Immunologic Features of Cornelia de Lange Syndrome

WHAT'S KNOWN ON THIS SUBJECT: Cornelia de Lange syndrome (CdLS) is a genetic syndrome with multisystem abnormalities. Infections are a significant cause of morbidity and mortality in affected patients and are typically attributed to anatomic abnormalities.

WHAT THIS STUDY ADDS: This study identified a high frequency of antibody immunodeficiency in CdLS subjects, indicating a critical need for screening and management of immunodeficiency in CdLS patients with a history of severe or recurrent infections.

abstract

OBJECTIVES: Cornelia de Lange syndrome (CdLS) is a genetic syndrome with multisystem abnormalities. Infections are a significant cause of morbidity and mortality. The goals of our study were to identify the frequency and types of infections in CdLS and to determine if underlying immunodeficiency contributes to the clinical spectrum of this syndrome.

METHODS: We assessed infectious histories in 45 patients with CdLS and evaluated conventional immunologic screening tests in 27 patients. Among these 27 subjects, additional phenotypic enumeration of T-cell subsets, expression of activation markers in T cells, and production of cytokines in response to T-cell stimulants were studied in 12 CdLS subjects compared with 12 normal case control subjects.

RESULTS: Recurrent infections were reported at high frequency in CdLS patients and included chronic ear infections (53%), chronic viral respiratory infections (46%), pneumonia (42%), sinus infections (33%), oral candidiasis (13%), sepsis (6%), and bacterial skin infections (4%). Full immune evaluation in 27 subjects led to identification of 9 cases of antibody deficiency syndrome in patients with severe forms of CdLS. Subjects with CdLS had decreased percentages of T regulatory cells and T follicular helper cells compared with normal control subjects ($P < .05$).

CONCLUSIONS: This study identified for the first time a high frequency of antibody deficiency in CdLS subjects, indicating a critical need for screening and management of immunodeficiency in CdLS patients with a history of well-documented severe or recurrent infections. Furthermore, our results indicate that impaired T-cell populations may be associated with antibody deficiency in CdLS. *Pediatrics* 2013;132:e484–e489

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KEY WORDS

Cornelia de Lange syndrome, cohesin, antibody, immunodeficiency

ABBREVIATIONS

CdLS—Cornelia de Lange syndrome
CVID—common variable immunodeficiency
Ig—immunoglobulin
ITP—idiopathic thrombocytopenic purpura
PB—peripheral blood
PBMC—peripheral blood mononuclear cell
SAD—specific antibody deficiency
Tfh—T follicular helper cell
Treg—T regulatory cell

Dr Jyonouchi conceptualized and designed the study, collected clinical data on patients, performed quantitative and qualitative studies of T cells in the laboratory, drafted the initial manuscript, and approved the final manuscript as submitted; Dr Orange was instrumental in the original conceptualization and design of the study, reviewed the manuscript, and approved the final manuscript as submitted; Dr Sullivan provided recommendations on study design, provided assistance with quantitative and qualitative studies of T cells in the laboratory, reviewed the manuscript, and approved the final manuscript as submitted; Dr Krantz provided key support for patient recruitment, assisted in clinical and molecular data collection, reviewed the manuscript, and approved the final manuscript as submitted; and Dr Deardorff provided key support for patient recruitment, assisted in clinical and molecular data collection, critically reviewed the manuscript, and approved the final manuscript as submitted.

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Cornelia de Lange syndrome (CdLS) is a genetic disorder affecting multiorgan systems, which is estimated to occur in 1:10 000 individuals.[1] Clinical findings typically include dysmorphic facial features (arched eyebrows, synophrys, long eyelashes, ptosis, long philtrum, thin upper lip, posteriorly rotated ears), growth and mental retardation, upper limb defects (clinodactyly, limb deficiencies), gastrointestinal complications (gastroesophageal reflux, pyloric stenosis, diaphragmatic hernia, malrotation, and volvulus), and heart defects (venticuloseptal and atrial septal defects).[1] In addition, chronic respiratory tract infections including sinusitis and pneumonia have been frequently described in this population. In fact, respiratory failure from infections and sepsis accounted for 13% of deaths in a large cohort study examining the causes of mortality in this syndrome.[2] Frequent infections observed in CdLS patients have classically been attributed to associated anatomic defects (cleft palate, severe gastroesophageal reflux, narrow ear canals). Here we present compelling new evidence to indicate that underlying immune dysfunction may also be critically contributing to recurrent infections in CdLS.

Sixty-five percent of CdLS patients have mutations in the cohesin regulatory protein NIPBL; the 3 core cohesin components SMC1A, SMC3, or RAD21; or the cohesin recycling enzyme histone deacetylase 8 (HDAC8).[3–7] A key function of the cohesin complex is to facilitate proper segregation of chromosomes by maintaining them in proximity from the point of sister chromatid synthesis until they are segregated at mitosis.[8] However, additional functions of cohesin relevant to immune functions have been increasingly described. These include transcriptional regulation, repair of double-strand DNA damage, and facilitation of long-range gene interactions such as those involved in V(D)J recombination, the process required for generation of a diverse repertoire of T and B lymphocytes.[9–11] Additionally, a conserved pattern of altered gene transcription profiles has also been described in CdLS patients, including decreased gene expression of NFATc2, a key transcription factor necessary for antigen-triggered T-cell activation.[12]

Given the role of cohesin protein complex in the immune system, altered expression of immune-related genes from CdLS patients, and the high frequency of infectious complications in CdLS patients, we hypothesized that immunodeficiency associated with CdLS gene mutations could exist as a feature of this syndrome. To test our hypothesis, we surveyed infectious histories and assessed humoral and cell-mediated immune functions in our cohorts of CdLS patients. The results revealed that a spectrum of antibody immunodeficiency (common variable immunodeficiency [CVID] and specific polysaccharide antibody deficiency) can be an important clinical feature in CdLS, resulting in severe recurrent infectious complications. These findings prompted us to investigate enumeration of peripheral blood (PB) T-cell subsets and functional analysis of T cells, given previous analysis indicated altered mRNA expression of T-cell transcription factors known to play a role in T-cell activation and differentiation (T-cell help is known to be critical for effective antibody production by B cells).[12–14] Our results revealed quantitative T-cell abnormalities in CdLS patients, indicating a role for impaired T-cell immunity in the development of antibody deficiency syndrome in CdLS patients.

METHODS

Study Subjects and Clinical History Evaluations

Informed consent was obtained from all individuals before enrollment into a study protocol approved by the internal review board of the Children's Hospital of Philadelphia and the Cornelia de Lange Syndrome Foundation. Infectious histories were collected from 45 CdLS patients by using a written questionnaire. The questionnaire was distributed to patients attending a national family meeting for CdLS and to patients evaluated at the genetics clinic of the Children's Hospital of Philadelphia. All respondents were diagnosed with CdLS clinically by geneticists experienced in the diagnosis (I.K. and M.D.).

Immunologic Screening Studies

Immune screening tests were performed for 21 CdLS patients followed at our institution, and results of immune workup were also available for an additional 6 patients followed at outside medical institutions (median age 10 years, range 1–42). Measurement of serum immunoglobulin (Ig)G, IgM, and IgA levels and antibody levels against protein (tetanus, diphtheria) and polysaccharide (pneumococcal) vaccine antigens were performed. Individuals with initial assessments indicating poor vaccine responses were reimmunized with appropriate vaccines, and antibody levels were reassessed 4 to 6 weeks postvaccination. Complete blood counts and flow cytometric enumeration of major lymphocyte subsets (T, B, and natural killer cells) were available for 24 of 27 patients. The results were compared with published age-specific reference values.[15]

T-cell Enumeration, Measurement of Activation, and Cytokine Production

Whole blood samples were obtained from 12 CdLS patients and 12 normal control subjects after obtaining signed informed consent forms approved by the Children's Hospital of Philadelphia Institutional Review Board. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient sedimentation (Amersham Pharmacia, Uppsala, Sweden). Enumeration of T regulatory

cell (Treg; CD4+CD25+FOXP3+) and peripheral T follicular helper cell (Tfh; CD4+CXCR5+) cells were conducted by flow cytometric analysis. For assessment of T-cell functions, PBMCs were cultured in a complete medium (AIM-V; 10% fetal calf serum) in the presence of anti-CD3/CD28 beads by using instructions for optimal concentrations provided by the manufacturer (Invitrogen, Carlsbad, CA) for 16 hours, followed by assessment of T-cell activation marker expression by staining cells with the antibodies against (CD69, CD38, CD25, CD40L, BD Bioscience, San Jose, CA). As for assessment of intracellular cytokine production, PBMCs were stimulated with anti-CD3/CD28 in the same way in the presence of Brefeldin-A (BD Bioscience), then fixed and permealized with Cytofix/Cytoperm, and stained with antibodies against T-cell cytokines (interleukin-2 and interferon-γ, BD Bioscience). The expression of these markers was assessed by flow cytometry (FACScalibur, Becton Dickinson, San Jose, CA). Data were analyzed by using FlowJo 8.3.3 software (Tree Star, Ashland, OR).

DNA Sequencing for Cohesin Mutations

Sequencing for Cohesin gene mutations was performed at the University of Chicago Genetic Services Laboratories or in a research setting. Testing was performed for cohesin genes for which mutations have been reported to cause CdLS (*NIPBL*, *SMC1A*, *SMC3*, *RAD21*, and *HDAC8*).

Statistical Methods

Statistical analyses were conducted by using PRISM software (GraphPad, San Diego, CA). The Wilcoxon test for matched pairs and the Mann-Whitney test for nonmatched pairs were used to compare the differences between groups. Statistical significance was defined as P values $<.05$.

RESULTS

Patient Infectious Histories

Infectious histories were collected from 45 CdLS patients by using a written questionnaire. Chronic sinopulmonary infections occurred at high frequency among surveyed patients and included recurrent otitis media (53%), recurrent viral respiratory infections (46%), recurrent pneumonia (42%), and recurrent sinus infections (33%). Additional infections included recurrent oral candidiasis (13%), bacterial sepsis (6%), and recurrent bacterial skin infections (4%; Table 1). There was a high frequency of gastroesophageal reflux disease (75%), consistent with previous reports. However, cleft palate was reported in only 11% of patients in this study.

Immunologic Findings

Blood-based immune screening evaluations were performed for 27 patients with CdLS. Of these patients, 6 were referred to an immunology subspecialty clinic and tested specifically due to a history of recurrent infections and the remaining 21 patients were tested as part of a comprehensive evaluation through a genetics clinic. Complete blood counts revealed mild thrombocytopenia (platelets 90 and 138 thousand/mL) in 2/27 patients but white blood cell, hemoglobin, absolute neutrophil counts, and absolute lymphocyte counts were within normal range in all 27 patients.

TABLE 1 Infection History in 45 CdLS Patients (Age Range 1–42 Years)

	Percentage
Ear infections	53[a]
Viral respiratory	46
Pneumonia	42[b]
Sinus infections	33[c]
Oral candidiasis	13
Herpesvirus infections	11
Bacterial sepsis	6
Skin infections	4
Varicella	4
Meningitis	2

[a] Average 4.2/y.
[b] Average 1.82/y.
[c] Average 3.46/y.

Total CD3 T-cell counts were normal in 23 of 23 patients but decreased numbers of CD4+ T, CD56/16+ natural killer, and CD19+ B cells were found in 1 of 23, 1 of 23, and 2 of 23 patients, respectively. Additional abnormalities included a reduced CD4:CD8 T-cell ratio (<2.0) in 7 of 23 patients. Defects in humoral immunity were detected in 9 of 27 patients (Table 2). These included low IgG (5 of 27), low IgM (3 of 27), low tetanus antibody titers (3 of 23), low diphtheria antibody titers (3 of 23), and low pneumococcal antibody titers (8 of 25) compared with age-appropriate controls (vaccine antibody levels were those measured post-immunization). Four patients fulfilled the diagnostic criteria for CVID (low IgG levels and reduced vaccine antibody responses), and 4 patients met the diagnostic criteria for specific antibody deficiency (SAD; normal IgG levels but reduced vaccine antibody responses against pneumococcal vaccines). One patient had markedly low IgG levels, but vaccine responses were not available before starting immunoglobulin replacement therapy. All 9 patients identified to have defects in humoral immunity had recurrent bacterial respiratory tract infections and a severe form of CdLS.

T-cell Activation, Cytokine Production, and Enumeration

T-cell activation markers and enumeration of T-cell subsets crucial for providing help for antibody production by B cells were further studied in selected CdLS patients. There were no statistically significant differences in expression of CD40L, CD25, CD69, and CD38 T-cell activation markers in response to anti-CD3/anti-CD28 stimulation of T cells in CdLS versus control subjects. Intracellular expression of T cell cytokines (interleukin-2 and interferon-γ) in stimulated T cells did not differ between CdLS and control groups. In contrast, enumeration of T-cell subsets revealed reduced percentage of Treg

TABLE 2 Humoral Immunity (CdLS Patients With Antibody Deficiency)

	Age, y[a]	IgG (mg/dL)	IgM (mg/dL)	IgA (mg/dL)	Tetanus Ab (IU/mL)	Diptheria Ab (IU/mL)	Pneumococcal Ab	CdLS Mutation
Reference		—[b]	—[b]	—[b]	>0.60 IU/mL	>0.10 IU/mL	(>1.3 ug/mL)	
Patient 1[c]	3	365 (L)	20 (L)	44	0.3.36	1.49	1/14 (L)	NIPBL 6402–6405del4 exon 37
Patient 6[c]	14	363 (L)	40 (L)	66	>3.0	>0.1	1/4 (L)	n/a
Patient 8[c]	0	30 (L)	6	45	<0.1 (L)	0.01 (L)	0/12 (L)	n/a
Patient 12[c]	6	319 (L)	47.6	55.5	0.15 (L)	0.07 (L)	6/14 (L)	n/a
Patient 18[d]	9	554 (L)	47	132	n/a	n/a	n/a	NIPBL frameshift 6407_6408insA
Patient 9[d]	2	464	42 (L)	100	n/a	n/a	6/14 (L)	NIPBL 1753delA frameshift
Patient 10[d]	16	142	92	313	n/a	n/a	5/14 (L)	n/a
Patient 14[d]	20	1090	116	486	0.51	0.179	5/14 (L)	n/a
Patient 23[d]	42	1144	140	248	6.3	n/a	5/14 (L)	n/a

Ab, antibody; n/a, not applicable.
[a] Age at the time of testing.
[b] Reference ranges vary with age.
[c] Patient diagnosed with CVID.
[d] Patient diagnosed with SAD.

cells in CdLS patients compared with controls (0.43% vs 1.1%, P = .016). Whereas Treg percentages were decreased for both CdLS patients without and with immunodeficiency, CdLS patients with immunodeficiency had a tendency toward lower percentages of Treg cells compared with CdLS patients with no immunodeficiency (0.42% vs 0.6%), although this was not statistically significant. We also observed decreased frequency of Tfh cells in the PB in CdLS patients compared with control subjects (1.87% vs 5.36%, P = .00021). Additionally, CdLS patients with immunodeficiency had a tendency toward lower percentages of Tfh than CdLS patients without immunodeficiency (1.45% vs 2.26%), although this was not statistically significant. Of these patients who had enumeration of Treg and Tfh cell subsets, 6 of 11 and 3 of 7 had a diagnosis of antibody deficiency, respectively.

Mutational Analysis

Of the 27 patients evaluated, 11 were found to have mutations in *NIPBL*, 2 patients were found to have mutations in *HDAC8*, 5 patients had no identified mutations to date, and 9 patients had testing pending at the time of publication.

DISCUSSION

CdLS is a well-known genetic disorder that affects many organ systems and recurrent infections in patients result in significant morbidity. In the largest CdLS cohort study evaluating causes of death (n = 295), 9% of patients had a direct cause of death that included pneumonia or viral respiratory infections, and another 4% died of sepsis.[2] To clarify the frequency of recurrent infection, we surveyed 45 individuals with CdLS and identified a high percentage with recurrent upper or lower respiratory tract infections. The infections most commonly reported include recurrent ear infections, viral respiratory infections, pneumonia, and sinus infections (Table 1).

To evaluate further the possible underlying immunodeficiency in CdLS, screening immune evaluation was sought. We were able to perform screening immune evaluations in 27 of 45 CdLS subjects. This led to identification of 9 CdLS patients with defects in humoral (antibody) immunity. This included 4 patients with CVID, 4 patients with SAD, and 1 patient with hypogammaglobulinemia. All CdLS patients diagnosed with CVID or SAD had a history of recurrent bacterial upper and lower respiratory tract infections. All 4 CdLS patients diagnosed with CVID and the patient with hypogammaglobulinemia were placed on intravenous or subcutaneous immunoglobulin replacement therapy, resulting in marked improvement in infectious complications. Patients with SAD were initially placed on a low dose daily prophylactic antibiotic regimen (eg, amoxicillin 20 mg/kg divided twice daily). One patient with SAD subsequently required immunoglobulin replacement therapy because of persistent severe infections despite prophylactic antibiotic therapy. The incidence of CVID and CdLS is estimated to be 1 in 50 000 and 1 in 10 000 individuals, respectively.[1,16] The likelihood of these 2 conditions occurring simultaneously by random chance alone would be predicted to be 1:500 000 000 births. Thus, our findings clearly indicate that antibody deficiency is overrepresented in the CdLS population.

All patients identified to have immunodeficiency had a classic severe form of CdLS characterized by marked developmental delay, dysmorphic facial features, severe gastroesophageal reflux disease, and limb abnormalities (3 of 9 patients had a confirmed *NIPBL* mutation, and the remaining 6 patients had pending genetic testing). Thus far, screening of patients with milder forms of CdLS has not revealed immunologic abnormalities. Our results identified a high frequency of impaired humoral immunity indicating that antibody deficiency syndrome is an important clinical feature in severe forms of CdLS.

To characterize the antibody deficiency identified in our CdLS cohort, we further addressed immune cell numbers and functions critical for antibody production. Tfh are a subset of CD4 T cells

that localize to germinal centers in secondary lymphoid organs to promote differentiation of B cells into memory B cells and plasma cells that produce antibodies.[17] CXCR5+CD4+ human PB T cells have previously been shown to share functional properties with germinal center Tfh cells; these cells are capable of stimulating naive and memory B cells to differentiate into antibody-producing cells.[18] Thus, these markers were used to identify PB Tfh cells in this study. Our results revealed that CdLS patients have lower percentages of PB Tfh cells. Given the critical role of Tfh cells in B-cell differentiation, our findings indicate that decreased numbers of Tfh cells may be associated with antibody deficiency found in our CdLS patient cohort.

In contrast to Tfh cells that play a crucial role in humoral immunity, Tregs are a subset of $CD4^+$ T cells that function to maintain peripheral tolerance and homeostasis through the inhibition of autoreactive and effector T lymphocytes.[19] Impaired numbers and functions of Treg cells could lead to higher frequency of autoimmunity. Interestingly, we found that CdLS patients had significantly lower Treg cell percentages compared with normal control subjects. In primary immune deficiencies characterized by reduced numbers or impaired functions of Tregs (eg, Wiskott-Aldrich syndrome, IPEX [immune dysregulation, polyendocrinopathy, enteropathy, X-linked] syndrome, 22q11.2 deletion syndrome, Omenn syndrome), there is a markedly increased incidence of autoimmune conditions such as idiopathic thrombocytopenic purpura (ITP) or autoimmune hemolytic anemia.[20–22] This raises the question of whether autoimmune conditions are more frequently observed in CdLS patients than in general population. Indeed, CdLS patients are known to have an increased incidence of ITP with detectable antiplatelet antibodies (relative risk of 30 in 633).[23] Thus, our findings indicate that higher frequency of ITP in CdLS patients may be associated with reduced frequency of Tregs.

Given the finding of reduced T-cell subsets in CdLS patients, we further addressed T-cell functions in CdLS patients. We did not find statistically significant differences in T-cell surface activation markers (CD25, CD40L, CD38, and CD69) after T-cell stimulation. Intracellular cytokine production for representative T-cell cytokines (interferon-γ and interleukin-2) after stimulation were similar between the CdLS and normal control groups, indicating that CdLS mutations do not affect T-cell cytokine production with these stimuli.

Several mechanisms may explain the clinical and laboratory findings in CdLS patients described here. Although the cohesin complex is best known for its role in maintaining proper chromosome segregation during cell division, more recently it has also been shown to have a role in regulation of gene expression.[9] Indeed, gene expression of NFATc2, a key transcription factor in T-cell activation and differentiation, has been shown to be decreased in CdLS patients.[12] In addition, cohesin has also been demonstrated to mediate key interactions between enhancer and promoter regions to control T-cell receptor gene rearrangement, the process through which a diverse repertoire of receptors capable of recognizing foreign antigens is generated.[24] Impaired T-cell receptor gene rearrangement in cohesin-deficient T cells has previously been shown to impair T-cell differentiation.[24] These mechanisms may explain our finding of reduced Treg and Tfh cell populations in CdLS patients. Additional studies are required to determine why these particular T-cell subsets were most affected.

Our study has a number of limitations, many of which are inherent to the evaluation of rare disorders. First, selection bias is present because 6 patients were included in our analysis from outside institutions. All 6 of these CdLS patients came to our attention because they had a history of recurrent infections requiring medical intervention (4 of these 6 patients were diagnosed with antibody deficiency). As a result of this selection bias, the true incidence of immunodeficiency in the CdLS population is difficult to estimate. However, our finding of 4 patients with antibody immunodeficiency after prospective screening of 19 CdLS patients followed in our general genetics clinic (regardless of infectious history) indicates that immunodeficiency is unlikely to be a rare clinical feature of this syndrome. Additionally, although no CdLS patients with mild forms of disease were found to have immunodeficiency, our sample size is too small to conclude whether this clinical feature applies only to patients with classic severe forms of CdLS.

In summary, this study indicated for the first time that immunodeficiency is an important clinical consideration in CdLS that may result in severe recurrent infections. We have identified a spectrum of antibody deficiency in CdLS patients ranging from CVID to SAD. Furthermore, our results indicate that quantitative differences are present in CdLS T cells, suggesting a potential mechanism for immunodeficiency in these patients. Clearly, additional studies are required to determine the exact mechanism of antibody deficiency in CdLS patients, and recruitment of more CdLS subjects for immune evaluation will be required to determine the full spectrum of immunodeficiency in CdLS.

CONCLUSIONS

Little attention has been paid to possible underlying immunodeficiency in CdLS, and patients with recurrent infections are seldom evaluated for immunodeficiency. Even given the limitations of our study, our results indicate a need for greater attention to identify possible underlying immunodeficiency

in CdLS patients. Namely, based on our results, screening immune evaluation for all CdLS patients (regardless of age) with a history of recurrent infections should be considered. Testing should include total Ig levels (IgG, IgM, IgA), antibody titers to vaccine antigens (tetanus, diphtheria, pneumococcus), and a complete blood count with differential (to calculate the absolute neutrophil and lymphocyte counts). Patients with abnormal findings should be referred to a clinical immunologist for additional evaluation and management.

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