pediatric autoimmune diseases. We isolated PBMCs from children with Henoch-Schönlein purpura (HSP), juvenile idiopathic arthritis (JIA), and systemic lupus erythematosus (SLE). We found that pcMSCs did not have a consistent influence on the activation, proliferation, and cytokine production of T cells isolated from children with autoimmune diseases (Figure 2). This was not consistent with previous

Abbreviations: CFSE, carboxyfluorescein succinimidyl ester; MSCs, mesenchymal stem cells; PBMCs, peripheral blood mononuclear cells; pcMSCs, placenta choriodecidual tissue MSCs.

Yih-Mei Liou and Teng-Yuan Wei contributed equally to this work.