

ORIGINAL ARTICLE

CD55 Deficiency, Early-Onset Protein-Losing Enteropathy, and Thrombosis

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ABSTRACT

BACKGROUND

Studies of monogenic gastrointestinal diseases have revealed molecular pathways critical to gut homeostasis and enabled the development of targeted therapies.

METHODS

We studied 11 patients with abdominal pain and diarrhea caused by early-onset protein-losing enteropathy with primary intestinal lymphangiectasia, edema due to hypoproteinemia, malabsorption, and less frequently, bowel inflammation, recurrent infections, and angiopathic thromboembolic disease; the disorder followed an autosomal recessive pattern of inheritance. Whole-exome sequencing was performed to identify gene variants. We evaluated the function of CD55 in patients' cells, which we confirmed by means of exogenous induction of expression of CD55.

RESULTS

We identified homozygous loss-of-function mutations in the gene encoding CD55 (decay-accelerating factor), which lead to loss of protein expression. Patients' T lymphocytes showed increased complement activation causing surface deposition of complement and the generation of soluble C5a. Costimulatory function and cytokine modulation by CD55 were defective. Genetic reconstitution of CD55 or treatment with a complement-inhibitory therapeutic antibody reversed abnormal complement activation.

CONCLUSIONS

CD55 deficiency with hyperactivation of complement, angiopathic thrombosis, and protein-losing enteropathy (the CHAPLE syndrome) is caused by abnormal complement activation due to biallelic loss-of-function mutations in *CD55*. (Funded by the National Institute of Allergy and Infectious Diseases and others.)

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GENETIC INQUIRY HAS CONTRIBUTED to our understanding of gastrointestinal diseases, associating at least 64 genes with early-onset or very-early-onset inflammatory bowel disease.¹ Deleterious gene variants affect the intestinal epithelial barrier, phagocytosis processes, immune regulation, and inflammation. Protein-losing enteropathy, or gastrointestinal protein wasting causing hypoproteinemia, edema, and pleural and pericardial effusions, has also been linked to monogenic disorders.² A loss-of-function variant in *PLVAP* (encoding plasmalemma vesicle associated protein) that disrupts endothelial fenestrated diaphragms and compromises barrier integrity is associated with severe protein-losing enteropathy.³ This condition can develop secondarily from systemic conditions that arrest lymph flow, such as congestive heart failure, or directly from gastrointestinal mucosal damage or impaired lymph drainage from primary intestinal lymphangiectasia (also known as Waldmann's disease).^{2,4} Although primary intestinal lymphangiectasia can be a component of multisystemic genetic syndromes, including the Hennekam syndrome (caused by biallelic loss-of-function variants in *CCBE1* or *FAT4*), the mechanisms of non-syndromic primary intestinal lymphangiectasia and protein-losing enteropathy remain largely unknown.^{5,6} Here, we define the molecular and clinical features of an autosomal recessive syndrome of early-onset protein-losing enteropathy characterized by primary intestinal lymphangiectasia, bowel inflammation, and thrombotic events.

METHODS

STUDY PARTICIPANTS

We enrolled 10 patients who were based in Turkey and 1 who was based in the Netherlands, along with their healthy parents and siblings when available. The 11 patients were from eight families, all of whom were of Moroccan, Syrian, or Turkish ancestry. Patients 3.1, 5.1, and 5.2 were assessed at the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases in Vienna and were from Istanbul or Ankara, Turkey. The remaining patients were all assessed at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, in Bethesda, Maryland, and were from Turkey, Syria, or Morocco. The ages of the patients ranged from 3 to 23 years as of February

2017. Patients were initially identified on the basis of a diagnosis of persistent protein-losing enteropathy and were included in the study after the identification of biallelic loss-of-function variants in *CD55*. For details regarding individual patients, see the Supplemental Patient Clinical Histories section in the Supplementary Appendix, available with the full text of this article at NEJM.org. All the study participants provided written informed consent for approved protocols at their respective institutions, with consent for minors being provided by the parents.

GENETIC AND FUNCTIONAL ANALYSIS

We performed whole-exome sequencing in the index patients and Sanger sequencing of *CD55* in the other participants. Complement assays were performed before and after lentiviral *CD55* reconstitution. Alexion Pharmaceuticals provided reagents. Details are provided in the Supplementary Appendix.

STATISTICAL ANALYSIS

We used the Mann–Whitney U test or Student's t-test to assess the significance between nonpaired samples and the Wilcoxon matched-pairs signed-rank test or a two-tailed paired t-test for the paired samples. Two-tailed P values of less than 0.05 were considered to indicate statistical significance. Statistical comparisons were made with the use of GraphPad Prism software, version 7.0a.

RESULTS

CLINICAL PHENOTYPE

We investigated 11 patients and 2 deceased relatives with a history of protein-losing enteropathy that was characterized by early-onset gastrointestinal symptoms, edema, malnutrition, hypoalbuminemia, and hypogammaglobulinemia, from eight consanguineous families with unaffected parents (Table 1 and Fig. 1A, and Fig. S1 in the Supplementary Appendix). Hypoproteinemia was always present, with minor variations, and, together with edema and gastrointestinal symptoms (abdominal pain, vomiting, and diarrhea), was alleviated by albumin infusion (Fig. 1B). Chronic malabsorption caused micronutrient deficiencies of iron, ferritin, calcium, magnesium, folate, and vitamins D and B₁₂ as well as anemia and growth retardation (Table 1). These condi-

Table 1. Demographic and Clinical Characteristics of 11 Patients with the CHAPLE Syndrome.*

Characteristic	No. of Patients
Sex	
Female	6
Male	5
Age at presentation <2 yr	8
Manifestations of gastrointestinal disease or inflammatory bowel disease	
Chronic or recurrent diarrhea	8
Abdominal pain	4
Vomiting	6
Features of protein-losing enteropathy	
Hypoalbuminemia	10
Hypogammaglobulinemia	11
Facial or extremity edema	9
Confirmed primary intestinal lymphangiectasia or Waldmann's disease†	5
Malabsorption features	
Growth retardation	8
Anemia	9
Vitamin or micronutrient deficiency‡	11
Features of thrombotic disease§	
Thrombocytosis	2
Thrombosis	3
Endoscopic findings†	
Mucosal ulcer	4
Lymphoid infiltrates in mucosa	6
Recurrent lung infection	5
Additional features	
Hypothyroidism¶	3
Arthritis or arthralgia	2
Finger clubbing	5

* The CHAPLE syndrome comprises CD55 (decay-accelerating factor) deficiency with hyperactivation of complement, angiopathic thrombosis, and protein-losing enteropathy.

† Two patients did not undergo endoscopic assessment because they did not have gastrointestinal symptoms.

‡ The micronutrients assessed included serum vitamin B₁₂, vitamin D, folate, iron, ferritin, zinc, calcium, and magnesium.

§ Thrombi were located in the deep veins in the abdominal sites, including the mesenteric and hepatic veins, sometimes with extension to the inferior vena cava and heart, and leading to pulmonary embolism.

¶ Tests for antithyroglobulin and anti-thyroid peroxidase antibodies were negative.

tions were improved by means of vitamin and micronutrient supplementation, a protein-rich diet with medium-chain triglycerides, albumin (ad-

ministered as an intravenous infusion), and blood transfusions. Details are provided in Table S1, Figures S1 and S2, and the Supplemental Patient Clinical Histories section in the Supplementary Appendix.

Histopathological assessment of intestinal-biopsy samples or resections revealed extensive lymphangiectasia, verified by lymphatic endothelial markers, which, together with the patients' young age, suggested the diagnosis of primary intestinal lymphangiectasia (Table 1 and Fig. 1C).^{2,4} Transmission electron microscopy of the duodenal-biopsy sample obtained from Patient 6.1 showed lymphatic dilatation, but, unlike in persons with PLVAP deficiency, we found normal capillary architecture. Surgical removal of the lymphangiectatic segments in Patients 2.1 (who had partial bowel obstruction), 5.1, and 5.2 ameliorated clinical symptoms and protein-losing enteropathy (although Patient 5.2 had a relapse), which raised the possibility of a causal relationship. Some patients had bowel inflammation (similar to that seen in persons with inflammatory bowel disease), exudates, and lymphocytic infiltrates without intestinal thromboses (Table 1 and Fig. 1D). Radiologic examinations revealed bowel-wall edema, thickening, or both in Patients 1.1, 2.1, and 6.1. Thus, protein-losing enteropathy and micronutrient deficiencies are probably caused by primary intestinal lymphangiectasia exacerbated by bowel inflammation. Details are provided in Table S1 and Figures S3 and S4 in the Supplementary Appendix.

Five patients had recurrent respiratory infections associated with hypogammaglobulinemia (Table 1 and Fig. 1B). Major immunologic cell subsets and antibody production were normal. Patient 1.1 had concomitant homozygous gene variants in *CD21*, and Patient 5.1 in *CD27*. In a finding consistent with the confirmed *CD21* deficiency, Patient 1.1 had decreased class-switched IgD-CD27+ memory B cells.⁷ Patient 5.1 had subclinical persistent Epstein-Barr virus infection (viral RNA level, 420 copies per milliliter) and, at the time of this writing, is being monitored closely, because *CD27* deficiency increases the risk of lymphoproliferative disease driven by the Epstein-Barr virus.⁸ Intravenous immune globulin reduced the frequency and severity of respiratory infections in Patients 1.1 and 2.1. Details are provided in Tables S1 and S3, Figures S2A and S5, and

the Supplemental Patient Clinical Histories section in the Supplementary Appendix.

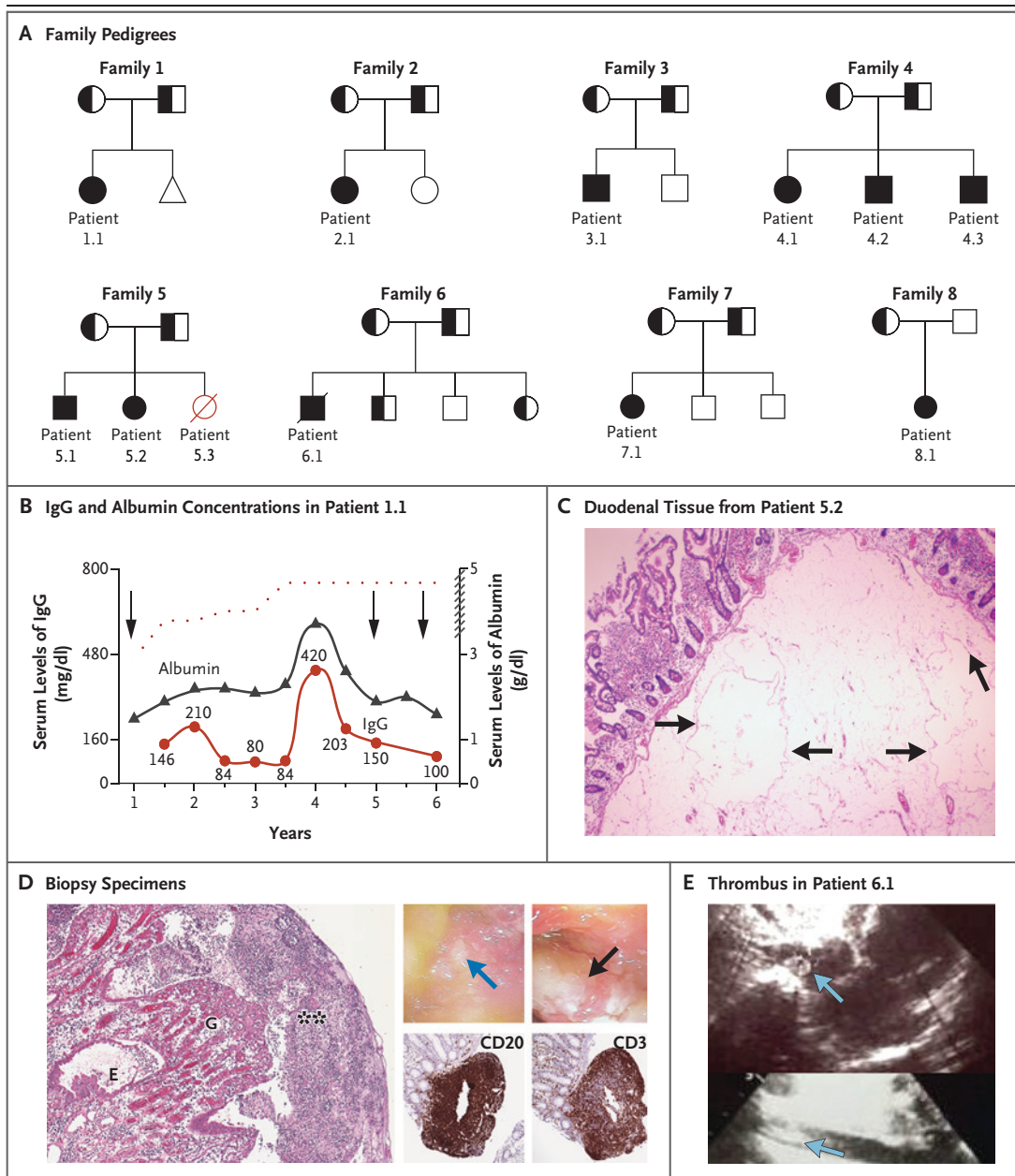
Three patients had severe thrombotic vascular occlusion (Table 1). Thrombi developed in the inferior vena cava, right atrium, and pulmonary arteries of Patient 6.1, causing arteriovenous malformations (Fig. 1E). Transmission electron microscopy of duodenal-biopsy samples revealed malformed erythrocytes binding abnormally to capillary walls and transmural migration. Patient 5.1 had multiple thromboses in the mesenteric and hepatic veins, heart, and cerebral veins that were associated with an intracranial hemorrhage. Thrombosis was unresolved, despite surgical removal of blood clots and anticoagulation. The Budd–Chiari syndrome developed in Patient 8.1, presumably owing to hepatic-vein thrombosis. Examination of extended family histories uncovered two additional patients, Patients 4.4 and 5.3, who had died before genotyping from thrombotic events associated with protein-losing enteropathy, lymphangiectasia, and malnutrition. Patient 6.1 died from pulmonary embolism during the course of the study. Hence, the natural history of the disease includes early death related to severe thrombotic events. Details are provided in Table S1, Figures S1 and S6A through S6D, and the Supplemental Patient Clinical Histories section in the Supplementary Appendix.

LOSS-OF-FUNCTION MUTATIONS IN CD55

Whole-exome sequence analysis in Patients 1.1, 2.1, 3.1, and 5.1 revealed novel homozygous variants in the gene encoding the complement regulatory protein CD55 (encoding for decay-accelerating factor) (Fig. 2A, and Table S3 and Figs. S1 and S7A in the Supplementary Appendix).⁹ These variants segregated recessively with disease, and heterozygous persons were unaffected. These variants were not present in the Exome Aggregation Consortium (ExAC) database and were predicted by bioinformatic analysis to be deleterious (Table S4 in the Supplementary Appendix). Further screening of 640 patients with inflammatory bowel disease (some with early-onset or very-early-onset disease) and a cohort of 239 samples obtained from Turkish persons revealed only 1 person heterozygous for a CD55 variant that was predicted to be only moderately deleterious (c.107delTGCCCGCGGCGC; Combined Annotation–Dependent Depletion [CADD] score, 13.23),

which indicates that CD55 loss-of-function variants are rare. Screening of the ExAC database of 60,000 unrelated persons revealed 53 persons who were heterozygous for loss-of-function CD55 variants and 1 who was homozygous for a loss-of-function CD55 variant, possibly from a cohort of persons with inflammatory bowel disease, although a lack of informed consent prevented us from identifying and contacting this person. CD55 had a probability of loss-of-function intolerance (pLI) score of 0.0, which indicates that heterozygous loss-of-function mutations are likely to be benign.¹⁰ Screening of additional patients with early-onset protein-losing enteropathy revealed 6 patients who were homozygous for CD55 loss-of-function variants in Families 4, 6, 7, and 8.

Patients 1.1 and 7.1 were homozygous for a dinucleotide deletion and a 4-nucleotide insertion at nucleotide positions 149 and 150. Patients 2.1, 3.1, 5.1, and 5.2 were homozygous for a single-nucleotide deletion in CD55 at position 109. Patient 8.1 was homozygous for a single-nucleotide insertion at position 367. All three variants resulted in a frameshift and were predicted to cause premature termination of CD55 messenger RNA (mRNA) translation. The variant common to Families 1 and 7 and the variant common to Families 2, 3, and 5 led to mRNA nonsense-mediated decay. In Family 4, a novel homozygous missense mutation in CD55 encodes a cysteine-to-serine substitution in the fourth short consensus repeat domain (c.800G→C, p.Cys267Ser); the wild-type Cys267 disulfide bond with Cys225 is presumably disrupted by the substituted serine at residue 267.¹¹ In Patient 6.1, a variant disrupting an exon 3 splice acceptor site probably caused alternative splicing. In all the patients, CD55 protein expression was lost, with only Patient 6.1 having minor residual expression (Fig. 2B, and Fig. S7D in the Supplementary Appendix). We observed that CD55 was normally expressed on capillary endothelial cells in the basal submucosa and lamina propria, the brush-border columnar epithelium, and infiltrating lymphocytes and was absent in tissues (Fig. 2C). These variants have strong (in Patient 6.1) or very strong (in all our other patients) evidence of pathogenicity, according to the guidelines of the American College of Medical Genetics and Genomics.¹² Altogether, we identified five distinct homozygous, novel, loss-of-function CD55 variants in nine patients from Turkey, one from Syria, and one



from Morocco (Fig. 2A). Details are provided in Figures S1, S6E, and S7 in the Supplementary Appendix.

COMPLEMENT ACTIVATION ON CD55-DEFICIENT CELLS

CD55 is attached to the surface by a glycosylphosphatidylinositol moiety and inhibits complement activation by destabilizing and preventing the formation of C3 and C5 convertases, which prevents complement damage.⁹ We therefore tested

whether CD55 deficiency accelerated complement activation.^{13,14} After incubation with human serum, we observed increased C3 fragment deposition on patients' CD4+ T-cell blasts by staining for an epitope common to C3, C3b, and C3d, which was increased by means of stimulation of the classical pathway by coating the cells with mouse IgG1 (Fig. 3A and 3B). We did not detect the C3b fragment (data not shown), which suggests rapid degradation, possibly by factor I and cofactor activity.¹⁵ CD55 reconstitution reduced complement

Figure 1 (facing page). Clinical Presentation of Eight Families with Familial Early-Onset Protein-Losing Enteropathy.

Panel A shows the pedigrees of the eight families. Affected persons who were homozygous for the mutant allele are indicated by solid symbols, heterozygous persons by half solid symbols, unaffected persons by open symbols, and an affected person with an unknown genotype by an open red symbol. Circles indicate female persons, and squares male persons; the triangle in Family 1 represents a miscarriage, and the slash in Family 5 indicates a person who had died. Patient 6.1 died during the course of the study (indicated by a slash). Affected members of the extended family are shown in Figure S1 in the Supplementary Appendix. Panel B shows the serum levels of IgG in relation to serum albumin concentrations as a function of age in years in Patient 1.1. The age-specific lower cutoff value for IgG is indicated by the red dotted curve, and the reference value for the albumin concentration is more than 3.5 g per deciliter (indicated by the dashed section on the right y axis). Each arrow denotes an episode of pneumonia. Panel C shows sections of resected duodenal tissue (hematoxylin and eosin staining) obtained from Patient 5.2, with markedly dilated lymphovascular spaces within the submucosa (arrows). Panel D shows sections from surgically resected material of the small intestine (hematoxylin and eosin) showing ulceration covered by fibrin with dense granulocytic infiltrate (left panel, double asterisks), granulation tissue (G) with edema in the lamina propria, and reactive epithelial changes (E) in Patient 2.1. Panel D also includes endoscopy photographs showing a mucosal ulcer (blue arrow) and exudate (black arrow) in the terminal ileum of Patient 1.1 (right side, top) and histopathological assessments of a biopsy sample obtained from the ileum of Patient 2.1 and immunohistochemical analysis showing the presence of B cells (CD20) and T cells (CD3) within lymphoid nodules (right side, bottom). Panel E shows an echocardiographic image from Patient 6.1 with thrombus in the right atrium (top, arrow) and inferior vena cava (bottom, arrow).

of either CD46-mediated C3b sensing or anaphylatoxin receptors.¹⁶⁻²⁰ Studies in knockout mice have confirmed a role for Cd55 in adaptive immune regulation, with *Cd55*^{-/-} mice producing more interferon- γ and less interleukin-10 in autoimmune models.^{21,22} CD4+ T cells from patients produced an increased level of tumor necrosis factor (TNF), reduced the interleukin-10 level (with a normal interferon- γ level), and had normal proliferation after T-cell-receptor engagement. Dual inhibition of the anaphylatoxin receptors C3aR and C5aR1 decreased TNF overproduction to control levels, primarily owing to the inhibition of C5aR1. Anaphylatoxin inhibition did not increase the interleukin-10 level, which suggests that this defect in interleukin-10 production is independently regulated.²¹ Inflammatory cytokines, including TNF, could instigate the severe thrombophilia in patients with CD55 deficiency by reducing thrombomodulin and augmenting tissue-factor expression on endothelial cells.²³ We found that TNF and interferon- γ induced procoagulatory decreases in thrombomodulin and increases in tissue factor. CD55 expression increased in human umbilical-vein endothelial cells after TNF treatment, which suggests that CD55 limits complement-mediated damage during inflammation.

CD55 can convey a costimulatory signal for T-cell activation and the production of interleukin-10, a cytokine that is inhibitory to intestinal inflammation.^{24,25} We found that patients' cells showed impaired proliferation and interleukin-10 production in response to an agonistic anti-CD55 antibody or recombinant CD97 together with T-cell-receptor stimulation. Details are provided in Figure S8 in the Supplementary Appendix.

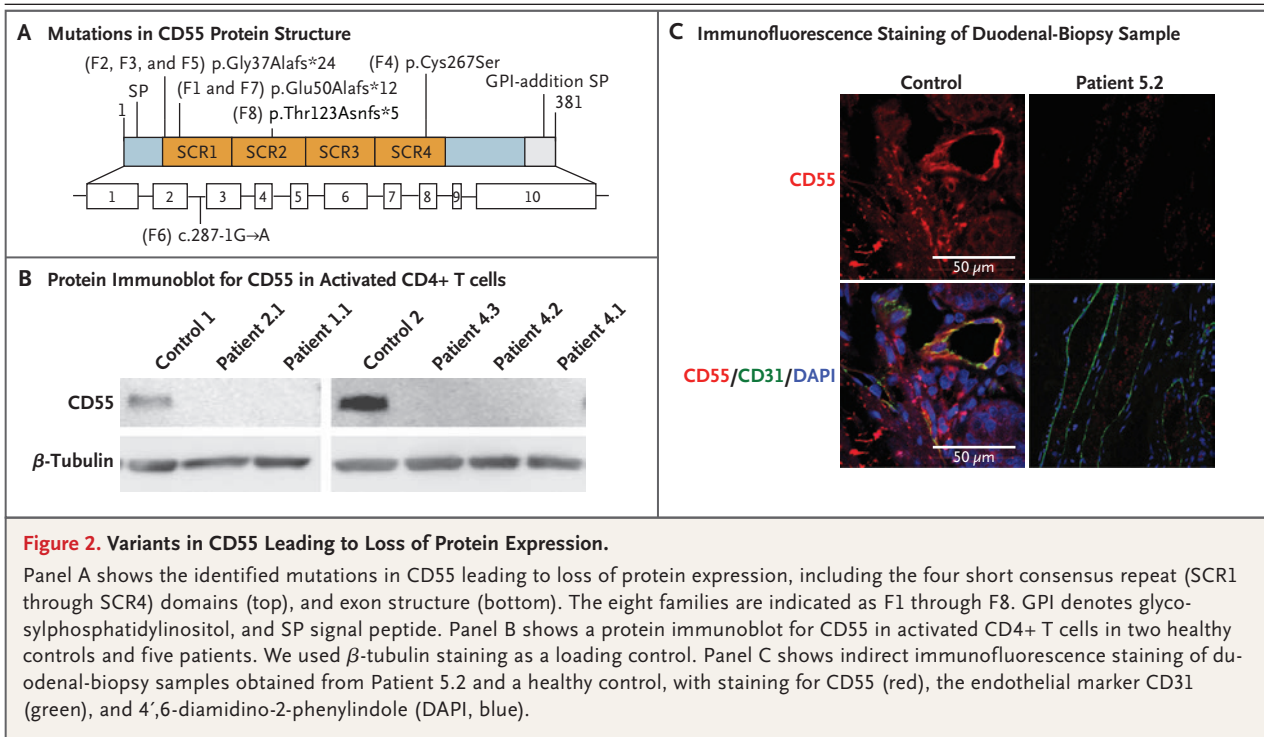
IN VITRO INHIBITION OF COMPLEMENT BY ECULIZUMAB FORMULATION

Finally, we investigated whether clinically available complement inhibitors could prevent the enhanced activation in samples obtained from patients. We observed that C5a production, which was elevated on incubation with patients' cells, was abrogated by coincubation with an experimental formulation of eculizumab, a complement-inhibitory therapeutic agent that is used to treat paroxysmal nocturnal hemoglobinuria (PNH) and the atypical hemolytic-uremic syndrome (Fig. 4).

deposition on T cells in patients (Fig. 3C). Immunohistochemical analysis of duodenal-biopsy samples revealed *in vivo* terminal complement activation (membrane attack complex [C5b-9]) in submucosal arterioles (Fig. 3D). Details are provided in Figure S7A and S7B in the Supplementary Appendix.

EXCESSIVE PRODUCTION OF INFLAMMATORY CYTOKINES BY CD55-DEFICIENT T CELLS

Complement proteins can provide costimulatory and differentiation signals to T cells by means



DISCUSSION

We define a genetic syndrome comprising CD55 deficiency with hyperactivation of complement, angioathic thrombosis, and protein-losing enteropathy (the CHAPLE syndrome). Protein-losing enteropathy is probably caused by primary intestinal lymphangiectasia, intestinal inflammation, and possibly thromboses (Fig. S9 in the Supplementary Appendix).^{2,4}

Complement is a system of interacting proteins that provides host defense by destroying microbes and modulating immunity with soluble anaphylatoxins governed by multiple regulators, including CD55.⁹ Genetic variants that increase complement activation cause PNH, the atypical hemolytic-uremic syndrome, C3 glomerulopathy, and age-related macular degeneration (Fig. S7A in the Supplementary Appendix).²⁶⁻²⁸ PNH results from somatic mutations that disable the glycosylphosphatidylinositol anchor that tethers CD55 and CD59 to the cell surface, leading to complement-mediated hemolysis and thrombosis.²⁹⁻³¹ Heterozygous germline loss-of-function variants affecting C3, factor H, factor I, or CD46 trigger the atypical hemolytic-uremic syndrome by inducing complement-mediated damage to glomerular micro-

vascular endothelial cells, hemolysis, and kidney failure.³² These genetic defects also cause complement-mediated retinal damage and age-related macular degeneration.³³ Unlike PNH or the atypical hemolytic-uremic syndrome, isolated CD55 deficiency causes early-onset protein-losing enteropathy owing to primary intestinal lymphangiectasia and bowel inflammation. We found that CD55 is up-regulated by retinoic acid, which is highly concentrated in the gut from the diet (Fig. S8G in the Supplementary Appendix). Persons with the CHAPLE syndrome and PNH are at increased risk for thrombosis, and thrombotic microangiopathy develops in patients with the atypical hemolytic-uremic syndrome. These findings indicate cross-regulation of the complement and coagulation cascades.

CD55 deficiency has been previously found in persons with sporadic gastrointestinal abnormalities who do not have Cromer blood group red-cell antigens (the Inab phenotype).³⁴⁻³⁷ The Inab phenotype can be transient (as has been observed in three cases) or persistent (as has been observed in nine cases)³⁴⁻³⁷ and is sometimes associated with gastrointestinal disease that has been variably diagnosed as Crohn's disease, capillary angioma, protein-losing enteropathy with intes-

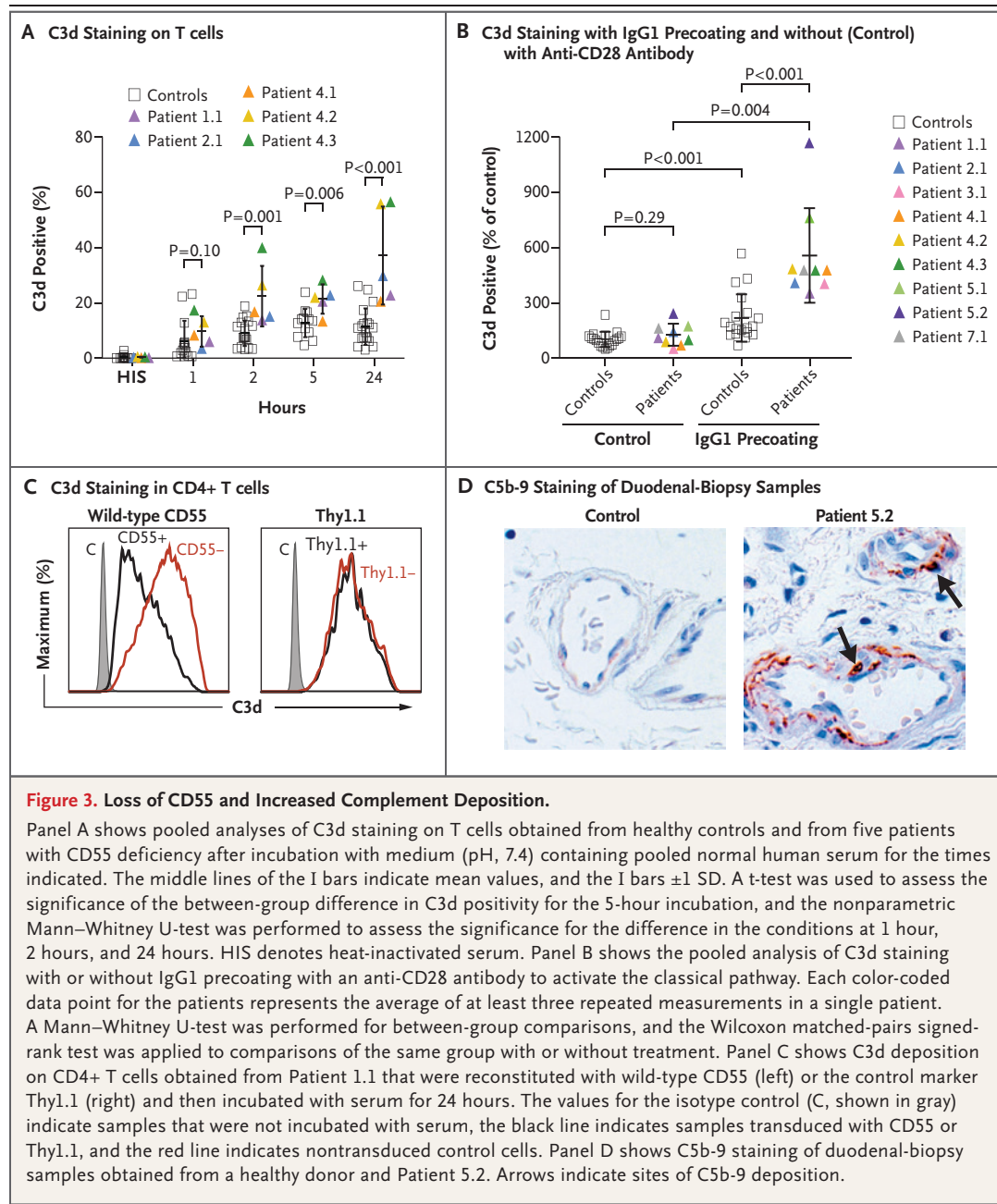


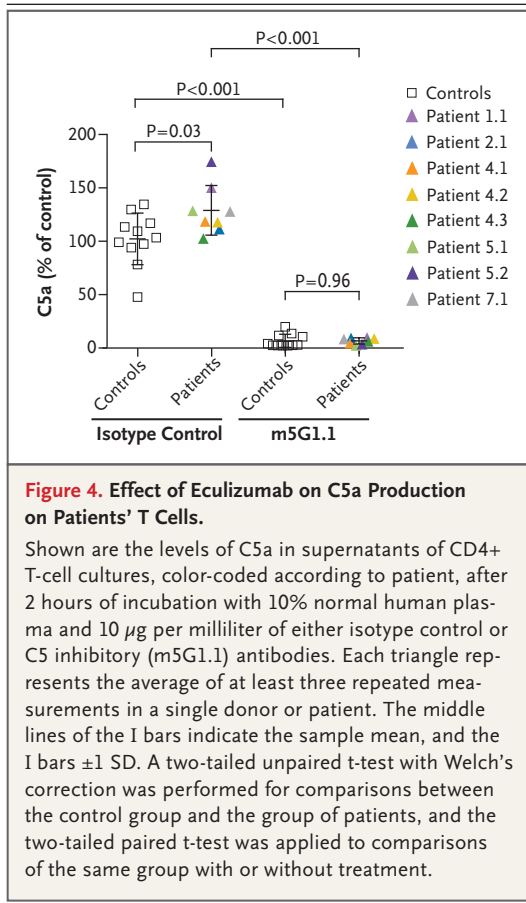
Figure 3. Loss of CD55 and Increased Complement Deposition.

Panel A shows pooled analyses of C3d staining on T cells obtained from healthy controls and from five patients with CD55 deficiency after incubation with medium (pH, 7.4) containing pooled normal human serum for the times indicated. The middle lines of the I bars indicate mean values, and the I bars ± 1 SD. A t-test was used to assess the significance of the between-group difference in C3d positivity for the 5-hour incubation, and the nonparametric Mann–Whitney U-test was performed to assess the significance for the difference in the conditions at 1 hour, 2 hours, and 24 hours. HIS denotes heat-inactivated serum. Panel B shows the pooled analysis of C3d staining with or without IgG1 precoating with an anti-CD28 antibody to activate the classical pathway. Each color-coded data point for the patients represents the average of at least three repeated measurements in a single patient. A Mann–Whitney U-test was performed for between-group comparisons, and the Wilcoxon matched-pairs signed-rank test was applied to comparisons of the same group with or without treatment. Panel C shows C3d deposition on CD4+ T cells obtained from Patient 1.1 that were reconstituted with wild-type CD55 (left) and the control marker Thy1.1 (right) and then incubated with serum for 24 hours. The values for the isotype control (C, shown in gray) indicate samples that were not incubated with serum, the black line indicates samples transduced with CD55 or Thy1.1, and the red line indicates nontransduced control cells. Panel D shows C5b-9 staining of duodenal-biopsy samples obtained from a healthy donor and Patient 5.2. Arrows indicate sites of C5b-9 deposition.

tinal tumor, and food intolerance. Three loss-of-function variants were identified (Fig. S7G in the Supplementary Appendix), although no definitive correlation with disease was made. Also, exacerbated dextran sulfate sodium–induced colitis develops in Cd55-deficient mice, which is consistent with the intestinal disease seen in our patients, as does T-cell–mediated autoimmunity in autoimmune models, probably owing to immunoregulatory abnormalities that are similar to those seen

in our patients.^{21,22,38} Only one patient with the CHAPLE syndrome presented with autoimmunity in the form of polyarthritis, and none had inflammatory markers or elevated levels of cytokine in the blood.

The disease in our patients showed variable expressivity, which was potentially attributable to background genetics, diet, microbiome composition, or other influences. Conventional treatments were only transiently effective, although



more sustained benefit occurred after the resection of lymphangiectatic intestinal segments. Recurrent infections were responsive to intravenous immune globulin. Eculizumab, which suppressed C5a production on patients' cells, warrants further investigation as a potential treatment of the CHAPLE syndrome.³⁹ In line with this, work by Kurolap et al., published in this issue of the *Journal*,⁴⁰ shows that eculizumab therapy in a family with the CHAPLE syndrome resulted in attenuated protein-losing enteropathy and reduced bowel-movement frequency within 100 days after the initiation of therapy.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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