Comèl-Netherton syndrome defined as primary immunodeficiency

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Background: Mutations in serine protease inhibitor Kazal-type 5 (*SPINK5*), encoding the serine protease inhibitor lympho-epithelial Kazal-type 5 related inhibitor (LEKTI), cause Comèl-Netherton syndrome, an autosomal-recessive disease characterized by congenital ichthyosis, bamboo hair, and atopic diathesis. Despite increased frequency of infections, the immunocompetence of patients with Comèl-Netherton syndrome has not been extensively investigated.

Objective: To define Comèl-Netherton syndrome as a primary immunodeficiency disorder and to explore the benefit of intravenous immunoglobulin replacement therapy.

Methods: We enrolled 9 patients with Comèl-Netherton syndrome, sequenced *SPINK5,* and analyzed LEKTI expression by immunohistochemistry. Immune function was assessed by measuring cognate immunity, serum cytokine levels, and natural killer cell cytotoxicity.

Results: All patients presented with recurrent skin infections caused predominantly by *Staphylococcus aureus.* All but 1 reported recurrent respiratory tract infections; 78% had sepsis and/or pneumonia; 67% had recurrent gastrointestinal disease and failure to thrive. Mutations in *SPINK5*—including 6 novel mutations—were identified in 8 patients. LEKTI expression was decreased or absent in all patients. Immunologic evaluation revealed reduced memory B cells and defective responses to vaccination with Pneumovax and bacteriophage phiX174, characterized by impaired antibody amplification and class-switching. Immune dysregulation was suggested by a skewed T_H1 phenotype and elevated proinflammatory cytokine levels, whereas serum concentrations of the chemokine (C-C motif) ligand 5 and natural killer cell cytotoxicity were decreased. Treatment with intravenous immunoglobulin resulted in remarkable clinical improvement and temporarily increased natural killer cell cytotoxicity.

Conclusion: These data provide new insights into the immunopathology of Comèl-Netherton syndrome and demonstrate that this multisystem disorder, characterized by lack of LEKTI expression in epithelial cells, is complicated by cognate and innate immunodeficiency that responds favorably to intravenous immunoglobulin therapy.

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Key words: *Comèl-Netherton syndrome, SPINK5, LEKTI, immune deficiency, NK-cell cytotoxicity, selective antibody deficiency, IVIG, ichthyosis, bamboo hair, atopic diathesis*

Comèl-Netherton syndrome is a rare autosomal recessive disease characterized by congenital ichthyosis, trichorrhexis invaginata (bamboo hair), and atopic diathesis,[1-4] with a 20% fatality rate in the first year of life.[5]

Patients present shortly after birth with generalized rashes that develop into severe ichthyosis.[5] Bamboo hair is pathognomonic, indicating a structural defect of the hair shaft.[6] A broad spectrum of atopic diseases includes eczema, reactive airway disease, food allergy, and angioedema.[3,7,8] Enteropathy, failure to thrive, hypernatremia, hypoalbuminemia, aminoaciduria, developmental delay, and recurrent infections have been reported.[5,7-10]

Most patients have mutations in the serine protease inhibitor Kazal-type 5 (*SPINK5*) gene, located on chromosome 5q32, resulting in a loss or reduced expression of the multidomain serine protease inhibitor lympho-epithelial Kazal-type 5 related inhibitor (LEKTI).[4,11] LEKTI has been proposed to downregulate desquamation and matrix maturation.[12] LEKTI is expressed by epithelial cells of skin, mucosa, and Hassall corpuscles,[13] raising the possibility that LEKTI affects T-cell maturation.

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Abbreviations used
CCL5: Chemokine (C-C motif) ligand 5
FITC: Fluorescein isothiocyanate
FOXP3: Forkhead box protein 3
IVIG: Intravenous immunoglobulin
LEKTI: Lympho-epithelial Kazal-type 5 related inhibitor
NK: Natural killer
SPINK5: Serine protease inhibitor Kazal-type 5
TCRBV: T-cell receptor β-chain variable
WAS: Wiskott-Aldrich syndrome

Several previous studies recognized an increased infection rate and postulated an underlying immune defect, but reported findings were not consistent with a well defined immune deficiency.[7-10,14] Thus, Comèl-Netherton syndrome is generally not listed as a primary immunodeficiency disorder.[15,16]

We studied 9 patients with Comèl-Netherton syndrome for *SPINK5* mutations, LEKTI expression, and immune abnormalities. Our results strongly suggest that Comèl-Netherton syndrome is a multisystem disorder associated with dysfunctional innate and cognate immunity.

METHODS

Subjects

Nine unrelated children (3 girls and 6 boys; age, 2-9 years) with diverse ethnic backgrounds were enrolled. Institutional Review Board approval and informed consent were obtained. Diagnostic criteria for Comèl-Netherton syndrome included the presence of congenital ichthyosis, bamboo hair, elevated serum IgE levels, allergies, mutations in *SPINK5*, and/or decreased or absent LEKTI expression by skin and/or buccal mucosal epithelial cells. None of the patients had systemic infections or received intravenous immunoglobulin (IVIG) infusions, systemic steroids, or other immunosuppressive treatments for at least 4 weeks before immunologic evaluation.

SPINK5 mutations

DNA was prepared from heparinized blood using QIAamp DNA Mini Kit (QIAGEN, Valencia, Calif). The 33 exons of the *SPINK5* gene including the intron-exon boundaries, the proximal promoter region (1000 bp upstream of the first exon), and the first polyadenylation site were sequenced by using the Big Dye Terminator kit (Applied Biosystems, Foster City, Calif) and analyzed with a 3730xl DNA Analyzer (Applied Biosystems) as previously described.[17] Mutations are reported as recommended.[18] Primer sequences are available on request.

Immunologic assessment

White blood cell and differential counts, lymphocyte subsets, serum immunoglobulin levels, and lymphocyte proliferation to mitogens and specific antigens were analyzed by using standard protocols. PBMCs were isolated by using Ficoll-Paque plus (Bioscience AB, Uppsala, Sweden). Lymphocyte subsets were identified by multicolor flow cytometry[19,20] using the following conjugated mAbs: anti-CD27-allophycocyanin (APC), anti-CD31-biotin, anti-CD8-Alexa700, anti–CD3–fluorescein isothiocyanate (FITC), anti-CD4-phycoerythrin (eBioscience, San Diego, Calif), anti-CD45RA-FITC, anti-IgD-Biotin, anti-CD19-ChyChr, anti-CD8-phycoerythrin-Cy7, anti–γδ–T cell receptor–APC (BD Bioscience, San Jose, Calif), anti-CD38-FITC, anti-CD4-CyC5 (Immunotech, Fullerton, Calif), anti-CD56-PE-Cy 5.5, anti-IgM-PE (Southern Biotechnology, Birmingham, Ala), and streptavidin APC-Cy7 and phycoerythrin-Cy7 (eBioscience). Regulatory T cells ($CD4^+CD25^+$ forkhead box protein 3 $[FOXP3]^+$) were assessed with anti-CD4-phycoerythrin-Cy5/CD25-phycoerythrin cocktail and Alexa Fluor 488 conjugated anti-FOXP3 mAb after exposure to fix/perm solution (all of BioLegend, San Diego, Calif). Where applicable, age-specific control ranges for lymphocyte populations were obtained from published sources.[21,22]

Samples were measured on an LSRII (BD Bioscience) and analyzed with FlowJo (TreeStar, Ashland, Ore). The T-cell receptor β-chain variable (TCRBV) gene repertoire on $CD4^+$ cells was determined with a panel of 22 antibodies.[23] Immunization with bacteriophage phiX174 was performed following a previously described protocol.[24] Serum cytokines were measured with the Luminex 100 system by using the Human Cytokine Twenty-Five-Plex Antibody Bead Kit (BioSource International, Inc, Camarillo, Calif). Natural killer (NK) cell cytotoxicity of Ficoll-Hypaque–isolated PBMCs was evaluated by ^{51}Cr-release assay using K562 erythroleukemia target cells.[25] Although a variety of other assessments of the NK-cell cytotoxic process are available, ^{51}Cr-release was used as a single measure to assess the complete process cytotoxicity. Lytic units per NK cell were calculated as the number of NK cells required to mediate 20% lysis of target cells expressed as the inverse normalized to 1×10^4 cells and were calculated by using the slopes of curves generated by ^{51}Cr-release assay over the range of effector-to-target cell ratios. To obtain lytic units per NK cell, the lytic K562 units of PBMC were corrected for the number of NK cells present in the different PBMCs to target cell ratios by dividing by the percentage of $CD56^+CD3^-$ NK cells present in that sample as determined by flow-cytometric analysis. Control donors included both adults and children as young as age 5 years.

LEKTI expression

Buccal mucosa epithelial cells were collected with a Cytobrush Plus GT (Medscand Medical AB, Malmoe, Sweden), spread on glass slides, fixed with acetone, permeabilized with 0.1% Triton-X 100 (Boehringer Mannheim, Mannheim, Germany) and 0.5% H_2O_2, and stained with anti-LEKTI mAb (Zymed Laboratories, Inc, San Francisco, Calif). Peroxidase-based immunohistochemical staining was performed with the Elite ABC Kit (Vector Laboratories, Burlingame, Calif) by using aminoethylcarbazol substrate-chromogen (DakoCytomation, Carpinteria, Calif). After counterstaining with hematoxylin (Sigma-Aldrich, St Louis, Mo), 200 cells from each subject were evaluated microscopically for LEKTI expression. Paraffin sections of tissues were similarly stained after pretreatment with CitriSolv (Decon Labs, Inc, King of Prussia, Pa) and ethanol.

Statistics

Statistical analyses of responses to bacteriophage phiX174 were performed on log-transformed control K values that are expressed as geometric means and 95% confidence limits. Cytokine levels of patients and 16 age-matched controls are shown as medians with interquartile ranges and analyzed with the nonparametric Mann-Whitney *U* test. A 2-sided *P* value <.01 was considered significant. For NK-cell cytotoxicity, serum immunoglobulin levels and LEKTI expression patient results were compared with the arithmetic mean ±2 SD of controls; lymphocyte subset values were compared with the geometric mean and the 95% confidence limit of normal control data. The student *t* test was used to compare the control versus the patient group.

RESULTS

Clinical presentation

The clinical triad of Comèl-Netherton syndrome—congenital ichthyosis, bamboo hair, and allergic diathesis—was found in all patients except 1 who lacked bamboo hair (Table I). Recurrent or persistent *Staphylococcus aureus* skin infections, frequently methicillin-resistant, occurred in all but 1 patient (patient 8) once skin lesions had developed. In patient 8, skin infections were caused by *Pseudomonas aeruginosa*.

All but 1 patient had a history of recurrent upper and lower respiratory tract infections, most frequently recurrent otitis

TABLE I. Clinical findings and gene analysis in 9 patients with Comèl-Netherton syndrome

Patient no.	Age[m] (y)	Sex	Congenital ichthyosis	Bamboo hair	Atopic findings	Recurrent/persistent infections of			Severe invasive infections[l]	Additional findings	Mutation in the gene *SPINK5*
						Skin	Gastro-intestinal tract	Respiratory tract			
1	0.5	F	Yes	Yes	Eczema, food allergy	Yes[a,b,c]	Yes[o]	Yes[c]	Sepsis[a,e]	Hypernatremic dehydration, failure to thrive	Compound heterozygous (1) 354_357delTTGT; C119AfsX25 (exon 5)[n] (2) 1431-12G>A (intron 15)[k]
2	3	M	Yes	No	Eczema	Yes[a,b,c,h]	Yes[o]	Yes[a,c]	Pneumonia, sepsis[a,h]	Hypernatremic dehydration, failure to thrive	Homozygous 1346_1352insT; C451LfsX5 (exon 15)[n]
3	6	M	Yes	Yes	Rhinitis, food allergy, eczema, asthma	Yes[a,b,c,d]	Yes[f]	Yes[a,c,g]	Pneumonia	Failure to thrive	Homozygous 2459-2468delA; K823RfsX100 (exon 26)
4	9	M	Yes	Yes	Rhinitis, food allergy, angioedema, eczema, asthma	Yes[a]	Yes[f]	Yes[a,c]	Sepsis[a]	Hypernatremic dehydration	Compound heterozygous (1) 1431-12G>A (intron 15)[k] (2) Not determined
5	2.5	M	Yes	Yes	Eczema, food allergy	Yes[a,b,c,d,e]	Yes[o]	Yes[a]	Sepsis[a,i]	Failure to thrive	Compound heterozygous (1) 377-8delAT; Y126X (exon 5) (2) 2473-4delGA; E825GfsX1 (exon 26)[n]
6	7	M	Yes	Yes	Rhinitis, eczema	Yes[a,c]	No	Yes[o]	No	No	Homozygous 2459-2468delA; K823RfsX100 (exon 26)
7	6	M	Yes	Yes	Rhinitis, food allergy, eczema	Yes[a]	Yes[o]	Yes[o]	Pneumonia, sepsis[a,j]	Cardiomyopathy with acute heart failure, failure to thrive	No mutation identified
8	1	F	Yes	Yes	Eczema, food allergy	Yes[c]	No	No	No	Hypernatremic dehydration, failure to thrive	Compound heterozygous (1) 410+1G>A (intron 5) (2) 2098G>T G700X (exon 22)[n]
9	7	F	Yes	Yes	Rhinitis, food allergy	Yes[o]	No	Yes[o]	Pneumonia	No	Compound heterozygous (1) 282+1G>A (intron 4)[n] (2) 603+1G>A (intron 8)[n]
Percent of patients positive for symptom of column			100	89	100	100	67	89	78	78	—

F, Female; *M*, male. Infectious agents: [a]*S aureus*, [b]*S aureus* methicillin-resistant, [c]*P aeruginosa*, [d]*Streptococcus viridans*, [e]*K oxytoca*, [f]rotavirus, [g]*Proteus mirabilis*, [h]*Enterococcus* spp., [i]*S cholerae-suis*., [j]*Acinetobacter*. [k]Putative splice site upstream (see Raghunath et al[26]). [l]Defined by requiring intravenous antibiotic treatment. [m]Age when studied. [n]Novel mutation. [o]No identification of pathogens.

media and/or externa. Four patients had recurrent lower respiratory tract disease, including multiple episodes of pneumonia and allergy or infection-related respiratory distress. Four infants developed *S aureus* sepsis with 1 also having *Salmonella choleraesuis* sepsis. At 6 years of age, patient 7 developed acute heart failure with cardiomyopathy possibly associated with recurrent *S aureus* sepsis. He recovered after removal of a central line, presumably the nidus for infection. PCR failed to detect common viruses during heart failure. Overall, *S aureus* was the most frequent infectious agent, followed by *P aeruginosa* and *Klebsiella oxytoca*.

The majority of our patients had recurrent acute gastroenteritis resulting in failure to thrive and requiring repeated hospitalization. Three patients developed hypernatremic dehydration, and 3 needed short-term parenteral nutrition. No patient had clinical signs of autoimmunity such as hemolytic anemia, thrombocytopenia, arthritis, or vasculitic skin lesions.

Mutations of *SPINK5* and LEKTI expression

SPINK5 mutations, including 6 novel mutations, were identified in all but patient 7 (Table I). Most mutations were located in the coding region and included short deletions or insertions of as many as 4 base pairs, resulting in frame shift and early termination of translation. Of the 4 intronic mutations, 3 (including 2 novel) were found at highly conserved intron-exon boundaries. The mutation 1431-12G>A in intron 15, observed in 2 patients, has been previously described and creates an alternative splice acceptor site leading to the insertion of 10 nucleotides upstream of exon 16 and premature termination within exon 16.[26] Two unrelated patients (patients 3 and 6), both of Polynesian origin but from different Pacific islands, had an identical homozygous mutation in exon 26, suggesting a founder effect in the Polynesian population. Patient 2, who was homozygous for a single nucleotide insertion, was the only patient with a family history of consanguinity.

LEKTI protein expression was absent or present as small immunoreactive foci in fewer than 2% of epithelial cells from skin biopsies and/or buccal mucosa from all 9 patients, including 1 (patient 4) with a detectable mutation in only 1 allele and 1 patient (patient 7) without detectable *SPINK5* mutations. In contrast, controls (n = 20) had a mean of 43% LEKTI-positive cells (95% confidence limit, 21% to 64%) that showed diffuse cytoplasmic staining. Heterozygous parents of 3 patients showed a mean of 42% (range, 40% to 49%) LEKTI-positive buccal mucosa cells.

Immunologic studies

Except for eosinophilia, leukocyte and differential counts were normal. There were no significant alterations in the numbers and percentages of T, B, and NK cells. The TCRBV repertoire was normally distributed in both patients investigated, and the percentage of recent thymic emigrant cells ($CD4^+CD31^+CD27^+CD45RA^+$) was within the 95% confidence limit of healthy controls in 6 patients investigated (data not shown).

As a group, the geometric mean of NK T cells ($CD3^+CD56^+$; patient geometric mean, 11.0% of lymphocytes; control geometric mean, 4.0%, with 95% confidence limit 1.4% to 10.7%) were significantly increased ($P < .001$). The geometric mean of unswitched memory B cells ($CD19^+CD27^+IgM^+IgD^+$) was significantly decreased ($P < .0001$) in the patient group compared with controls (patient geometric mean, 3.0% of $CD19^+$ cells; control geometric mean, 8.7%; 95% CI, 2.6% to 29.3%), with 3 of 7 patients below the 95% confidence limit. Similarly, switched memory B cells ($CD19^+CD27^+IgM^-IgD^-$) were decreased ($P < .01$), with 2 of 7 patients below the 95% confidence limit (patient geometric mean, 4.5% of $CD19^+$ cells; control geometric mean, 7.3%; 95% CI, 2.2% to 24.0%). Mean percentages of γδ-T cells and regulatory T cells ($CD4^+CD25^+FOXP3^+$) were within the 95% confidence limit of healthy controls (data not shown).

Lymphocyte proliferation to mitogens and antigens was normal, and random serum antibody titers to tetanus and diphtheria were protective in the 6 patients investigated (data not shown). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity of neutrophils by dihydrorhodamine studies was normal in the 4 patients evaluated (data not shown).

Serum IgE levels were significantly elevated in all, and IgM and IgA were elevated in 3 and 4 patients, respectively (Fig 1, *A*). Serum IgG levels were elevated in 2 patients and reduced in 1 patient; IgG subclasses were normal in the 5 patients studied (data not shown).

Primary and/or secondary antibody responses to bacteriophage phiX174 were quantitatively depressed in all 4 patients studied, with impaired isotype switching in 3 (Fig 1, *B*). Protective antipneumoccocal polysaccharide antibody titers (protection defined as ≥1 ug/mL) in random samples were reduced in 5 patients tested (range, 0% to 36% of as many as 12 serotypes tested; mean, 21%). Both patients (patients 6 and 7) immunized with a polyvalent pneumococcal polysaccharide vaccine (Pneumovax 23, Merck & Co) failed to mount a positive response—defined as a 4-fold increase in antibody titer—to the 14 or 12 serotypes tested.

Although the absolute numbers of NK cells ($CD3^-CD56^+$) were normal or elevated in all patients, NK-cell cytotoxicity, measured in at least 3 independent experiments in total PBMCs from 3 patients before monthly IVIG infusion, was consistently below the 95% confidence limit of healthy controls. To account for any potential variation in NK-cell percentage among PBMCs, the lytic units per NK cell were calculated. This is additionally important because the percentage of NK cells in PBMCs normally changes as a feature of age, with the highest found between the ages of 10 and 17 years.[21] Lytic units per NK cell were 1884 ± 428 (1SD) in controls and 567 ± 284 (1SD) in patients, suggesting that decreased cytotoxic activity is not explained by variations in the size of the NK-cell population.

Serum analysis revealed significantly increased proinflammatory (IL-1β, IL-12, TNF-α, GM-CSF, IL-1 receptor antagonist) and anti-inflammatory cytokines (IL-2 and soluble IL-2 receptor) compared with age-matched controls (Fig 1, *D*). In both patients and controls, the T_H2-associated cytokines IL-4 and IL-5 were undetectable or detected at low amounts (data not shown). Only the chemokine (C-C motif) ligand 5 (CCL5), which is regulated on activation and mainly expressed and secreted by T cells, was significantly diminished in patients compared to controls (Fig 1, *D*).

Response to IVIG therapy

Symptomatic treatment with allergy control, moisturizers, steroid creams, and antibiotics had uniformly limited effect. IVIG replacement therapy (0.4 g/kg/mo) was initiated because of

A

Patient ID	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Patient #6	Patient #7	Patient #8	Patient #9
Immunoglobulin levels at age (y)	1.5	0.2	0.5	1	0.5	6	4	1	7
IgE, IU/mL	885 ↑	213 ↑	43 *	2072 ↑	287 ↑	10,000↑	2334 ↑	1172 ↑	1109 ↑
IgM, mg/dL	155 ↑	205 ↑	58	81	114 ↑	102	79	73	92
IgA, mg/dL	97	159 ↑	51	27	53	263 ↑	346 ↑	134 ↑	174
IgG, mg/dL	577	678 ↑	586	182 ↓	522	1370	549	1125 ↑	882

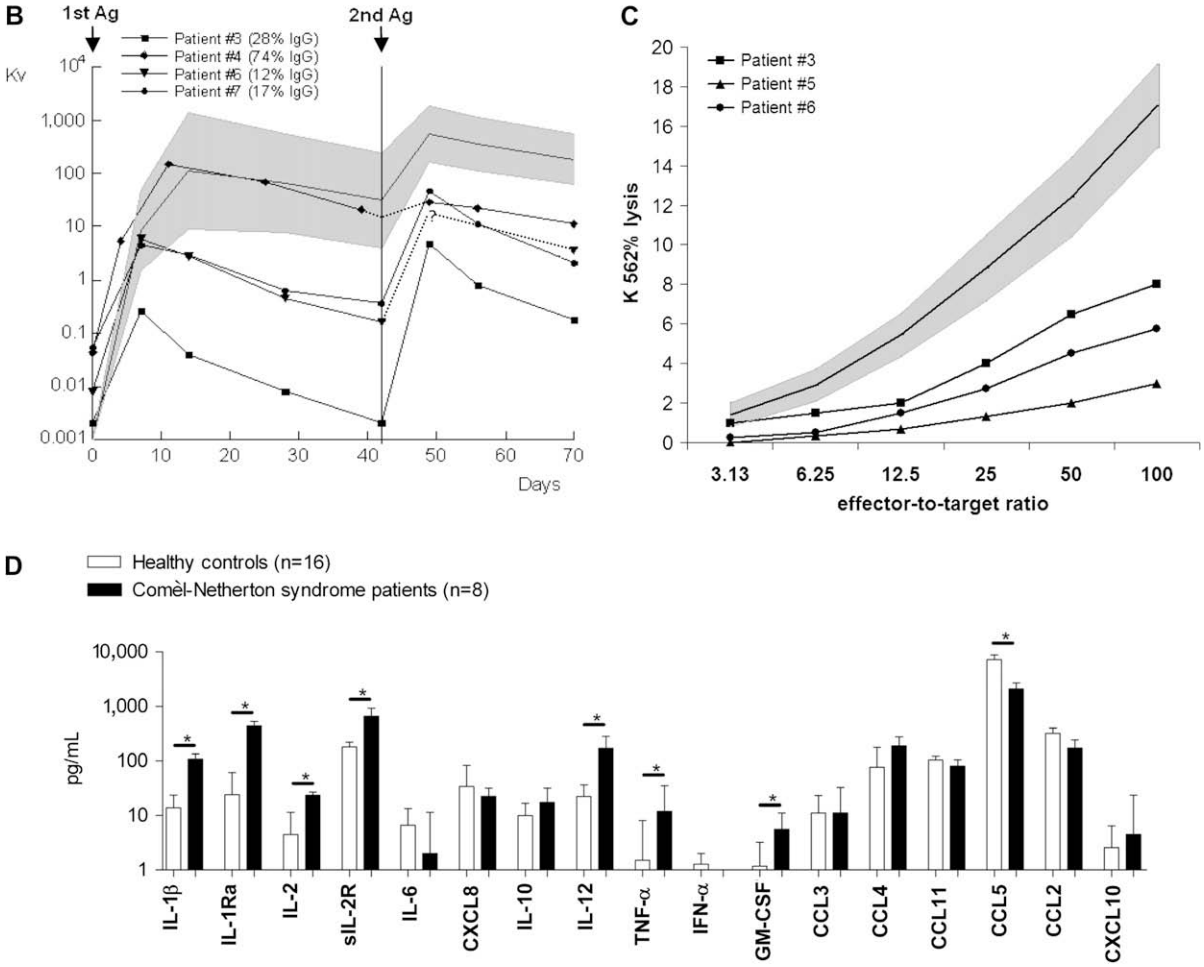

FIG 1. Immunologic assessment of patients with Comèl-Netherton syndrome. **A,** Serum immunoglobulin levels measured before IVIG was started; *arrows* indicate 2 SD below *(↓)* or above *(↑)* age-matched control value.[32] *Patient 3 had a serum IgE level of 1119 IU/mL (2 SD above age-matched controls) at 6 years of age. **B,** Decreased antibody responses after primary and secondary immunization with the neoantigen, bacteriophage phiX174, which was injected twice 6 weeks apart. Neutralizing antibody titers were determined in serially obtained serum samples and expressed as rate of phage inactivation or K value *(K_v)*[22] plotted on a log scale (*solid line in gray area,* geometric mean and 95% confidence limit measured in 50 normal controls). The mean percentage of phage-specific IgG antibody in serum collected 2 weeks after the second immunization was identified as being resistant to treatment with 2-mercaptoethanol (*2-ME*; values of 50 normal controls, 48% ± 23% 1 SD). For patient 6, only 1 sample was collected after secondary immunization. **C**, NK-cell cytotoxicity of Ficoll-Hypaque–isolated PBMCs against K562 cells measured before IVIG treatment. The mean percent of lysis observed in at least 3 independent experiments performed in each of the 3 patients studied (*solid line in gray area,* mean and ± 2 SD measured in 18 normal controls). **D,** Median ± interquartile ranges of serum cytokine levels observed in 8 patients with Comèl-Netherton syndrome *(filled columns)* are compared with those of 16 age-matched normal controls *(open columns)*; *P values <.01. Median values of IL-4, IL-5, IL-7, IL-13, IL-15, IL-17, INF-α, INF-γ, and monokine induced by INF-γ were below detection limit in the majority of patients and control subjects (data not shown). *ID,* (Patient) Identification; *IL-1Ra*, IL-1 receptor antagonist; *sIL-2R,* soluble IL-2 receptor.

abnormal antibody responses to bacteriophage phiX174 in the 4 patients studied (patients 3, 4, 6, 7; Fig 1, *B*) and in 1 additional patient (patient 5) because of severe failure to thrive. All 5 families reported remarkable clinical benefit, including decreased inflammation and itching of the skin, thicker hair with less breaking of hair shafts, and healthier scalps, compared with conventional treatment. The most dramatic improvement occurred in patient 5, the youngest and most severely affected child (Fig 2). In response to a survey on the utility of IVIG, parents of the 5 treated children estimated that the numbers of missed school days and

FIG 2. Toddler (patient 5) with Comèl-Netherton syndrome before and 3 months after IVIG treatment.

doctors' visits were reduced, infections were lessened, and overall quality of life was improved. Over 2 years' observation on IVIG, patient 3 advanced from under the 3rd to grow along the 3rd percentile for height, and from the 3rd to the 75th percentile in weight. One year after starting IVIG, patient 5, whose height and weight were far below the 3rd percentile, is approaching the 3rd percentile for length and reached it for weight. Patient 6 is reaching the 3rd percentile for height, and his weight increased from the 50th to the 90th percentile 6 months after IVIG was started.

To evaluate objectively the impact of IVIG therapy on the immune system, we explored the effect on NK-cell function before, immediately after, and 6 to 7 days after monthly infusions. NK-cell cytotoxicity has been reported to increase after IVIG therapy.[27] We found that NK-cell cytotoxicity did not improve within 15 minutes after IVIG infusion but was temporarily restored 6 to 7 days later to the normal range in patient 3 and increased to 80% of the normal range in patient 6. In patient 5, there was no baseline NK-cell cytotoxicity detectable before receiving monthly IVIG infusion, but 6 days after infusion, NK-cell cytotoxicity was present, although reduced. There was a 26% ± 18% increase in the percentage of NK cells in PBMCs after IVIG treatment, but this alone did not account for the increase in cytotoxicity. To account for the changes in the percentage of NK cells among PBMCs after IVIG treatment, NK-cell lytic units were calculated and increased in all 3 patients to a mean of 1156 ± 442 (1SD) six to seven days after infusion. Thus, IVIG increased NK-cell cytotoxicity in 3 patients with Comèl-Netherton syndrome independently of any alterations in NK-cell percentages.

DISCUSSION

The initial presentation of Comèl-Netherton syndrome is early-onset severe chronic skin disease requiring expert dermatologic care. However, Comèl-Netherton syndrome has been recognized not only as a disorder of skin and hair but also as a complex systemic disease including atopic diathesis, recurrent infections, failure to thrive, and a high fatality rate in early childhood.[3,5]

The observation of recurrent infections has suggested an associated immune defect, which led to limited investigations.[7-10,14,16] The findings of these studies, however, were too inconsistent to define Comèl-Netherton syndrome as a primary immunodeficiency disease, and it is generally not listed as such.[15] Unlike earlier reports that estimated a rate of recurrent infections as high as 30%,[7,8] we observed this complication in every one of our patients. Bacterial infections involving the skin, respiratory, and gastrointestinal tracts often resulted in severe failure to thrive and were life-threatening in 78% (Table I). Infections included neonatal sepsis as reported by others.[9,28,29] Cardiomyopathy, which developed in 1 of our patients after *S aureus* sepsis, is a complication with unknown etiology, reported previously in 2 patients.[30]

All patients with sepsis were culture positive for *S aureus*, a pathogen present in normal skin flora. Disruption of the skin and gut barrier in Comèl-Netherton syndrome likely contributes to susceptibility to *S aureus* skin infections and sepsis, and *Salmonella* sepsis, respectively.[31] However, multiple immune defects reported here, from antibody deficiency to impaired NK-cell function, and the favorable response to IVIG treatment imply that a defective skin barrier cannot entirely explain the susceptibility to infections.

The consistently abnormal antibody responses to bacteriophage are similar to those observed in other primary immunodeficiencies affecting both cognate[32-35] and innate[36,37] immunity, suggesting abnormal T-cell or B-cell development or defective costimulatory signaling resulting in reduced isotype switching and defective immunologic memory.

As a group, memory B cells, especially IgM^+ memory B cells, were significantly decreased compared with a normal control group. IgM^+ memory B cells, also referred to as unswitched memory B cells or splenic marginal zone B cells, play a role in defense against encapsulated bacteria.[38] This observation is supported by the finding that splenectomized/asplenic patients have reduced numbers of IgM^+ memory B cells and decreased responses to pneumococcal polysaccharide vaccines.[39] As reported by others,[7,14] protective type-specific antipolysaccharide antibodies were reduced in the 5 patients tested, and the 2 patients challenged with Pneumovax failed to respond to most serotypes.

Reports of skin cancer in young adults with Comèl-Netherton syndrome[40-42] raised consideration of defective cellular immunity. None of our pediatric patients, all younger than 10 years, developed a malignancy, nor was a clearly defined T-cell defect demonstrated. Although LEKTI is expressed in normal Hassall corpuscles,[13] those patients tested (n = 2) had normal TCRBV repertoires, and all but 1 exhibited normal thymic output judged by the presence of a normal circulating pool of recent thymic emigrant cells ($CD4^+CD31^+CD27^+CD45RA^+$). Increased T-cell activation is suggested by elevated serum levels of proinflammatory cytokines with compensatory elevated anti-inflammatory cytokines. There was no skewing toward T_H2 cytokines, unlike in other diseases associated with allergies and elevated serum IgE.[43]

CCL5, the only chemokine significantly decreased in patient sera, is secreted by T cells, monocytes, and especially by NK cells activated in the context of FcR engagement on antibody exposure.[43,44] Because the numbers of peripheral blood NK cells were normal in all but 1 patient, reduced levels of CCL5 cannot be accounted for by quantitatively low NK cells. More likely, the reduced CCL5 concentration is the direct result of the decreased NK-cell function observed in all patients studied. This CCL5 deficiency may directly affect NK-cell migration, preventing NK cells from reaching their destination in peripheral lymphoid tissue where final maturation occurs.[45]

Natural killer cells are critical in host defense, and impaired NK-cell function has been observed in several primary immunodeficiencies.[46] The combination of NK-cell and specific

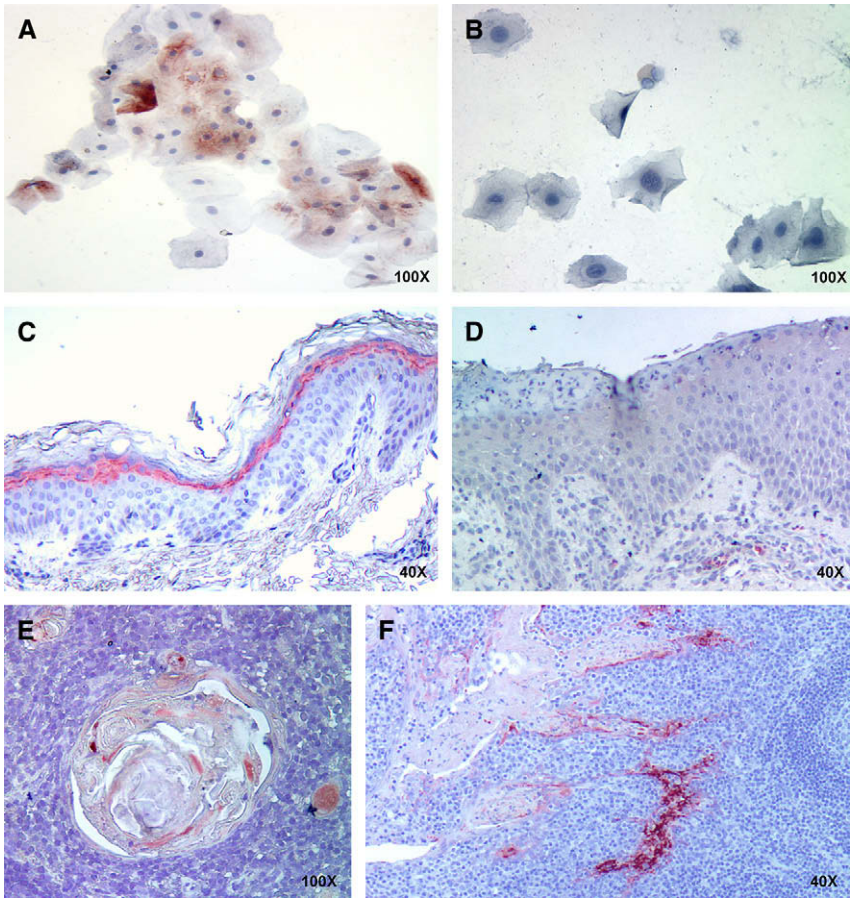

FIG 3. Expression of LEKTI in different human tissues. Immunohistochemistry counterstained with hematoxylin shows LEKTI protein in *red*, if present. Buccal mucosal cells of a healthy individual **(A)** and patient 3 **(B)**; skin biopsies of a healthy child with LEKTI expression in superficial epidermal squamous layer **(C)** and patient 4 **(D)** lacking LEKTI; LEKTI expression in Hassall corpuscles **(E)** and tonsillar crypts **(F)** of normal controls.

antibody deficiency is exemplified in the Wiskott-Aldrich syndrome (WAS). The increased susceptibility to infections and malignancies observed in patients with WAS[25,47] are also characteristic of Comèl-Netherton syndrome. Unlike in WAS, the diminished NK cytotoxicity observed in patients with Comèl-Netherton syndrome is less likely caused by an intrinsic cellular defect, but rather may reflect impaired NK-cell maturation, which typically requires contact with epithelial cells.[48] The detrimental effect on epithelium by LEKTI deficiency may result in aberrant NK cell–epithelial cell interaction. However, the fact that IVIG increased NK-cell cytotoxicity suggests that the patient NK cells at least maintain the capacity (and lytic machinery) to mount a cytotoxic response under the right conditions. This may explain why patients do not develop the hallmark infections of NK-cell deficiencies.[46] Further studies of individual components of the cytolytic process as well as of NK cells isolated from patients with Comèl-Netherton syndrome are planned and warranted. Although the mechanisms by which LEKTI mutations result in the Comèl-Netherton phenotype are unknown, the strong LEKTI expression in normal epidermis and in epithelial cells within tonsils and thymus (Fig 3) suggests that LEKTI secondarily affects development of the cognate immune system, underscoring the critical link between epithelial cells and the development and function of the immune system.

The decision to initiate IVIG treatment was based on abnormal antibody responses to bacteriophage phiX174 and Pneumovax and abnormal numbers of memory B cells. Monthly IVIG infusions may improve the clinical course of Comèl-Netherton syndrome by providing high affinity neutralizing and opsonizing antibody required for phagocytosis and killing of bacteria. In addition, IVIG is known to reduce inflammation in patients with chronic inflammatory disorders[49] and under certain circumstances to increase NK cytotoxicity temporarily.[27] Although regular IVIG infusions at a dose recommended for replacement therapy in primary immunodeficient patients (0.4 g/kg/mo) benefited all 5 patients treated to date, only controlled clinical trials will define the role of IVIG treatment in Comèl-Netherton syndrome.

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Clinical implications: Comèl-Netherton Syndrome, a multisystem disorder, is associated with abnormal antibody responses and decreased NK-cell function. IVIG substitution reduces infections, increases NK-cell function, and improves quality of life

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