Original Article

Smith-Magenis Syndrome Patients Often Display Antibody Deficiency but Not Other Immune Pathologies

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What is already known about this topic? Smith-Magenis syndrome (SMS) is a complex neurobehavioral disorder associated with otitis. Most SMS cases result from chromosome 17p11.2 deletions that encompass the intellectual disability gene *retinoic acid-induced 1* and also genes associated with immunodeficiency, autoimmunity, and/or malignancy.

What does this article add to our knowledge? Description of the immunopathologies and laboratory immunological features of a large cohort of 76 patients with SMS reveals a consistent susceptibly to sinopulmonary infections, including pneumonia, but not to autoimmune, allergic, or malignant diseases.

How does this study impact current management guidelines? As with other genetic syndromes associated with antibody deficiency listed in the American Academy of Allergy, Asthma, and Immunology practice parameters for diagnosis and management of primary immunodeficiency, all SMS patients should receive an immunologic evaluation. Infectious prophylaxis should be considered in selected SMS patients.

BACKGROUND: Smith-Magenis syndrome (SMS) is a complex neurobehavioral disorder associated with recurrent otitis. Most SMS cases result from heterozygous interstitial chromosome 17p11.2 deletions that encompass not only the intellectual disability gene *retinoic acid-induced 1* but also other genes associated with immunodeficiency, autoimmunity, and/or malignancy.

OBJECTIVES: The goals of this study were to describe the immunological consequence of 17p11.2 deletions by determining the prevalence of immunological diseases in subjects with SMS and by assessing their immune systems via laboratory methods.

METHODS: We assessed clinical histories of 76 subjects with SMS with heterozygous 17p11.2 deletions and performed in-depth immunological testing on 25 representative cohort members. Laboratory testing included determination of serum antibody concentrations, vaccine titers, and lymphocyte subset frequencies. Detailed reactivity profiles of SMS serum

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This work was supported by grant number K23AI115001 from National Institutes of Health-National Institute of Allergy and Infectious Diseases (NIH-NIAID), K12HD0141401-10 from UL1, TR000142 from NIH-National Institutes of Health-National Center for Advancing Translational Sciences, and the Jeffrey Modell Foundation (to N.R.); the Donald E. and Delia B. Baxter Foundation Career Development Award, a gift from The Floren Family Trust, U19-AI1110491, R01 AI125197 both from the NIH-NIAID, and Alliance for Lupus Research Grant No. 296550 (to P.J.U.); and AI061093 from NIH-NIAID (to E.M.).

Conflicts of interest: R. J. Antaya has received consultancy fees from Pierre Fabre and Anacor Pharma; has provided expert testimony for Hoffmann LaRoche; has received research support from Leo Pharma/Astellas Pharma; and received a gift to study from Fermdale Labs. S. H. Elsea has received travel support from Patients and the professional advisory board of Researchers Interested in Smith-Magenis Syndrome (PRISMS); is employed by Baylor College of Medicine; and has received research support from Smith-Magenis Syndrome Research Foundation. P. J. Utz has received research support from the National Institutes of Health (NIH). E. Meffre has received research support from the NIH (7 P01 AI061093-07), Yale AbbVie-003, and Biogen Idec, Inc. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication November 4, 2016; revised January 3, 2017; accepted for publication January 21, 2017.

Available online March 9, 2017.

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2213-2198

http://dx.doi.org/10.1016/j.jaip.2017.01.028

Abbreviations used
BHDS- Birt-Hogg-Dubé syndrome
CVID- Common variable immune deficiency
EMR- Electronic medical records
FLCN- Folliculin
HiB- Haemophilus influenza *type B*
MFI- Mean fluorescence intensity
PRISMS- Patients and the professional advisory board of Researchers Interested in Smith-Magenis Syndrome
RAI1- Retinoic acid-induced 1
SAM- Significance analysis of microarrays
SMS- Smith-Magenis syndrome
TNFRSF13B- Tumor necrosis factor receptor superfamily member 13b
TOM1L2- Target of myb1 like 2 membrane trafficking protein

antibodies were performed using custom-made antigen microarrays.
RESULTS: Of 76 subjects with SMS, 74 reported recurrent infections including otitis (88%), pneumonia (47%), sinusitis (42%), and gastroenteritis (34%). Infections were associated with worsening SMS-related neurobehavioral symptoms. The prevalence of autoimmune and atopic diseases was not increased. Malignancy was not reported. Laboratory evaluation revealed most subjects with SMS to be deficient of isotype-switched memory B cells and many to lack protective antipneumococcal antibodies. SMS antibodies were not more reactive than control antibodies to self-antigens.
CONCLUSIONS: Patients with SMS with heterozygous 17p.11.2 deletions display an increased susceptibility to sinopulmonary infections, but not to autoimmune, allergic, or malignant diseases. SMS sera display an antibody reactivity profile favoring neither recognition of pathogen-associated antigens nor self-antigens. Prophylactic strategies to prevent infections may also provide neurobehavioral benefits to selected patients with SMS. **(J Allergy Clin Immunol Pract 2017;5:1344-50)**

Key words: *Smith-Magenis syndrome; Chromosome 17p11.2 deletion; Immune deficiency; Autoantibody;* TNFRSF13B*;* FLCN*;* TOM1L2*; B-cell tolerance*

Smith-Magenis syndrome (SMS; Online Mendelian Inheritance in Man [OMIM] #182290; *607642) is a complex genetic disorder, estimated prevalence 1:15,000-25,000. SMS is characterized by intellectual disability, sleep disturbances, self-injurious behaviors, and skeletal abnormalities.[1-3] Although ear infections are commonly described in patients with SMS,[4] it is unclear if the predisposition is due to an anatomic or immunologic abnormality. Diminished antipneumococcal antibodies have been described in SMS sera,[5] but neither a comprehensive clinical evaluation of the SMS immune system nor a detailed account of the full spectrum of infections experienced by patients with SMS has been reported. Similarly, it is unknown if the SMS immune system is prone to the development of autoimmune, malignant, and/or atopic diseases as is the case in many primary immunodeficiencies.[6-9]

Approximately 90% of SMS cases are caused by the heterozygous 3.7 Mb interstitial deletion of 17p11.2, a region encompassing the retinoic acid-induced 1 (*RAI1*) gene locus.[3] In rare cases, SMS may be caused by deleterious *RAI1* point mutations, without deletion of 17p11.2, suggesting that *RAI1* is the gene primarily responsible for the neurodevelopmental features of SMS.[10] *RAI1* serves no known immunologic function,[11] but proximate genes also lost to 17p11.2 deletion, including tumor necrosis factor receptor superfamily member 13b (*TNFRSF13B*), folliculin (*FLCN*), and target of myb1 like 2 membrane trafficking protein (*TOM1L2*), do. *TNFRSF13B* encodes transmembrane activator and CAML interactor (TACI), which controls T-independent humoral responses and B-cell tolerance.[12-15] Heterozygous missense *TNFRSF13B* mutations are associated with common variable immune deficiency (CVID),[16,17] an antibody deficiency disorder often complicated by autoantibody production and hematologic malignancy.[18] Autoimmune disease occurs in 41% of patients with CVID with heterozygous *TNFRSF13B* missense mutations.[19] *FLCN* is a tumor suppressor gene mutated in Birt-Hogg-Dubé syndrome (BHDS).[20] Patients with BHDS accumulate both benign and malignant tumors.[20] *TOM1L2* is not implicated in a human disease, but *Tom1l2*-deficient mice are susceptible to infections and tumors.[21]

Because many patients with SMS are hemizygous for multiple genes associated with immunodeficiency, autoimmunity, and/or malignancy, we hypothesized that they may also be susceptible to these diseases. To test this hypothesis, we surveyed medical histories, spanning 970 person-years, from a large cohort of 76 subjects with SMS aged 6 months to 37 years (mean, 13.8 years) with 17p11.2 deletions. We obtained peripheral blood samples on 25 representative subjects from our cohort, all with deletions encompassing *RAI1, TNFRSF13B, FLCN*, and *TOM1L2*, to create in-depth immunologic profiles via laboratory assessments that included serum immunoglobulin quantification, vaccine titers, lymphocyte flow-cytometry, and custom-made antigen microarrays. Our results indicate that patients with SMS are antibody deficient and frequently experience sinopulmonary infections, including severe bacterial illnesses like pneumonia. Unlike many patients with primary immunodeficiency, subjects with SMS were not more susceptible to autoimmune, allergic, or malignant diseases, nor did they frequently display increased serum autoantibodies or elevated IgE.

METHODS

Study subjects and clinical history evaluations

We enrolled 76 subjects with SMS with heterozygous chromosome 17p11.2 deletions. Subjects ranged from 6 months to 37 years in age (mean, 13.8 years); 52% were female (Table I). Informed consent was obtained for all individuals before study enrollment. The study protocol was approved by the Human Subjects Committee of Yale University, the Institutional Review Board of the Children's Hospital of Philadelphia, and the professional advisory board of Parents and Researchers Interested in Smith-Magenis Syndrome (PRISMS). An immunological diseases questionnaire was distributed to families of subjects with SMS at the international PRISMS family meeting, on the PRISMS website, or in the course of the authors' clinical practice. When subjects were identifiable ($n = 12$), survey responses were secondarily confirmed for accuracy using electronic medical records (EMR). Overall good concordance between survey and EMR data was observed. For survey responses, a recurrent infection was defined as at least 4 infections per year. Peripheral blood samples, paired to survey data, were obtained either at the 2014 International PRISMS Conference and family meeting

TABLE I. Clinical serum antibody testing and infectious histories of 25 SMS subjects not receiving antibody replacement therapy

	Age*	Sex	IgM, mg/dL	IgA, mg/dL	IgG, mg/dL	IgG2, mg/dL	Tetanus Ab, IU/mL	Hib Ab, μg/mL	Pneumococcal Ab, μg/mL 14 serotypes	IgE, ng/mL	Infections
Reference			−†	−†	−†	−†	≥0.15	≥0.15	≥0.35	−†	
SMS1	4	M	41 (L)	76	863	114	>7	0.4	6/14 (L)	158 (H)	O, P, S, G, U
SMS2	6	M	82	106	1040	122	>7	1.5	11/14	<4	P, U
SMSY2	6	F	113	124	891	117	<0.1 (L)	6.25	11/14	<2	O
SMS4‡	7	M	44 (L)	38 (L)	508 (L)	95	0.7	0.8	3/14§ (L)	3	O
SMS5	7	F	51	12 (L)	650	42 (L)	3.2	1.1	3/14§ (L)	<2	W, O
SMSY6	9	M	134	303	490 (L)	194	0.37	0.5	9/14	43	O
SMS7	10	M	113	91	928	93	0.23	0.6	7/14	3	O, S, P, G, U
SMS8	10	F	83	67 (L)	675	119	0.26	0.4	11/14	<2	P, U
SMS9	11	M	72	73	879	190	0.75	0.5	11/14	14	O
SMSY3	12	F	51	12 (L)	680	121	>7	1.1	6/14 (L)	ND	O, W, U
SMS10	13	F	118	54 (L)	642 (L)	114	1.76	1.4	12/14	32	O, S, G, W,
SMS11‡	15	F	53 (L)	97	585 (L)	176	2.02	0.7	4/14§ (L)	38	O, S, P
SMS12	16	F	99	32 (L)	718	112	1.06	4.3	6/14 (L)	5	O, S, P, G
SMS13	16	M	77	126	1470	304	1	3.6	14/14	15	O
SMS14	20	F	62	217	1210	404	0.1 (L)	0.7	8/14	5	C, O, S, G, W
SMSY5	20	F	72	114	1400	377	0.53	0.9	14/14	<2	O, G
SMSY7	20	M	18 (L)	<7 (L)	1050	ND	0.23	19.5	13/14	45	O, S, P, B
SMS17	20	F	59 (L)	83	720	257	0.4	1.1	13/14	5	O, S, P
SMS18	21	F	26 (L)	195	925	178	2.55	1.4	11/14	5	O, S, P
SMS19	22	M	48	181	1150	214	3.7	1.5	5/14 (L)	7	O, OE
SMS20	22	F	71	90	1280	351	2.05	4.1	11/14	42	O, U, G
SMS21	23	M	119	71	1250	148	1.64	4.4	14/14	22	O, P, O, U
SMS22	23	F	174	140	1500	190	1.24	2.1	11/14	5	O, S
SMSY4	26	F	83	278	1360	67 (L)	5.36	4.3	4/14§ (L)	5	O, C, W
SMS23	27	M	68	82	904	170	1	4.8	13/14	8	O, U

Ab, Antibody; *B*, bacteremia; *C*, cellulitis; *F*, female; *G*, gastroenteritis; *H*, higher than normal range; *Hib*, *Haemophilus influenza* type B; *L*, below normal/protective range; *M*, male; *ND*, not determined; *O*, otitis; *OE*, osteomyelitis; *P*, pneumonia; *S*, sinusitis; *SMS*, Smith-Magenis syndrome; *U*, upper respiratory tract infection; *W*, warts.
*Age (y) at the time of testing.
†Reference range varies with age.[20]
‡Patient meets diagnostic criteria for common variable immune deficiency including vaccine challenge failure.
§Patient failed a challenge with the 23-valent pneumococcal vaccine. The number of protective serotypes displayed reflect prechallenge values.

(n = 18), at Yale New Haven Hospital (n = 5), or the Children's Hospital of Philadelphia (n = 2). Blood-based screening evaluations were performed on 25 subjects with SMS; all possessed a chromosome 17p11.2 deletion spanning *TNFRSF13B, RAI1, FLCN*, and *TOM2L1* as determined by florescence *in situ* hybridization or chromosomal microarray (see Table E1 in this article's Online Repository at www.jaci-inpractice.org); all had completed a primary vaccination series; none were receiving antibody replacement or immunosuppressive therapies. Healthy control adult serum samples were obtained from 3 first-degree relatives of subjects with SMS and 6 unrelated adult donors after obtaining informed consent. Serum samples from 8 healthy unrelated children were purchased as comparators (Biodesign International Inc., Saco, Maine).

Quantitative and qualitative antibody testing

Measurement of IgM, IgA, IgE, IgG, IgG subclass 1-4 concentrations and antibody responses to tetanus toxoid, *Haemophilus influenza* type B (HiB), and 14 serotypes of *Streptococcus pneumonia* (1, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 12F, 14, 18C, 19F, 23F) were performed on 20 serum samples by the Yale New Haven Hospital clinical laboratory. Results from 5 additional patients with SMS, performed by other clinical reference laboratories, were also included. Age-specific normal value ranges were used to assess if a subject's laboratory assessments were abnormal.[22] Anti-HiB and antitetanus toxoid antibody concentrations were considered protective at concentrations of ≥0.15 μg/mL and ≥0.15 IU/mL, respecitively.[23] Antipneumococcal antibodies were considered protective at concentrations >0.35 μg/mL.[24] For the subset of 6 patients challenged with the 23-valent pneumococcal vaccine, an adequate vaccine response was defined as antipneumococcal antibody concentrations of 1.3 μg/mL to >50% serotypes assessed.[25]

Flow cytometry

Flow cytometry sample acquisition was performed on an LSRFortessa (BD Biosciences, Mountain View, Calif). The following antibodies were used for flow cytometric stainings: anti-TACI PE (clone 1A1), anti-CD19 APC-Cy7, anti-CD27 AF700, anti-CD4 APC-Cy7, anti-CD8 BV711, anti-CD25 Pe/Dazzle 594, anti-CD127 PerCPCy5.5 (all from BioLegend, San Diego, Calif), anti-IgM PerCPCy5.5 and anti-CD3 eFluor 605NC (BD Biosciences). Intracellular staining for FOXP3 Alexa Fluor 488 (clone 150D; Biolegend) was performed using the FOXP3/Transcription Factor Staining Buffer Set (eBioscience, San Diego, Calif) in accordance with the manufacturer's instructions. Subset analysis was performed with FlowJo software (Tree Star, Ashland, Ore).

TABLE II. Infections reported in 76 subjects with SMS (aged 6 mo to 37 y)

	Percentage (n)
Recurrent ear infections	88.2 (67)
Recurrent viral respiratory	60.5 (46)
Pneumonia	47.4 (36)
Recurrent sinus infections	42.1 (32)
Recurrent gastroenteritis	34.2 (26)
Bacterial cellulitis	17.1 (13)
Warts	15.8 (12)
Bacteremia	2.6 (2)
Osteomyelitis	1.3 (1)

SMS, Smith-Magenis syndrome.

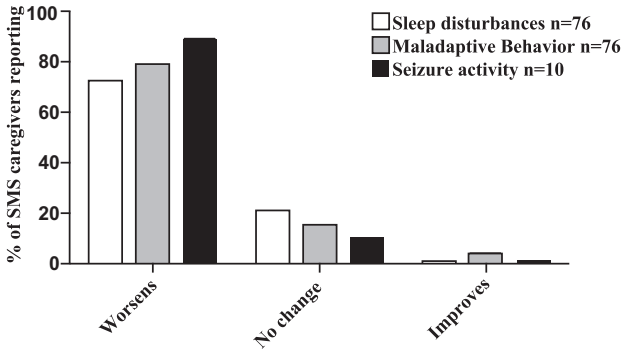

FIGURE 1. Most SMS caregivers perceive acute infections to worsen SMS-associated sleep disturbances, maladaptive behaviors, and seizures. *SMS*, Smith-Magenis syndrome.

Antigen microarrays

Antigen microarrays were generated using a VersArray ChipWriter Pro microarrayer (Bio-Rad, Hercules, Calif) and using customized printheads from Silicon Microarray Spotting Pins (Parallel Synthesis Technologies, Santa Clara, Calif) as previously described.[26] Briefly, 337 purified biomolecules including autoantigens, cytokines, and chemokines were purchased from multiple vendors and printed in triplicate at dilutions of 200 μg/mL onto Nexterion E epoxysilane-coated glass slides (Schott, Duryea, Pa). A complete list of molecules printed can be found in Figure E1 (available in this article's Online Repository at www.jaci-inpractice.org). Arrays were blocked, and then washed in 7% fetal bovine serum in PBS plus 0.1% Tween (PBST). Arrays were probed for 1 hour with sera, diluted 1:150 in 30% FBS 1% PBS, from 18 subjects with SMS with heterozygous 17p11.2 deletions, 3 of their healthy first-degree relatives, and 14 healthy unrelated pediatric controls. After washing in PBST, serum reactivity was detected using an Alexa-fluor 647-conjugated goat antihuman IgG (Fc-specific) secondary antibody (Jackson, West Grove, Pa). After washing, arrays were dried under negative pressure and scanned using an Agilent microarray scanner. Data were bioinformatically processed using GenePix 6 software. Mean fluorescence intensity (MFI) values for each antigen were calculated by taking the mean of median fluorescence intensity for each feature. From this value was subtracted the value of MFI reactivity by probing with the secondary antibody alone.

Statistical methods

Linear regression modeling was conducted using PRISM software (GraphPad, San Diego, Calif). Significance analysis of microarrays (SAM), a permutation-based algorithm for determining statistically significant differences in large datasets, was used to determine differences in antibody reactivities between SMS and control serum samples.[27] For SAM analyses, a false discovery rate of <0.001 was accepted and an adjusted P value of $<.05$ was considered statistically significant. Antigen microarray analyses were powered (>0.8) to detect at least 1.5-fold reactivity differences between the subject and control sera.

RESULTS

Subjects with SMS are susceptible to sinopulmonary infections

A history of recurrent and/or severe infections was reported in 72 of 76 (95%) subjects with SMS (Table II). Sinopulmonary infections were most commonly described and included recurrent otitis media (88% of subjects), recurrent upper respiratory tract infections (61%), pneumonia (47%), and recurrent sinusitis (42%). Recurrent gastroenteritis was described by 34% of respondents. Skin infections were also reported, including bacterial cellulitis (17%) and warts (16%) primarily affecting the hands and feet (Figure E2, available in this article's Online Repository at www.jaci-inpractice.org). A history of bacteremia was reported in 2 subjects; hematogenously seeded osteomyelitis in another. No cases of abscesses, deep tissue infections, or joint infections were identified. Excluding warts, other opportunistic infections such as mucocutaneous *Candidiasis*, *Pneumocystis* pneumonia, *Cryptococcosis*, *Cryptosporidiosis*, molluscum, and cytomegalovirus infections were not identified.

A total of 25 (33%) subjects with SMS surveyed had previously received an immunological evaluation. Of those evaluations, 68% were performed by allergists/clinical immunologists, 21% by clinical geneticists, and 8% by infectious disease physicians. A total of 67 (87%) subjects with SMS had received a complete primary vaccine series, and 6 (9%) subjects with SMS were currently receiving, or had at one time received, antibody replacement therapy. Altogether, these results show that patients with SMS display an increased susceptibility to sinopulmonary infections.

Infections negatively impact SMS-associated sleep disturbances, maladaptive behaviors, and seizures

SMS-associated sleep disturbances and maladaptive behaviors, which included self-injury by onychotillomania or polyembolokoilamania, temper tantrums, and attention deficit/hyperactivity, were described by all respondents. During acute infections, 72% of SMS caregivers perceived a worsening of sleep disturbances and 79% perceived a worsening of behavioral issues (Figure 1). Of those reporting a negative impact, the majority described the effect of infections to be "significant." Seizures were reported in 10 subjects with SMS; infections increased seizure frequency and severity in 8 of these (Figure 1).

Autoimmune, atopic, and malignant diseases do not occur frequently in subjects with SMS

Autoimmune diseases, which included autoimmune thyroiditis (n = 2), autoimmune neutropenia (n = 1), and pernicious anemia (n = 1), were reported in 5% of subjects with SMS

(Table E2, available in this article's Online Repository at www.jaci-inpractice.org). This frequency was identical to that reported in subjects' siblings suggesting that autoimmunity was not increased in our young SMS cohort. Atopy was reported in 27% of subjects with a prevalence and variety (Table E3, available in this article's Online Repository at www.jaci-inpractice.org) similar to large national health surveys.[28,29] A modestly elevated serum IgE concentration was identified in 1 subject (Table I). Malignant diseases were not reported in our cohort.

Antibody responses are impaired in SMS

We assessed SMS serum for immunoglobulin isotypes and IgG subclass concentrations and identified at least 1 abnormal result in the majority of subjects (60%) (Table I). IgM, IgA, and IgG concentrations were beneath age-adjusted institutional normal ranges in 22%, 16%, and 28% of serum samples, respectively. Two subjects were selectively IgG2 deficient. All subjects possessed protective anti-HiB antibody concentrations and most possessed protective concentrations to tetanus toxoid (92%). In contrast, 32% lacked protective antibody concentrations (>0.35 μg/mL) to the majority of the 14 *Streptococcus pneumonia* serotypes tested. Vaccine challenges with the 23-valent pneumococcal vaccine were performed on 6 subjects with SMS; 4 of these were unable to generate appropriate anti-pneumococcal antibody responses. Two vaccine nonresponders met CVID diagnostic criteria (Table I). Although there was a trend of improving pneumococcal vaccine titers with advancing age, we conclude that many SMS subjects not on antibody replacement therapy suffer from decreased antibody production and impaired pneumococcal responses.

Many primary antibody deficiency diseases are associated with diminished class-switched memory B cells.[30,31] In our SMS cohort, total B-cell and total memory B-cell frequencies were not diminished compared with age-matched institutional normal ranges, whereas isotype-switched memory B cells were diminished in 17 of 19 subjects with SMS. This is consistent with our previous study analyzing fewer subjects.[32] Enumeration of T-cell subsets including CD4 T cells, CD8 T cells, and T regulatory cells in our cohort revealed no consistent abnormal trends (Table E4, available in this article's Online Repository at www.jaci-inpractice.org). Significant T-cell lymphopenia was identified in only 1 subject with SMS (SMS2), a boy with a history of partial thymectomy secondary to surgical correction of a congenital heart defect. He did not experience opportunistic infections. Natural killer cell deficiency was not identified in our SMS cohort.

SMS antibody reactivities to pathogens and to self-antigens are limited

To create an unbiased and in-depth reactivity profile of SMS protective IgG antibodies and autoantibodies, we designed and fabricated antigen microarrays and probed them with sera from 18 subjects with SMS and 17 healthy controls. Antigen microarrays contained a total of 337 antigens including 19 pathogen-specific antigens and autoantigens, including cytokines, chemokines, and growth factors.[33] Serum reactivities measured by microarray and by conventional laboratory testing linearly correlated ($R^2 = 0.54$, $P < .0005$) and were generally concordant (Figure E3, available in this article's Online Repository at www.jaci-inpractice.org). For instance, 10 of 10 of the most reactive SMS serum samples to veterinary tetanus vaccine also demonstrated protective tetanus-specific IgG levels (≥0.15μg/mL) by conventional clinical laboratory testing.

To measure differences in the levels of antibodies between patients with SMS and age-matched, related, and unrelated controls, we performed SAM, a permutation-based algorithm for determining statistically significant differences in large datasets.[27] SAM analysis of the 19 pathogen-associated antigens on the array demonstrated that antibodies against 4 pathogens were decreased in sera from patients with SMS compared with healthy controls. These antigens were the HiB-conjugate vaccine, hepatitis B surface antigen, bacterial flagellin, and horse tetanus vaccine (Figure 2). No antipathogen antibodies tested were significantly elevated in SMS sera compared with controls.

Antigen microarrays have previously identified diverse and numerous autoantibodies in the sera from patients with autoimmune diseases including systemic lupus erythematosus, juvenile dermatomyositis, and recombination-activating gene enzyme (RAG) deficiency compared with healthy controls.[34-36] We therefore assessed autoantibody profiles between SMS and healthy control sera by performing SAM a second time using array values for autoantibody reactivity against classical self-antigens. Of the 94 self-antigens tested, no autoantibody was significantly increased in the SMS population. There were 3 autoantibodies found at lower levels in SMS sera than healthy sera; among these was antithyroid peroxidase (Figure 2). Given the newly recognized importance of anticytokine autoantibodies in primary immunodeficiencies,[33,36,37] we also performed SAM for sera reactivities to cytokines, chemokines, and growth factors. No statistically significant differences were identified. Taken together, these data demonstrate a deficiency of pathogen protective antibodies in subjects with SMS without an increased presence of autoantibodies.

DISCUSSION

Herein, we report that patients with SMS display an increased susceptibility to sinopulmonary infections including otitis but also invasive bacterial infections. During our study, a 35-year-old female subject succumbed to pneumonia, an infection affecting nearly half our cohort, underscoring the potential infectious acuity of SMS. Our results are consistent with the limited number of previously published reports on the immunologic phenotype of patients with SMS that were either more limited in scope[38] or were mechanistic and not clinical.[5,32] Here, we provide a unique and detailed analysis of SMS antibody reactivities to pathogen-associated antigens and self-antigens by traditional clinical laboratory testing and by an in-depth antigen microarray. We have demonstrated decreased pathogen-specific antibodies, impaired pneumococcal responses, and fewer isotype-switched memory B cells to be general features of SMS. Such immune defects mirror those of patients with CVID and reinforce the essential role TACI in mediating T-independent humoral responses and regulating late-stage B-cell differentiation.[15,32] Yet, despite such dysfunction, SMS B cells do not preferentially produce serum autoantibodies as CVID B cells do.[14] Why are patients with SMS and *TNFRSF13B*-mutated patients with CVID similarly susceptible to infections but not to autoimmune diseases? One possible explanation is that the single unmutated *TNFRSF13B* allele possessed by most patients with SMS is sufficient to establish B-cell tolerance, whereas a CVID-associated *TNFRSF13B* mutant allele, likely encoding a

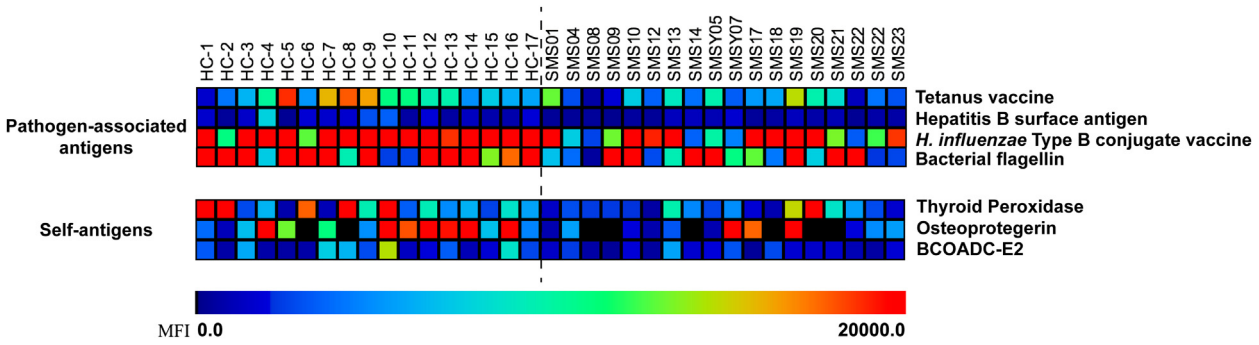

FIGURE 2. SMS sera are less reactive than control sera to pathogen-associated and self-antigens. The heat map displays sera reactivities to 4 pathogen-associated antigens (upper panel) and 3 self-antigens (lower panel) lower in 18 SMS serum samples than 17 healthy related and unrelated control samples. Colorimetric differences corresponding to array MFIs are indicated. *SMS*, Smith-Magenis syndrome; *MFI*, mean fluorescence intensity.

dominant-negative product, interferes with it.[14,32] Unlike tolerance formation, optimal antibody production requires B cells with 2 functional *TNFRSF13B* alleles. Hence, patients with SMS and CVID with *TNFRSF13B* mutations are both susceptible to antibody deficiency-associated sinopulmonary infections.

Several subjects with SMS reported warts, an uncharacteristic finding for pure antibody deficiency diseases, but one that may be explained by "skin picking," a compulsive SMS behavior. Warts may also be related to fibrofolliculomas, benign hair follicle tumors pathognomonic for BHDS. However, because 30% of patients with BHDS also develop renal cancer, and cancer was not reported in our cohort, it appears that heterozygous BHDS-associated *FLCN* mutations and SMS-associated *FLCN* hemizygosity are not equivalent.[20] A longitudinal study of our relatively young cohort may provide further confirmation of this hopeful finding.

Despite a significant infectious burden, family members of subjects with SMS consistently rate behavioral issues and sleep disturbances to be the most challenging aspects of the disease.[39] Such prioritization is understandable and may partially explain why only 35% of our cohort received a prior immunological evaluation. Yet, we report here that SMS caregivers also perceive infections to significantly aggravate SMS-associated neurobehavioral problems. As many laboratory abnormalities we identified in subjects with SMS, including hypogammaglobulinemia, IgG subclass deficiency, and specific antibody deficiency, are indications for prophylactic antibody replacement, a trial of this therapy in selected patients with SMS may improve both infectious and noninfectious outcomes.

Acknowledgments

We thank the PRISMS (Parents and Researchers Interested in Smith-Magenis Syndrome) organization for assistance recruiting subjects and obtaining blood samples. We also acknowledge the Yale New Haven Hospital Department of Laboratory Medicine and the Children's Hospital of Philadelphia's Center for Applied Genomics for assistance with clinical and genetic testing.

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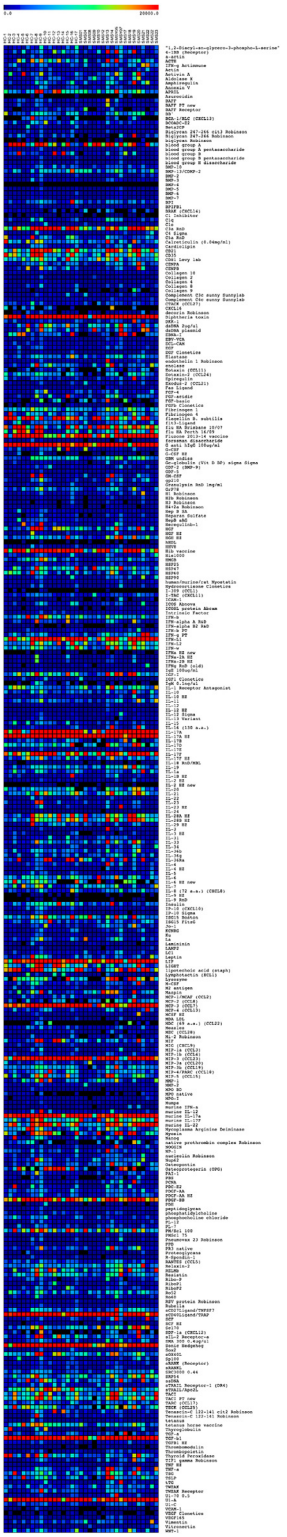

FIGURE E1. A detailed account of the reactivities of SMS sera vs control sera to 337 antigens. A heat map displays reactivities of sera from 18 subjects with SMS, 14 healthy unrelated controls, and 3 related controls to the 337 listed antigens. Colorimetric differences on the heat map correspond. *SMS*, Smith-Magenis syndrome.

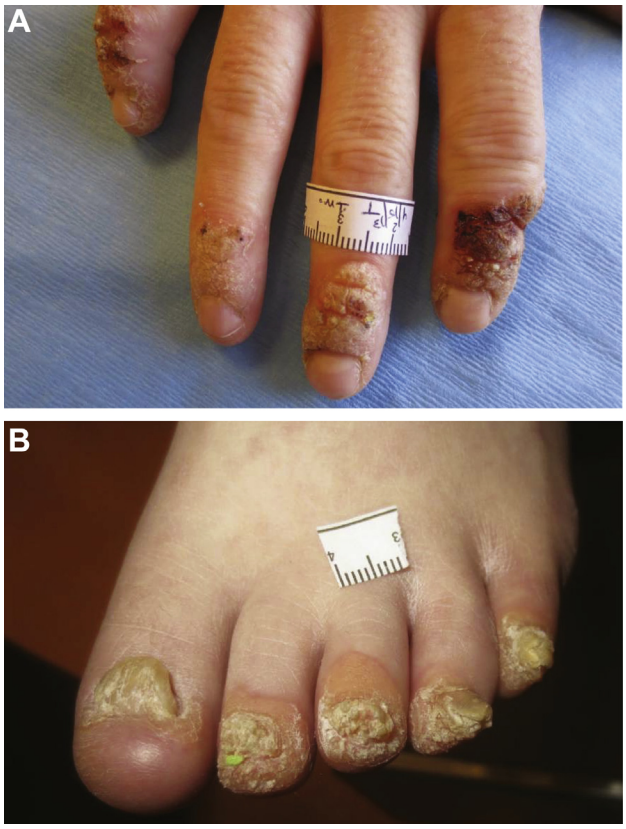

FIGURE E2. Persistent verrucous disease of the (**A**) hand and (**B**) foot of SMSY4. *SMS*, Smith-Magenis syndrome.

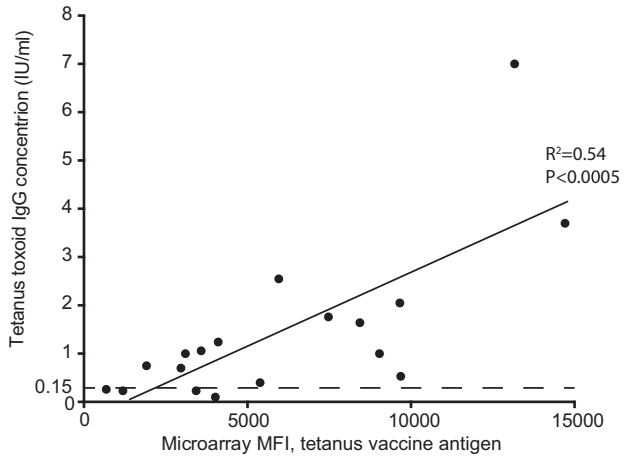

FIGURE E3. Bivariate analysis of serum reactivity to tetanus toxoid by conventional laboratory testing vs antigen microarray reveals fair concordance. Linearity (R^2) and statistical significance (P) were calculated via linear regression analysis. *MFI*, Mean florescence intensity.

TABLE E1. Location, size, and relevant genes contained within the deleted regions of 25 blood donors with SMS

	Deleted element		Deleted element encompasses			
	Location	Size	*RAI11*	*TOM1L2*	*FLCN*	*TNFRSF13B*
SMS1	17p11.2*	1.5 Mb	Y	Y	Y	Y
SMS2	17p11.2†	3.6 Mb	Y	Y	Y	Y
SMSY2	17p11.2†	3.7 Mb	Y	Y	Y	Y
SMS4	17p11.2*	3.4 Mb	Y	Y	Y	Y
SMS5	17p11.2†	6.3 Mb	Y	Y	Y	Y
SMSY6	17p11.2*	3.4 Mb	Y	Y	Y	Y
SMS7	17p11.2*	1.5Mb	Y	Y	Y	Y
SMS8	17p11.2*	3.1 Mb	Y	Y	Y	Y
SMS9	17p11.2†	1.2 Mb	Y	Y	Y	Y
SMSY3	17p11.2†	3.7 Mb	Y	Y	Y	Y
SMS10	17p11.2†	3.4 Mb	Y	Y	Y	Y
SMS11	17p11.2*	6.4 Mb	Y	Y	Y	Y
SMS12	17p11.2*	3.7 Mb	Y	Y	Y	Y
SMS13	17p11.2†	3.6 Mb	Y	Y	Y	Y
SMS14	17p11.2*	3.6 Mb	Y	Y	Y	Y
SMSY5	17p11.2*	6.3 Mb	Y	Y	Y	Y
SMSY7	17p11.2*	3.3 Mb	Y	Y	Y	Y
SMS17	17p11.2*	3.6 Mb	Y	Y	Y	Y
SMS18	17p11.2*	3.2 Mb	Y	Y	Y	Y
SMS19	17p11.2*	3.4 Mb	Y	Y	Y	Y
SMS20	17p11.2*	3.7 Mb	Y	Y	Y	Y
SMS21	17p11.2*	3.4 Mb	Y	Y	Y	Y
SMS22	17p11.2*	2.1 Mb	Y	Y	Y	Y
SMSY4	17p11.2*	3.5 Mb	Y	Y	Y	Y
SMS23	17p11.2*	3.5 Mb	Y	Y	Y	Y

FLCN, Folliculin; *RAI1*, retinoic acid-induced 1; *SMS*, Smith-Magenis syndrome; *TNFRSF13B*, tumor necrosis factor receptor superfamily member 13b; *TOM1L2*, target of myb1 like 2 membrane trafficking protein; *Y*, yes; the indicated gene is deleted.
*Determined by fluorescence *in situ* hybridization.
†Determined by microarray.

TABLE E2. History of autoimmune diseases in 76 subjects with SMS (aged 6 mo to 37 y)

	Percentage (n)
Autoimmune thyroiditis	2.6 (2)
Autoimmune neutropenia	1.3 (1)
Pernicious anemia	1.3 (1)

SMS, Smith-Magenis syndrome.

TABLE E3. Atopic history in 76 subjects with SMS (aged 6 mo to 37 y)

	Percentage (n)
Allergic rhinitis	15.8 (12)
Atopic dermatitis	9.2 (7)
Food allergy	6.6 (5)
Asthma	7.9 (6)
Drug allergy	2.6 (2)

SMS, Smith-Magenis syndrome.

TABLE E4. Relative frequencies of lymphocyte subsets in the peripheral blood of 19 subjects with SMS

	Age*	Sex	$CD3^+$ (% PBLs)	$CD3^+CD4^+$ (% PBLs)	$CD3^+CD8^+$ (% PBLs)	$CD3^+CD4^+CD25hi$ $FOXP3^+CD127^-$ (% $CD4^+$ T cells)	$CD19^+$ (% PBLs)	$CD19^+CD27^+$ (% B cells)	$CD19^+CD27^+$ IgM^- (% B cells)	$CD3^-CD16^+CD56^+$ (% PBLs)
Reference			–†	–†	–†	–†	–†	–†	–†	–†
SMS2	6	M	33.5 (L)	14.4 (L)	18.9	4.6	41.8 (H)	13.6	1.2 (L)	3.4 (L)
SMS4	7	M	56.7	38.3	15.6	2.7 (L)	15.5	23.9	2.5 (L)	19.1
SMS7	10	M	68.4	28.7	18.0	5.5	19.5	11.8 (L)	1.7 (L)	4.7
SMS8	10	F	48.0	29.4	17.4	5.4	36.1 (H)	41.2 (H)	12.0	11.1
SMS9	11	M	63.4	31.1	25.4	5.9	18.6	16.3	3.9 (L)	8.1
SMS10	13	F	68.6	28.6	28.0	ND	30.5 (H)	9.5 (L)	2.8 (L)	6.2
SMS11	15	F	54.1	35.9	14.7	4.1	48.0 (H)	22.8	4.9 (L)	3.6
SMS12	16	F	61.0	37.5	19.1	4.9	26.7	20.1	4.1 (L)	4.7
SMS13	16	M	58.9	34.2	21.9	4.5	26.2	29.3	3.5 (L)	8.2
SMS14	20	F	63.4	38.8	23.0	5.7	25.3	27.6	7.6 (L)	5.2
SMSY5	20	F	56.8	22.2	32.1	6.5‡	20.1	11.8 (L)	1.7 (L)‡	15.7
SMSY7	20	F	52.2	36.5	14.9	4.0‡	31.6 (H)	20.6	3.5 (L)‡	9.4
SMS17	20	F	57.1	33.8	16.8	4.5	33.0 (H)	21.6	3.3 (L)	5.3
SMS18	21	F	67.9	45.1	19.1	5.4	7.8	5.1 (L)	2.6 (L)	9.7
SMS19	22	M	69.9	50.3	18.7	4	9.2	20.2	7.0 (L)	3.7
SMS20	22	F	65.9	37.4	25.9	4.1	21.5	22.6	4.9 (L)	8.6
SMS21	23	M	48.2	22.8	22.1	8.6	39.7 (H)	18.1	3.8 (L)	11.6
SMSY4	26	F	58.9	33.4	19.6	5.4‡	20.6	22.5	12.1‡	5.7
SMS23	27	M	51.1	30.1	19.4	4.8	32.7 (H)	24.3	6.5 (L)	12.8

F, Female; *H*, higher than normal range; *L*, below normal range; *M*, male; *ND*, not done; *PBL*, peripheral blood lymphocyte; *SMS*, Smith-Magenis syndrome.
*Age (y) at the time of testing.
†Institutional reference ranges vary by age.
‡Value previously published.[3]