TABLE IV. Diseases of immune dysregulaton

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Genetic defects, presumed Pathogenesis	Relative frequency among PIDs
1. Immunodeficiency with hypopigmentation							
(a) Chediak-Higashi syndrome	Normal	Normal	Normal	Partial albinism, giant lysosomes, low NK and CTL activities, heightened acute-phase reaction, late-onset primary encephalopathy	AR	Defects in *LYST*, impaired lysosomal trafficking	Rare
(b) Griscelli syndrome, type 2	Normal	Normal	Normal	Partial albinism, low NK and CTL activities, heightened acute phase reaction, encephalopathy in some patients	AR	Defects in *RAB27A* encoding a GTPase in secretory vesicles	Rare
(c) Hermansky-Pudlak syndrome, type 2	Normal	Normal	Normal	Partial albinism, neutropenia, low NK and CTL activity, increased bleeding	AR	Mutations of AP3B1 gene, encoding for the β subunit of the AP-3 complex	Extremely rare
2. Familial hemophagocytic lymphohistiocytosis (FHL) syndromes							
(a) Perforin deficiency	Normal	Normal	Normal	Severe inflammation, fever, decreased NK and CTL activities	AR	Defects in *PRF1*; perforin, a major cytolytic protein	Rare
(b) UNC13D 13-D deficiency	Normal	Normal	Normal	Severe inflammation, fever, decreased NK and CTL activities	AR	Defects in *UNC13D* required to prime vesicles for fusion	Rare
(c) Syntaxin 11 (STX11) deficiency	Normal	Normal	Normal	Severe inflammation, fever, decreased NK activity	AR	Defects in *STX11*, involved in vesicle trafficking and fusion	Very rare
3. Lymphoproliferative syndromes							
(a) XLP1, SH2D1A deficiency	Normal	Normal or reduced	Normal or low immunoglobulins	Clinical and immunologic abnormalities triggered by EBV infection, including hepatitis, aplastic anemia, lymphoma	XL	Defects in *SH2D1A* encoding an adaptor protein regulating intracellular signals	Rare
(b) XLP2, XIAP deficiency	Normal	Normal or reduced	Normal or low immunoglobulins	Clinical and immunologic abnormalities triggered by EBV infection, including splenomegaly, hepatitis, hemophagocytic syndrome, lymphoma	XL	Defects in *XIAP*, encoding an inhibitor of apoptosis	Very rare
(c) ITK deficiency	Modestly decreased	Normal	Normal or decreased	EBV-associated lymphoproliferation	AR	Mutations in *ITK*	Extremely rare
4. Syndromes with autoimmunity							
(a) Autoimmune lymphoproliferative syndrome (ALPS)							

(Continued)

TABLE IV. *(Continued)*

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Genetic defects, presumed Pathogenesis	Relative frequency among PIDs
(i) CD95 (Fas) defects, ALPS type Ia	Increased $CD4^-$ $CD8^-$ double negative (DN) T cells	Normal	Normal or increased	Splenomegaly, adenopathy, autoimmune blood cytopenias, defective lymphocyte apoptosis increased lymphoma risk	AD (rare severe AR cases)	Defects in *TNFRSF6*, cell surface apoptosis receptor; in addition to germline mutations, somatic mutations cause a similar phenotype	Rare
(ii) CD95L (Fas ligand) defects, ALPS type Ib	Increased DN T cells	Normal	Normal	Splenomegaly, adenopathy, autoimmune blood cytopenias, defective lymphocyte apoptosis, SLE	AD AR	Defects in *TNFSF6*, ligand for CD95 apoptosis receptor	Extremely rare
(iii) Caspase 10 defects, ALPS type 2a	Increased DN T cells	Normal	Normal	Adenopathy, splenomegaly, autoimmune disease, defective lymphocyte apoptosis	AR	Defects in *CASP10*, intracellular apoptosis pathway	Extremely rare
(iv) Caspase 8 defects, ALPS type 2b	Slightly increased DN T cells	Normal	Normal or decreased	Adenopathy, splenomegaly, recurrent bacterial and viral infections, defective lymphocyte apoptosis and activation;	AR	Defects in *CASP8*, intracellular apoptosis and activation pathways	Extremely rare
(v) Activating N-Ras defect, N-Ras-dependent ALPS	Increased DN T cells	Elevation of CD5 B cells	Normal	Adenopathy, splenomegaly, leukemia, lymphoma, defective lymphocyte apoptosis after IL-2 withdrawal	AD	Defect in *NRAS* encoding a GTP binding protein with diverse signaling functions, activating mutations impair mitochondrial apoptosis	Extremely rare
(b) APECED, autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy	Normal	Normal	Normal	Autoimmune disease, particularly of parathyroid, adrenal and other endocrine organs plus candidiasis, dental enamel hypoplasia and other abnormalities	AR	Defects in *AIRE*, encoding a transcription regulator needed to establish thymic self-tolerance	Rare
(c) IPEX, immune dysregulation, polyendocrinopathy, enteropathy (X-linked)	Lack of $CD4^+CD25^+$ $FOXP3^+$ regulatory T cells	Normal	Elevated IgA, IgE	Autoimmune diarrhea, early onset diabetes, thyroiditis, hemolytic anemia, thrombocytopenia, eczema	XL	Defects in *FOXP3*, encoding a T cell transcription factor	Rare
(d) CD25 deficiency	Normal to modestly decreased	Normal	Normal	Lymphoproliferation, autoimmunity, impaired T-cell proliferation	AR	Defects in IL-2Ra chain	Extremely rare

AD, Autosomal-dominant; *AIRE*, autoimmune regulator; *AP3B1*, adaptor protein complex 3 beta 1 subunit; *AR*, autosomal-recessive; *CASP*, caspase; *CTL*, cytotoxic T lymphocyte; *DN*, double-negative; *FOXP3*, forkhead box protein 3; *LYST*, lysosomal trafficking regulator; *NRAS*, neuroblastoma Ras protein; *PRF1*, perforin 1; *RAB27A*, Ras-associated protein 27A; *SH2D1A*, SH2 domain protein 1A; *TNFRSF6*, tumor Necrosis Factor Receptor Soluble Factor 6; *TNFSF6*, tumor Necrosis Factor Soluble Factor 6; *IAP*, X-linked inhibitor of apoptosis; *XL*, X-linked; *XLP*, X-linked lymphoproliferative disease

TABLE V. Congenital defects of phagocyte number, function, or both

Disease	Affected cells	Affected function	Associated features	Inheritance	Gene defect—presumed pathogenesis	Relative frequency among PIDs
1.-2. Severe congenital neutropenias	N	Myeloid differentiation	Subgroup with myelodysplasia	AD	*ELA2*: mistrafficking of elastase	Rare
	N	Myeloid differentiation	B/T lymphopenia	AD	*GFI1*: repression of elastase	Extremely rare
3. Kostmann disease	N	Myeloid differentiation	Cognitive and neurological defects*	AR	*HAX1*: control of apoptosis	Rare
4 Neutropenia with cardiac and urogenital malformations	N + F	Myeloid differentiation	Structural heart defects, urogenital abnormalities, and venous angiectasias of trunks and limbs	AR	*G6PC3*: abolished enzymatic activity of glucose-6-phosphatase and enhanced apoptosis of N and F	Very rare
5 Glycogen storage disease type 1b	N + M	Killing, chemotaxis, O_2^- production	Fasting hypoglycemia, lactic acidosis, hyperlipidemia, hepatomegaly, neutropenia	AR	*G6PT1*: Glucose-6-phosphate transporter 1	Very rare
6. Cyclic neutropenia	N	?	Oscillations of other leukocytes and platelets	AD	*ELA2*: mistrafficking of elastase	Very rare
7. X-linked neutropenia/ myelodysplasia	N + M	?	Monocytopenia	XL	*WAS:* Regulator of actin cytoskeleton (loss of autoinhibition)	Extremely rare
8. P14 deficiency	N+L Mel	Endosome biogenesis	Neutropenia Hypogammaglobulinemia ↓CD8 cytotoxicity Partial albinism Growth failure	AR	*MAPBPIP*: Endosomal adaptor protein 14	Extremely rare
9. Leukocyte adhesion deficiency type 1	N + M + L + NK	Adherence Chemotaxis Endocytosis T/NK cytotoxicity	Delayed cord separation, skin ulcers Periodontitis Leukocytosis	AR	*ITGB2*: Adhesion protein	Very rare
10. Leukocyte adhesion deficiency type 2	N + M	Rolling chemotaxis	Mild LAD type 1 features plus hh-blood group plus mental and growth retardation	AR	*FUCT1*: GDP-Fucose transporter	Extremely rare
11. Leukocyte adhesion deficiency type 3	N + M + L + NK	Adherence	LAD type 1 plus bleeding tendency	AR	*KINDLIN3*: Rap1-activation of β1-3 integrins	Extremely rare
12. Rac 2 deficiency	N	Adherence Chemotaxis O_2^- production	Poor wound healing, leukocytosis	AD	*RAC2*: Regulation of actin cytoskeleton	Extremely rare: Regulation of actin cytoskeleton
13. β-Actin deficiency	N + M	Motility	Mental retardation, short stature	AD	*ACTB*: Cytoplasmic actin	Extremely rare
14. Localized juvenile periodontitis	N	Formylpeptide-induced chemotaxis	Periodontitis only	AR	*FPR1*: Chemokine receptor	Very rare
15. Papillon-Lefèvre syndrome	N + M	Chemotaxis	Periodontitis, palmoplantar hyperkeratosis†	AR	*CTSC*: Cathepsin C activation of serine proteases	Very rare
16. Specific granule deficiency	N	Chemotaxis	N with bilobed nuclei	AR	*CEBPE*: myeloid transcription factor	Extremely rare
17. Shwachman-Diamond syndrome	N	Chemotaxis	Pancytopenia, exocrine pancreatic insufficiency, chondrodysplasia	AR	*SBDS*	Rare
18. X-linked chronic granulomatous disease (CGD)	N + M	Killing (faulty O_2^- production)	McLeod phenotype in a subgroup of patients	XL	*CYBB*: Electron transport protein (gp91phox)	Relatively common

(Continued)

TABLE V. *(Continued)*

Disease	Affected cells	Affected function	Associated features	Inheritance	Gene defect—presumed pathogenesis	Relative frequency among PIDs
19.- 21. Autosomal CGDs	N + M	Killing (faulty O_2^- production)		AR	*CYBA*: Electron transport protein (p22phox) *NCF1*: Adapter protein (p47phox) *NCF2*: Activating protein (p67phox)	Relatively common
22. IL-12 and IL-23 receptor β1 chain deficiency	L + NK	IFN-γ secretion	Susceptibility to mycobacteria and *Salmonella*	AR	*IL12RB1*: IL-12 and IL-23 receptor β1 chain	Rare
23. IL-12p40 deficiency	M	IFN-γ secretion	Susceptibility to mycobacteria and *Salmonella*	AR	*IL12B:* subunit of IL12/IL23	Very rare
24. IFN-γ receptor 1 deficiency	M + L	IFN-γ binding and signaling	Susceptibility to mycobacteria and *Salmonella*	AR, AD	*IFNGR1*: IFN-γR ligand binding chain	Rare
25. IFN-γ receptor 2 deficiency	M + L	IFN-γ signaling	Susceptibility to mycobacteria and *Salmonella*	AR	*IFNGR2*: IFN-γR accessory chain	Very rare
26. STAT1 deficiency (2 forms)	M + L	IFN α/β, IFN-γ, IFN-λ, and IL-27 signaling	Susceptibility to mycobacteria, *Salmonella* and *viruses*	AR	*STAT1*	Extremely rare
27. AD hyper-IgE	L+M+N+ epithelial	IFN-γ signaling	Susceptibility to mycobacteria and *Salmonella*	AD	*STAT1*	Extremely rare
28. AR hyper-IgE (TYK2 deficiency)	L+M+N+ others	IL-6/10/22/23 signaling IL-6/10/12/ 23/IFN-α/ IFN-β signaling	Distinctive facial features (broad nasal bridge); eczema; osteoporosis and fractures; scoliosis; failure/delay of shedding primary teeth; hyperextensible joints; bacterial infections (skin and pulmonary abscesses/ pneumatoceles) caused by *Staphylococcus aureus*; candidiasis Susceptibility to intracellular bacteria (mycobacteria, *Salmonella*), *Staphylococcus*, and viruses.	AD AD	*STAT3* *TYK2*	Rare Extremely rare
29. Pulmonary alveolar proteinosis	Alveolar macrophages	GM-CSF signaling	Alveolar proteinosis	biallelic mutations in pseudoautosomal gene	*CSF2RA*	extremely rare

ACTB, Actin beta; *AD*, autosomal-dominant; *AR*, autosomal-recessive inheritance; *CEBPE*, CCAAT/Enhancer-binding protein epsilon; *CTSC*, cathepsin C; *CYBA*, cytochrome b alpha subunit; *CYBB*, cytochrome b beta subunit; *ELA2*, elastase 2; *IFN*, interferon; *IFNGR1*, interferon-gamma receptor subunit 1; *IFNGR2*, interferon-gamma receptor subunit 2; *IL12B*, interleukin-12 beta subunit; *IL12RB1*, interleukin-12 receptor beta 1; *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; *MAPBPIP*, MAPBP-interacting protein; *Mel*, melanocytes; *N*, neutrophils; *NCF1*, neutrophil cytosolic factor 1; *NCF2*, neutrophil cytosolic factor 2; *NK*, natural killer cells; *SBDS*, Shwachman-Bodian-Diamond syndrome; *STAT*, signal transducer and activator of transcription; *XL*, X-linked inheritance.
*Cognitive and neurologic defects are observed in a fraction of patients.
†Periodontitis may be isolated.

TABLE VI. Defects in innate immunity

Disease	Affected cell	Functional defect	Associated features	Inheritance	Gene defect/presumed pathogenesis	Relative frequency among PIDs
Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID)	Lymphocytes + monocytes	NF-κB signaling pathway	Anhidrotic ectodermal dysplasia + specific antibody deficiency (lack of antibody response to polysaccharides) Various infections (mycobacteria and pyogenic bacteria)	XL	Mutations of *NEMO (IKBKG)*, a modulator of NF-κB activation	Rare
EDA-ID	Lymphocytes + monocytes	NF-κB signaling pathway	Anhidrotic ectodermal dysplasia + T-cell defect + various infections	AD	Gain-of-function mutation of *IKBA*, resulting in impaired activation of NF-κB	Extremely rare
IL-1 receptor associated kinase 4 (IRAK4) deficiency	Lymphocytes + monocytes	TIR-IRAK signaling pathway	Bacterial infections (pyogens)	AR	Mutation of *IRAK4*, a component of TLR and IL-1R-signaling pathway	Very rare
MyD88 deficiency	Lymphocytes + monocytes	TIR-MyD88 signaling pathway	Bacterial infections (pyogens)	AR	Mutation of MYD88, a component of the TLR and IL-1R signaling pathway	Very rare
WHIM (warts, hypogammaglobulinemia infections, myelokathexis) syndrome	Granulocytes + lymphocytes	Increased response of the CXCR4 chemokine receptor to its ligand CXCL12 (SDF-1)	Hypogammaglobulinemia, reduced B-cell number, severe reduction of neutrophil count, warts/ HPV infection	AD	Gain-of-function mutations of *CXCR4*, the receptor for CXCL12	Very rare
Epidermodysplasia verruciformis	Keratinocytes and leukocytes	?	HPV (group B1) infections and cancer of the skin	AR	Mutations of *EVER1, EVER2*	Extremely rare
Herpes simplex encephalitis (HSE)	Central nervous system resident cells, epithelial cells and leukocytes	UNC-93B-dependent IFN-α, IFN-β, and IFN-λ induction	Herpes simplex virus 1 encephalitis and meningitis	AR	Mutations of *UNC93B1*	Extremely rare*
HSE	Central nervous system resident cells, epithelial cells, dendritic cells, cytotoxic lymphocytes	TLR3-dependent IFN-α, IFN-β, and IFN-λ induction	Herpes simplex virus 1 encephalitis and meningitis	AD	Mutations of *TLR3*	Extremely rare*
Chronic mucocutaneous candidiasis	Macrophages	Defective Dectin-1 signaling	Chronic mucocutaneous candidiasis	AR	Mutations of *CARD9* leading to low number of Th17 cells	Extremely rare**
Trypanosomiasis		APOL-I	Trypanosomiasis	AD	Mutation in APOL-I	Extremely rare*

AD, Autosomal-dominant; *AR*, autosomal-recessive; *EDA-ID*, ectodermal dystrophy immune deficiency; *EVER*, epidermodysplasia verruciformis; *HPV*, human papilloma virus; *IKBA*, inhibitor of NF-kB alpha; *IRAK4*, interleukin-1 receptor associated kinase 4; *MYD88*, myeloid differentiation primary response gene 88; *NEMO*, NF-kB essential modulator; *NF-κB*, nuclear factor-κB; *SDF-1*, stromal-derived factor 1; *TIR*, toll and IL-1 receptor; *TLR*, toll-like receptor; *XL*, X-linked.
*Only a few patients have been genetically investigated, and they represented a small fraction of all patients tested, but the clinical phenotype being common, these genetic disorders may actually be more common.
**Mutations in CARD9 have been identified only in one family. Other cases of chronic mucocutaneous candidiasis remain genetically undefined.

TABLE VII. Autoinflammatory disorders

Disease	Affected cells	Functional defects	Associated features	Inheritance	Gene defects	Relative frequency among PIDs
Familial Mediterranean fever	Mature granulocytes, cytokine-activated monocytes	Decreased production of pyrin permits ASC-induced IL-1 processing and inflammation after subclinical serosal injury; macrophage apoptosis decreased	Recurrent fever, serositis and inflammation responsive to colchicine Predisposes to vasculitis and inflammatory bowel disease	AR	Mutations of *MEFV*	Common

(Continued)

TABLE VII. *(Continued)*

Disease	Affected cells	Functional defects	Associated features	Inheritance	Gene defects	Relative frequency among PIDs
TNF receptor-associated periodic syndrome (TRAPS)	PMNs, monocytes	Mutations of 55-kD 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	AD	Mutations of *TNFRSF1A*	Rare
Hyper IgD syndrome		Mevalonate kinase deficiency affecting cholesterol synthesis; pathogenesis of disease unclear	Periodic fever and leukocytosis with high IgD levels	AR	Mutations of *MVK*	Rare
Muckle-Wells syndrome*	PMNs, monocytes	Defect in cryopyrin, involved in leukocyte apoptosis and NF-κB signaling and IL-1 processing	Urticaria, SNHL, amyloidosis Responsive to IL-1R/ antagonist	AD	Mutations of *CIAS1* (also called PYPAF1 or NALP3)	Rare
Familial cold autoinflammatory syndrome*	PMNs, monocytes	Same as above	Nonpruritic urticaria, arthritis, chills, fever, and leukocytosis after cold exposure Responsive to IL-1R/ antagonist (Anakinra)	AD	Mutations of *CIAS1* Mutations of *NLRP12*	Very rare
Neonatal onset multisystem inflammatory disease (NOMID) or chronic infantile neurologic cutaneous and articular syndrome (CINCA)*	PMNs, chondrocytes	Same as above	Neonatal onset rash, chronic meningitis, and arthropathy with fever and inflammation responsive to IL-1R antagonist (Anakinra)	AD	Mutations of *CIAS1*	Very rare
Pyogenic sterile arthritis, pyoderma gangrenosum, acne (PAPA) syndrome	Hematopoietic tissues, upregulated in activated T cells	Disordered actin reorganization leading to compromised physiologic signaling during inflammatory response	Destructive arthritis, inflammatory skin rash, myositis	AD	Mutations of *PSTPIP1* (also called C2BP1)	Very rare
Blau syndrome	Monocytes	Mutations in nucleotide binding site of CARD15, possibly disrupting interactions with LPSs and NF-κB signaling	Uveitis, granulomatous synovitis, camptodactyly, rash and cranial neuropathies, 30% develop Crohn disease	AD	Mutations of *NOD2* (also called CARD15)	Rare
Chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia (Majeed syndrome)	Neutrophils, bone marrow cells	Undefined	Chronic recurrent multifocal osteomyelitis, transfusion-dependent anemia, cutaneous inflammatory disorders	AR	Mutations of *LPIN2*	Very rare
DIRA (deficiency of the IL-1 receptor antagonist)	PMNs, monocytes	Mutations in the IL-1 receptor antagonist allows unopposed action of IL-1	Neonatal onset of sterile multifocal osteomyelitis, periostitis and pustulosis	AR	Mutations of *IL1RN*	Very rare

AD, Autosomal dominant inheritance; *AR*, autosomal-recessive inheritance; *ASC*, apoptosis-associated specklike protein with a caspase recruitment domain; *CARD*, caspase recruitment domain; *CD2BP1*, CD2 binding protein 1; *CIAS1*, cold-induced autoinflammatory syndrome 1; *LPN2*, lipin-2; *MEFV*, Mediterranean fever; *MVK*, mevalonate kinase; *NF-κB*, nuclear factor-κB; *PMN*, polymorphonuclear cell; *PSTPIP1*, proline/serine/threonine phosphatase-interacting protein 1; *SNHL*, sensorineural hearing loss.
*All 3 syndromes associated with similar CIAS1 mutations; disease phenotype in any individual appears to depend on modifying effects of other genes and environmental factors.

TABLE VIII. Complement deficiencies

Disease	Functional defect	Associated features	Inheritance	Gene defects	Relative frequency among PIDs
C1q deficiency	Absent C hemolytic activity, defective MAC* Faulty dissolution of immune complexes Faulty clearance of apoptotic cells	SLE–like syndrome, rheumatoid disease, infections	AR	C1q	Very rare
C1r deficiency*	Absent C hemolytic activity, defective MAC Faulty dissolution of immune complexes	SLE–like syndrome, rheumatoid disease, infections	AR	C1r*	Very rare
C1s deficiency	Absent C hemolytic activity	SLE-like syndrome; multiple autoimmune diseases	AR	C1s*	Extremely rare
C4 deficiency	Absent C hemolytic activity, defective MAC Faulty dissolution of immune complexes Defective humoral immune response	SLE–like syndrome, rheumatoid disease, infections	AR	C4A and C4B†	Very rare
C2 deficiency‡	Absent C hemolytic activity, defective MAC Faulty dissolution of immune complexes	SLE–like syndrome, vasculitis, polymyositis, pyogenic infections	AR	C2‡	Rare
C3 deficiency	Absent C hemolytic activity, defective MAC Defective bactericidal activity Defective humoral immune response	Recurrent pyogenic infections	AR	C3	Very rare
C5 deficiency	Absent C hemolytic activity, defective MAC Defective bactericidal activity	Neisserial infections, SLE	AR	C5	Very rare
C6 deficiency	Absent C hemolytic activity, defective MAC Defective bactericidal activity	Neisserial infections, SLE	AR	C6	Rare
C7 deficiency	Absent C hemolytic activity, defective MAC Defective bactericidal activity	Neisserial infections, SLE, vasculitis	AR	C7	Rare
C8a deficiency§	Absent C hemolytic activity, defective MAC Defective bactericidal activity	Neisserial infections, SLE	AR	C8α	Very rare
C8b deficiency	-Absent C hemolytic activity, defective MAC Defective bactericidal activity	Neisserial infections, SLE	AR	C8β	Very rare
C9 deficiency	-Reduced C hemolytic activity, defective MAC Defective bactericidal activity	Neisserial infections‖	AR	C9	Rare

(Continued)

TABLE VIII. *(Continued)*

Disease	Functional defect	Associated features	Inheritance	Gene defects	Relative frequency among PIDs
C1 inhibitor deficiency	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	AD	C1 inhibitor	Relatively common
Factor I deficiency	Spontaneous activation of the alternative complement pathway with consumption of C3	Recurrent pyogenic infections, glomerulonephritis, hemolytic-uremic syndrome	AR	Factor I	Very rare
Factor H deficiency	Spontaneous activation of the alternative complement pathway with consumption of C3	Hemolytic-uremic syndrome, membranoproliferative glomerulonephritis	AR	Factor H	Rare
Factor D deficiency	Absent hemolytic activity by the alternate pathway	Neisserial infection	AR	Factor D	Very rare
Properdin deficiency	Absent hemolytic activity by the alternate pathway	Neisserial infection	XL	Properdin	Rare
MBP deficiency¶	Defective mannose recognition Defective hemolytic activity by the lectin pathway.	Pyogenic infections with very low penetrance, mostly asymptomatic	AR	MBP¶	Relatively common
MASP2 deficiency	Absent hemolytic activity by the lectin pathway	SLE syndrome, pyogenic infection	AR	MASP2	Extremely rare
Complement receptor 3 (CR3) deficiency	See LAD1 in Table V		AR	*ITGB2*	Rare
Membrane cofactor protein (CD46) deficiency	Inhibitor of complement alternate pathway, decreased C3b binding	Glomerulonephritis, atypical hemolytic uremic syndrome	AD	MCP	Very rare
Membrane attack complex inhibitor (CD59) deficiency	Erythrocytes highly susceptible to complement-mediated lysis	Hemolytic anemia, thrombosis	AR	CD59	Extremely rare
Paroxysmal nocturnal hemoglobinuria	Complement-mediated hemolysis	Recurrent hemolysis	Acquired X-linked mutation	PIGA	Relatively common
Immunodeficiency associated with ficolin 3 deficiency	Absence of complement activation by the ficolin 3 pathway	Recurrent severe pyogenic infections mainly in the lungs	AR	FCN3	Extremely rare

AD, Autosomal-dominant inheritance; *AR*, autosomal-recessive inheritance; *MAC*, membrane attack complex; *MASP-2*, MBP associated serine protease 2; *MBP*, mannose binding protein; *PIGA*, phosphatidylinositol glycan class A; *SLE*, systemic lupus erythematosus; *XL*, X-linked inheritance.

*The C1r and C1s genes are located within 9.5 kb of each other. In many cases of C1r deficiency, C1s is also deficient.

†Gene duplication has resulted in 2 active C4A genes located within 10 kb. C4 deficiency requires abnormalities in both genes, usually the result of deletions.

‡Type 1 C2 deficiency is in linkage disequilibrium with HLA-A25, B18, and -DR2 and complotype, SO42 (slow variant of Factor B, absent C2, type 4 C4A, type 2 C4B) and is common in Caucasian subjects (about 1 per 10,000). It results from a 28-bp deletion resulting in a premature stop codon in the C2 gene; C2 mRNA is not produced. Type 2 C2 deficiency is very rare and involves amino acid substitutions, which result in C2 secretory block.

§C8α deficiency is always associated with C8γ deficiency. The gene encoding C8γ maps to chromosome 9 and is normal. C8γ is covalently bound to C8α.

‖Association is weaker than with C5, C6, C7, and C8 deficiencies. C9 deficiency occurs in about 1 per 1,000 Japanese.

¶Population studies reveal no detectable increase in infections in MBP-deficient adults.

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Hemophagocytosis after bone marrow transplantation for JAK3-deficient severe combined immunodeficiency

Hashii Y, Yoshida H, Kuroda S, Kusuki S, Sato E, Tokimasa S, Ohta H, Matsubara Y, Kinoshita S, Nakagawa N, Imai K, Nonoyama S, Oshima K, Ohara O, Ozono K. Hemophagocytosis after bone marrow transplantation for JAK3-deficient severe combined immunodeficiency. Pediatr Transplantation 2009. © 2009 John Wiley & Sons A/S.

Abstract: HSCT is the optimal treatment for patients with SCID. In particular, HSCT from a HLA-identical donor gives rise to successful engraftment with long survival. We report a six-month-old girl with JAK3-deficient SCID who developed hemophagocytosis after BMT without conditioning from her HLA-identical father. She had suffered from pneumonia and hepatitis before BMT. Prophylaxis for GVHD was short-term methotrexate and tacrolimus. On day 18 after BMT, the patient developed hemophagocytosis in bone marrow when donor lymphocytes were increasing in peripheral blood. Analysis of chimerism confirmed host origin of macrophages and donor origin of lymphocytes. Thus, host macrophage activation was presumably induced in response to donor lymphocytes through immunoreaction to infections and/or alloantigens. HSCT for SCID necessitates caution with respect to hemophagocytosis.

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Key words: bone marrow transplantation – hemophagocytosis – JAK3 mutation – severe combined immunodeficiency

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SCID is a uniformly fatal disease unless promptly treated with HSCT, which reconstitutes a normal immune system (1–3). Patients with SCID have often been affected by various kinds of infections prior to HSCT and the presence of pulmonary infection is a powerful predictor of death after HSCT (1). In addition, hemophagocytosis has been reported as an important complication early after HSCT (4–7). This phenomenon is in many cases triggered by infections (4, 5) and in some cases by an alloimmune response (6, 7). We report a girl with JAK3-deficient SCID who developed hemophagocytosis after BMT without conditioning from her HLA-identical father, where donor lymphocytes presumably activated host macrophages.

Abbreviations: γc, γ chain; BCG, Bacille de Calmette et Guérin; BM, bone marrow; BMT, bone marrow transplantation; CMV, cytomegalovirus; EBV, Epstein-Barr virus; FISH, fluorescence *in situ* hybridization; GVHD, graft-versus-host disease; HHV, human herpes virus; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; HSV, herpes simplex virus; IFN, interferon; IL, interleukin; m-PSL, methyl PSL; NK, natural killer; PCR, polymerase chain reaction; PSL, prednisolone; RT, reverse transcription; SCID, severe combined immunodeficiency; TNF, tumor necrosis factor; TRECs, T-cell-receptor excision circles; VNTR, variable number of tandem repeat.

Case report

A five-month-old girl, born to consanguineous Chinese parents, had repeatedly developed viral and bacterial bronchitis and oral candidiasis

from two months of age. She had received no BCG vaccination. White blood cell count was 2690/μL (63.5% neutrophils, 27.1% lymphocytes, 1.6% eosinophils, 0% basophils, 7.8% monocytes). Serum IgG, IgA, and IgM levels were 213, 1, and 34 mg/dL, respectively. Lymphocyte subset analysis showed absence of T lymphocytes (0.6% $CD3^+$, 0.3% $CD4^+$, 1.2% $CD8^+$) and NK cells (1.6% $CD16^+$, 0.6% $CD56^+$) with normal numbers of B lymphocytes (96.9% $CD19^+$, 97.3% $CD20^+$). A diagnosis of $T^-B^+NK^-$SCID was made, and genetic analysis revealed a novel homozygous non-sense mutation of JAK3: a C to T point mutation at nucleotide 623 that changed amino acid 175 in the JH6 domain from arginine to a stop codon (C623T; R175X) (Fig. 1).

The clinical course of the patient is summarized in Fig. 2. When she was referred to our hospital, she suffered from severe interstitial pneumonia and liver dysfunction. No sign of infection was observed on studies by RT-PCR of her serum for CMV, HHV6, HHV7, adenovirus, HSV-1 or -2, and EBV genomes. *Aspergillus* spp. and *Pneumocystis jiroveci* were not detected in her sputa by PCR analysis. β-D-glucan was not detected in her serum. She received oxygen therapy and infusion of hyperalimentation because of poor feeding.

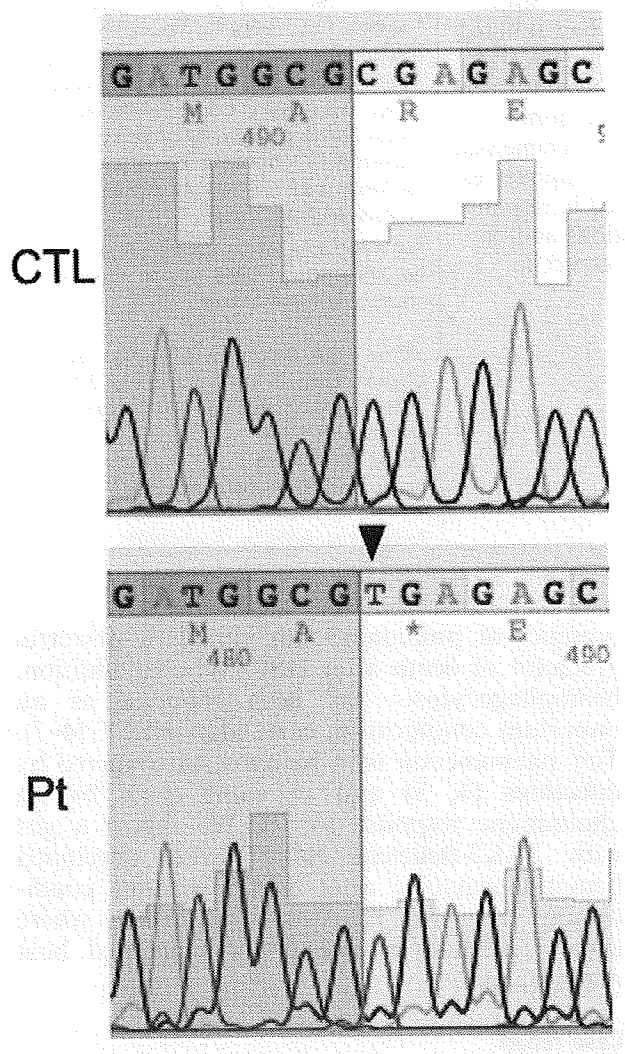

Fig. 1. Sequence analysis showing a non-sense mutation. The JAK3 gene of the patient showed a C to T point mutation (C623T) as shown by arrowhead. CTL, control; Pt, patient.

At the age of six months, she underwent unmanipulated BMT from her genotypically HLA-identical father without conditioning. Both the patient and her father had the same HLA genotype: HLA-A*0101/3001-B*1302/3701-C*0602-DRB1*0701/1501.

Prophylaxis for GVHD was short-term methotrexate (days 1, 3, 6, and 11) and tacrolimus. She developed grade 3 acute GVHD with watery diarrhea (stage 2) and skin eruption (stage 1) on day 9 after BMT, for which she was treated with 2 mg/kg/day of PSL. On day 16, her interstitial pneumonia deteriorated in both lungs on chest X-ray. RT-PCR analysis of sputa showed negative results of CMV, *Aspergillus* spp., and *P. jiroveci.*

Her WBC count decreased to 330/μL on day 18 and BM aspiration revealed hypoplastic marrow with hemophagocytosis by activated macrophages (nuclear cell count, 4000/μL; megakaryocyte count, 0/μL) (Fig. 3). Serum ferritin level was 715 ng/mL and serum soluble IL-2 receptor level was 3295 U/mL. Hemophagocytosis improved three days after administration of etoposide 30 mg/m^2 and pulsed m-PSL 30 mg/kg/day on day 18. VNTR analysis revealed that donor cells were almost completely absent from whole cells and macrophages ($CD14^+$ cells) of the BM cells on days 18 and 20, respectively (Fig. 4). Meanwhile, donor cells were detected in peripheral blood cells on days 20 and 24, including T lymphocytes ($CD3^+$ cells) on day 24 (Fig. 4). A serial flow cytometric analysis of lymphocyte-gated cells also demonstrated that $CD3^+$ cells with predominance of $CD4^+$ cells, most likely donor cells, increased to 3.59% and 5.03% on days 18 and 21, respectively (Table 1). Furthermore, FISH analysis of sex chromosome detected donor cells in 10.8% and 8.6% of peripheral blood cells on days 20 and 28, respectively (data not shown).

As her respiratory condition deteriorated, she received repeated courses of pulsed m-PSL (30 mg/kg/day) therapy and underwent mechanical ventilation on day 20. Despite intensive therapy, she died on day 32 due to respiratory failure. Lung necropsy showed necrotized cells

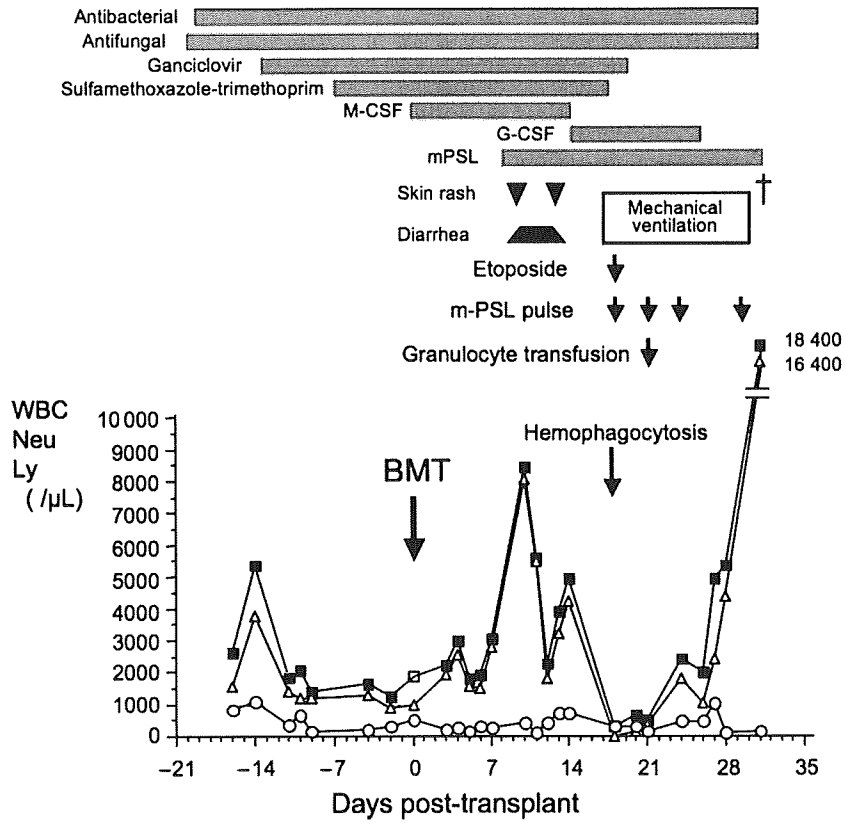

Fig. 2. Clinical course and changes in white blood cell counts. WBC, white blood cells (solid squares); Neu, neutrophil (open triangles); Ly, lymphocyte (open circles); M-CSF, macrophage-colony stimulating factor; G-CSF, granulocyte-colony stimulating factor.

without inflammatory cells. No bacterial, viral, or fungal components were detected in the tissue.

Discussion

SCID is a rare syndrome with heterogeneous genetic inheritance. Common γc mutations have been identified in X-linked SCID, characterized by lack of T cells and NK cells with presence of B cells ($T^-B^+NK^-$SCID). JAK3 mutations have been identified in some patients of autosomal SCID, which shares similar clinical features to X-linked SCID but with normal γc (8–10). JAK3,

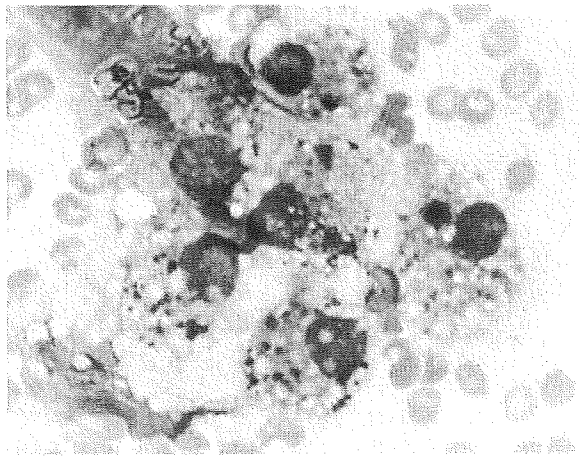

Fig. 3. Bone marrow aspiration on day 18 showing aggregate of activated macrophages. On the far right an erythroblast appears to be undergoing endocytosis.

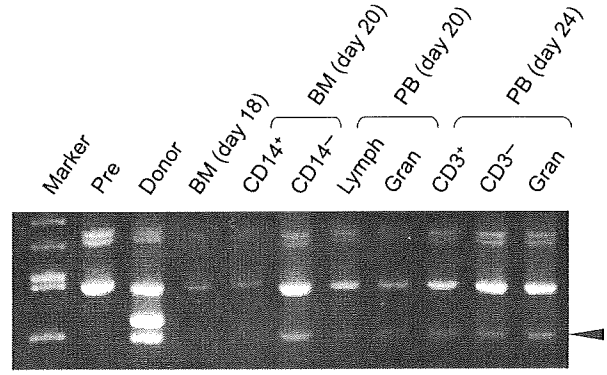

Fig. 4. VNTR analysis. Specific primers designed to flank the repetitive unit, D1S80, were used for the PCR (17). Amplified DNA was electrophoresed and visualized with ethidium bromide. $CD14^+$ or $CD3^+$ cells were purified by magnetic cells sorting enrichment kit (MACS: Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Arrowhead indicates a donor-specific band. Prespecific bands cannot be separated from donor-specific bands.

Table 1. Flow cytometric analysis of lymphocyte-gated cells in peripheral blood

	Lymphocytes (/μL)	CD19 (%)	CD3 (%)	CD4 (%)	CD8 (%)	CD56 (%)
Pre (Day −15)	838	96.7	0.55	NE	NE	1.46
Day +7	243	87.9	2.82	1.31	0.44	0.28
Day +11	111	98.4	0.53	NE	NE	0.34
Day +13	707	98.6	0.39	NE	NE	0.15
Day +18	330	91.6	3.59	3.28	ND	0.24
Day +21	167	88.3	5.03	4.15	0.52	0.88

NE, not evaluable; ND, not done.

a member of the Janus family intracellular protein kinases, associates with intracellular domain of γc and is required for signal transduction from γc-containing receptors (8–10). To date, more than 30 mutations of JAK3 have been reported according to RAPID (Resource of Asian Primary Immunodeficiency Database) (http://rapid.rcai.riken.jp/RAPID/mutation?pid_id=AGID_86); most of them are sporadic and lacking preferential hot spots.

The JAK3 gene has an open reading frame of 3372 bp that is translated into a 1124 amino acid protein (10). In our patient, we identified a novel non-sense homozygous mutation (C623T; Arg157X) leading to a premature stop codon in the JH6 domain. Although we did not evaluate protein expression, this non-sense mutation, nearer to the amino-terminus, probably resulted in abrogated protein expression. The homozygosity was in line with other reported cases with parental consanguinity (8).

Prompt HSCT is an effective life-saving treatment modality for reconstitution of T-cell immunity in this defect (1–3). Our patient therefore underwent BMT immediately after diagnosis from her genotypically HLA-identical father without conditioning. A large European study (1), which analyzed 475 HSCTs for SCID from 1968 to 1999, showed 81% and 72% three-yr survival after HSCT in patients after HSCT from genotypically and phenotypically HLA-identical related donors, respectively. This study furthermore reported 96% sustained engraftment from HLA-identical HSCT, and better engraftment at 93% in SCID with B-cell-positive phenotype, i.e., γc- or JAK3-deficient SCID, compared with 88% in SCID with B-cell-negative phenotype. Recent studies also showed successful HSCT outcome with >90% survival with engraftment in SCID including γc- or JAK3-deficient SCID (2, 3).

Hemophagocytosis early after HSCT has been reported as an important complication (4–7), which is thought to be caused by infections (4, 5) or an alloimmune response (6, 7). The previous reports did not show any detailed analysis of macrophage origin, and the exact mechanism of macrophage activation remains unclear. Moreover, hemophagocytosis after HSCT for SCID as the cause of the graft failure has been reported in only some cases. Norris et al. (11) reported hemophagocytosis after three months HSCT in a T^-B^+ SCID patient who had received T cell-depleted HSCT from an HLA-haploidentical donor without a conditioning regimen. They demonstrated that the hemophagocytosis occurred as a result of donor T-cell engraftment with incomplete immune function, since B-cell reconstitution and tri-lineage hematopoiesis including macrophages showed host type. In our case, hemophagocytosis also occurred after donor T-cell engraftment.

Our patient developed hemophagocytosis and respiratory distress, accompanied by unexpected slow and low engraftment of donor cells. Hemophagocytosis was caused by host macrophages when donor lymphocytes were increasing. Since the patient congenitally had no functioning T cells, it is most probable that donor lymphocytes responded to host cells or resident infectious organisms, leading to IFN-γ production and to activation of host macrophages (12, 13). In SCID patients, maternal engraftment of T cells can lead to GVHD of the skin and liver. Dvorak et al. (14) reported that the T(-)B(+) NK(-) SCID patient with complete CD132 deficiency represented hemophagocytosis without GVHD and that hemophagocytosis was most likely caused by maternal perforin-expressing CD8 T cells. In our case, maternal T cells were not detected pre-SCT (Table 1), which suggests that paternal CD8 T cells or NK cells were involved in hemophagocytosis.

Monocyte function in JAK3-deficient SCID patients has been reported to be intact with respect to cytokine production in response to stimulation (15). The activated macrophages, in turn, probably produced the pro-inflammatory cytokines, TNFα, IL-1β, and IL-6 (12, 13), which might have caused the lung injury as no organism was detected by post-mortem examination.

A conditioning regimen is generally not administered to SCID patients during HSCT from HLA-identical related donors (1–3). However, in our patient, residual macrophages would appear to play an important role in causing hemophagocytosis, which might have led to poor engraftment. Furthermore, Cavazzana et al. (16) analyzed primary T-cell-immunodeficient patients who had undergone HSCT and demonstrated that all patients having undergone full myeloablation had donor myeloid cells and persistent

thymopoiesis, as evidenced by the presence of naive T cells carrying TRECs, which indicates the importance of the complete absence of thymic progenitors by myeloablative conditioning in providing a favorable environment for thymic seeding by early progenitor cells. Our results lead us to surmise that, even when transplanted from an HLA-identical donor, some kind of immunosuppressive conditioning is needed to prevent hemophagocytosis.

In conclusion, we describe a child with JAK3-deficient SCID who developed hemophagocytosis after HSCT from her HLA-identical father. Host macrophage activation would appear to be induced by donor lymphocytes through immune reaction to alloantigen or infectious organisms. HSCT for SCID necessitates caution with respect to hemophagocytosis.

Acknowledgment

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Identification of Severe Combined Immunodeficiency by T-Cell Receptor Excision Circles Quantification Using Neonatal Guthrie Cards

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Objective To assess the feasibility of T-cell receptor excision circles (TRECs) quantification for neonatal mass screening of severe combined immunodeficiency (SCID).

Study design Real-time PCR based quantification of TRECs for 471 healthy control patients and 18 patients with SCID with various genetic abnormalities (*IL2RG*, *JAK3*, *ADA*, *LIG4*, *RAG1*) were performed, including patients with maternal T-cell engraftment (n = 4) and leaky T cells (n = 3).

Results TRECs were detectable in all normal neonatal Guthrie cards (n = 326) at the levels of 10^4 to 10^5 copies/μg DNA. In contrast, TRECs were extremely low in all neonatal Guthrie cards (n = 15) and peripheral blood (n = 14) from patients with SCID, including those with maternal T-cell engraftment or leaky T cells with hypomorphic RAG1 mutations or LIG4 deficiency. There were no false-positive or negative results in this study.

Conclusion TRECs quantification can be used as a neonatal mass screening for patients with SCID. *(J Pediatr 2009;155:829-33).*

See related article, p 834

Severe combined immunodeficiency (SCID) is a genetic disorder characterized by the absence of T-cells and adaptive immunity.[1,2] Affected infants usually have severe infections due to opportunistic pathogens in the first months of life. Hematopoietic stem cell transplantation can reconstitute immune function, although severe infections before hematopoietic stem cell transplantation can be fatal to the patients within the first year of life.[3,4] Thus, early diagnosis before the occurrence of severe infection is essential.[5-7]

Four different mechanisms have been identified as a cause of SCID, including purine metabolism defects, defective signaling of the common γ-chain–dependent cytokine receptors, defective V(D)J recombination, and defective pre-TCR/TCR signaling.[1,2] Although human SCID is caused by mutations of at least 10 different genes, all patients have a characteristic decreased number of newly

BCG	Bacillus Calmette-Guérin
BMT	Bone marrow transplantation
CMV	Cytomegalovirus
FISH	Fluorescent in situ hybridization
HSCT	Hematopoietic stem cell transplantation
PB	Peripheral blood
PCR	Polymerase chain reaction
sjTRECs	Signal joint TRECs
SCID	Severe combined immunodeficiency
TCR	T-cell receptor
TRECs	T-cell receptor excision circles
UCB	Umbilical cord blood

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Table. Genotype, lymphocyte subset, and TRECs of patients with SCID

Patient	Sex	Genotype	Age at onset of symptoms	Age at SCID diagnosis	Lymphocytes (/μL)	CD3+ (%)	CD3+ (/μL)	CD19+ (%)	CD45RO+ / CD4 + CD3 + (%)	Maternal lymphocyte engraftment	Guthrie cards TRECs (/μg DNA)	PB Pre-HSCT TRECs (/μg DNA)	PB Pre-HSCT Age
1	M	*IL2RG*	3 mo	3 mo	720	0.0	0	-	86.0	-	<10	-	-
2	M	*IL2RG*	-	0 mo	780	0.0	0	-	94.0	-	<10	<10	0 y, 0 mo
3	M	*IL2RG*	-	0 mo	920	0.0	0	-	91.0	-	<10	<10	0 y, 0 mo
4	M	*IL2RG*	4 mo	5 mo	2550	0.2	5	NA	99.4	-	<10	<10	0 y, 5 mo
5	M	*IL2RG*	10 mo	10 mo	1035	0.0	0	-	94.7	-	<10	<10	0 y, 10 mo
6	M	*IL2RG*	4 mo	5 mo	3560	0.0	0	-	95.8	-	<10	<10	0 y, 5 mo
7	M	*IL2RG*	-	0 mo	966	0.7	7	95.3	77.5	-	<10	<10	0 y, 0 mo
8	M	*JAK3*	4 mo	4 mo	3810	0.0	0	-	87.0	-	<10	-	-
9	F	*JAK3*	2 mo	5 mo	2495	0.0	0	-	89.8	-	<10	<10	0 y, 6 mo
10	M	*ADA*	1 mo	4 mo	90	40.0	36	99.5	4.4	-	<10	<10	0 y, 2 mo
11	M	*ADA*	1 mo	2 m	100	6.8	7	89.9	0.9	-	6.2×10^2	<10	0 y, 1 mo
12	M	*IL2RG*	8 mo	8 mo	3250	40.8	1326	89.8	65.5	T + NK+	-	<10	1 y
13	M	*IL2RG*	-	0 mo	950	4.2	40	NA	68.6	T+	<10	-	-
14	M	*IL2RG*	9 mo	10 mo	860	7.0	60	99.6	85.9	T + NK+	<10	<10	0 y, 10 mo
15	M	*IL2RG*	3 mo	3 mo	300	36.5	110	NA	53.5	T+	<10	-	-
16	F	*LIG4*	-	0 mo	550	38.7	213	97.6	0.3	-	-	<10	2 y
17	M	*LIG4*	1 y, 6 mo	4 y	300	44.3	133	25.2	0.1	-	<10	<10	4 y
18	F	*RAG1*	8 mo	1 y 9 mo	550	53.1	292	91.6	12.0	-	-	8.0×10^1	2 y

NA, Not available.

developed T cells.[1,2,8,9] T-cell receptor excision circles (TRECs) are small circular DNA fragments formed through rearrangement of the T-cell receptor (TCR) α locus and do not multiply during cell division.[9-13] Therefore, TRECs quantification is reportedly useful for determining recent thymic emigrants. Two reports of a method for neonatal screening of SCID using TRECs quantification by real-time PCR have been published.[6,7] Both studies quantified TRECs of patients with SCID using peripheral blood and found significantly lower levels of TRECs than those of control neonates. In addition, Guthrie cards from 2 patients with SCID retrospectively had undetectable TRECs.[6] Most control neonates had high amounts of TRECs. However, TRECs were undetectable in some samples. To increase specificity, 1 study[7] proposed a 2-tiered strategy using a combination of quantified TRECs and IL-7.

We have evaluated blood from Guthrie cards and peripheral blood from control patients and patients with SCID for detecting TRECs.

Methods

Peripheral blood samples were obtained from 112 healthy volunteers (median age, 14 years; range, 0.1 to 51 years). Thirty-three umbilical cord blood samples (median gestational age, 38.9 weeks) were collected at the National Defense Medical College Hospital. Dried blood spots of umbilical cord blood were obtained by applying 50 μL of residual blood to the 11-mm circles on filter-paper cards (PKU-S, Toyoroshi, Tokyo, Japan). Twenty-six neonatal Guthrie cards with dried blood spots were donated from surplus routine samples for newborn mass screening from neonates born at National Defense Medical College Hospital during this study period (January 2005 to December 2007). In addition, 300 neonatal Guthrie cards, stored at −20°C for less than 5 years in a neonatal mass screening center at Shimane University, were analyzed.

Eighteen patients with SCID were analyzed for TRECs (Table). All patients were genetically diagnosed using genomic DNA sequencing. Mutations of either *IL2RG* (n = 11), *JAK3* (n = 2), *RAG1* (n = 1), *ADA* (n = 2), or *LIG4* (n = 2) were identified in the patients (Table).

Peripheral blood samples of 14 patients before hematopoietic stem cell transplantation were used. In addition, neonatal Guthrie cards of 15 patients that had been stored in newborn mass screening centers were obtained.

Maternal T and NK lymphocyte engraftment was diagnosed by fluorescent in situ hybridization (FISH) using X and Y chromosome–specific probes after purification of each compartment by specific monoclonal antibodies and immunomagnetic beads.

The study protocol was approved by the National Defense Medical College Institutional Review Board, and informed consent was obtained from the parents of patients with SCID and healthy control patients, as well as adult control patients, in accordance with the Declaration of Helsinki.

Quantification of TRECs by Real-Time PCR

We used 100 μL of whole blood (EDTA anticoagulated peripheral blood and heparinized cord blood) or 2 punches of 6 mm in diameter from Guthrie card to extract genomic DNA.

Concentrations of DNA from peripheral blood, fresh dried blood punches from normal neonates (n = 26), and stored dried blood spots from normal neonates (n = 300) were

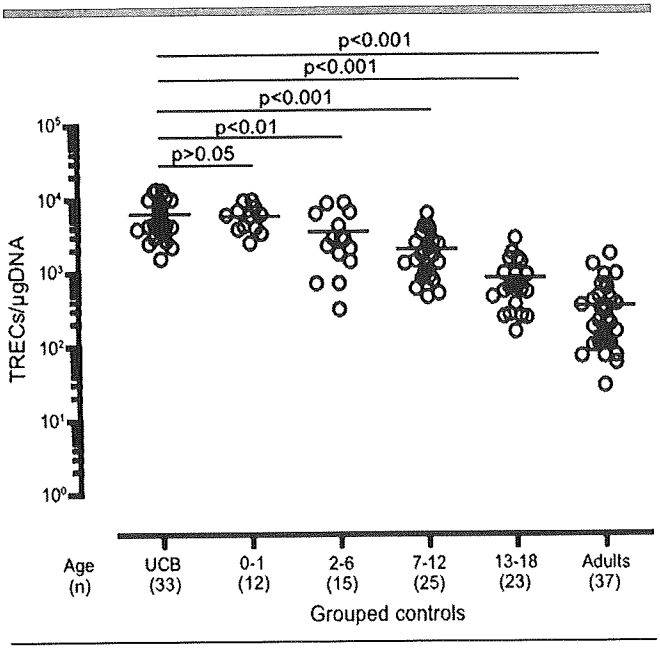

Figure 1. Umbilical cord blood (UCB) (n = 33) and peripheral blood (n = 112, 0 to 51 years) samples were analyzed. TRECs in different age groups are shown. TRECs were significantly higher in umbilical cord blood ($6.2 \pm 3.2 \times 10^3$ copies/µg DNA) and infants ($5.8 \pm 2.3 \times 10^3$ copies/µg DNA) as compared with other age groups of children ($3.5 \pm 2.8 \times 10^3$ copies/µg DNA in 2 to 6 years old, $2.0 \pm 1.4 \times 10^3$ copies/µg DNA in 7 to 12 years old, $8.2 \pm 6.3 \times 10^2$ copies/µg DNA in 13 to 18 years old) and adults ($3.4 \pm 3.6 \times 10^2$ copies/µg DNA).

40.6 ± 2.3 ng/µL (mean ± SEM) (range, 13 to 167 ng/µL), 7.8 ± 2.8 ng/µL (2.9 to 13.0 ng/µL), and 5.3 ± 0.2 ng/µL (0.6 to 20.2 ng/µL), respectively.

Quantitative real-time PCR for δRec-ψJα sjTRECs was performed using the same primers and δRec probes as reported by Hazenberg et al.[14]

As an internal control, RNaseP gene was amplified in each sample tested using TaqMan RNaseP Primer-Probe (VIC dye) Mix (Applied Biosystems, Foster City, California).

Statistical Analysis

An exponential regression model was used to quantify the relationship between age and TRECs levels (per µg DNA and per RNaseP). Goodness-of-fit of the model was evaluated by R^2. The Dunnett multiple comparison test was conducted to test the differences of each age group (0 to 1, 2 to 6, 7 to 12, 13 to 18 years and adults) versus umbilical cord blood comparisons serving as a control group (Figure 1). RNaseP and TRECs levels of patients with SCID and control patients were compared by an unpaired Student *t* test (if the variance was equal) or Welch *t* test (if the variance was different).

All statistical analyses were performed using GraphPad Prism Version 4.00 (GraphPad Software, San Diego, California). $P < .05$ denotes a statistically significant difference.

Results

TRECs were detectable in all DNA samples from whole blood of normal control patients, including umbilical cord blood (n = 33), healthy infants (0 to 1 year old, n = 12), children (2 to 18 years old, n = 63), and adults (n = 37). TRECs in whole blood were found to decline with increasing age ($r = 0.851$). TRECs of umbilical cord blood were significantly higher than those of children and adults but were not significantly different from those of infants (Figure 1). We found a strong correlation between TRECs copies/µg DNA and TRECs/RNaseP ratio ($r = 0.979$).

TRECs of peripheral blood samples from all 14 patients with SCID before hematopoietic stem cell transplantation were below detectable levels (<10 copies/µg DNA) with the exception of 1 (P18, see below), in contrast to high levels of control infants ($5.8 \pm 0.7 \times 10^3$/µg DNA, n = 12) ($P < .0001$) (Figure 2, A).

Next, we analyzed TRECs of dried blood spots from normal control neonates using simulated Guthrie cards from cord blood (n = 31), neonatal Guthrie cards obtained during this study period (January 2005 to December 2007) (n = 26), and those stored in a neonatal mass screening center for less than 5 years (n = 300). TRECs were detectable in all dried blood spots: in cord blood ($1.3 \pm 0.1 \times 10^4$ copies/µg DNA, mean ± SEM), in neonatal Guthrie cards ($2.3 \pm 0.2 \times 10^4$ copies/µg DNA), and in stored neonatal Guthrie cards ($3.6 \pm 0.2 \times 10^5$ copies/µg DNA).

To determine whether this method can identify patients with SCID, we quantified TRECs using 15 stored neonatal Guthrie cards from patients with SCID (patients 1 through 11, 13 through 15, and patient 17). RNaseP levels were high in all neonatal Guthrie cards from patients with SCID ($1.8 \pm 0.3 \times 10^6$ copies/µg DNA, n = 15), which were similar to control levels ($2.3 \pm 0.1 \times 10^6$ copies/µg DNA, n = 26) ($P = .184$), indicating an appropriate amount of genomic DNA was extracted from the neonatal Guthrie cards (Figure 2, B). In contrast, TRECs were below detection levels in all patients ($P < .0001$) except 1 (patient 11). This patient with SCID had compound heterozygous mutations of *ADA* (Gln119Stop/Arg34Ser). He had detectable but significantly lower levels of TRECs (6.2×10^2 copies/µg DNA) than those of control neonates ($2.3 \pm 0.2 \times 10^4$ copies/µg DNA, n = 26) (Figure 2, B). At 1 month of age, the TRECs from the peripheral blood of patient 11 were below detectable levels (Table and Figure 2, A).

These results indicate that neonatal mass screening of SCID by quantitative real-time PCR for TRECs using neonatal Guthrie cards is feasible.

We analyzed TRECs in 4 patients with SCID with maternal T-cell engraftment (patients 12 through 15, $CD3^+$ cells: 40 to 1326/µL). We found that all patients had undetectable levels of TRECs in neonatal Guthrie cards (patients 13 through 15) and peripheral blood (patients 12 and 14). Patient 12 had a normal lymphocyte count (3250/µL) on admission as well as a significant number of T, B, and NK cells (Table). His peripheral blood TRECs level was below the detection

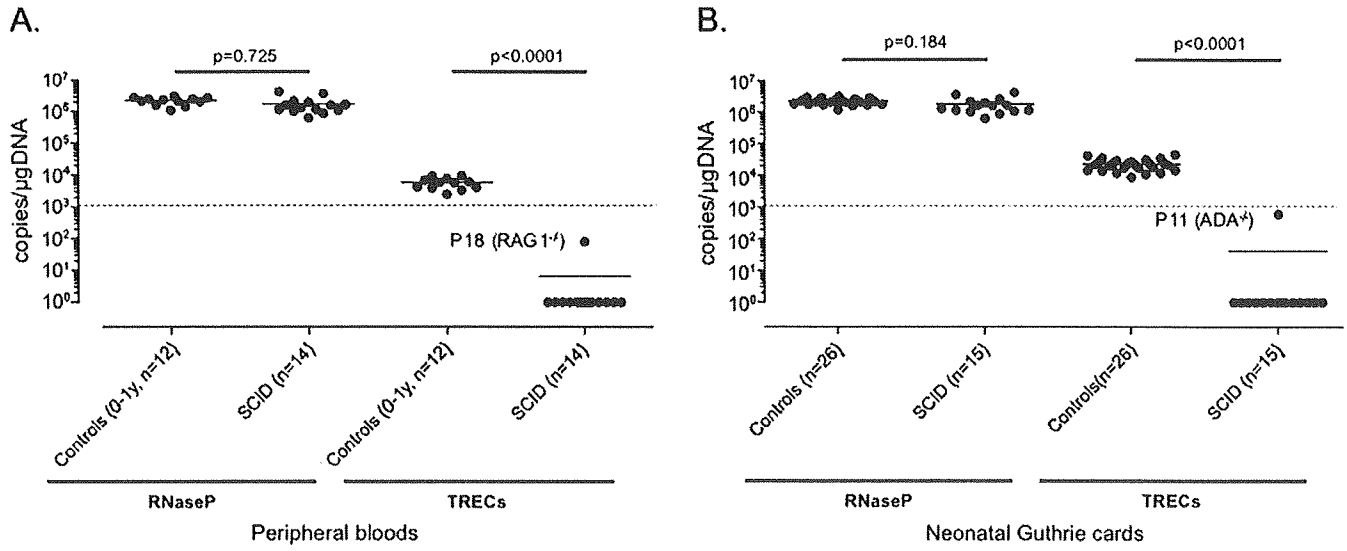

Figure 2. **A,** RNaseP levels in peripheral blood from patients with SCID ($2.1 \pm 0.2 \times 10^6$ copies/μg DNA, n = 14) were comparable with those from control infants ($2.2 \pm 0.2 \times 10^6$ copies/μg DNA, n = 12) ($P = .725$). Peripheral blood TRECs from all typical patients with SCID were undetectable (<10 copies/μg DNA) as compared with the high copy number of peripheral blood TRECs from control neonates ($5.8 \pm 0.7 \times 10^3$ copies/μg DNA, n = 12) ($P < .0001$). Patient 18, with hypomorphic RAG1 mutations, had detectable but extremely low TRECs in peripheral blood (8.0×10^1 copies/μg DNA). **B,** RNaseP levels in neonatal Guthrie cards from patients with SCID ($1.8 \pm 0.3 \times 10^6$ copies/μg DNA, n = 15) were comparable with those from control neonates ($2.3 \pm 0.1 \times 10^6$ copies/μg DNA, n = 26) ($P = .184$). TRECs of Guthrie cards from 14 of 15 patients with SCID during the early neonatal period were undetectable (<10 copies/μg DNA). An ADA-deficient patient ($ADA^{-/-}$, patient 11) had detectable but significantly low TRECs levels (6.2×10^2 copies/μg DNA) compared with control neonates ($2.3 \pm 0.2 \times 10^4$ copies/μg DNA, n = 26).

levels despite the presence of peripheral T cells. FISH analysis revealed that all circulating $CD3^+$ cells (1326/μL) were derived from his mother. Similarly, TRECs of other patients (patients 13 through 15) were also undetectable despite the maternal T cells (Table).

These results confirm the findings of Patel et al[9] indicating that TRECs quantification is both effective and reliable for the screening of SCID even in the presence of maternal T cells.

Patient 18 with hypomorphic *RAG1* mutations was lymphopenic (550/μL) at 21 months of age, but T (53.1%), B (12.0%), and NK cells (31.2%) were present in peripheral blood. Both T cells with TCR$\alpha\beta$ chain and TCR$\gamma\delta$ chain were derived from the patient, as determined by FISH analysis of the sex chromosome.

The peripheral blood TRECs from this patient (8.0×10^1 copies/μg DNA) (Figure 2, A) was significantly lower than age-matched control patients ($5.2 \pm 3.2 \times 10^3$ copies/μg DNA, n = 5). We purified T cells with TCR$\alpha\beta$ chain by FACS sorting. T cells with TCR$\alpha\beta$ from patient 18 had very low TRECs/cells (4.0×10^1 copies/10^5 cells) than those of age-matched control patients (5.1×10^3 copies/10^5 cells).

We also analyzed TRECs in 2 LIG4-deficient patients (patients 16 and 17) who had leaky T cells (Table). Peripheral blood TRECs were undetectable in both patients (Figure 2, A), and TRECs in the neonatal Guthrie card were also undetectable in patient 17 (Figure 2, B).

These results indicate that TRECs are extremely low in patients with SCID, even if leaky T cells are present.

Discussion

We demonstrated that TRECs were undetectable, or were significantly lower (10^1 to 10^2 copies/μg DNA) than healthy neonates and infants (10^4 to 10^5 copies/μg DNA), in both neonatal Guthrie cards and peripheral blood samples obtained from SCID. All types of SCID tested, including IL2RG, JAK3, ADA, RAG1, LIG4 deficiencies, were identified by measuring TRECs. This finding was consistent with the previous reports that showed the usefulness of TRECs for the identification of SCID[6,7,9] and further indicates that TRECs can identify SCID with maternal T cells and SCID with leaky T cells. In 2 LIG4-deficient patients with leaky T cells (CD3 + 38.7% and 44.3%) and in 1 patient with hypomorphic mutations in *RAG1* gene, who had immunodeficiency and autoimmunity with residual memory T cells,[15,16] TRECs were undetectable. These results indicate that TRECs is a good marker to identify the defect of V(D)J recombination, in which LIG4 and RAG1 play essential roles. We observed progressive loss of TRECs in a patient with SCID and ADA deficiency (patient 11). This observation is compatible with the report that the loss of naive T lymphocytes occurs after birth in patients with ADA deficiency.[17,18]

No false-negative was observed in this study (TRECs were below normal range in all cases of SCID), although sample size was too small to determine the exact false-negative rate.

Consistently, there were no false-positive (TRECs were all positive) in the control samples in this study. The previous studies showed 2.9% and 7.7% of false-positive rates.[6,7] The primers and probe used in this study [14] were different from previously studies.[6,7] However, both probes were found to result in equivalent quantities (data not shown). Thus, the reason of the different false-positive rate between this and the previous studies is currently undetermined. Mass screening using larger population will disclose the exact false-positive and false-negative rate.

The cost to test 1 sample in our study is $5 per sample, which was reported to be cost-effective.[19] We are now trying to further reduce the cost.

Early diagnosis of SCID can prevent severe and recurrent infection, which is often fatal and makes stem cell transplantation difficult. Identification of SCID by newborn screening by TRECs will improve the prognosis and quality of life of patients with SCID. ■

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Generation of transplantable, functional satellite-like cells from mouse embryonic stem cells

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ABSTRACT **Satellite cells are myogenic stem cells responsible for the postnatal regeneration of skeletal muscle. Here we report the successful *in vitro* induction of Pax7-positive satellite-like cells from mouse embryonic stem (mES) cells. Embryoid bodies were generated from mES cells and cultured on Matrigel-coated dishes with Dulbecco's modified Eagle medium containing fetal bovine serum and horse serum. Pax7-positive satellite-like cells were enriched by fluorescence-activated cell sorting using a novel anti-satellite cell antibody, SM/C-2.6. SM/C-2.6-positive cells efficiently differentiate into skeletal muscle fibers both *in vitro* and *in vivo*. Furthermore, the cells demonstrate satellite cell characteristics such as extensive self-renewal capacity in subsequent muscle injury model, long-term engraftment up to 24 wk, and the ability to be secondarily transplanted with remarkably high engraftment efficiency compared to myoblast transplantation. This is the first report of transplantable, functional satellite-like cells derived from mES cells and will provide a foundation for new therapies for degenerative muscle disorders.—Chang, H., Yoshimoto, M., Umeda, K., Iwasa, T., Mizuno, Y., Fukada, S., Yamamoto, H., Motohashi, N., Yuko-Miyagoe-Suzuki, Takeda, S., Heike, T., Nakahata, T. Generation of transplantable, functional satellite-like cells from mouse embryonic stem cells. *FASEB J.* 23, 1907–1919 (2009)**

Key Words: long-term engraftment · secondary transplantation · high engraftment efficiency · self-renewal

DUCHENNE MUSCULAR DYSTROPHY (DMD; ref. 1) is a progressive, lethal muscular disorder (2) with no effective cure despite extensive research efforts. DMD results from mutations in the X-linked *dystrophin* gene (3). Dystrophin and its associated proteins function to link the intracellular actin cytoskeleton of muscle fibers to laminin in the extracellular matrix (4), thereby protecting myofibers from contraction-induced damage (5). Skeletal muscle fibers are continuously regenerated following exercise and injuries when satellite cells (6) are induced to differentiate into myoblasts that form myotubes and replace the damaged myofibers (7, 8). This muscular regeneration is observed at a much higher frequency in DMD patients (9). Continuous damage to myofibers and constant activation of resident satellite cells due to loss of dystrophin leads to the exhaustion of the satellite cells (10, 11), and the eventual depletion of satellite cells is primarily responsible for the onset of DMD symptoms.

Successful transplantation of normal satellite cells into the skeletal muscle of DMD patients may enable *in situ* production of normal muscle tissue and create a treatment option for this otherwise fatal disease. A recent report has shown that the transplantation of satellite cells collected from mouse muscle tissues can produce muscle fibers with normal dystrophin expression in mdx mice (12-14), a model mouse for DMD (15). This study suggests that stem cell transplantation may be a viable therapeutic approach for the treatment of DMD (16).

Satellite cells are monopotent stem cells that have the ability to self-renew and to differentiate into myoblasts and myotubes to maintain the integrity of skeletal muscle (17). Satellite cells lie dormant beneath the basal lamina and express transcription factors such as Pax3 (13, 18) and Pax7 (19). Pax7, a paired box transcription factor, is particularly important for satellite cell function. A recent study of *Pax7-null* mice revealed that Pax7 is essential for satellite cell formation (19) and that the *Pax7-null* mice exhibit a severe deficiency in muscle fibers at birth and premature mortality with complete depletion of the satellite cells. Surface markers such as M-cadherin and c-met (20) are also expressed by satellite cells. However, these markers are not specific to satellite cells because they are also expressed in the cerebellum (21) and by hepatocytes (22). To specifically identify quiescent satellite cells, a

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novel monoclonal antibody, SM/C-2.6, has recently been established (23). Satellite cells purified with this antibody regenerate muscle fibers on implantation into mdx mice (15).

The use of satellite cells for clinical therapies would require the establishment of a reliable source of these cells. Embryonic stem (ES) cells are totipotent stem cells that are able to differentiate into various types of somatic cells *in vitro*. While mouse embryonic stem (mES) cells can be readily induced to differentiate into muscle fibers (24, 25) and the myogenicity of human ES cells was recently validated (26), the induction of mES cells into functional satellite cells has not been reported. Here we have successfully induced mES cells to generate cells expressing Pax7 *in vitro* by forming embryoid bodies (EBs). These ES cell-derived (ES-derived) Pax7-positive cells can be enriched using the SM/C-2.6 antibody (23) and possess a great potential for generating mature skeletal muscle fibers both *in vitro* and *in vivo*. The Pax7-positive cells display a self-renewal ability that can repopulate Pax7-positive cells *in vivo* in the recipient muscles following an injury. Furthermore, these ES-derived Pax7-positive cells could engraft in the recipient muscle for long periods, up to 24 wk, and could also be serially transplanted. These results indicate that ES-derived Pax7-positive cells possess satellite cell characteristics. This is the first report of effective induction of functional satellite cells from mES cells, and these novel findings may provide a new therapeutic approach for treatment of DMD.

MATERIALS AND METHODS

Cell culture

D3 cells, mES cells (27) that ubiquitously express the *EGFP* gene under the *CAG* promoter (28) (a gift from Dr. Masaru Okabe, Osaka University, Osaka, Japan), were used in this study. ES cells were maintained on tissue culture dishes (Falcon) coated with 0.1% gelatin (Sigma, Oakville, CA, USA), in DMEM (Sigma) supplemented with 15% fetal bovine serum (FBS; Thermo Trace, Melbourne, Australia), 0.1 mM 2-mercaptoethanol (Nakalai Tesque, Japan), 0.1 mM nonessential amino acids (Invitrogen, Burlington, CA, USA), 1 mM sodium pyruvate (Sigma), penicillin/streptomycin (50 μg/mL), and 5000 U/ml leukemia inhibitory factor (Dainippon Pharmaceutical Co., Japan).

In vitro differentiation of ES cells into a muscle lineage

To induce EB formation, undifferentiated ES cells were cultured in hanging drops for 3 d at a density of 800 cells/20 μl of differentiation medium, which consisted of DMEM supplemented with penicillin/streptomycin, 0.1 mM nonessential amino acids, 0.1 mM 2-mercaptonethanol, 5% horse serum (HS), and 10% FBS. EBs were transferred to suspension cultures for an additional 3 d (d 3+3). Finally, the EBs were plated in differentiation medium in 48-well plates (Falcon) coated with Matrigel (BD Bioscience, Bedford, MA, USA). The medium was changed every 5 d.

Immunofluorescence and immunocytochemical analysis

Immunostaining of cultured cells and recipient mouse tissues were carried out as described previously (29). Briefly, the left tibialis anterior (LTA) muscle of the recipient mouse was fixed with 4% paraformaldehyde and cut into 6 μm cross sections using a cryostat, and samples were fixed for 5 min in 4% paraformaldehyde (PFA) in PBS and permeabilized with 0.1% Triton X-100 in PBS for 10 min. After incubation in 5% skim milk for 10 min at room temperature to block nonspecific antibody binding, cells were incubated for 12 h at 4°C with anti-mouse monoclonal antibodies. Antibodies used in this study were mouse anti-Pax7, which was biotinylated using a DSB-X Biotin Protein Labeling Kit (D20655; Molecular Probes, Eugene, OR, USA), mouse anti-Pax3 (MAB1675, MAB2457; R&D Systems, Minneapolis, MN, USA), rabbit anti-mouse Myf5 (sc-302; Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse anti-mouse M-cadherin (205610; Calbiochem, San Diego, CA, USA), mouse anti-myosin heavy chain (MHC; 18-0105; Zymed Laboratories, San Francisco, CA, USA; reacts with human, rabbit, rat, mouse, bovine, and pig skeletal MHC), mouse anti-mouse myogenin and mouse anti-mouse Myo-D1 (M3559, M3512; Dako, Carpinteria, CA, USA), monoclonal rabbit anti-mouse laminin (LB-1013; LSL, Tokyo, Japan), and mouse anti-mouse dystrophin (NCL-DYS2; Novocastra Laboratories, Newcastle-upon-Tyne, UK). Cy3-labeled antibodies to mouse or rabbit IgG, fluorescein isothiocyanate-labeled antibodies to mouse or rabbit IgG (715-005-150, 711-165-152; Jackson ImmunoResearch Laboratory, Bar Harbor, ME, USA), or Alexa 633-labeled goat anti-rabbit IgG (A21070; Invitrogen, Molecular Probes) were applied as secondary antibodies. Hoechst 33324 (H3570; Molecular Probes) was used for nuclear staining. The samples were examined with a fluorescence microscope (Olympus, Tokyo, Japan) or an AS-MDW system (Leica Microsystems, Wetzlar, Germany). Micrographs were obtained using an AxioCam (Carl Zeiss Vision, Hallbergmoos, Germany) or the AS-MDW system (Leica Microsystems). In sections of muscles transplanted with ES-derived satellite cells, the number of GFP-positive muscle fascicles and GFP/Pax7-double-positive cells were counted, per field, at ×100. More than 10 fields in each tissue sample were observed. To prevent nonspecific secondary antibody binding to Fc receptors, all immunostaining of frozen sections used the Vector® M.O.M™ Immunodetection Kit (BMK-2202; Vector Laboratories, Burlingame, CA, USA).

PCR analysis

Total RNA was isolated from cultured cells in 48-well plates, using TRIzol reagent (Invitrogen). The following specific primers were used for PCR:

Pax3, sense, 5′-AACACTGGCCCTCAGTGAGTTCTAT-3′, and antisense, 5′-ACTCAGGATGCCATCGATGCTGTG-3′; Pax7, sense, 5′-CATCCAGTGCTGGTACCCCACAG-3′, and antisense, 5′-CTGTGGATGTCACCTGCTTGAA-3′; Myf5, sense, 5′-GAGCTGCTGAGGGAACAGGTGG-3′, and antisense, 5′-GTTCTTTCGGGACCAGACAGGG-3′; MyoD, sense, 5′-AGGCTCTGCTGCGCGACCAG-3′, and antisense, 5′-TGCAGTCGATCTCTCAAAGC-3′; myogenin, sense, 5′-TGAGGGAGAAGCGCAGGCTCAAG-3′, and antisense, 5′-ATGCTGTCCACGATGGACGTAAGG-3′; M-cadherin, sense, 5′-CCACAAACGCCTCCCCTACCC-3′, and antisense, 5′-GTCGATGCTGAAGAACTCAGGGC-3′; C-met, sense, 5′-GAATGTCGTCCTACACGGCCAT-3′, and antisense, 5′-CACTACACAGTCAGGACACTGC-3′; GAPDH, sense, 5′-TGAAGGTCGGTGTGAACGGATTTGGC-3′, and antisense, 5′-TGTTGGGGGCCGAGTTGGGATA-3′. AmpliTaqGold (Applied