Table 5 | Continued

Disease	Genetic defect/ presumed pathogenesis	Inheritance	Affected cells	Affected function	Associated features	OMIM number
(f) Macrophage gp91 phox deficiency[a]	Mutation in *CYBB*: electron transport protein (gp 91 phox)	XL	Mf only	Killing (faulty O_2^- production)	Isolated susceptibility to *Mycobacteria*	306400
(g) IRF8-deficiency (AD form)[a]	Mutation in *IRF8*: IL-12 production by CD1c$^+$ MDC	AD	CD1c+ MDC	Differentiation of CD1c+ MDC subgroup	Susceptibility to *Mycobacteria*	601565
(h) ISG15	Mutation in *ISG15*; an interferon (IFN) α/β-inducible, ubiquitin-like intracellular protein	AR	M + N + L	IFN-γ secretion	Susceptibility to *Mycobacteria*	14751
5. Other defects						
(a) IRF 8-deficiency (AR form)[a]	Mutation in *IRF8*: IL-12 production	AR	Monocytes periph-eral DC	Cytopenias	Susceptibility to *Mycobacteria*, *Candida*, myeloproliferation	614893
(b) GATA2 deficiency (Mono MAC syndrome)	Mutation in *GATA2*: loss of stem cells	AD	Monocytes periph-eral DC + NK + B	Multilineage cytopenias	Susceptibility to *Mycobacteria*, papilloma viruses, histoplasmosis, alveolar proteinosis, MDS/AML/CMML	137295
(c) Pulmonary alveolar proteinosis[a]	Mutation in *CSF2RA*	Biallelic mutations in pseudo-autosomal gene	Alveolar macro-phages	GM-CSF signaling	Alveolar proteinosis	306250

XL, X-linked inheritance; AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; ACTB, actin beta; B, B lymphocytes; CEBPE, CCAAT/enhancer-binding protein epsilon; CMML, chronic myelomonocytic leukemia; CTSC, cathepsin C; CYBA, cytochrome b alpha subunit; CYBB, cytochrome b beta subunit; DC, dendritic cells; ELANE, elastase neutrophil-expressed; GATA2, GATA binding protein 2; IFN, interferon; IFNGR1, interferon-gamma receptor subunit 1; IFNGR2, interferon-gamma receptor subunit 2; IL-12B, interleukin-12 beta subunit; IL-12RB1, interleukin-12 receptor beta 1; IFR8, interferon regulatory factor 8; F, fibroblasts; FPR1, formylpeptide receptor 1; FUCT1, fucose transporter 1; GFI1, growth factor independent 1; HAX1, HLCS1-associated protein X1; ITGB2, integrin beta-2; L, lymphocytes; M, monocytes–macrophages; MDC, myeloid dendritic cells; MDS, myelodysplasia; Mel, melanocytes; Mϕ, macrophages; MSMD, Mendelian susceptibility to mycobacterial disease; N, neutrophils; NCF1, neutrophil cytosolic factor 1; NCF2, neutrophil cytosolic factor 2; NCF4, neutrophil cytosolic factor 4; NK, natural killer cells; ROBLD3: roadblock domain containing 3; SBDS, Shwachman–Bodian–Diamond syndrome; STAT, signal transducer and activator of transcription.

[a]*Ten or fewer unrelated cases reported in the literature.*

***Table 5** includes seven newly described genetic defects of phagocyte number and/or function including Barth syndrome, Cohen syndrome, and poikiloderma with neutropenia. In these three clinically well-known diseases, the genetic defects have been elucidated, although their molecular pathogenesis remains ill-defined. A new cause of autosomal recessive chronic granulomatous disease, namely a deficiency of the cytosolic activating protein p40 phox, has now been found in two CGD patients and is included under defects of respiratory burst. Under the heading of Mendelian susceptibility of mycobacterial disease (MSMD), two new entities were added: (a) a subgroup of X-linked gp91 phox deficiency with isolated susceptibility to mycobacteria and a defect of the respiratory burst in macrophages only; (b) an autosomal dominant form of IRF8-deficiency, resulting from a lack of CD1c+ myeloid dendritic cells that would normally secrete IL-12. The clinical phenotype of MSMD may vary, depending on the nature of the genetic defect. Finally, GATA2 deficiency was recently identified as the cause of the Mono MAC syndrome, with multilineage cytopenias (of monocytes, peripheral dendritic cells, NK- and B-lymphocytes) resulting in opportunistic infections (including mycobacteria), alveolar proteinosis, and malignancy.*

Table 6 | Defects in innate immunity.

Disease	Genetic defect/ presumed pathogenesis	Inheritance	Affected cell	Functional defect	Associated features	OMIM number
1. Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID)						
(a) EDA-ID, X-linked (NEMO deficiency)	Mutations of *NEMO (IKBKG)*, a modulator of NF-κB activation	XL	Lymphocytes + monocytes	NF-κB signaling pathway	Various infections (bacteria, *Mycobacteria*, viruses, and fungi) Colitis EDA (not in all patients) Hypogammaglobulinemia to specific antibody polysaccharides deficiency	300248
(b) EDA-ID, autosomal-dominant[a]	Gain-of-function mutations of *IKBA*, resulting in impaired activation of NF-κB	AD	Lymphocytes + monocytes	NF-κB signaling pathway	Various infections (bacteria, viruses, and fungi) EDA T cell defect	612132
2. TIR signaling pathway deficiency						
(a) IRAK-4 deficiency	Mutations of *IRAK-4*, a component of TLR- and IL-1R-signaling pathway	AR	Lymphocytes + granulocytes + monocytes	TIR–IRAK signaling pathway	Bacterial infections (pyogenes)	607676
(b) MyD88 deficiency	Mutations of *MYD88*, a component of the TLR and IL-1R signaling pathway	AR	Lymphocytes + granulocytes + monocytes	TIR–MyD88 signaling pathway	Bacterial infections (pyogenes)	612260
3. HOIL1 deficiency[a]	Mutation of *HOIL1*, a component of LUBAC	AR	Lymphocytes + granulocytes + monocytes	NF-κB signaling pathway	Bacterial infections (pyogenes) Autoinflammation Amylopectinosis	Not assigned
4. WHIM (Warts, hypogammaglobulinemia, infections, myelokathexis) syndrome	Gain-of-function mutations of *CXCR4*, the receptor for CXCL12	AD	Granulocytes + lymphocytes	Increased response of the CXCR4 chemokine receptor to its ligand CXCL12 (SDF-1)	Warts/human papilloma virus (HPV) infection Neutropenia Reduced B cell number Hypogammaglobulinemia	193670
5. Epidermodysplasia verruciformis						
EVER1 deficiency	Mutations of *EVER1*	AR	Keratinocytes and leukocytes	EVER proteins may be involved in the regulation of cellular zinc homeostasis in lymphocytes	HPV (group B1) infections and cancer of the skin (typical EV)	226400
EVER2 deficiency	Mutations of *EVER2*	AR	Keratinocytes and leukocytes	EVER proteins may be involved in the regulation of cellular zinc homeostasis in lymphocytes	HPV (group B1) infections and cancer of the skin (typical EV)	226400
6. Predisposition to severe viral infection						
(a) STAT2 deficiency[a]	Mutations of *STAT2*	AR	T and NK cells	STAT2-dependent IFN-α and -β response	Severe viral infections (disseminated vaccine-strain measles)	Not assigned

(Continued)

Table 6 | Continued

Disease	Genetic defect/ presumed pathogenesis	Inheritance	Affected cell	Functional defect	Associated features	OMIM number
(b) MCM4 deficiency[a]	Mutations in *MCM4*	AR	NK cells	DNA repair disorder	Viral infections (EBV, HSV, VZV) Adrenal failure Short stature	609981
7. Herpes simplex encephalitis (HSE)						
(a) TLR3 deficiency[a]	(b) Mutations of *TLR3*	AD AR	Central nervous system (CNS) resident cells and fibroblasts	TLR3-dependent IFN-α, -β, and -λ induction	Herpes simplex virus 1 encephalitis (incomplete clinical penetrance for all etiologies listed here)	613002
(b) UNC93B1 deficiency[a]	(a) Mutations of *UNC93B1*	AR	CNS resident cells and fibroblasts	UNC-93B-dependent IFN-α, -β, and -λ induction	Herpes simplex virus 1 encephalitis	610551
(c) TRAF3 deficiency[a]	(c) Mutations of *TRAF3*	AD	CNS resident cells and fibroblasts	TRAF3-dependent IFN-α, -β, and -λ induction	Herpes simplex virus 1 encephalitis	614849
(d) TRIF deficiency[a]	(c) Mutations of *TRIF*	AD AR	CNS resident cells and fibroblasts	TRIF-dependent IFN-α, -β, and -λ induction	Herpes simplex virus 1 encephalitis	614850
(e) TBK1 deficiency[a]	(c) Mutations of *TBK1*	AD	CNS resident cells and fibroblasts	TBK1-dependent IFN-α, -β, and -λ induction	Herpes simplex virus 1 encephalitis	Not assigned
8. Predisposition to invasive fungal diseases[a]						
CARD9 deficiency	Mutations of *CARD9*	AR	Mononuclear phagocytes	CARD9 signaling pathway	Invasive candidiasis infection Deep dermatophytoses	212050
9. Chronic mucocutaneous candidiasis (CMC)						
(a) IL-17RA deficiency[a]	(a) Mutations in *IL-17RA*	AR	Epithelial cells, fibroblasts, mononuclear phagocytes	IL-17RA signaling pathway	CMC Folliculitis	605461
(b) IL-17F deficiency[a]	(b) Mutations in *IL-17F*	AD	T cells	IL-17F-containing dimers	CMC Folliculitis	606496
(c) STAT1 gain-of-function	(c) Gain-of-function mutations in *STAT1*	AD	T cells	Gain-of-function STAT1 mutations that impair the development of IL-17-producing T cells	CMC Various fungal, bacterial, and viral (HSV) infections Autoimmunity (thyroiditis, diabetes, cytopenia) Enteropathy	614162
(d) ACT1 deficiency[a]	(c) Mutations in *ACT1*	AR	T cells, fibroblasts	Fibroblasts fail to respond to IL-17A and IL-17F, and their T cells to IL-17E	CMC Blepharitis, folliculitis, and macroglossia	615527
10. Trypanosomiasis[a]	Mutations in *APOL-I*	AD		APOL-I	Trypanosomiasis	603743

(Continued)

Table 6 | Continued

Disease	Genetic defect/ presumed pathogenesis	Inheritance	Affected cell	Functional defect	Associated features	OMIM number
11. Isolated congenital asplenia (ICA)	Mutations in *RPSA*	AD	Spleen	RPSA encodes ribosomal protein SA, a component of the small subunit of the ribosome	Bacteremia (encapsulated bacteria) No spleen	271400

XL, X-linked inheritance; AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; NF-κB, nuclear factor kappa B; TIR, Toll and interleukin 1 receptor; IFN, interferon; HVP, human papilloma virus; TLR, Toll-like receptor; IL, interleukin.

[a]Ten or fewer unrelated cases reported in the literature.

*Eight new disorders have been added to **Table 6**. Three new entries have been added in the table. One is a new PID with the association of recurrent bacterial infections, autoinflammation, and amylopectinosis caused by AR HOIL1 mutations found in two kindreds. The second is severe viral infection, for which three genetic etiologies have been discovered. AR-STAT2 deficiency and AR-CD16 deficiency have been found in one kindred each. AR MCM4 deficiency has been found in several Irish kindreds. The third is isolated congenital asplenia identified in 18 patients from 8 kindreds.*

XR-EDA-ID is highly heterogeneous clinically, both in terms of developmental features (some patients display osteopetrosis and lymphedema, in addition to EDA, while others do not display any developmental features) and infectious diseases (some display multiple infections, viral, fungal, and bacterial, while others display a single type of infection). The various OMIM entries correspond to these distinct clinical diseases.

Table 7 | Autoinflammatory disorders.

Disease	Genetic defect/ presumed pathogenesis	Inheritance	Affected cells	Functional defects	Associated features	OMIM number
1. Defects effecting the inflammasome						
(a) Familial Mediterranean fever	Mutations of *MEFV (lead to gain of pyrin function, resulting in inappropriate IL-1β release)*	AR	Mature granulocytes, cytokine-activated monocytes	Decreased production of pyrin permits ASC-induced IL-1 processing and inflammation following subclinical serosal injury; macrophage apoptosis decreased	Recurrent fever, serositis, and inflammation responsive to colchicine. Predisposes to vasculitis and inflammatory bowel disease	249100
(b) Mevalonate kinase deficiency (hyper IgD syndrome)	Mutations of *MVK (lead to a block in the mevalonate pathway). Interleukin-1beta mediates the inflammatory phenotype*	AR		Affecting cholesterol synthesis; pathogenesis of disease is unclear	Periodic fever and leukocytosis with high IgD levels	260920
(c) Muckle–Wells syndrome	Mutations of *CIAS1 (also called PYPAF1 or NALP3) lead to constitutive activation of the NLRP3 inflammasome*	AD	PMNs monocytes	Defect in cryopyrin, involved in leukocyte apoptosis and NF-κB signaling and IL-1 processing	Urticaria, SNHL, amyloidosis	191900
(d) Familial cold autoinflammatory syndrome	Mutations of *CIAS1* (see above) Mutations of *NLRP12*	AD	PMNs, monocytes	Same as above	Non-pruritic urticaria, arthritis, chills, fever, and leukocytosis after cold exposure	120100

(Continued)

Table 7 | Continued

Disease	Genetic defect/ presumed pathogenesis	Inheritance	Affected cells	Functional defects	Associated features	OMIM number
5. Neonatal onset multisystem inflammatory disease (NOMID) or chronic infantile neurologic cutaneous and articular syndrome (CINCA)	Mutations of *CIAS1* (see above)	AD	PMNs, chondrocytes	Same as above	Neonatal onset rash, chronic meningitis, and arthropathy with fever and inflammation	607115
2. Non inflammasome-related conditions						
(a) TNF receptor-associated periodic syndrome (TRAPS)	Mutations of *TNFRSF1* (resulting in increased TNF inflammatory signaling)	AD	PMNs, monocytes	Mutations of 55-kDa TNF receptor leading to intracellular receptor retention or diminished soluble cytokine receptor available to bind TNF	Recurrent fever, serositis, rash, and ocular or joint inflammation	142680
(b) Early-onset inflammatory bowel disease	Mutations in *IL-10 (results in increase many proinflammatory cytokines)*	AR	Monocyte/ macrophage, activated T cells	IL-10 deficiency leads to increase of TNFγ and other proinflammatory cytokines	Early-onset enterocolitis enteric fistulas, perianal abscesses, chronic folliculitis	124092
(b) Early-onset inflammatory bowel disease	Mutations in *IL-10RA (see above)*	AR	Monocyte/ macrophage, activated T cells	Mutation in IL-10 receptor alpha leads to increase of TNFγ and other proinflammatory cytokines	Early-onset enterocolitis enteric fistulas, perianal abscesses, chronic folliculitis	146933
(b) Early-onset inflammatory bowel disease	Mutations in *IL-10RB (see above)*	AR	Monocyte/ macrophage, activated T cells	Mutation in IL-10 receptor beta leads to increase of TNFγ and other proinflammatory cytokines	Early-onset enterocolitis enteric fistulas, perianal abscesses, chronic folliculitis	123889
(c) Pyogenic sterile arthritis, pyoderma gangrenosum, acne (PAPA) syndrome	Mutations of *PSTPIP1* (also called C2BP1) (affects both pyrin and protein tyrosine phosphatase to regulate innate and adaptive immune responses)	AD	Hematopoietic tissues, upregulated in activated T cells	Disordered actin reorganization leading to compromised physiologic signaling during inflammatory response	Destructive arthritis, inflammatory skin rash, myositis	604416
(d) Blau syndrome	Mutations of *NOD2* (also called CARD15) (involved in various inflammatory processes)	AD	Monocytes	Mutations in nucleotide binding site of CARD15, possibly disrupting interactions with lipopolysaccharides and NF-κB signaling	Uveitis, granulomatous synovitis, camptodactyly, rash, and cranial neuropathies, 30% develop Crohn's disease	186580
10. Chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia (Majeed syndrome)[a]	Mutations of *LPIN2* (increased expression of the proinflammatory genes)	AR	Neutrophils, bone marrow cells	Undefined	Chronic recurrent multifocal osteomyelitis, transfusion-dependent anemia, cutaneous inflammatory disorders	609628

(Continued)

Table 7 | Continued

Disease	Genetic defect/ presumed pathogenesis	Inheritance	Affected cells	Functional defects	Associated features	OMIM number
11. DIRA (deficiency of the interleukin 1 receptor antagonist)[a]	Mutations of *IL1RN* (see functional defect)	AR	PMNs, monocytes	Mutations in the IL-1 receptor antagonist allow unopposed action of Interleukin 1	Neonatal onset of sterile multifocal osteomyelitis, periostitis, and pustulosis	612852
12. DITRA – deficiency of IL-36 receptor antagonist	Mutation in *IL36RN* (see functional defect)	AR	Keratinocyte leukocytes	Mutations in IL-36RN leads to increase IL-8 production	Pustular psoriasis	614204
13. SLC29A3 mutation	Mutation in SLC29A3 (?)	AR	Leukocyte, bone cells	Macrophage activation?	Hyperpigmentation hypertrichosis	602782
14. CAMPS (CARD14 mediated psoriasis)	Mutation in *CARD14* (see functional defect)	AD	Mainly in keratinocyte	Mutations in CARD14 activate the NF-κB pathway and production of IL-8	Psoriasis	173200
15. Cherubism	Mutation in *SH3BP2* (see functional defect)	AD	Stroma cells, bone cells	Hyperactivated macrophage and increased NF-κB	Bone degeneration in jaws	11840
16. CANDLE (chronic atypical neutrophilic dermatitis with lipodystrophy)	Mutation in *PSMB8* (see functional defect)	AD	Keratinocyte, B cell adipose cells	Mutations cause increase IL-6 production	Dystrophy, panniculitis	256040
17. HOIL1 deficiency	Mutation in *HOIL1* (see functional defect)	AR	PMNs, fibroblast	Mutation in *HOIL1* leads to IL-1β dysfunction	Immunodeficiency autoinflammation amylopectinosis	610924
18. PLAID (PLCγ2 associated antibody deficiency and immune dysregulation)	Mutation in *PLCG2* (see functional defect)	AD	B cells, NK, mast cells	Mutations cause activation of IL-1 pathways	Cold urticaria hypogammaglobulinemia	614878

AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; PMN, polymorphonuclear cells; ASC, apoptosis-associated speck-like protein with a caspase recruitment domain; CARD, caspase recruitment domain; CD2BP1, CD2 binding protein 1; PSTPIP1, proline/serine/threonine phosphatase-interacting protein 1; SNHL, sensorineural hearing loss; CIAS1, cold-induced autoinflammatory syndrome 1.

[a]*Ten or fewer unrelated cases reported in the literature.*

Autoinflammatory diseases are clinical disorders marked by abnormally increased inflammation, mediated predominantly by the cells and molecules of the innate immune system, with a significant host predisposition. While the genetic defect of one of the most common autoinflammatory conditions, PFAPA, is not known, recent studies suggest that it is associated with activation of IL-1 pathway and response to IL-1beta antagonists.

Muckle–Wells syndrome, familial cold autoinflammatory syndrome and neonatal onset multisystem inflammatory disease (NOMID), which is also called chronic infantile neurologic cutaneous and articular syndrome (CINCA) are caused by similar mutations in CIAS1 mutations. The disease phenotype in any individual appears to depend on modifying effects of other genes and environmental factors.

Table 8 | Complement deficiencies.

Disease	Genetic defect; presumed pathogenesis	Inheritance	Functional defect	Associated features	OMIM number
1. C1q deficiency	Mutation in *C1QA, C1QB, C1QC*: classical complement pathway components	AR	Absent CH50 hemolytic activity, defective activation of the classical pathway Diminished clearance of apoptotic cells	SLE, infections with encapsulated organisms	120550; 601269; 120575
2. C1r deficiency	Mutation in *C1R*: classical complement pathway component	AR	Absent CH50 hemolytic activity, defective activation of the classical pathway	SLE, infections with encapsulated organisms	216950
3. C1s deficiency	Mutation in *C1S*: classical complement pathway component	AR	Absent CH50 hemolytic activity, defective activation of the classical pathway	SLE, infections with encapsulated organisms	120580
4. C4 deficiency	Mutation in *C4A, C4B*: classical complement pathway components	AR	Absent CH50 hemolytic activity, defective activation of the classical pathway, defective humoral immune response to carbohydrate antigens in some patients	SLE, infections with encapsulated organisms	120810; 120820
5. C2 deficiency	Mutation in *C2*: classical complement pathway component	AR	Absent CH50 hemolytic activity, defective activation of the classical pathway	SLE, infections with encapsulated organisms, atherosclerosis	217000
6. C3 deficiency	Mutation in *C3*: central complement component	AR, gain-of-function AD	Absent CH50 and AH50 hemolytic activity defective opsonization Defective humoral immune response	Infections; glomerulonephritis Atypical hemolytic–uremic syndrome with gain-of-function mutations	120700
7. C5 deficiency	Mutation in *C5*: terminal complement component	AR	Absent CH50 and AH50 hemolytic activity; defective bactericidal activity	Neisserial infections	120900
8. C6 deficiency	Mutation in *C6*: terminal complement component	AR	Absent CH50 and AH50 hemolytic activity; defective bactericidal activity	Neisserial infections	217050
9. C7 deficiency	Mutation in *C7*: terminal complement component	AR	Absent CH50 and AH50 hemolytic activity; defective bactericidal activity	Neisserial infections	217070
10. C8 α–γ deficiency	Mutation in *C8A, C8G*: terminal complement components	AR	Absent CH50 and AH50 hemolytic activity; defective bactericidal activity	Neisserial infections	120950
11. C8b deficiency	Mutation in *C8B*: Terminal complement component	AR	Absent CH50 and AH50 hemolytic activity; defective bactericidal activity	Neisserial infections	120960
12. C9 deficiency	Mutation in *C9*: Terminal complement component	AR	Reduced CH50 and AP50 hemolytic activity; deficient bactericidal activity	Mild susceptibility to Neisserial infections	613825

(Continued)

Table 8 | Continued

Disease	Genetic defect; presumed pathogenesis	Inheritance	Functional defect	Associated features	OMIM number
13. C1 inhibitor deficiency	Mutation in *SERPING1*: regulation of kinins and complement activation	AD	Spontaneous activation of the complement pathway with consumption of C4/C2 Spontaneous activation of the contact system with generation of bradykinin from high molecular weight kininogen	Hereditary angioedema	138470
14. Factor B[a]	Mutation in *CFB*: activation of the alternative pathway	AD	Gain-of-function mutation with increased spontaneous AH50	aHUS	138470
15. Factor D deficiency	Mutation in *CFD*: regulation of the alternative complement pathway	AR	Absent AH50 hemolytic activity	Neisserial infections	134350
16. Properdin deficiency	Mutation in *CFP*: regulation of the alternative complement pathway	XL	Absent AH50 hemolytic activity	Neisserial infections	312060
17. Factor I deficiency	Mutation in *CFI*: regulation of the alternative complement pathway	AR	Spontaneous activation of the alternative complement pathway with consumption of C3	Infections, Neisserial infections, aHUS, preeclampsia, membranoproliferative glomerulonephritis (MPGN)	610984
18. Factor H deficiency	Mutation in *CFH*: regulation of the alternative complement pathway	AR	Spontaneous activation of the alternative complement pathway with consumption of C3	Infections, Neisserial infections, aHUS, preeclampsia, membranoproliferative glomerulonephritis (MPGN)	609814
19. Factor H-related protein deficiencies	Mutation in *CFHR1-5*: bind C3b	AR	Normal CH50, AH50, autoantibodies to Factor H	aHUS	235400
20. Thrombomodulin[a]	Mutation in *THBD*: regulates complement and coagulant activation	AD	Normal CH50, AH50	aHUS	188040
21. MASP1 deficiency	Mutation in *MASP1*: cleaves C2 and activates MASP2	AR	Deficient activation of the lectin activation pathway, cell migration	Infections, 3MC syndrome	600521
22. MASP2 deficiency[a]	*MASP2*: cleavage of C2 and C4	AR	Deficient activation of the lectin activation pathway	Pyogenic infections; inflammatory lung disease, autoimmunity	605102
23. 3MC syndrome COLEC11 deficiency[a]	Mutation in *COLEC11*: binds MASP1, MASP3	AR	Loss of neural crest cell migration signals	A developmental syndrome of facial dysmorphism, cleft lip and/or palate, craniosynostosis, learning disability, and genital, limb, and vesicorenal anomalies (3MC syndrome)	612502

(Continued)

Table 8 | Continued

Disease	Genetic defect; presumed pathogenesis	Inheritance	Functional defect	Associated features	OMIM number
24. Complement receptor 2 (CR2) deficiency[a]	Mutation in *CD21*	AR	See CD21 deficiency in **Table 3**		120650
25. Complement receptor 3 (CR3) deficiency	Mutation in *ITGB2*	AR	See LAD1 in **Table 5**		116920
Membrane cofactor protein (CD46) deficiency	Mutation in *CD46*: dissociates C3b and C4b	AD	Inhibitor of complement alternate pathway, decreased C3b binding	aHUS, infections, preeclampsia	120920
Membrane Attack Complex inhibitor (CD59) deficiency[a]	Mutation in *CD59*: regulates the membrane attack complex formation	AR	Erythrocytes highly susceptible to complement-mediated lysis	Hemolytic anemia, polyneuropathy	107271
Ficolin 3 deficiency[a]	Mutation in *FCN3*: activates the classical complement pathway	AR	Absence of complement activation by the Ficolin 3 pathway	Respiratory infections, abscesses	604973

XL, X-linked inheritance; AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; MAC, membrane attack complex; SLE, systemic lupus erythematosus; MBP, mannose-binding protein; MASP2, MBP-associated serine protease 2.

[a]Ten or fewer unrelated cases reported in the literature.

*New entities added to **Table 8** demonstrate the important role of complement regulators in a group of well-described inflammatory disorders. In particular, we have added mutations in membrane bound as well as surface attached soluble complement regulatory proteins recognized in hemolytic–uremic syndrome, age-related macular degeneration, and preeclampsia. The connecting theme of these otherwise unrelated clinical events is excessive activation or insufficient regulation of C3; these events lead to recruitment of leukocytes and permit secretion of inflammatory and anti-angiogenic mediators that disrupt the vascular bed of the target organ. Alterations in the genes for Factor B (CFB), Factor I (CFI), Factor H (CFH), and CD46 act as susceptibility genes rather than disease causing mutations. Population studies reveal no detectable increase in infections in MBP (also known at mannose-binding lectin – MBL) deficient adults. The 3MC syndrome, a developmental syndrome, has been variously called Carnevale, Mingarelli, Malpuech, and Michels syndrome.*

Table 9 | Phenocopies of PID.

Disease	Genetic defect/ presumed pathogenesis	Circulating T cells	Circulating B cells	Serum Ig	Associated features/ similar PID
Associated with somatic mutations					
(a) Autoimmune lymphoproliferative syndrome (ALPS–SFAS)	Somatic mutation in *TNFRSF6*	Increased $CD4^-$ $CD8^-$ double negative (DN) T alpha/beta cells	Normal, but increased number of $CD5^+$ B cells	Normal or increased	Splenomegaly, lymphadenopathy, autoimmune cytopenias Defective lymphocyte apoptosis/*ALPS–FAS (=ALPS type Im)*
(b) RAS-associated autoimmune leukoproliferative disease (RALD)	Somatic mutation in *KRAS* (gain-of-function)	Normal	B cell lymphocytosis	Normal or increased	Splenomegaly, lymphadenopathy, autoimmune cytopenias, granulocytosis, monocytosis/*ALPS-like*

(Continued)

Table 9 | Continued

Disease	Genetic defect/ presumed pathogenesis	Circulating T cells	Circulating B cells	Serum Ig	Associated features/ similar PID
(c) RAS-associated autoimmune leukoproliferative disease (RALD)	Somatic mutation in *NRAS* (gain-of-function)	Increased $CD4^-$ $CD8^-$ double negative (DN) T alpha/beta cells	Lymphocytosis		Splenomegaly, lymphadenopathy, autoantibodies/*ALPS-like*
Associated with autoantibodies					
(a) Chronic mucocutaneous candidiasis (isolated or with APECED syndrome)	Germline mutation in *AIRE* AutoAb to IL-17 and/or IL-22	Normal	Normal	Normal	Endocrinopathy, chronic mucocutaneous candidiasis/*CMC*
(b) Adult-onset immunodeficiency	AutoAb to IFN gamma	Decreased naive T cells	Normal	Normal	Mycobacterial, fungal, *Salmonella* VZV infections/*MSMD, or CID*
(c) Recurrent skin infection	AutoAb to IL-6	Normal	Normal	Normal	Staphylococcal infections/*STAT3 deficiency*
(d) Pulmonary alveolar proteinosis	AutoAb to GM-CSF	Normal	Normal	Normal	Pulmonary alveolar proteinosis, cryptococcal meningitis/*CSF2RA deficiency*
(e) Acquired angioedema	AutoAb to CI inhibitor	Normal	Normal	Normal	Angioedema/*C1 INH deficiency* (hereditary angioedema)

The rapid advances in gene identification technology, including the widespread use of whole exome and whole genome sequencing, has meant that the ability to identify gene defects in affected families and even single individuals with inherited diseases has grown enormously. In this report, over 30 new gene defects have been added that were identified since the previous classification in November, 2011. These defects can be found in all major groups of PIDs included in this report. In many cases, the mutations are not necessarily in genes formally implicated in immune cell function but are genes involved in essential cell processes. The more detailed analysis and functional consequences of such defects as illustrated by these PIDs will increase our understanding of the interplay between different cellular processes in the development and function of the immune system.

Among the newly identified, gene defects are many that are to date particular to a single pedigree or individual; such defects may prove exceedingly rare, or indeed may not necessarily be found to recur in other individuals. We have marked conditions for which there are 10 or fewer reported individuals with an asterisk, although historically, following the description of the first few cases, additional individuals with a similar PID phenotype and genotype have often been recognized. It is likely that we will uncover many more "personal" or very rare gene defects over time and that the spectrum of PIDs will become increasingly diverse and complex, due to contributions of both environmental exposures and genetic modifiers to each affected individual. The value of this report therefore to capture and catalog the full spectrum at any one time point becomes increasingly important.

The goal of the IUIS Expert Committee on PIDs is to increase awareness, facilitate recognition, and promote optimal treatment for patients with PIDs. In addition to the current report and previous "classification table" publications, the committee has also produced a "Phenotypic Approach for IUIS PID Classification and Diagnosis: Guidelines for Clinicians at the Bedside," which aims to lead physicians to particular groups of PIDs starting from clinical features and combining routine immunological investigations. Together, these contributions will hopefully allow a practical clinical framework for PID diagnosis. The committee also aims to establish a classification of PIDs based on other aspects and will work on publishing further guidelines in due course.

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Analysis of somatic hypermutations in the IgM switch region in human B cells

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Background: The molecular mechanism of class-switch recombination (CSR) in human subjects has not been fully elucidated. The CSR-induced mutations occurring in the switch region of the IgM gene (Smu-SHMs) in *in vitro* CSR-activated and *in vivo* switched B cells have been analyzed in mice but not in human subjects.

Objective: We sought to better characterize the molecular mechanism of CSR in human subjects.

Methods: Smu-SHMs were analyzed *in vitro* and *in vivo* by using healthy control subjects and patients with molecularly defined CSR defects.

Results: We found that Smu-SHMs can be induced *in vitro* by means of CSR activation in human subjects. We also found large amounts of Smu-SHMs in *in vivo* class-switched memory B cells, smaller (although significant) amounts in unswitched memory B cells, and very low amounts in naive B cells. In class-switched memory B cells a high frequency of Smu-SHMs was found throughout the Smu. In unswitched memory B cells, the Smu-SHM frequency was significantly decreased in the 5′ part of the Smu. The difference between switched and unswitched B cells suggests that the extension of somatic hypermutation (SHM) to the 5′ upstream region of the Smu might be associated with the effective induction of CSR. The analysis of the pattern of mutations within and outside the WRCY/RGYW (W, A/T; R, A/G; and Y, C/T) motifs, as well as the Smu-SHMs, in CD27$^+$ B cells from CD40 ligand (CD40L)–, activation-induced cytidine deaminase (AID)–, and uracil-DNA glycosylase (UNG)–deficient patients revealed the dependence of Smu-SHM on CD40L, AID, UNG, and the mismatch repair system in human subjects.

Conclusion: CD40L-, AID-, UNG-, and mismatch repair system–dependent Smu-SHMs and extension to the 5′ region of Smu are necessary to accomplish effective CSR in human subjects. (J Allergy Clin Immunol 2014;134:411-9.)

Key words: Activation-induced cytidine deaminase, somatic hypermutation, antibody maturation, uracil-DNA glycosylase, class-switch recombination, immunoglobulin switch region

Several single gene defects are known to cause immunoglobulin class-switch recombination (CSR) defects, resulting in hyper-IgM syndromes. The affected patients have severely impaired IgG, IgA, and IgE production but normal-to-high serum IgM levels associated with recurrent bacterial infections. Of these, 2 gene defects, namely CD40 ligand (CD40L) deficiency and CD40 deficiency, are associated with bacterial and opportunistic infections and unfavorable prognosis. In contrast, patients with B cell–intrinsic CSR defects, including activation-induced cytidine deaminase (AID) deficiency[1] and uracil-DNA glycosylase (UNG) deficiency,[2] have a milder clinical course, particularly when treated with prophylactic immunoglobulin infusions, but frequently have autoimmune diseases.[3]

CSR is the mechanism that induces functional diversity into immunoglobulin molecules. The first step of CSR is the germline transcription of and R-loop formation in the immunoglobulin switch (S) region. In the second step DNA lesions and single-strand DNA breaks are introduced. In the third step the single-strand DNA break leads to double-strand DNA breaks through mechanisms that are not yet fully understood. In the fourth step double-strand DNA breaks are joined by nonhomologous end-joining enzymes, most likely through alternative end-joining using microhomology[4] and not homologous recombination.[5]

In the second step uracil is introduced through the deoxycytidine deamination induced by AID and is then removed by base excision repair molecules to form single-strand DNA breaks. This process requires uracil DNA glycosylase (UNG) and AP-endonuclease, as well as several mismatch repair (MMR) proteins, including PMS2, MSH2, MSH6, and EXO1.[5] During this process, a somatic hypermutation (SHM) is introduced into the S region (S-SHM).[6,7] This S-SHM is likely to be closely associated with CSR but has not been well investigated, particularly in human B cells.

An SHM is also introduced into the variable (V) region of the immunoglobulin gene (V-SHM), which results in production of high-affinity immunoglobulin.[8] Several molecules are known to be used by both V-SHM and S-SHM, but the molecular mechanisms are different and need to be clarified. In AID-deficient

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Abbreviations used
AID: Activation-induced cytidine deaminase
CD40L: CD40 ligand
CSR: Class-switch recombination
MMR: Mismatch repair
SHM: Somatic hypermutation
Smu: Switch region of IgM
UNG: Uracil-DNA glycosylase

human subjects and mice, the frequency of V-SHM is extremely decreased.[1,9] In UNG deficiency the frequency of V-SHM is normal, but the introduced DNA sequences are severely skewed.[2] However, although data on S-SHM in dominant-negative AID and UNG-deficient human B cells have been reported,[10] no data are available on purified B-cell populations. After CSR activation in mice, an SHM has also been found in the S region not only near the recombination site but also in the far upstream 5′ region of the highly repetitive S-core region. S-SHMs are induced *in vitro* on CSR activation in Smu regions, as well as in Sgamma, Salpha, and Sepsilon regions, in mice.[6,7,11]

In this study we analyzed SHMs in the Smu region (Smu-SHM, Fig 1) in purified human B cells. We found that an Smu-SHM can be induced by CD40 and IL-4 stimulation, which vigorously induces CSR *in vitro*. The Smu-SHM frequency was different depending on the B-cell maturation stage and was defective in cases of CD40L, AID, and UNG deficiency. The analysis of the mutation pattern of the nucleotides suggests the involvement of a MMR in CSR.

METHODS

Patients and control subjects

Peripheral blood samples were obtained from 4 healthy adults between 32 and 36 years of age (C1-C4) and 3 age-matched healthy children (1, 8, and 11 years old [C5-C7]). P1 was a patient with newly diagnosed AID deficiency (homozygous nonsense mutation leading to W80X) with typical hyper-IgM syndrome (IgM, 386 mg/dL; IgA, <6 mg/dL; and IgG, <10 mg/dL). The other 4 AID-deficient patients (P2-P5) and the 2 UNG-deficient patients (P6 and P7) have been previously reported: P2 and P3 were P5 and P4, respectively, in the study conducted by Revy et al[1]; P4 was the younger brother of P10 and P5 was P12 in the study conducted by Zhu et al[12]; and P6 and P7 were P2 and P3 in the study conducted by Imai et al.[2] P8 to P11 were CD40L-deficient patients. The study was approved by the Institutional Review Board of the National Defense Medical College. Informed consent from each participant or their parents was obtained in accordance with the Declaration of Helsinki.

Detection of SHM in the Smu region of the immunoglobulin gene

The genomic DNA from PBMCs was extracted by using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany). The upstream flanking region to the highly repetitive Smu core region was amplified with *PfuTurbo* DNA polymerase (Stratagene, La Jolla, Calif) and the following primers (Fig 1): Imu-1S, 5′-AGATTCTGTTCCGAATCACCGATG-3′; and Smu-1AS, 5′-CCATCTGAGTCCATTTCTGA-3′. A 1128-bp fragment was sequenced from position 12,880 to position 13,794 of NT_010168.8. Within this region, the G/C content and the RGYW/WRCY (W, A/T; R, A/G; and Y, C/T) motifs are equally distributed, as shown in Fig 1.

The GeneAmp PCR System 9700 (Applied Biosystems, Foster City, Calif) was used for the PCR reaction by using the following temperature program: 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 2 minutes. After the PCR products were subcloned with the Topo TA cloning kit for Sequencing (Invitrogen, Carlsbad, Calif), the plasmid was extracted by using Perfectprep Plasmid 96Vac Direct Bind (Eppendorf AG, Hamburg, Germany). The sequencing reaction of the obtained plasmid was conducted by using the BigDye Terminator V1.1 cycle sequencing kit (Applied Biosystems) and analyzed with Genetic analyzer 3130XL (Applied Biosystems). More than 18 unique clones (20,304 bases) were analyzed for each control.

In vitro induction of SHM in Smu-SHM in control B cells after CSR activation

PBMCs were separated by means of Ficoll-Hypaque density centrifugation with Lymphoprep (Axis-Shied PoC) and were cultured at a final concentration of 2.0×10^6 cells per milliliter under standard conditions (37°C and 5% CO_2) in RPMI 1640 medium (Invitrogen) containing 10% heat-inactivated FCS (Invitrogen), penicillin (100 U/mL), streptomycin (100 μg/mL; Cellgro, Manassas, Va), anti-CD40–stimulating mAb (anti-CD40 mAb; G28-5, 1 μg/mL), and recombinant IL-4 (100 U/mL; R&D Systems, Minneapolis, Minn). The induction of AID expression was confirmed by means of relative quantification with TaqMan Gene Expression Assays (Applied Biosystems) with a 7300 Fast Real Time PCR System (Applied Biosystems, data not shown). The genomic DNA from B cells was extracted, and the presence of Smu-SHM was analyzed as described above.

In vivo detection of SHM in the switch region of IgM in control subjects and CD40L-, AID-, and UNG-deficient patients

B cells were purified from the peripheral blood of 4 control subjects (C1-C4) by using the RosetteSep Human B Cell Enrichment Cocktail (StemCell Technologies, Vancouver, British Columbia, Canada). The enriched B cells (>90% purity) were stained with anti-IgM–phycoerythrin-Cy5 (BD Biosciences, San Jose, Calif), anti-CD19–allophycocyanin, anti-CD27–phycoerythrin, and anti-IgD–fluorescein isothiocyanate (Beckman Coulter, Fullerton, Calif), according to the manufacturer's suggestions. The $CD19^+$ cells were gated, and the $IgD^+IgM^+CD27^-$, $IgD^+IgM^+CD27^+$, and $IgD^-IgM^-CD27^+$ cells were sorted by using FACSVantage (BD Biosciences). The mean purities of these fractions were 95.6%, 86.9%, and 94.8%, respectively. Because of the absence of class-switched B cells ($IgD^-IgM^-CD27^+CD19^+$) in immunoglobulin CSR–deficient patients (P1-P11), we purified only the $CD27^+$ and $CD27^-$ fractions from the B cells of these patients and their age-matched control subjects (C5-C7, >90% purity). The genomic DNA from the B cells was extracted, and the presence of the switch region of IgM (Smu-SHM) was analyzed, as described above.

RESULTS

In vitro induction of SHM in Smu-SHM in human B cells following CSR activation

To examine whether Smu-SHMs are indeed linked to the induction of CSR in human B cells, we stimulated the PBMCs of control subjects (C1-C4) with anti-CD40 mAb and IL-4, which is a combination that powerfully stimulates the induction of *in vitro* CSR, but not V-SHM,[13,14] in human B cells. This stimulation, as reported previously, induced AID expression, functional transcripts of IgE (as determined by using RT-PCR), and IgE production (as determined by using ELISA) in the supernatants of all (C1-C4) human B-cell cultures (data not shown).

The unstimulated PBMCs presented very few Smu-SHMs (0.006% ± 0.004% bp, mean ± SD; Fig 2, *A*). On CSR-inducing anti-CD40 mAb and IL-4 stimulation, the Smu-SHM frequency significantly increased (0.045% ± 0.002% bp, $P = .0361$). The induced Smu-SHMs were equally targeted to GC and AT (the GC target was 47.4%). The transition type of the mutations was increased at the G and C nucleotides (GC transition, 72.2%) but was not altered at the A and T nucleotides (AT transition,

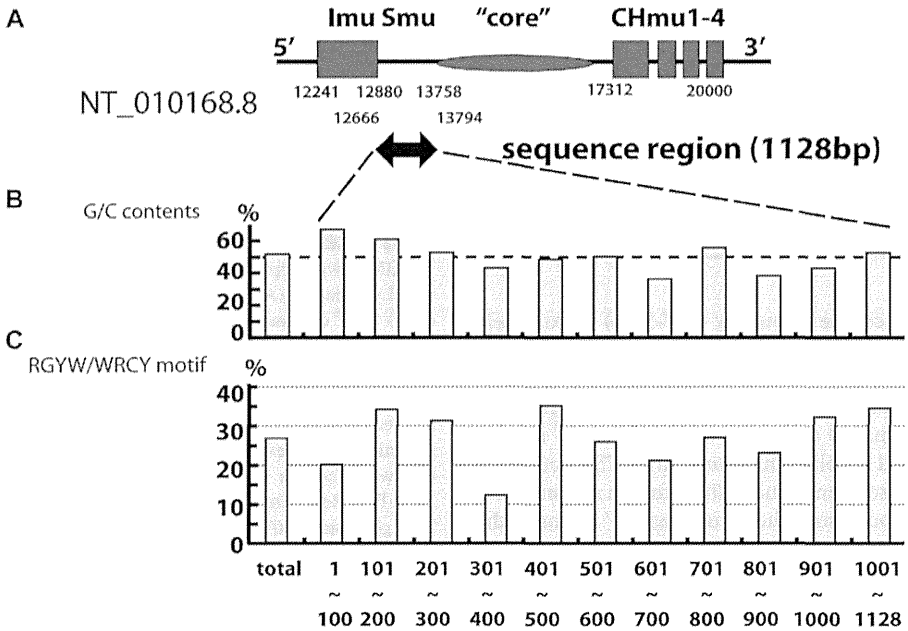


FIG 1. Sequence region for the analysis of mutations in Smu-SHM. **A,** We amplified, subcloned, and sequenced the 5′ region of the highly repetitive GC-rich region of the IgM gene (Smu core) using Pfu polymerase. **B,** Percentages of G and C nucleotides (G/C contents) are equally distributed throughout the sequence region. **C,** Percentages of RGYW/WRCY motifs in each 100-bp fragment are not significantly different throughout the sequence region. *CHmu1-4,* Exons of the constant region of IgM; *Imu,* enhancer exon of the IgM gene; *R,* purines (A/G); *W,* A/T; *Y,* pyrimidines (C/T).

50.0%; Fig 2, *B*). In contrast, the VH-SHMs at the JH4-JH5 region were not changed because of stimulation (Fig 2, *A*). These results indicate that Smu-SHMs can be induced *in vitro* by means of CSR activation.

In vivo induction of Smu-SHM in human B cells

We separated human B cells into 3 subpopulations (naive B cells, unswitched memory B cells, and switched memory B cells) using fluorescence-activated cell sorting based on the expression of IgM, IgD, CD27, and CD19[15] from the PBMCs of healthy adults (n = 4; Fig 3, *A*). We purified the genomic DNA from each purified B-cell subpopulation and amplified the Smu region (5′ to the Smu core region containing repetitive sequences) with the high-fidelity *Pfu* DNA polymerase and Smu-specific primers (Fig 1, *A*).

We found that the frequency of Smu-SHMs in the $IgD^-IgM^-CD27^+$ switched memory B cells was 20-fold higher (0.21% ± 0.014% bp) than that found in the $IgD^+IgM^+CD27^-$ naive B cells (0.008% ± 0.003% bp, $P < .001$). The frequency of Smu-SHMs in the $IgD^+IgM^+CD27^+$ unswitched memory B cells (0.13% ± 0.023% bp) was also significantly higher than that found in naive B cells ($P < .001$) and significantly lower than that found in switched memory B cells ($P < .001$; Fig 3, *B*). The mutations were mainly point mutations (Fig 3, *C*), but some small deletions were also observed in each fraction (Table I).

Distribution of Smu-SHMs within the 5′ upstream region of Smu core

We then compared the frequency of SHMs among the 1- to 400-bp, 401- to 800-bp, and 801- to 1128-bp regions of the 5′ upstream region of the Smu core region.

In all 5′ regions (1-400 bp, 401-800 bp, and 801-1128 bp), the switched memory B-cell subpopulation exhibited a significantly higher frequency of SHMs than the unswitched memory and naive B cells (Fig 4).

In the 1- to 400-bp region (Fig 4, *A*), which is the most distant from the Smu core region, the SHM frequencies found in the unswitched memory and naive B cells were not significantly different. However, the switched memory B cells (0.15% ± 0.05% bp) presented a higher frequency of SHMs than the unswitched memory B cells (0.06% ± 0.04% bp, $P < .05$) and naive B cells (0.00% ± 0.00% bp, $P < .001$).

In the 401- to 800-bp region (Fig 4, *B*) the switched memory B cells (0.19% ± 0.02% bp) exhibited a significantly higher frequency of SHMs than the unswitched memory B cells (0.07% ± 0.03% bp, $P < .001$) and naive B cells (0.01% ± 0.01% bp), and the unswitched memory B cells presented a higher frequency of SHMs than the naive B cells ($P < .01$).

In the 801- to 1128-bp region (Fig 4, *C*), which is the closest to the Smu core region, both switched (0.29% ± 0.02% bp) and unswitched (0.27% ± 0.05% bp) memory B-cell subpopulations exhibited significantly higher SHM frequencies than naive B cells (0.02% ± 0.02% bp, $P < .001$). The SHM frequencies obtained for the switched and unswitched memory B cells were not significantly different.

These differences were independent of the distributions of G and C nucleotides (Fig 1, *B*) and WRCY/RGYW motifs (Fig 1, *C*) because these motifs are equally distributed in these regions.

Sequence analysis of Smu-SHMs in memory B cells

The Smu region analyzed in this study (1128 bp) contains 40 WRCY motifs (160 bp, 14.2% of the length of the Smu region)

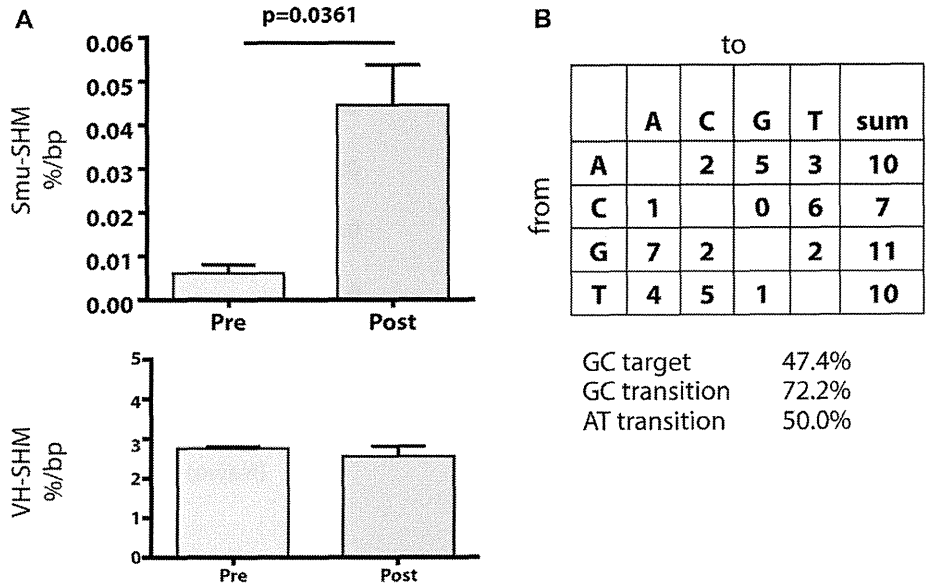


FIG 2. Smu-SHMs induced by anti-CD40 mAb and IL-4 *in vitro* in PBMCs of the control subjects. **A,** Smu-SHMs were analyzed before and after stimulation of PBMCs from control subjects (n = 4) with anti-CD40 mAb and IL-4 for 5 days *in vitro*. Mutations were rarely found before stimulation (*Pre*, 0.006% ± 0.004% bp) but were significantly increased after stimulation with anti-CD40 mAb and IL-4 *in vitro* (*Post*, 0.04% ± 0.02% bp, $P = .0361$). The VH-SHMs at the JH4-JH5 region were analyzed, but no significant difference was observed because of stimulation. **B,** The Smu-SHM pattern observed after stimulation with anti-CD40 mAb and IL-4 is shown. The germline nucleotides are shown on the left *(from)*, and the mutated nucleotides are shown on the top *(to)*. The *right column* indicates the sum of the mutated nucleotides for each row. The *GC target* indicates the mutations at G or C. The *GC transition* indicates the frequency of transition mutations at G and C (G>A and C>T) of the total mutations at G and C. The *AT transition* indicates the frequency of transition mutations at A and C (A>G and T>C) of the total mutations at A and T.

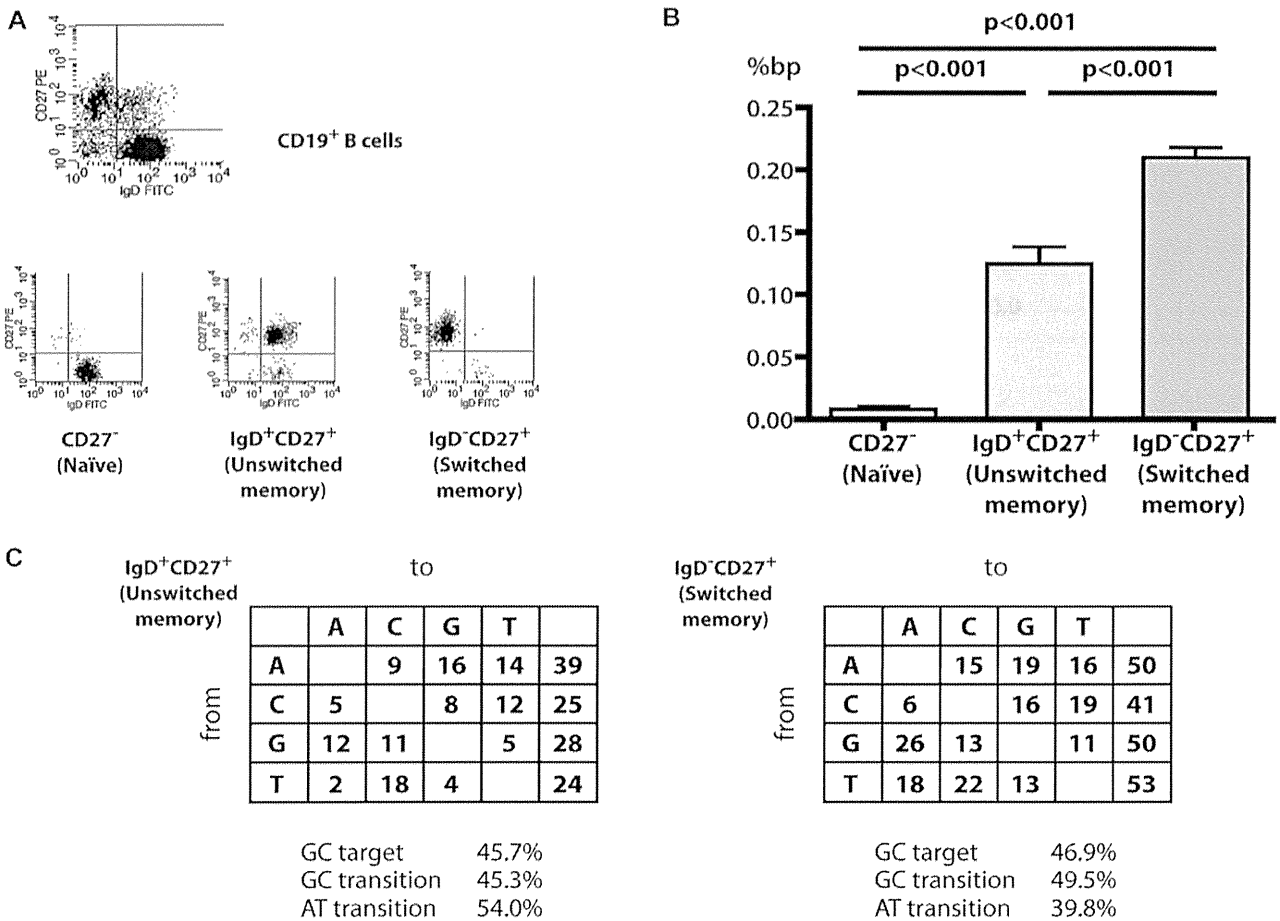
and 43 RGYW motifs (172 bp, 15.2% of the length of the Smu region). In addition, 73.0% of the region is independent of either of the 2 motifs (824 bp, Fig 5).

We found 18 (unswitched memory B cells) and 41 (switched memory B cells) mutations in the WRCY motifs, which correspond to 1.12- and 1.35-fold increases in mutations compared with the expected number, respectively. Similar to the findings obtained in the analysis of the WRCY motif, we found 26 and 64 mutations in the RGYW motif, which correspond to 1.51- and 1.97-fold more mutations than expected, respectively. Thus Smu-SHMs tended to be observed within the WRCY/RGYW motifs. The C nucleotide was the preferential target in the WRCY motif because this nucleotide was the target in 52% and 51% of the mutations that were observed in unswitched memory and switched memory B cells, respectively. The G nucleotide was preferentially targeted in the RGYW motifs because it was the target in 50% and 51% of the mutations observed in unswitched memory and switched memory B cells, respectively. Outside the WRCY/RGYW motifs, the A and T nucleotides were predominantly mutated in both switched (60%) and unswitched (62%) memory B cells. The mutations at A were as frequent as those at T. In unswitched memory B cells 75.6% of the mutations at A/T were found within the WA/TW motifs, and WA/TW to WG/TC mutations accounted for 52.9% of the mutations in WA/TW motifs. In switched memory B cells 55.7% of the mutations at A/T were found within the WA/TW motifs, and WA/TW to WG/TC mutations accounted for 43.6% of the mutations in WA/TW motifs.

Smu-SHM in CD40L-, AID-, and UNG-deficient patients

To assess the CD40L, AID, and UNG dependency of the CSR process in human B cells, we analyzed the Smu-SHMs in peripheral blood purified B cells from age-matched control subjects (C1-C7) and 5 AID-deficient (P1-P5), 2 UNG-deficient (P6 and P7), and 4 CD40L-deficient (P8-P11) patients. There was no significant difference in the frequency of Smu-SHMs in $CD27^+$ memory B cells from control adults (C1-C4) and control children (C5-C7; Fig 6, *A*).

The frequencies of Smu-SHM were decreased, and small deletions were not observed (Table I) in both $CD27^+$ and $CD27^-$ B cells (Fig 6, *A*, and data not shown) in the AID- and CD40L-deficient patients, indicating the dependence of Smu-SHM on AID and CD40L. The mutation pattern of the CD40L-deficient patients was not significantly different from that of the control subjects (Fig 6, *B*). Of the 2 UNG-deficient patients,[2] P6, who has a homozygous missense mutation, presented a significantly decreased frequency of Smu-SHMs in both $CD27^+$ and $CD27^-$ B cells, and P7, who has homozygous 2-bp deletions, exhibited an increased Smu-SHM frequency in $CD27^+$ B cells but a decreased frequency in $CD27^-$ B cells (Fig 6, *A*, and data not shown). This discrepancy cannot be related to the patient's age because no difference in the Smu-SHM frequency was observed according to age (Fig 6, *A*). None of the UNG-deficient patients had small deletions in the Smu region (Table I). An important finding is that Smu-SHMs exhibited significant and characteristic bias to transitions at G and C nucleotides in both patients



C

IgD+CD27+ (Unswitched memory) to

from	A	C	G	T	
A		9	16	14	39
C	5		8	12	25
G	12	11		5	28
T	2	18	4		24

GC target	45.7%
GC transition	45.3%
AT transition	54.0%

IgD-CD27+ (Switched memory) to

from	A	C	G	T	
A		15	19	16	50
C	6		16	19	41
G	26	13		11	50
T	18	22	13		53

GC target	46.9%
GC transition	49.5%
AT transition	39.8%

FIG 3. *In vivo* Smu-SHMs in control subjects. **A,** We purified $IgD^+IgM^+CD27^-$ (naive), $IgD^+IgM^+CD27^+$ (unswitched memory), and $IgD^-IgM^-CD27^+$ (switched memory) B cells from control subjects (n = 4) using fluorescence-activated cell sorting. Representative fluorescence-activated cell sorting data are shown. **B,** We found a higher frequency of Smu-SHMs in $IgD^-IgM^-CD27^+$ B cells, which are class-switched, and V region–mutated B cells, than in $IgD^+IgM^+CD27^+$ B cells, which are V region mutated but not class-switched B cells. The $IgD^+IgM^+CD27^-$ (naive) B cells presented the lowest frequency of Smu-SHMs. **C,** The mutation patterns of unswitched *(left)* and switched *(right)* memory B cells are shown.

(Fig 6, *B*). In contrast, A/T-targeted mutations were observed in P7, similarly to the control subjects. These results demonstrate the contribution of UNG to the formation of Smu-SHMs during CSR.

DISCUSSION

We demonstrated that SHMs occur in the Smu region in not only *in vivo* CSR-induced human B cells but also B cells activated *in vitro* with anti-CD40 mAb and IL-4, a combination that induces AID expression and CSR but not V-SHM.[14] Because the V and S regions are both transcribed on stimulation, the discrepancy between V-SHM and S-SHM occurrence *in vitro* might be related to the R-loop structure of the S (but not V) region, likely because of differences in G/C content or secondary structure.[8]

In fact, we found a significant amount of mutations in the Smu region of switched memory B cells. This amount was more than 4-fold higher than the frequency observed in B cells stimulated *in vitro*. We also found that unswitched memory B cells presented more Smu-SHMs than naive B cells, suggesting that the unswitched memory B-cell subpopulation contains a fraction that has unsuccessfully undergone CSR after the introduction of AID deamination and error-free DNA repair. These results indicate that Smu-SHMs are closely associated with CSR both *in vivo* and *in vitro* and suggest that Smu-SHMs precede the completion of CSR.

We observed significantly more mutations in the 3′ part than the 5′ part of the Smu region, even though the distribution of all of the nucleotides and the WRCY/RGYW motifs are equivalent throughout the region. Although the sequenced region is outside of the Smu core region with highly repetitive, G/C-rich sequences, this region is also known to form the R-loop with single-stranded DNA and a DNA-RNA hybrid.[16] As observed in the Smu core region, this R-loop structure might allow the accessibility of DNA to AID-induced deamination. The preferential targeting of Smu-SHMs to the 3′ part of the Smu region might reflect the frequency of the deamination of AID during CSR. Furthermore, we found more Smu-SHMs in the 5′ part of the Smu region of $IgD^-IgM^-CD27^+$ class-switched memory B cells than in the Smu region of $IgD^+IgM^+CD27^+$ unswitched memory B cells

TABLE I. *In vivo* Smu-SHMs in control subjects and patients

		IgD$^+$IgM$^+$CD27$^+$ (unswitched memory)						IgD$^-$IgM$^-$CD27$^+$ (switched memory)					
		Total clones	Mutated clones	Percentage	Clones with small deletions	Percentage	Length of small deletions (bp)	Total clones	Mutated clones	Percentage	Clones with small deletions	Percentage	Length of small deletions (bp)
C1	Adult control subjects	23	5	22	1	20	1	21	15	71	2	13	30, 72
C2		21	8	38	3	38	1, 1, 11	21	15	71	1	7	1
C3		19	7	37	1	14	8	20	10	50	1	10	5
C4		21	11	52	0	0	—	21	14	67	1	7	8, 28
				CD27$^+$									
C5	Age-matched control subjects	18	5	28	0	0	—						
C6		19	11	58	0	0	—						
C7		19	8	42	4	50	2, 11, 12, 25						
P1	AID deficiency	22	2	9	0	0	—						
P2		18	0	0	0	0	—						
P3		21	3	14	0	0	—						
P4		11	0	0	0	0	—						
P5		13	0	0	0	0	—						
P6	UNG deficiency	34	4	12	0	0	—						
P7		20	17	85	0	0	—						
P8	CD40L deficiency	20	10	50	0	0	—						
P9		20	3	15	0	0	—						
P10		23	1	4	0	0	—						
P11		22	4	18	0	0	—						

The genomic DNA from the purified unswitched memory and switched memory B cells of adult control subjects (C1-C4) and CD27$^+$ memory B cells from age-matched control subjects (C5-C7) and patients with AID (P1-P5), UNG (P6-P7), and CD40L (P8-P11) deficiency was analyzed for Smu-SHMs. *Total clones* indicates analyzed clones. *Mutated clones* indicates clones with at least 1 mutation. The percentage indicates the proportion of mutated clones in the total clones. The number and proportion of clones with small deletions are also shown. The length of small deletions (in base pairs) is indicated for each subject.

(Fig 3, *A*). This finding might suggest that the R-loop extension in the 5′ region of the S region contributes to CSR by opening the chromatin structure of this region to allow access for AID (see Fig E1 in this article's Online Repository at www.jacionline.org).[16] The R-loop is a characteristic structure of the S region. Our observation is consistent with the finding that the R-loop is wider in the 5′ region than formerly considered. This widespread R-loop formation might be important for CSR by opening the chromatin structure or some other unknown mechanism. These results indicate that Smu-SHMs start in the 3′ region and then spread to the 5′ upstream region during the CSR phase and that the R-loop might be larger in switched memory B cells than unswitched and naive B cells (see Fig E1). Thus the switched memory cells might have more chance of successful CSR than unswitched memory and naive B cells. Further study is needed to analyze the R-loop formation in the S region during the CSR stimulation by using the bisulfite modification assay[16] to prove this hypothesis.

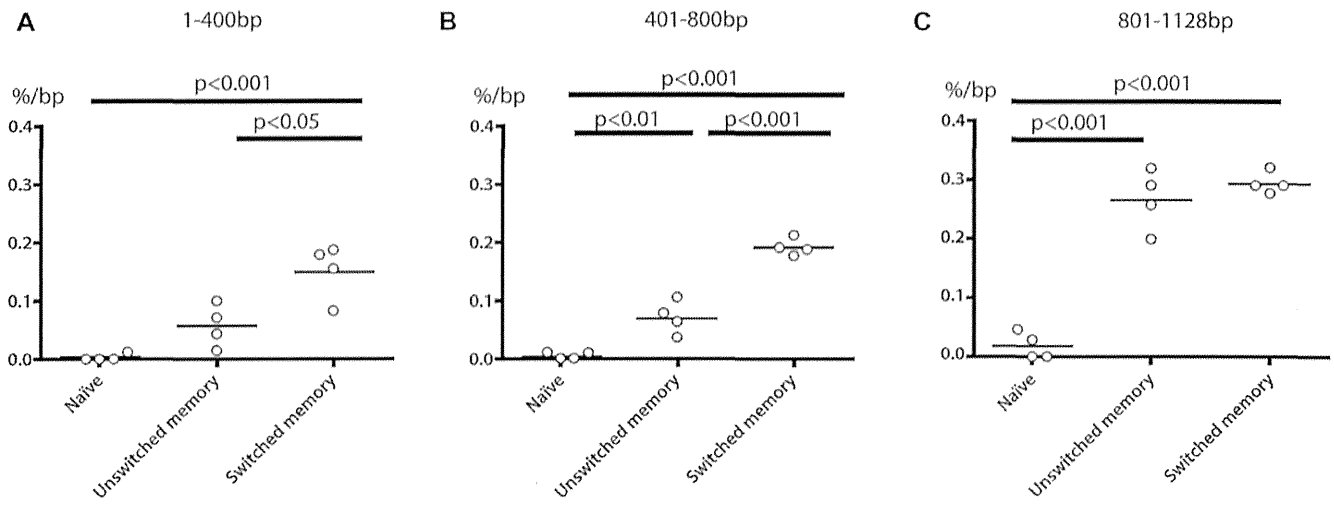
We compared the nucleotide mutation pattern of Smu-SHMs with that of V-SHMs in memory B cells and found that the mutations at A were as frequent as those at T in the Smu-SHM both *in vivo* and *in vitro* (Figs 2, *C*, and 6, *B*). This finding for Smu-SHMs is different from that observed for V-SHMs because the frequency of V-SHMs at A residues is 2-fold higher than that found for T residues.[17] Smu-SHMs at A and T residues are dependent on MMR and the DNA polymerase eta.[18,19] In V-SHM the preference of A residues over T residues is explained by the preference for MMR molecules on the top strand of opened double-stranded DNA.[20] Our results might suggest that AID deamination and MMR contribute equally to both the top and bottom strands of the S region, which is different from the findings found for the V region and characteristic for Smu-SHMs. Because the number of mutations analyzed in this study is limited, further studies using next-generation sequencing would be helpful to elucidate these questions.[21]

We found that Smu-SHMs at C and G were limited within WRCY and RGYW motifs, respectively, whereas mutations at A and T were observed outside of these motifs *in vivo* (Fig 4). Because WRCY/RGYW motifs are known as target motifs of SHM in the V region and as a target sequence of RPA-binding phosphorylated AID,[22] this result indicates that AID is involved in Smu-SHMs, as well as V-SHMs, by inducing deamination on Smu-SHM in human B cells *in vivo*.

Stimulation with anti-CD40 mAb and IL-4, which induces Smu-SHM, does not induce V-SHM. This discrepancy might be due to the R-loop formation in the Smu region but not in the V region on stimulation *in vitro*. AID favors the single-strand DNA observed in the R-loop of Smu region, which is not formed in V regions by CSR stimulation.

To further investigate the roles of CD40L, AID, and UNG in Smu-SHMs in human B cells, we studied the B-cell subpopulations (naive and memory) from CD40L-deficient (n = 4), AID-deficient (n = 5), and UNG-deficient (n = 2) patients. The results show that Smu-SHMs were reduced in both CD40L- and AID-deficient patients, even in their CD27$^+$ B cells. This observation, which demonstrates the essential role for AID in Smu-SHM generation, similarly to that observed in V-SHM,[1] confirms data previously reported for successfully recombined Smu regions from unseparated peripheral blood B-cell populations of patients with heterozygous dominant negative AID mutations.[10]

The analysis of the UNG-deficient patients showed that the Smu-SHM frequency was decreased in one patient (P6) but increased in the other patient (P7). Because both patients lack expression of the UNG protein,[2,23] this discrepancy could not be explained by their different genotypes. In addition, the difference



D

	Nucleotide number		
	1-400	401-800	801-1128
Naïve ($IgD^+IgM^+CD27^-$)	0.00±0.00	0.01±0.01	0.02±0.02
Unswitched memory ($IgD^+IgM^+CD27^+$)	0.06±0.04	0.07±0.03	0.27±0.05
Switched memory ($IgD^-IgM^-CD27^+$)	0.15±0.05	0.19±0.02	0.29±0.02

FIG 4. Distribution of Smu-SHMs in each fraction of control B cells *in vivo*. **A-C,** The frequency of Smu-SHMs was higher in the 3′ region than in the 5′ region in both IgD^- (switched) and IgD^+ (unswitched) fractions of $CD27^+$ B cells. **D,** Means ± SDs of Smu-SHMs in each region are shown (n = 4).

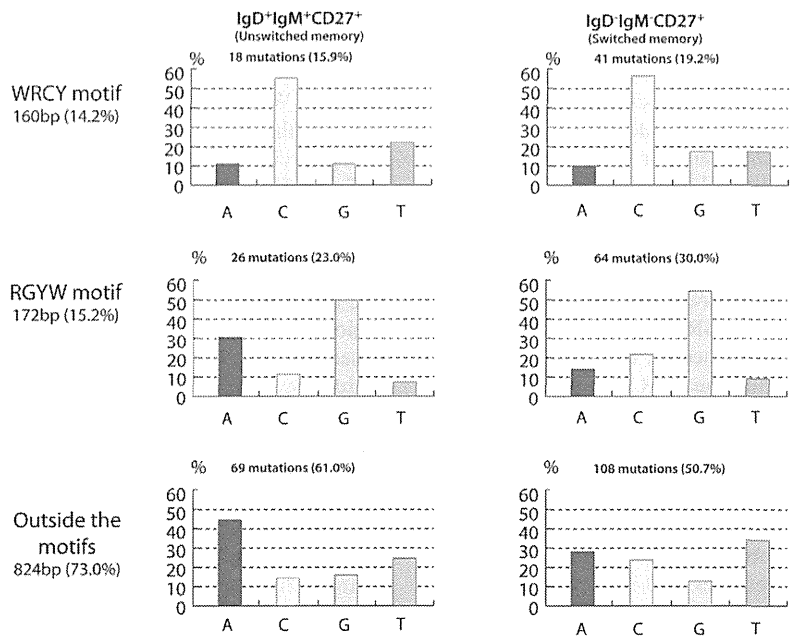
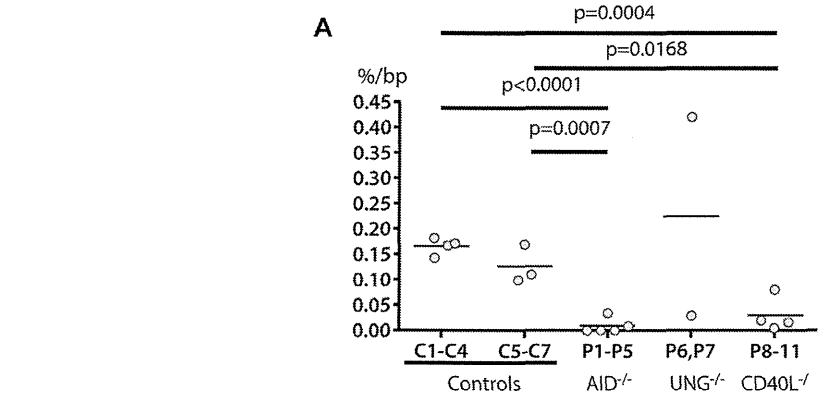


FIG 5. Target motifs of Smu-SHMs in each fraction of control B cells *in vivo*. The Smu-SHMs of the control subjects *in vivo* (n = 4) were analyzed according to their target motifs in each fraction of B cells ($IgD^+IgM^+CD27^+$ and $IgD^-IgM^-CD27^+$ B cells). *R*, Purines (A/G); *W*, A/T; *Y*, pyrimidines (C/T).

does not appear to be related to age because we did not observe any discrepancy between B cells from children and adult control subjects (Fig 6, *A*). Neither of the 2 UNG-deficient patients had small deletions in the Smu region. Smu-SHMs exhibited a skewed pattern similar to that seen in V-SHM[2] (ie, an excess of transitions on G and C nucleotides [G to A, 100%; C to T, 98.4%]) and an



B

C1-C4 (control adults)

from \ to	A	C	G	T	
A		24	35	30	89
C	11		24	31	66
G	38	24		16	78
T	20	40	17		77

GC target 46.5%
GC transition 47.9%
AT transition 45.2%

C5-C7 (control children)

from \ to	A	C	G	T	
A		6	10	8	24
C	1		4	8	13
G	20	8		1	29
T	6	7	2		15

GC target 51.9%
GC transition 66.7%
AT transition 43.6%

P6,7 (UNG-/-)

from \ to	A	C	G	T	
A		4	8	6	18
C	1		0	28	29
G	45	0		0	45
T	4	8	2		14

GC target 69.8%
GC transition 98.6%
AT transition 50.0%

P8-11 (CD40L-/-)

from \ to	A	C	G	T	
A		2	3	2	7
C	0		1	2	3
G	9	3		2	14
T	1	2	0		3

GC target 63.0%
GC transition 64.7%
AT transition 50.0%

FIG 6. Smu-SHMs in patients with CD40L, AID, and UNG deficiency *in vivo*. **A,** Smu-SHMs in the $CD27^+$ fraction of B cells from control adults (C1-C4), control children (C5-C7), and AID-deficient (P1-P5), UNG-deficient (P6 and P7), and CD40L-deficient (P8-11) patients. The frequency of Smu-SHMs was decreased in patients with AID (P1-P5) and CD40L (P8-11) deficiency. In patients with UNG deficiency, the Smu-SHM frequency was decreased in P6 but increased in P7. **B,** The mutations were significantly targeted and biased to transition at G and C in UNG-deficient patients (P6 and P7).

increased targeting of G and C in both patients. This result indicates that in the absence of UNG, CSR-induced AID-dependent deaminated cytidines on both strands of the S region lead to transition mutations during their replications.

In contrast, we found normal mutation patterns at A and T in both UNG-deficient patients. The U/G mismatch introduced by AID in the S region, similarly to that observed in the V region, can be recognized by the MMR pathway. The MMR, particularly the MSH2/MSH6 complex, has been shown to be essential in the absence of UNG for both CSR and SHM in mice.[11,19] The MLH1/PMS2 complex is also reported to be important for CSR.[24] MMR deficiencies (PMS2 and MSH6 biallelic mutations) are associated with mild CSR deficiency in human subjects.[25,26] In the V regions the recruitment of the DNA polymerase eta (POLH) by the MSH2/MSH6 complex is responsible for mutations on A/T residues.[27] However, this alternative pathway, which compensates for UNG deficiency in V-SHM and Smu-SHM, is not sufficient in human subjects to induce full CSR, as shown by the marked immunoglobulin CSR deficiency and the complete lack of small deletions in Smu regions observed in both patients. In the absence of UNG, CSR-induced AID-dependent lesions in the S regions lead to mutations occurring during their repair but are inefficient for the generation of the double-strand DNA breaks necessary for CSR in human subjects.

At present, most of the hyper-IgM syndromes caused by an intrinsic B-cell defect remain molecularly undefined.[28,29] An analysis of the frequency and occurrence of CSR-induced Smu-SHMs might be a useful tool to better understand the molecular defect in these patients and disclose the CSR mechanisms.

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Key messages

- **CSR-induced mutations occurring in Smu-SHM were observed in both *in vitro* and *in vivo* switched and unswitched human memory B cells.**
- **The extension of SHM to the 5′ upstream region of Smu might be associated with the effective induction of CSR.**
- **Smu-SHM depends on CD40L, AID, UNG, and the MMR system.**

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