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Serum sphingosine-1-phosphate is elevated in atopic dermatitis and associated with the severity

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To the Editor

Atopic dermatitis (AD) is the most common inflammatory skin disease with a strong impact on the quality of life.¹ Sphingosine-1-phosphate (S1P) is a bioactive lipid mediator that impacts immunological processes and S1P receptors (S1PRs) are considered as therapeutic targets for different disorders including AD.² However, although multiple studies have addressed stratum corneum lipids in the context of epidermal barrier dysfunction,³ S1P in serum has not been evaluated in patients with AD. In this study, we evaluated the serum levels of S1P in specimens from 179 patients with AD, 22 non-AD patients with atopic comorbidities (i.e., allergic rhinitis, bronchial asthma and/or food allergy) and 47 healthy controls.

In AD, serum S1P levels were increased when compared to other groups (Figure 1a). AD with eczema area and severity index (EASI) ≥ 16 , currently the cut-off for indication for systemic therapy,⁴ exhibited higher serum S1P levels than AD with EASI < 16 (Figure 1b). Serum S1P levels in patients with AD showed a positive correlation with EASI (Figure 1c), body surface area (BSA, Figure 1d), and thymus and activation regulated chemokine (TARC, Figure S2c), although the correlation coefficients were low. Next, we evaluated the AD severity and serum protein biomarkers in patients with AD by serum S1P levels every 10th percentile (Figure 1e-h). The AD subgroup with the highest S1P levels (i.e., with S1P levels ≥ 90 th percentile) showed high BSA, EASI, and serum concentration of TARC compared to other subgroups. To investigate possible distinguishing phenotypic characteristics of AD in patients with very high serum S1P levels, two subgroups of AD were defined based on serum S1P levels: a Normal-S1P-AD group, in which serum S1P levels fell within the 25th-75th percentiles (513.7-675.7 nM); and a High-S1P-AD group, in which serum S1P levels fell within the 90th-100th percentiles (757.3-956.2 nM). The severity scores for AD and serum concentrations of TARC and interleukin-22 in High-S1P-AD group were higher compared to the Normal-S1P-AD subgroup (Table 1). The High-S1P-AD group exhibited a trend for the high frequency of facial pallor/erythema and ear rhagades compared to the Normal-S1P-AD group (Table 1).

The present study showed that serum S1P levels were elevated in AD and identified that the AD

subgroup with very high serum S1P levels exhibited high severity of AD and high frequency of facial pallor/erythema and ear rhagades. Although the evaluation of serum S1P is still not a standard technique in hospitals, these observations could provide new insight into AD's pathogenesis. The mechanism(s) of increased serum S1P in AD are unclear. On the other hand, increased serum S1P could affect the immune system function by several ways such as establishing a sharper gradient of S1P across various tissues, a phenomenon that may contribute to inflammation in AD.^{5,6} Agents targeting S1PRs have been shown to have immunomodulatory effects and several clinical trials with S1PRs modulators are ongoing for the treatment of multiple inflammatory disorders.² Currently, the safety and efficacy of the systemic treatment with Etrasimod, targeting S1PRs 1, 4, and 5, has been confirmed in patients with moderate to severe AD in a Phase 2 clinical trial (NCT04162769), opening the door for this compound to the Phase 3 program.

Limitations: This is an observational study that does not provide a mechanism behind the study. Additionally, atopic comorbidities were diagnosed solely on the basis of the participants' self-reported diagnoses, and the number of non-AD patients with atopic comorbidities was small compared to the numbers of participants in the other groups.

In summary, this study showed that serum S1P levels were elevated in AD and associated with the severity. These observations support the concept of S1PRs being interesting therapeutic targets in AD.

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Author contributions

TS, NH, and TB designed the work; LM recruited the participants and created the clinical database; TS, NH, LM, and TJN acquired the data; TS, NH, LM, TJN, and TW analyzed the data; TS, RAC, and MHG performed the lipid analyses; TS interpreted the data and wrote the manuscript; TS, NH and TB edited the manuscript; TB obtained funding.

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This work was supported by the Christine Kühne-Center for Allergy Research and Education (CK-CARE). The funding source did not participate in study design, collection and interpretation of data, in the writing of the report and in the decision to submit the article for publication.

Conflict of Interest

The study, Laura Maintz, Nadine Herrmann, and Thomas Welchowski were supported by the Christine Kühne-Center for Allergy Research and Education (CK-CARE).

Thomas Bieber was speaker, and/or consultant, and/or investigator for: AbbVie, Allmiral, AnaptysBio, Arena, Asana Biosciences, Astellas, BioVerSys, Böhringer-Ingelheim, Celgene, Daichi-Sankyo, Dermavant/Roivant, DermTreat, DS Pharma, RAPT/FLX Bio, Galapagos/MorphoSys, Galderma, Glenmark, GSK, Incytes, Kymab, LEO, Lilly, L'Oréal, MenloTx, Novartis, Pfizer, Pierre Fabre, Sanofi/Regeneron, UCB. Thomas Bieber is founder of the non-profit biotech company "Davos Biosciences".

All other authors have no conflicts of interest to declare.

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Disclosure Statement (copy and paste from each COI form)

Dr. Sakai has nothing to disclose.

Dr. Herrmann has nothing to disclose.

Dr. Maintz reports grants from Christine Kühne-Center for Allergy Research and Education, during the conduct of the study.

Dr. Nümm has nothing to disclose.

Dr. Welchowski has nothing to disclose.

Dr. Claus has nothing to disclose.

Dr. Gräler has nothing to disclose.

Dr. Bieber reports personal fees from AbbVie, personal fees from Allmiral, personal fees from AnaptysBio, personal fees from Arena, personal fees from Asana Biosciences, personal fees from Böhringer-Ingelheim, personal fees from Dermavant/Roivant, personal fees from Galapagos/MorphoSys, personal fees from Galderma, personal fees from Incytes, personal fees from Kymab, personal fees from LEO, personal fees from Lilly, personal fees from MenloTx, personal fees from Novartis, personal fees from Pfizer, personal fees from Pierre Fabre, personal fees from Sanofi/Regeneron, personal fees from UCB, outside the submitted work.

Table 1. The severity and clinical phenotype of AD patients with high serum S1P levels

	Normal-S1P-AD	High-S1P-AD	<i>P</i> value
Number	90	17	N/A
Female/Male, n	51/39	11/6	.6014
Age (years)	35.5 (25.0-51.0)	34.0 (22.5-50.0)	.7787
BMI (kg/m2)	23.35 (21.31-26.03)	24.17 (21.60-26.51)	.6437
BSA (%)	16.25 (6.800-37.00)	39.50 (28.75-50.25)	.0042**
EASI	7.200 (2.975-17.75)	18.90 (10.35-25.60)	.0055**
SCORAD	36.70 (27.05-52.03)	49.00 (38.90-65.45)	.0259*
TARC (pg/ml)	549.1 (245.9-1493)	1474 (543.4-2651)	.0151*
IL-22 (pg/ml)	0.7200 (0.2825-1.723)	1.660 (0.9650-4.090)	.0126*
Eosinophils (G/L)	0.23 (0.14-0.43)	0.34 (0.18-0.67)	.2306
Total IgE (IU/ml)	1151 (148.8-3225)	1059 (268.5-5979)	.5907
Pruritus (score: 1-10)	3.0 (2.0-6.0)	5.0 (2.5-7.0)	.0985
Sleeplessness (score: 1-10)	0.0 (0.0-4.0)	1.0 (0.0-6.0)	.6076
AD with any atopic comorbidities	82/90 (91.1%)	14/17 (82.4%)	.3761
AD with allergic rhinitis	75/90 (83.3%)	13/17 (76.5%)	.4977
AD with bronchial asthma	49/90 (54.4%)	9/17 (52.9%)	>.9999
AD with food allergy	59/90 (65.6%)	12/17 (70.6%)	.7851
Xerosis	82/90 (91.1%)	17/17 (100%)	.3505
Palmar hyperlinearity	72/90 (80.0%)	14/17 (82.4%)	>.9999
Keratosis pilaris	22/90 (24.4%)	3/17 (17.6%)	.7569
Nipple eczema	12/90 (13.3%)	2/17 (11.8%)	>.9999
Cheilitis sicca	45/90 (50.0%)	8/17 (47.1%)	>.9999
Dennie-Morgan fold	50/90 (55.6%)	10/17 (58.8%)	>.9999

Facial pallor/erythema	46/90 (51.1%)	13/17 (76.5%)	.0656
Pityriasis alba	14/89 (15.7%)	4/17 (23.5%)	.4825
Anterior neck fold	46/90 (51.1%)	9/17 (52.9%)	>.9999
Dirty neck	25/90 (27.8%)	4/17 (23.5%)	>.9999
Ear rhagades	34/90 (37.8%)	11/17 (64.7%)	.0593
Periorbital darkening	74/90 (82.2%)	15/17 (88.2%)	.7317
Dermographismus albus	56/90 (62.2%)	13/17 (76.5%)	.4075
Hertoghe sign	46/89 (51.7%)	10/17 (58.8%)	.6094
Perleche	32/90 (35.6%)	8/17 (47.1%)	.4183

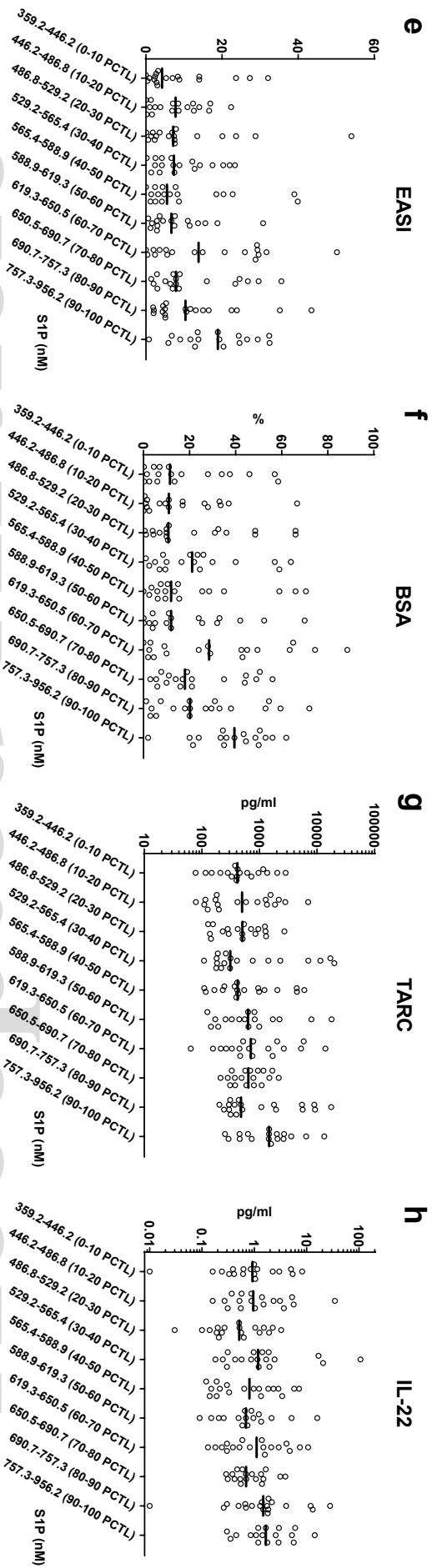
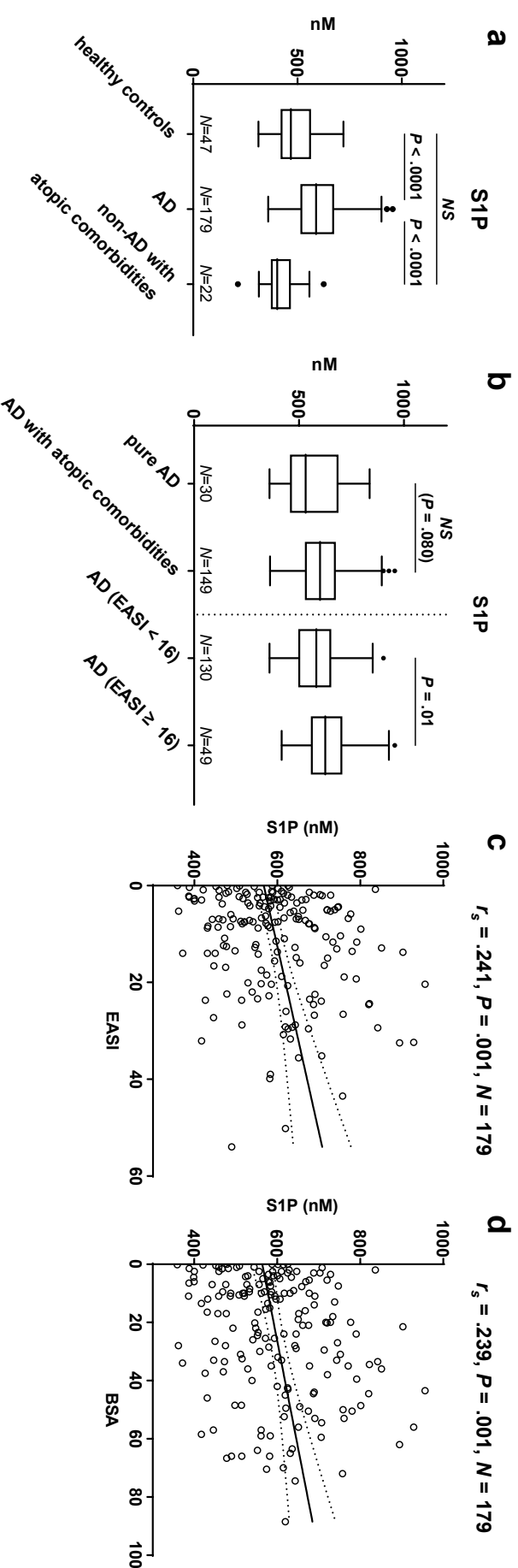
Table 1 legend.Two AD subgroups were defined by serum S1P levels: Normal-S1P-AD (serum S1P levels ranged between the 25th and 75th percentiles) and High-S1P-AD (serum S1P levels ranged between the 90th and 100th percentiles) to evaluate the phenotypic characteristics of AD patients with high serum S1P levels. Data are shown as paired values indicating the median and first to third quartiles (Q1-Q3), or frequency. *, $P<.05$; **, $P<.01$; N/A, not applicable.

Figure Legends

Figure 1. Serum S1P levels are elevated in AD and correlated with the severity

We analyzed S1P concentrations in serum from healthy controls, AD patients, and non-AD patients with atopic comorbidities(a). Serum S1P levels were also evaluated in AD subgroups stratified by the presence/absence of atopic comorbidities, and severity (b). The correlations between serum S1P levels and EASI or BSA are shown in (c,d). Next, we evaluated the AD severity (EASI, BSA)and serum protein biomarkers(TARC, IL-22) in AD subgroups stratified by serum S1P levels every 10th percentile (e-h). PCTL, percentile; NS, not significant.

Figure 1



a

	healthy controls (A)	AD (B)	non-AD with atopic comorbidities (C)	<i>P</i> -Value (A vs. B)	<i>P</i> -Value (B vs. C)	<i>P</i> -Value (A vs. C)
Number	47	179	22	N/A	N/A	N/A
Female/Male, n	34/13	112/67	18/4	<i>NS</i>	<i>NS</i>	<i>NS</i>
Age (years)	35 (28-44)	35 (25-49)	34.5 (29.5-42)	<i>NS</i>	<i>NS</i>	<i>NS</i>
BMI (kg/m ²)	22.15 (20.72-25.83)	23.15 (21.22-25.51)	23.86 (19.68-28.34)	<i>NS</i>	<i>NS</i>	<i>NS</i>

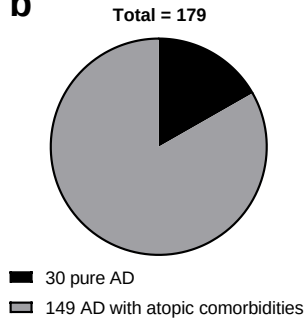
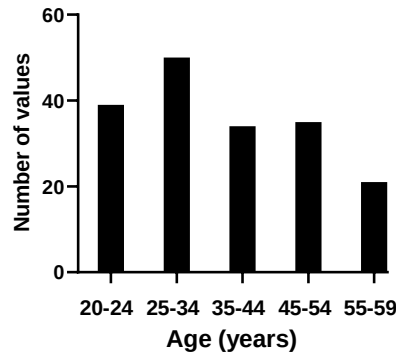
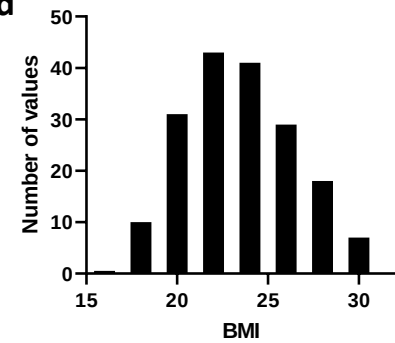
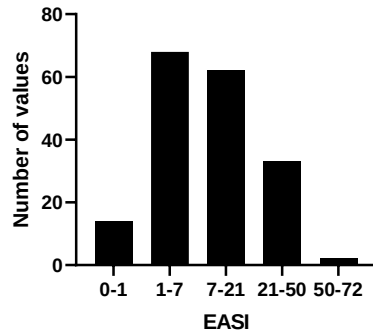
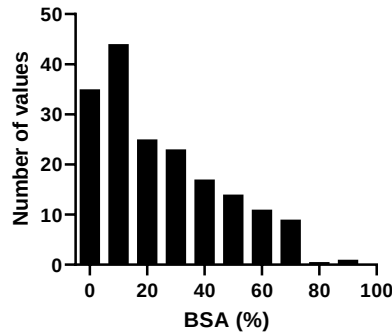
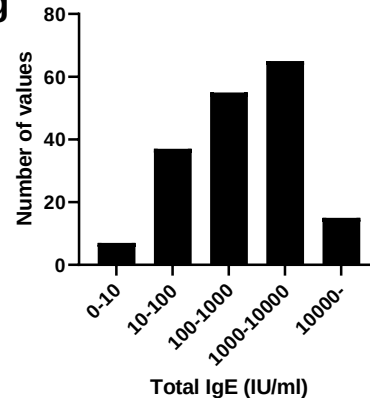
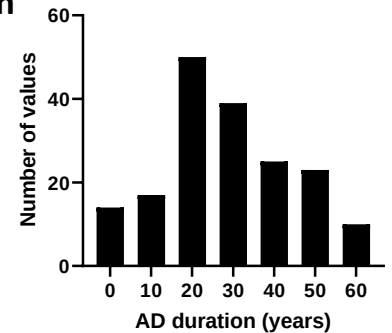
b**c****d****e****f****g****h**

Figure S1. Characteristics of study groups:

This study consisted of 3 groups: healthy controls, patients with AD, and non-AD with atopic comorbidities, i.e., allergic rhinitis, bronchial asthma, and/or food allergy (a). Data are shown as the median and first to third quartiles (Q1-Q3, respectively). Significances were calculated with the Bonferroni method or Fisher's exact test (a). Pie chart shows the rate of pure AD (AD without atopic comorbidities [allergic rhinitis, bronchial asthma, and/or food allergy]) and AD with atopic comorbidities (b). Histograms show distributions of age (c), BMI (d), EASI (e), BSA (f), total IgE levels (g), and AD duration (h) in patients with AD. AD, atopic dermatitis; BMI, body mass index; BSA, body surface area; EASI, eczema area and severity index; Ig, immunoglobulin; N/A, not applicable; *NS*, not significant.

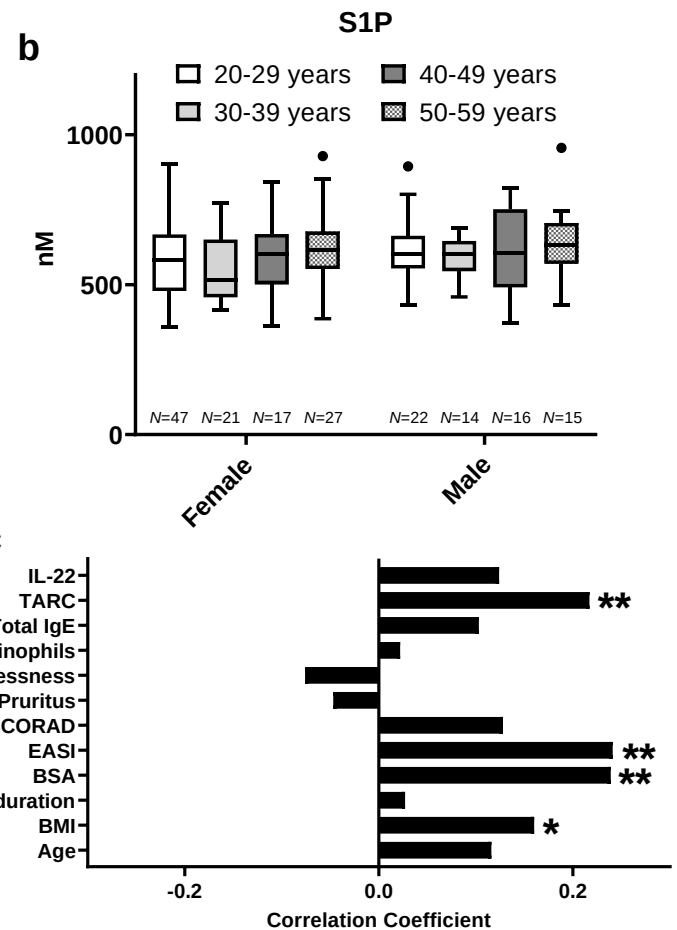
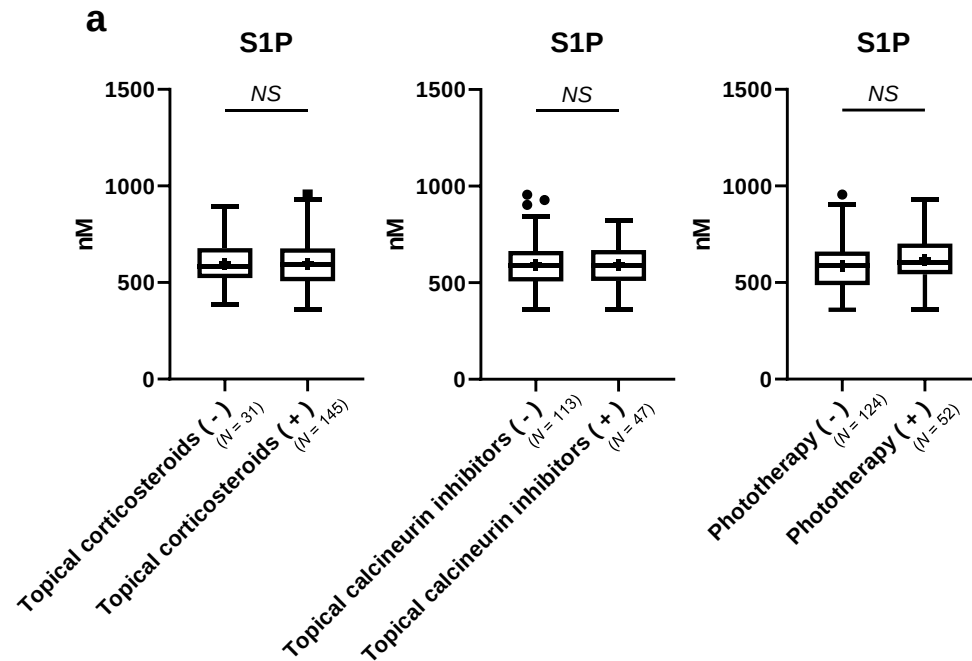


Figure S2 (a) Topical treatments and phototherapy do not affect serum levels of S1P in patients with AD:

Serum S1P levels were evaluated in patients with AD, with or without each treatment within the preceding year. Significances were calculated with the Mann-Whitney U test. NS, not significant.

Figure S2 (b) Serum S1P levels in the AD subgroups stratified by age/gender:

Serum S1P levels in the AD subgroups stratified by age/gender were evaluated.

Figure S2 (c) Correlation between serum S1P and clinical and laboratory data in patients with AD:

The correlation between serum S1P levels and clinical and laboratory data in patients with AD was analyzed by Spearman's correlation test. The right bar of the bar graph indicates a positive correlation; the left bar indicates a negative correlation. $N=178-179$, *, $P < .05$; **, $P < .01$.

AD, atopic dermatitis; BMI, body mass index; BSA, body surface area; EASI, eczema area and severity index; Ig, immunoglobulin; IL, interleukin; SCORAD, severity scoring of atopic dermatitis; S1P, sphingosine-1-phosphate; TARC, thymus and activation regulated chemokine.

Serum sphingosine-1-phosphate is elevated in atopic dermatitis and associated with the severity

Takashi Sakai, MD, PhD,^{1,2,*} Nadine Herrmann, PhD,¹ Laura Maintz, MD,¹ Tim Joachim Nümm, PhD,¹ Thomas Welchowski, PhD,^{1,3} Ralf A. Claus, PhD,⁴ Markus H. Gräler, PhD,⁴ Thomas Bieber, MD, PhD, MDRA.¹

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Methods

Participants and groups

The participants were recruited in the Christine Kühne-Center for Allergy Research and Education (CK-CARE) program. They were part of an atopic dermatitis (AD)-focused registry program with a comprehensive collection of phenotypic and epidemiologic information as well as biobanking.^{S1} Since circulating sphingosine-1-phosphate (S1P) levels can be affected by several diseases, patients with any systemic or chronic disorder other than AD and atopic comorbidities (allergic rhinitis, bronchial asthma, or food allergy), were excluded. Furthermore, participants were limited to the ages of 20-59 years, because in that range, there is no difference in serum S1P levels among healthy controls stratified by age and gender.^{S2} This study enrolled a total of 248 participants: 179 patients with AD (AD group), including 30 patients with AD only (pure AD) and 149 patients with AD with atopic comorbidities (allergic rhinitis, bronchial asthma, and/or food allergy); 22 non-AD patients with allergic rhinitis, bronchial asthma, and/or food allergy (non-AD with atopic comorbidities group); and 47 healthy controls (healthy controls group). The study was approved by the ethics committee of the University of Bonn and performed in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from each participant in the study. The diagnosis of AD was performed according to the criteria of Hanifin and Rajka.^{S3} Clinical scoring of AD (severity scoring of atopic dermatitis [SCORAD], eczema area and severity index [EASI], and body surface area [BSA, %]), and the assessment of clinical phenotypes of AD were performed by experienced dermatologists in the Dermatology Department at the University Hospital Bonn.^{S4-S6} Information on pruritus and sleeplessness were extracted from the subjective SCORAD. Diagnosis of allergic rhinitis, bronchial asthma, or food allergy was conducted based on information provided by the subjects on a self-reported diagnosis. Blood was collected from each participant and immediately processed to serum using a standardized procedure; the resulting sera were stored frozen at -80 °C until assessment. There were no differences among the groups with regard to age, body mass index (BMI), or gender (Figure S1a). There was no apparent bias in the AD group with regard to age, BMI, EASI, BSA, total

immunoglobulin (Ig) E levels, or AD duration (Figure S1b-h). None of the patients with AD had received systemic treatment for AD within the preceding month. None of the topical therapies or UV therapy affected serum S1P levels (Figure S2a). Patients with AD showed no significant difference in serum S1P levels when stratified by age or gender (Figure S2b) as shown in healthy individuals.^{S2}

Lipid analyses

Serum lipid analyses for determination of S1P were performed in all participants using liquid chromatography coupled to tandem mass spectrometry. Protein precipitation was performed in 96-well plates from Sarstedt (Nuremberg, Germany; K-series) from 20.0 μ L serum samples by addition of 200 μ L methanol (99.9%, Chromasolv LC-MS grade, Honeywell Riedel-de Haën, Seelze, Germany) supplemented with 10.0 μ L of internal standard mixture (*inter alia* 15.0 pmol C17-Sphingosine-1-Phosphate per injection volume) as an internal reference for normalization and quantification. Following vigorous mixing and precipitation overnight at -80 °C, supernatants were transferred to a fresh plate after centrifugation for 10 min at 2000xg at 4 °C. Following evaporating the solvent, pellets were reconstituted in 200.0 μ L DMSO/MeOH (1:1, v/v) and stored at -80 °C until further analysis. C17-Sphingosine-1-Phosphate was purchased from Avanti Polar Lipids, Alabaster, AL, U.S.A. The HPLC system consisted of an autosampler AS-100 (Biorad, München, Germany), a G1322A degassing unit, a G1212A binary pump (Series 1100), and a L-2300 column oven (Merck Hitachi) with a 60x2 mm MultoHigh 100 RP 18 column with 3 μ m particle size (CS-Chromatographie Service, Langerwehe, Germany), which was maintained at 50 °C. Mobile phase A consisted of 1.0% (v/v) formic acid in ddH₂O, and mobile phase B of 100% methanol. The column was equilibrated in 10% B with a flow rate of 0.5 mL min⁻¹. The mobile phase switched to 100% B after sample injection. The flow rate increased linearly from 0.5 mL min⁻¹ at 5 min to 1.0 mL min⁻¹ at 7 min and remained constant until 10 min. Subsequently, the mobile phase changed to 10% B, and the flow rate decreased linearly from 1.0 mL min⁻¹ at 10 min to 0.5 mL min⁻¹ at 10.5 min and remained constant until the end of the program at 11.3 min. Detection took place between 2 to 10 min. The injection volume per sample was 30.0 μ L, samples were cooled at 4 °C. The HPLC system was coupled to the API 2000 triple-quadrupole mass spectrometer (Sciex, Foster City, CA, USA) equipped either with an ESI source, operating in positive mode under following source parameters: Source temperature 450 °C, curtain gas 40, collision gas “low”, ion spray voltage 5500, ion source gas1 60, ion source gas2 30. Target ions Q1>Q3 were 366.4>250.4 for d17:1 So1P (internal standard) and 380.4>264.4 for d18:1 So1P, respectively. The analytical results were quantified with Analyst 1.6.2 (AB Sciex, Forster City, CA, USA) based on internal standard samples and an external standard curve.

Protein biomarker analyses

Serum levels of thymus and activation-regulated chemokine (TARC) and interleukin (IL) -22 were measured by MSD U-PLEX Multiplex Assays (Meso Scale Diagnostics, LLC, USA) according to the manufacturer's instructions. These biomarkers were measured in all patients with AD.

Statistical analyses

We applied the two-tailed Mann-Whitney U test, Fisher's exact test, Spearman's correlation test, or linear regression test for statistical analyses. Multiple comparisons were corrected by the Bonferroni method (i.e., Figure 1: Significances were calculated with the Bonferroni method (a) or the Mann-Whitney U test (b). r_s and P values in (c,d) were calculated with the Spearman's correlation test. Solid lines represent curves fitted by linear regression; dotted lines represent 95% confidence bands for the best-fit lines (c,d). The black bars in the graphs indicate each median value (e-h). Table 1: Significances were calculated with the Mann-Whitney U test or Fisher's exact test.) P values of $<.05$ were considered significant. All analyses were performed using SPSS Statistics, ver. 25 (IBM, USA), or Prism, ver. 9.0.0 (GraphPad Software, LLC., USA).

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