TABLE III. *(Continued)*

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Genetic defects/ presumed Pathogenesis	Relative frequency among PIDs
(b) Ataxia-telangiectasia like disease (ATLD)	Progressive decrease	Normal	Antibodies variably decreased	Moderate ataxia; pulmonary infections; severely increased radiosensitivity	AR	Hypomorphic mutations in *MRE11*; disorder of cell cycle checkpoint and DNA double-strand break repair	Very rare
(c) Nijmegen breakage syndrome	Progressive decrease	Variably reduced	Often decreased IgA, IgE, and IgG subclasses; increased IgM; antibodies variably decreased	Microcephaly; birdlike face; lymphomas; solid tumors; ionizing radiation sensitivity; chromosomal instability	AR	Hypomorphic mutations in *NBS1* (*Nibrin*); disorder of cell cycle checkpoint and DNA double-strand break repair	Rare
(d) Bloom syndrome	Normal	Normal	Reduced	Short stature; birdlike face; sun-sensitive erythema; marrow failure; leukemia; lymphoma; chromosomal instability	AR	Mutations in *BLM*; RecQ like helicase	Rare
(e) Immuno-deficiency with centromeric instability and facial anomalies (ICF)	Decreased or normal	Decreased or normal	Hypogammaglobulinemia; variable antibody deficiency	Facial dysmorphic features; macroglossia; bacterial/ opportunistic infections; malabsorption; multiradial configurations of chromosomes 1, 9, 16; no DNA breaks	AR	Mutations in DNA methyltransferase *DNMT3B*, resulting in defective DNA methylation	Very rare
(f) PMS2 deficiency (class-switch recombination [CSR] deficiency caused by defective mismatch repair)	Normal	Switched and nonswitched B cells are reduced	Low IgG and IgA, elevated IgM, abnormal antibody responses	Recurrent infections; café-au-lait spots; lymphoma, colorectal carcinoma, brain tumor	AR	Mutations in PMS2, resulting in defective CSR-induced DNA double strand breaks in Ig switch regions	Very rare
3. Thymic defects							
DiGeorge anomaly (chromosome 22q11.2 deletion syndrome	Decreased or normal	Normal	Normal or decreased	Conotruncal malformation; abnormal facies; large deletion (3Mb) in 22q11.2 (or rarely a deletion in 10p)	*De novo* defect or AD	Contiguous gene defect in 90% affecting thymic development; mutation in *TBX1*	Common

(Continued)

TABLE III. *(Continued)*

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Genetic defects/ presumed Pathogenesis	Relative frequency among PIDs
4. Immune-osseous dysplasias							
(a) Cartilage hair hypoplasia	Decreased or normal; impaired lymphocyte proliferation*	Normal	Normal or reduced Antibodies variably decreased	Short-limbed dwarfism with metaphyseal dysostosis, sparse hair, bone marrow failure, autoimmunity, susceptibility to lymphoma and other cancers, impaired spermatogenesis, neuronal dysplasia of the intestine	AR	Mutations in *RMRP* (RNase MRP RNA) Involved in processing of mitochondrial RNA and cell cycle control	Rare
(b) Schimke syndrome	Decreased	Normal	Normal	Short stature, spondiloepiphyseal dysplasia, intrauterine growth retardation, nephropathy; bacterial, viral, fungal infections; may present as SCID; bone marrow failure	AR	Mutations in *SMARCAL1* Involved in chromatin remodeling	Very rare
5. Comel-Netherton syndrome	Normal	Switched and nonswitched B cells are reduced	Elevated IgE and IgA Antibody variably decreased	Congenital ichthyosis, bamboo hair, atopic diathesis, increased bacterial infections, failure to thrive	AR	Mutations in *SPINK5* resulting in lack of the serine protease inhibitor LEKTI, expressed in epithelial cells	Rare
6. Hyper-IgE syndromes (HIES)							
(a) AD-HIES (Job syndrome)	Normal T_H17 cells decreased	Normal	Elevated IgE; specific antibody production decreased	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	AD Often *de novo* defect	Dominant-negative heterozygous mutations in *STAT 3*	Rare

(Continued)

TABLE III. *(Continued)*

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Genetic defects/ presumed Pathogenesis	Relative frequency among PIDs
(b) AR-HIES				No skeletal and connective tissue abnormalities;	AR		
	Normal	Normal	Elevated IgE	i) susceptibility to intracellular bacteria (mycobacteria, *Salmonella*), fungi and viruses		Mutation in *TYK2*	Extremely rare
	Reduced	Reduced	Elevated IgE, low IgM	ii) recurrent respiratory infections; extensive cutaneous viral and staphylococcal infections, increased risk of cancer, severe atopy with anaphylaxis		Mutation in *DOCK8*	Very rare
	Normal	Normal	Elevated IgE	iii) CNS hemorrhage, fungal and viral infections		Unknown	Extremely rare
7. Chronic mucocutaneous candidiasis	Normal (defect of Th17 cells in CARD9 deficiency)	Normal	Normal	Chronic mucocutaneous candidiasis, impaired delayed-type hypersensitivity to *Candida* antigens, autoimmunity, no ectodermal dysplasia	AD, AR, sporadic	Mutations in *CARD9* in one family with AR inheritance: defect unknown in other cases	Very rare
8. Hepatic veno-occlusive disease with immunodeficiency (VODI)	Normal (decreased memory T cells)	Normal (decreased memory B cells)	Decreased IgG, IgA, IgM	Hepatic veno-occlusive disease; Pneumocystis jiroveci pneumonia; thrombocytopenia; hepatosplenomegaly	AR	Mutations in *SP110*	Extremely rare
9. XL-dyskeratosis congenita (Hoyeraal-Hreidarsson syndrome)	Progressive decrease	Progressive decrease	Variable	Intrauterine growth retardation, microcephaly, nail dystrophy, recurrent infections, digestive tract involvement, pancytopenia, reduced number and function of NK cells	XL	Mutations in dyskerin *(DKC1)*	Very rare

AD, Autosomal-dominant inheritance; *AR*, autosomal-recessive inheritance; *ATM*, ataxia-telangiectasia mutated; *BLM*, Bloom syndrome; *DNMT3B*, DNA methyltransferase 3B; *MRE11*, meiotic recombination 11; *NBS1*, Nijmegen breakage syndrome 1; *TBX1*, T-box 1; *TYK2*, tyrosine kinase 2; *XL*, X-linked inheritance.
*Patients with cartilage-hair hypoplasia can also present with typical SCID or with Omenn syndrome.

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 1a	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 1b	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; *LI2B*, 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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Ex vivo expanded cord blood CD4 T lymphocytes exhibit a distinct expression profile of cytokine-related genes from those of peripheral blood origin

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Summary

With an increase in the importance of umbilical cord blood (CB) as an alternative source of haematopoietic progenitors for allogenic transplantation, donor lymphocyte infusion (DLI) with donor CB-derived activated $CD4^+$ T cells in the unrelated CB transplantation setting is expected to be of increased usefulness as a direct approach for improving post-transplant immune function. To clarify the characteristics of activated $CD4^+$ T cells derived from CB, we investigated their mRNA expression profiles and compared them with those of peripheral blood (PB)-derived activated $CD4^+$ T cells. Based on the results of a DNA microarray analysis and quantitative real-time reverse transcriptase–polymerase chain reaction (RT-PCR), a relatively high level of forkhead box protein 3 (Foxp3) gene expression and a relatively low level of interleukin (IL)-17 gene expression were revealed to be significant features of the gene expression profile of CB-derived activated $CD4^+$ T cells. Flow cytometric analysis further revealed protein expression of Foxp3 in a portion of CB-derived activated $CD4^+$ T cells. The low level of retinoic acid receptor-related orphan receptor γ isoform t (RORγt) gene expression in CB-derived activated $CD4^+$ T cells was speculated to be responsible for the low level of IL-17 gene expression. Our data indicate a difference in gene expression between $CD4^+$ T cells from CB and those from PB. The findings of Foxp3 expression, a characteristic of regulatory T cells, and a low level of IL-17 gene expression suggest that CB-derived $CD4^+$ T cells may be a more appropriate source for DLI.

Keywords: CD4; cord blood; donor lymphocyte infusion; forkhead box protein 3; interleukin 17; T cell

Abbreviations: BIM, BCL2-like 11; CB, cord blood; CTLA-4, cytotoxic T-lymphocyte antigen-4; CDKN, cyclin-dependent kinase inhibitor; DLI, donor lymphocyte infusion; Foxp3, forkhead box protein 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GM-CSF, granulocyte–macrophage colony-stimulating factor; GVHD, graft-versus-host disease; GVL, graft-versus-leukaemia; HSCT, haematopoietic stem cell transplantation; ICOS, inducible T-cell co-stimulator; IFNG, interferon γ; IL, interleukin; PB, peripheral blood; RORγt, retinoic acid receptor-related orphan receptor γ isoform t; RT, reverse transcriptase; TCR, T-cell receptor; Th, T helper cell; Treg, regulatory T cell.

Introduction

Donor lymphocyte infusion (DLI) is a direct and useful approach for improving post-transplant immune function. DLI has been shown to exert a graft-versus-leukaemia (GVL) effect and has emerged as an effective strategy for the treatment of patients with leukaemia, especially chronic myelogenous leukaemia, who have relapsed after unrelated haematopoietic stem cell transplantation (HSCT).[1] In addition, DLI has been successfully used for some life-threatening viral infections, including Epstein–Barr virus and cytomegalovirus infections after HSCT.[2]

Although DLI frequently results in significant acute and/or chronic graft-versus-host disease (GVHD), several groups have demonstrated that depletion of CD8 T cells from DLIs efficiently reduces the incidence and severity of GVHD while maintaining GVL activity.[3,4] Therefore, selective CD4 DLI is expected to provide an effective and low-toxicity therapeutic strategy for improving post-transplant immune function. Actually, selective CD4 DLI based on a recently established method for *ex vivo* T-cell expansion using anti-CD3 monoclonal antibody and interleukin (IL)-2 is now becoming established as a routine therapeutic means of resolving post-transplant immunological problems in Japan.[5]

The importance of umbilical cord blood (CB) as an alternative source of haematopoietic progenitors for allogenic transplantation, mainly in patients lacking a human leucocyte antigen (HLA)-matched marrow donor, has increased in recent years. Because of the naïve nature of CB lymphocytes, the incidence and severity of GVHD are reduced in comparison with the allogenic transplant setting. In addition, CB is rich in primitive $CD16^-$ $CD56^+$ natural killer (NK) cells, which possess significant proliferative and cytotoxic capacities, and so have a substantial GVL effect.[6]

In contrast, a major disadvantage of CB transplantation is the low yield of stem cells, resulting in higher rates of engraftment failure and slower engraftment compared with bone marrow transplantation. In addition, it was generally thought to be difficult to perform DLI after CB transplantation using donor peripheral blood (PB), with the exception of transplantations from siblings. However, the above-described method for the *ex vivo* expansion of activated T cells can produce a sufficient amount of cells for therapy using the CB cell residues in an infused bag, which has solved this problem and made it possible to perform DLI with donor CB-derived activated $CD4^+$ T cells in the unrelated CB transplantation setting.[5] It has also been reported that CB-derived T cells can be expanded *ex vivo* while retaining the naïve and/or central memory phenotype and polyclonal T-cell receptor (TCR) diversity,[7] and thus potential utilization for adoptive cellular immunotherapy post-CB transplantation has been suggested.[8]

There are functional differences between CB and PB lymphocytes, although the details remain unclear. In an attempt to clarify the differences in characteristics between activated $CD4^+$ T cells derived from CB and those derived from PB, we investigated gene expression profiles. In this paper we present evidence that CB-derived $CD4^+$ T cells are distinct from PB-derived $CD4^+$ T cells in terms of gene expression.

Materials and methods

Cell culture and preparation

CB was distributed by the Tokyo Cord Blood Bank (Tokyo, Japan). The CB was originally collected and stored for stem cell transplantation. Stocks that were inappropriate for transplantation because they contained too few cells were distributed for research use with informed consent, with the permission of the ethics committee of the bank. In addition, all of the experiments in this study using distributed CB were performed with the approval of the local ethics committee. The mononuclear cells were isolated by Ficoll-Paque centrifugation and cultured in the presence of an anti-CD3 monoclonal antibody and interleukin (IL)-2 using TLY Culture Kit 25 (Lymphotec Inc., Tokyo, Japan) as described previously.[5] Although several different methods for T-cell stimulation have been reported, this method is currently being used clinically in Japan. Thus we selected this method in this study. After 14 days of culture, $CD4^+$ cells were isolated using a magnetic-activated cell sorting (MACS) system (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. As a control, mononuclear cells isolated from the peripheral blood of healthy volunteers were similar examined.

Polymerase chain reaction (PCR)

Total RNA was extracted from cells using an RNeasy kit (Qiagen, Valencia, CA) and reverse-transcribed using a First-Strand cDNA synthesis kit (GE Healthcare Bio-Science Corp., Little Chalfont, Buckinghamshire, UK) according to the manufacturer's instructions. Using cDNA synthesized from 150 ng of total RNA as a template for one amplification, real-time reverse transcriptase (RT)-PCR was performed using SYBR® Green PCR master mix, TaqMan® Universal PCR master mix and TaqMan® gene expression assays (Applied Biosystems, Foster City, CA), and an inventoried assay carried out on an ABI PRISM® 7900HT sequence detection system (Applied Biosystems) according to the instructions provided. Either the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene or the β-actin gene was used as an internal control for normalization. The sequences of gene-specific primers for real-time RT-PCR are listed in Table 1.

DNA microarray analysis

The microarray analysis was performed as previously described.[9] Total RNA isolated from cells was reverse-

Table 1. The sequences of gene-specific primers for reverse transcriptase–polymerase chain reaction (RT-PCR) and real-time RT-PCR used in this study

Primer	Sequence
IL-4 forward	CACAGGCACAAGCAGCTGAT
IL-4 reverse	CCTTCACAGGACAGGAATTCAAG
IL-6 forward	GTAGCCGCCCCACACAGA
IL-6 reverse	CCGTCGAGGATGTACCGAAT
IL-10 forward	GCCAAGCCTTGTCTGAGATGA
IL-10 reverse	CTTGATGTCTGGGTCTTGGTTCT
IL-17 forward	GACTCCTGGGAAGACCTCATTG
IL-17 reverse	TGTGATTCCTGCCTTCACTATGG
IL-17F forward	GCTTGACATTGGCATCATCAA
IL-17F reverse	GGAGCGGCTCTCGATGTTAC
IL-23 forward	GAGCCTTCTCTGCTCCCTGATAG
IL-23 reverse	AGTTGGCTGAGGCCCAGTAG
IL-23R forward	AACAACAGCTCGGCTTTGGTATA
IL-23R reverse	GGGACATTCAGCAGTGCAGTAC
IFNG forward	CATCCAAGTGATGGCTGAACTG
IFNG reverse	TCGAAACAGCATCTGACTCCTTT
GM-CSF forward	CAGCCCTGGAGCATGTG
GM-CSF reverse	CATCTCAGCAGCAGTGTCTCTAC
RORγt forward	TGGGCATGTCCCGAGATG
RORγt reverse	GCAGGCTGTCCCTCTGCTT
STAT-3 forward	GGAGGAGGCATTCGGAAAGT
STAT-3 reverse	GCGCTACCTGGGTCAGCTT
FOXP3 forward	GAGAAGCTGAGTGCCATGCA
FOXP3 reverse	GCCACAGATGAAGCCTTGGT

IL, interleukin; *IFNG*, interferon γ; *FOXP3*, forkhead box protein 3; *GM-CSF*, granulocyte–macrophage colony-stimulating factor; *RORγt*, retinoic acid receptor-related orphan receptor γ isoform t; *STAT*, signal transducer and activator of transcription.

transcribed and labelled using One-Cycle Target Labeling and Control Reagents as instructed by the manufacturer (Affymetrix, Santa Clara, CA). The labelled probes were hybridized to a Human Genome U133 Plus 2·0 Array (Affymetrix). The arrays were used in a single experiment and analysed with GENECHIP operating software 1.2 (Affymetrix). Background subtraction and normalization were performed using GENESPRING GX 7.3 software (Agilent Technologies, Santa Clara, CA). The signal intensity was pre-normalized based on the positive control genes (GAPDH and β-actin) for all measurements on that chip. To account for differences in detection efficiency between spots, the pre-normalized signal intensity of each gene was normalized to the median of pre-normalized measurements for that gene. The data were filtered as follows. (i) Genes that were scored as absent in all samples were eliminated. (ii) Genes with a signal intensity of < 90 were eliminated. (iii) Genes that exhibited increased (fold-change > 2) or decreased (fold-change > 2) expression in CB-derived $CD4^+$ T cells compared with PB-derived $CD4^+$ T cells were selected by comparing the mean value of signal intensities in each condition.

Immunofluorescence study

After periods of cultivation, cells were collected and stained with fluorescence-labelled monoclonal antibodies and analysed by flow cytometry (FC500; Beckman/Coulter, Fullerton, CA). A four-colour immunofluorescence study was performed with a combination of fluorescein isothiocyanate (FITC)-conjugated anti-CD3, phycoerythrin (PE)-conjugated anti- forkhead box protein 3 (Foxp3), phycoerythrin-cyanine-5 (PC5)-conjugated anti-CD4 and PC7-conjugated anti-CD8 (Beckman/Coulter). After staining of cell surface antigens, cells were permeabilized with IntraPrep (Dako, Glostrup, Denmark) and intracellular antigen (Foxp3) was further stained.

Statistical analysis

The statistical analysis was performed using a Student's *t*-test and a *P*-value < 0·05 was considered to be statistically significant.

Results

Expression profiles of activated $CD4^+$ T cells derived from human CB and PB

To compare the gene expression patterns of CB-derived $CD4^+$ cells and PB-derived $CD4^+$ cells, we performed DNA microarray analysis using the Affymetrix Human Genome U133 Plus 2·0 Array. After background subtraction, comparison of the gene expression profiles of two independent CB-derived $CD4^+$ samples and PB-derived $CD4^+$ samples was performed using a gene cluster analysis. The genes differentially expressed (fold-change > 2) between the activated $CD4^+$ T cells derived from CB and those derived from PB were selected, and 396 probes were found to exhibit higher levels of expression in CB-derived $CD4^+$ samples while 131 probes exhibited higher levels in PB-derived $CD4^+$ samples. Parts of the data are summarized and presented in Fig. 1a and Tables 2–4.

Among these genes, those closely correlated to T-cell function and development were selected (Fig. 1b). The genes exhibiting higher levels of expression in CB-derived $CD4^+$ samples included those encoding cell cycle regulators, including cyclin-dependent kinase (CDKN)2A and 2B, transcriptional regulators and signal transduction factors (Tables 2 and 3). The genes for cytokines, chemokines and their receptors such as Interferon γ (IFNG), granulocyte-macrophage colony-stimulating factor (GM-CSF) and for T-cell transcriptional regulators (*FOXP3*) as well as the genes related to T-cell development including CD28, cytotoxic T lymphocyte antigen-4 (CTLA4) and inducible T-cell co-stimulator (ICOS) were also found among the genes exhibiting higher levels of expression in CB-derived $CD4^+$ samples (Fig. 1b). The factors reported

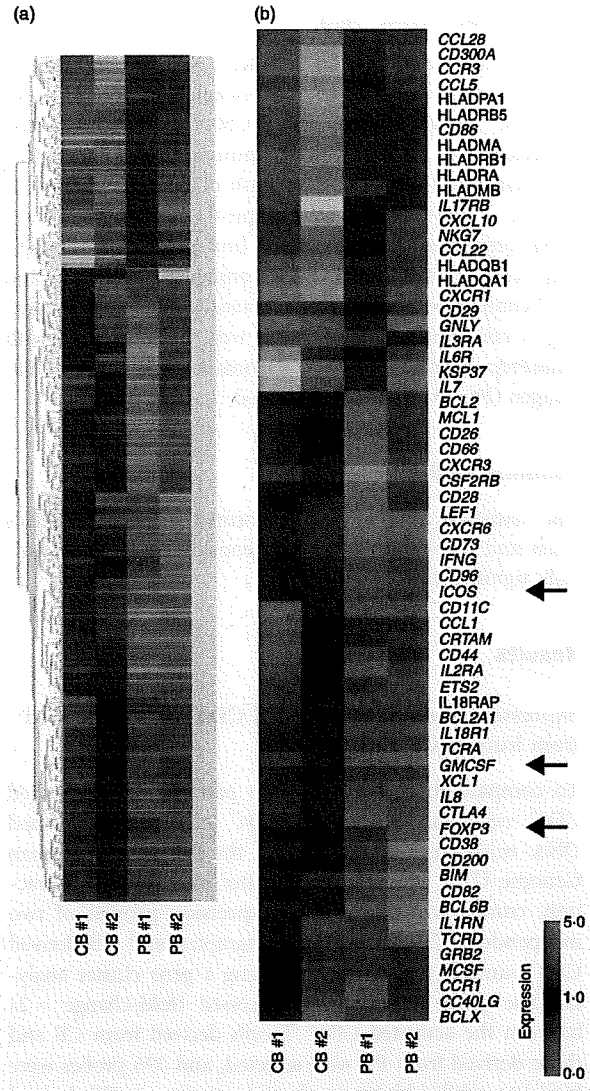

Figure 1. Comparison of the gene expression profiles of cord blood (CB)- and peripheral blood (PB)-derived $CD4^+$ T cells. Hierarchical clustering of results from a microarray analysis for CB- and PB-derived $CD4^+$ T cells is indicated. (a) A total of 529 genes characterizing $CD4^+$ T cells (396 genes for CB-derived $CD4^+$ T cells and 131 genes for PB-derived $CD4^+$ T cells) were used to create the gene tree. The gene list is presented in Tables 3 and 4. (b) Genes related to T-cell development (40 genes for CB-derived $CD4^+$ T cells and 26 genes for PB-derived $CD4^+$ T cells) are presented. The arrows indicate the expression pattern of T-cell lineage-specific genes including inducible T-cell co-stimulator (*ICOS*), granulocyte-macrophage colony-stimulating factor (*GM-CSF*) and forkhead box protein 3 (*FOXP3*).

to be essential for negative selection in $CD4^+$ $CD8^+$ thymocytes such as BCL2-like 11 (BIM)[10] as well as other apoptotic regulators were also found among the genes exhibiting higher expression levels in CB-derived $CD4^+$ samples.

The genes with a higher level of expression in the PB-derived $CD4^+$ T cells included those encoding transcriptional regulators, signal transduction factors, major histocompatibility complex (MHC) class II molecules (*HLADMA*, *HLADMB*, *HLADPA1*, *HLADQB1*, *HLADRA*, *HLADRB1* and *HLADRB5*), and cytokines, chemokines and their receptors (*IL-7*, *IL-17RB*), as well as genes that characterize the T-cell lineage (*CD29*, *CD86*) (Fig. 1b, Tables 2, 4).

Notably, microarray studies showed that the expression of several regulatory T cell (Treg)-related genes was significantly higher in the CB-derived T cells. Foxp3 is an important T-cell transcription factor and is considered to be a marker of Tregs. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) and ICOS, which belong to the CD28 family of receptors and play a crucial role in the activation of T cells, were reported to be highly expressed in activated Tregs.[11,12] All of the above genes were expressed at higher levels in the CB-derived CD4 T cells (Fig. 1).

The microarray results for major genes related to the development of the T-cell lineage, including those not appeared in Fig. 1, are summarized in Table 2. As shown in Table 2, the expression of T-cell lineage master regulator genes, such as *TBX21*, *GATA3* and *MAF*, and T cell-related cytokines, such as *IL-4*, *IL-5*, *IL-13*, *IL-22* and *TGFB1*, revealed no significant difference between CB-derived $CD4^+$ cells and PB-derived $CD4^+$ cells. However, other T cell-related genes, including *IL-2*, *IL-6*, *IL-9*, *IL-10* and *IL-17*, were eliminated from the list in the course of background subtraction because the signal intensity of each gene was low (< 90 as raw data) in all of the samples.

Differences in the expression patterns of T-cell lineage-specific genes between CB-derived and PB-derived $CD4^+$ T cells

To further confirm the characteristic gene expression in CB- and PB-derived $CD4^+$ T cells, we performed a real-time RT-PCR analysis. Consistent with the microarray data, when the mRNA levels of the genes related to the T helper type 1 (Th1) and Th2 phenotypes were examined, higher levels of GM-CSF and IFNG were observed in CB-derived T cells, while IL-4 revealed no significant tendency (Fig. 2). We also examined IL-6 and IL-10 and no significant tendency was observed either in the expression of these genes (Fig. 2).

Next we examined the expression of the genes related to Tregs and observed a higher level of Foxp3, but lower levels of retinoic acid receptor-related orphan receptor γ isoform t (RORγt); and IL-17F, in CB-derived T cells (Fig. 3). In contrast, there was no significant tendency in the expression of genes encoding signal transducer and activator of transcription 3 (STAT-3), IL-23 and IL-23 receptors. In the case of the *IL-17* gene, clear amplifica-

Table 2. The microarray results for T-cell-related genes

Description	Gene	Gene ID	CB-I		CB-2		PB-1		PB-2	
			Normalized	Raw	Normalized	Raw	Normalized	Raw	Normalized	Raw
Master regulation										
Thl	*TBX21*	220684_at	1·1382915	305·7	0·7851455	247·1	1·045663	230·5	0·954337	261·4
Th2	*GATA3*	209602_s_at	1·471558	1204	0·7742825	742·1	1·0740323	721·1	0·9259675	772·5
	GATA3	209603_at	1·265932	416·5	0·53335179	205·7	1·0535141	284·5	0·9464856	317·6
	GATA3	209604_s_at	1·350573	5300	0·6415387	2950	1·0573606	3406	0·9426395	3773
	MAF	206363_at	0·7447395	672·7	0·8744312	925·6	1·1255689	834·5	1·2704437	1170
	MAF	209348_s_at	1·0320604	2078	0·8329663	1965	0·9679398	1600	1·8301903	3758
	MAF	229327_s_at	0·9099149	569·7	0·6089576	446·8	1·090085	560·2	1·4076804	898·9
Treg	*FOXP3*	221334_s_at	1·8893701	100·6	1·4199468	88·6	0·4988136	21·8	0·5800531	31·5
	FOXP3	224211_at	1·6205869	152·3	1·4101433	155·3	0·5898568	45·5	0·2347433	22·5
Cytokines										
Thl	*IFNG*	210354_at	1·4801383	2000	1·9182948	3037	0·457517	507·4	0·5198616	716·4
	GM-CSF	210229_s_at	1·2802086	1293	2·6726868	3163	0·6906437	572·5	0·7197912	741·4
Th2	*IL-4*	207538_at	2·0291064	687·2	0·3361219	133·4	0·9317174	259	1·0682826	369
	IL-4	207539_s_at	2·8263247	965	0·3561467	142·5	0·8481774	237·7	1·1518226	401·1
	IL-5	207952_at	1·3380713	810	0·0610382	43·3	1·0097023	501·7	0·9902797	611·4
	IL-13	207844_at	3·9835246	1712	0·8117443	408·8	1·1453367	404	0·8691162	452·9
Treg	*TGFB1*	203085_s_at	1·5166419	774·9	0·9012154	539·6	1·0987847	460·8	0·8546632	374·6
Others	*IL-22*	222974_at	0·1272062	5·2	4·325279	207·2	0·5632869	18·9	1·4367131	59·9
Surface molecules										
Treg	*CTLA4*	231794_at	1·3871489	336·9	1·2560804	357·5	0·7439196	148·3	0·4444751	110·1
	CTLA4	236341_at	1·2573498	905·7	1·6210791	1368	0·6800935	402·1	0·7426501	545·6
Others	*IL-2RA*	206341_at	1·5216751	3569	1·2715347	3494	0·7284654	1402	0·6569936	1571
	IL-2RA	211269_s_at	1·1563299	4436	1·3173387	5923	0·8436702	2657	0·560745	2194
	ICOS	210439_at	1·378036	619·8	1·343834	708·3	0·567216	209·4	0·656166	301
	CD28	211856_x_at	1·3887135	144·9	1·2905376	157·8	0·3292731	28·2	0·7094624	75·5
	CD28	211861_x_at	1·350062	183·3	1·4109998	224·5	0·4863549	54·2	0·649938	90

The microarray results for major genes related to the development of the T-cell lineage are summarized. The normalized and raw data for four samples are indicated for each gene. Those for which differential expression was found between cord blood (CB)- and peripheral blood (PB)-derived $CD4^+$ T cells in a gene cluster analysis (fold-change > 2) are highlighted in grey. Genes exhibiting low signal intensity (< 90 as raw data) in all of the four samples were eliminated from the list beforehand in the process of background subtraction, and thus do not appeared in this table.

CTLA-4, cytotoxic T-lymphocyte antigen-4; *FOXP3*, forkhead box protein 3; GATA, GATA family of zinc finger trancription factors; *GM-CSF*, granulocyte–macrophage colony-stimulating factor; ICOS, inducible T-cell co-stimulator; IFNG, interferon γ; *IL*, interleukin; MAF, macrophage-activating factor; TBX21, T-box protein 21; TGFB1, transforming growth factor, beta 1; Th1, T helper type 1; Treg, regulatory T cell.

tion was detected in PB-derived T cells whereas no amplification was observed in the samples of CB-derived T cells (data not shown).

To further investigate whether increased expression of the *FOXP3* gene is a general feature of CB-derived $CD4^+$ T cells, we tested four samples of CB-derived $CD4^+$ T cells by real-time RT-PCR analysis and compared the results with those for equivalent numbers of PB-derived samples. As shown in Fig. 4, two CB-derived samples (CB 4 and 5, at 2 weeks) revealed significantly increased gene expression of *FOXP3* when compared with PB-derived samples, whereas the remaining two samples (CB 3 and 6; termed 'additional' samples below) did not. We also tested *FOXP3* gene expression at an earlier time-point in the same samples and observed no significant increase of *FOXP3* gene expression in CB-derived $CD4^+$ T cells at 1 week (Fig. 4). When the data were analysed statistically, expression of the *FOXP3* gene was found to be significantly higher in CB-derived $CD4^+$ T cells in comparison with equivalent PB-derived $CD4^+$ T cells at both 1 week ($P < 0{\cdot}05$) and 2 weeks ($P < 0{\cdot}05$) (Fig. 4).

Next we assessed the expression of the Foxp3 protein in CB-derived $CD4^+$ T cells. When the same samples as described above were examined by flow cytometry using a specific antibody, the Foxp3 protein was certainly detected in a portion of cells in all of four CB-derived samples while not detected in any of the PB-derived samples tested (Fig. 5). Inconsistent with the results of real-time RT-PCR, expression level of Foxp3 proteins was higher in CB-derived $CD4^+$ T cells at 1 week than at 2 weeks.

Table 3. Genes up-regulated in $CD4^+$ T cells from cord blood samples 1 and 2 (CB 1 and CB 2, respectively)

		Fold change				
Affi ID	Gene abbreviation	CB 1	CB 2	PB 1	PB 2	Gene name
Apoptosis						
1555372_at	*BimL*	1·39	1·52	0·61	0·42	BCL2-like 11 (apoptosis facilitator)
237837_at	*BCL2*	1·27	1·32	0·49	0·73	B-cell CLL/lymphoma 2
205681_at	*BCL2A1*	1·91	1·53	0·39	0·47	BCL2-related protein A1
1558143_a_at	*BCL2L11*	1·68	1·74	0·32	0·32	BGL2-like 11 (apoptosis facilitator)
228311_at	*BCL6B*	1·36	3·39	0·64	0·26	B-cell CLL/lymphoma 6, member B (zinc finger protein)
215037_s_at	*BCLX*	2·56	1·27	0·73	0·56	BCL2-like 1
224414_s_at	*CARD6*	2·65	1·34	0·56	0·66	Caspase recruitment domain family, member 6
201631_s_at	*IER3*	1·62	2·95	0·38	0·31	Immediate early response 3
218000_s_at	*PHLDA1*	2·34	1·21	0·53	0·79	Pleckstrin homology-like domain, family A, member 1
209803_s_at	*PHLDA2*	2·87	1·32	0·31	0·68	Pleckstrin homology-like domain, family A. member 2
203063_at	*PPMIF*	1·26	1·53	0·74	0·64	Protein phosphatase IF (PP2C domain containing)
205214_at	*STK17B*	1·78	1·26	0·74	0·71	Serine/threonine kinase 17b (apoptosis-inducing)
217853_at	*TENS1*	1·63	6·00	0·04	0·37	Tensin 1
B- and T-cell development						
211861_x_at	*CD28*	1·35	1·41	0·49	0·65	CD28 antigen(Tp44)
207892_at	*CD40LG*	3·67	1·32	0·45	0·68	C040 ligand (TNF superfamily, member 5, hyper-IgM syndrome)
206914_at	*CRTAM*	2·76	1·60	0·40	0·36	Class I MHC-restricted T-cell-associated molecule
210557_x_at	*CSF1*	3·79	1·22	0·78	0·70	Colony-stimulating factor 1 (macrophage)
210229_s_at	*CSF2*	1·28	2·67	0·69	0·72	Colony-stimulating factor 2 (granulocyte–macrophage)
205159_at	*CSF2RB*	2·33	1·60	0·18	0·40	Colony-stimulating factor 2 receptor
231794_at	*CTLA4*	1·39	1·26	0·74	0·44	Cytotoxic T-lymphocyte-associated protein 4
204232_at	*FCER1G*	1·63	2·14	0·28	0·37	Fc fragment of IgE, high affinity 1, receptor for; gamma polypeptide
210439_at	*ICOS*	1·38	1·34	0·57	0·66	Inducible T-cell costimulator
210354_at	*IFNG*	1·48	1·92	0·46	0·52	Human mRNA for HuIFN -gamma interferon
230536_at	*PBX4*	1·48	1·26	0·50	0·74	Pre-B-cell leukaemia transcription factor 4
215540_at	*TCRA*	1·25	1·87	0·67	0·75	T-cell antigen receptor alpha
234440_al	*TCRD*	7·51	1·48	0·50	0·52	Human T-cell receptor delta-chain
Cell growth and maintenance						
213497_at	*ABTB2*	2·06	1·34	0·66	0·63	Ankyrin repeat and BTB (POZ) domain containing 2
201236_s_at	*BTG2*	1·60	1·23	0·60	0·77	BTG family, member 2
235287_at	*CDK6*	1·50	1·32	0·44	0·68	Cyclin-dependent kinase 6
209644_x_at	*CDKN2A*	2·90	1·21	0·67	0·79	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)
236313_at	*CDKN2B*	3·24	1·28	0·58	0·72	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
241984_at	*CHES1*	1·38	1·34	0·66	0·63	Checkpoint suppressor 1
202552_s_at	CRIM1	1·94	1·39	0·32	0·61	Cysteine-rich transmembrane BMP regulator 1 (chordin-like)
204844_at	*ENPEP*	1·64	1·75	0·09	0·36	Glutamyl aminopeptidase (aminopeptidase A)
205418_at	*FES*	1·39	1·80	0·61	0·25	Feline sarcoma oncogene
228572_at	*GRB2*	4·69	1·21	0·79	0·78	Growth factor receptor-bound protein 2
207688_s_at	*INHBC*	1·46	1·25	0·51	0·75	Inhibin, beta C
209744_x_at	*ITCH*	1·30	1·47	0·63	0·70	Itchy homolog E3 ubiquitin protein ligase (mouse)
201548_s_at	*JARID1B*	1·27	1·92	0·73	0·46	Jumonji, AT-rich interactive domain IB (RBP2-like)
203297_s_at	*JARID2*	1·42	1·28	0·54	0·72	Jumonji, AT-rich interactive domain 2
41387_r_at	*JMJD3*	1·82	1·24	0·76	0·65	Jumonji domain containing 3
205569_at	*LAMP3*	2·32	1·24	0·76	0·50	Lysosomal-associated membrane protein 3
214039_s_at	*LAPTM4B*	1·41	1·49	0·49	0·59	Lysosomal-associated protein transmembrane 4 beta
205857_x_at	*MSH3*	1·79	1·28	0·58	0·72	MutS homolog 3 (*E. coli*)
209550_at	*NDN*	3·42	1·38	0·17	0·62	Necdin homolog (mouse)
207943_x_at	*PLAGL1*	1·37	1·43	0·57	0·63	Pleiomorphic adenoma gene-like 1
204748_at	*PTGS2*	1·65	1·78	0·14	0·35	Prostaglandin-endoperoxide synthase 2
201482_at	*QSCN6*	1·32	1·23	0·38	0·77	Quiescin Q6
203743_s_at	*TDG*	1·47	1·23	0·54	0·77	Thymine-DNA glycosylase
204227_s_at	*TK2*	2·12	1·26	0·56	0·74	Thymidine kinase 2, mitochondrial

Table 3. Continued

Affi ID	Gene abbreviation	Fold change CB 1	CB 2	PB 1	PB 2	Gene name
Cytokines and chemokines						
207533_at	*CCL1*	1·67	1·48	0·52	0·49	Chemokine (C-C motif) ligand 1
205099_s_at	*CCR1*	4·70	1·21	0·61	0·79	Chemokine (C-C motif) receptor 1
207681_at	*CXCR3*	1·51	1·33	0·41	0·67	Chemokine (C-X-C motif) receptor 3
211469_s_at	*CXCR6*	1·58	1·95	0·32	0·42	Chemokine (C-X-C motif) receptor 6
206613_at	*IL-18R1*	2·32	1·38	0·61	0·62	Interleukin-18 receptor 1
207072_at	*IL-18RAP*	2·16	1·44	0·46	0·56	Interleukin-18 receptor accessory protein
212657_s_at	*IL-1RN*	1·44	3·12	0·56	0·37	Interleukin 1 receptor
206341_at	*IL-2RA*	1·52	1·27	0·73	0·66	Interleukin-2 receptor alpha
202859_x_at	*IL-8*	1·31	3·75	0·38	0·69	Interleukin-8
202643_s_at	*TNFAIP3*	1·61	1·25	0·67	0·75	Tumour necrosis factor, alpha-induced protein 3
202687_s_at	*TNFSF10*	2·83	1·23	0·67	0·77	Tumour necrosis factor (ligand) superfamily member 10
205599_at	*TRAF1*	2·25	1·32	0·68	0·61	Tumour necrosis factor receptor-associated factor 1
202871_at	*TRAF4*	1·43	1·58	0·57	0·48	Tumour necrosis factor receptor-associated factor 4
206366_x_at	*XCL1*	1·24	2·66	0·46	0·76	Chemokine (C motif) ligand 1
Signal transduction						
210538_s_at	*AIP1*	1·35	1·54	0·65	0·61	Baculoviral IAP repeat-containing 3
209369_at	*ANXA3*	1·39	6·82	0·61	0·05	Annexin A3
1554343_a_at	*BRDG1*	1·45	1·67	0·52	0·55	BCR downstream signalling 1
225946_at	*C12orf2*	3·20	1·77	0·23	0·23	Ras association (RaIGDS/AF-6) domain family 8
204392_at	*CAMK1*	1·26	1·62	0·74	0·54	Calcium/calmodulin-dependent protein kinase I
231042_s_at	*CAMK2D*	1·31	1·63	0·25	0·69	Calcium/calmodulin-dependent protein kinase (CaM kinase) II delta
205692_s_at	*CD38*	1·37	1·29	0·71	0·48	CD38 antigen (p45)
231747_at	*CYSLTR1*	3·16	1·45	0·55	0·43	Cysteinyl leukotriene receptor 1
211272_s_at	*DGKA*	1·43	1·23	0·77	0·54	Diacylglycerol kinase alpha 80 kDa
200762_at	*DPYSL2*	1·35	1·40	0·37	0·65	Dihydropyrimtdinase-like 2
208370_s_at	*DSCR1*	1·23	1·90	0·63	0·77	Down syndrome critical region gene 1
204794_at	*DUSP2*	1·55	2·57	0·39	0·45	Dual specificity phosphatase 2
204015_s_at	*DUSP4*	1·35	2·66	0·65	0·39	Dual specificity phosphatase 4
211333_s_at	*FASLG*	1·20	1·37	0·49	0·80	Fas ligand (TNF superfamily, member 6)
211535_s_at	*FGFR1*	1·23	2·79	0·70	0·77	Fibroblast growth factor receptor 1
224148_at	*FYB*	1·50	1·21	0·45	0·79	FYN binding protein (FYB-120/130)
209304_x_at	*GADD45B*	1·55	1·29	0·65	0·71	Growth arrest and DNA-damage-inducible beta
234284_at	*GNG8*	1·50	3·16	0·50	0·35	Guanine nucleotide binding protein (G protein), gamma 8
224285_at	*GPR174*	1·91	1·42	0·56	0·58	G protein-coupled receptor 174
223767_at	*GPR84*	4·41	1·44	0·05	0·56	G protein-coupled receptor 84
211555_s_at	*GUCY1B3*	1·66	1·73	0·34	0·03	Guanylate cyclase 1, soluble, beta 3
38037_at	*HBEGF*	1·54	1·36	0·55	0·64	Heparin-binding EGF-like growth factor
203820_s_at	*IMP-3*	1·83	2·18	0·17	0·17	IGF-II-mRNA-binding protein 3
203006_at	*INPP5A*	1·40	1·86	0·60	0·52	Inositol polyphosphate-5-phosphatase, 40 kDa
231779_at	*IRAK2*	1·93	1·46	0·46	0·54	Interleukin-1 receptor associated kinase 2
32137_at	*JAG2*	1·58	1·29	0·71	0·64	Jagged 2
203904_x_at	*KAI1*	1·65	1·59	0·41	0·25	CD82 antigen
235252_at	*KSR*	1·72	1·56	0·43	0·44	Kinase suppressor of ras 1
210948_s_at	*LEF1*	1·21	1·64	0·41	0·79	Hypothetical protein LOC641518
203236_s_at	*LGALS9*	1·48	1·27	0·73	0·51	Lectin, galactoside-binding, soluble, 9 (galectin 9)
220253_s_at	*LRP12*	1·27	1·30	0·31	0·73	Low-density lipoprotein-related protein 12
206637_at	*P2RY14*	1·32	1·48	0·39	0·68	Purinergic receptor P2Y, G-protein coupled, 14
210837_s_at	*PDE4D*	1·35	1·31	0·62	0·69	Phosphodiesterase 4D, cAMP-specific
206726_at	*PGDS*	6·45	1·40	0·60	0·43	Prostaglandin D2 synthase, haematopoietic
210617_at	*PHEX*	1·53	4·08	0·21	0·47	Phosphate regulating endopeptidase homologue, X-linked
206370_at	*PIK3CG*	1·23	1·32	0·50	0·77	Phosphoinositide-3-kinase, catalytic, gamma polypeptide
205632_s_at	*PIP5K1B*	1·32	1·42	0·64	0·68	Phosphalidylinositol-4-phosphate 5-kinase, type 1 beta

Table 3. Continued

Affi ID	Gene abbreviation	Fold change CB 1	CB 2	PB 1	PB 2	Gene name
215195_at	*PRKCA*	2·17	1·36	0·64	0·61	Protein kinase C, alpha
210832_x_at	*PTGER3*	4·44	1·47	0·07	0·53	Prostaglandin E receptor 3 (subtype EP3)
1553535_a_at	*RANGAP1*	1·58	1·39	0·58	0·61	Ran GTPase activating protein 1
234344_at	*RAP2C*	1·75	1·26	0·46	0·74	RAP2C, member of RAS oncogene family
223809_at	*RGS18*	2·12	1·67	0·15	0·33	Regulator of G-protein signalling 18
209882_at	*RIT1*	1·74	1·32	0·63	0·68	Ras-like without CAAX 1
209451_at	*TANK*	1·34	1·20	0·42	0·80	TRAF family member-associated NFKB activator
204924_at	*TLR2*	1·60	2·52	0·36	0·40	Toll-like receptor 2
217979_at	*TM4SF13*	1·21	2·47	0·30	0·79	Tetraspanin 13
209263_x_at	*TM4SF7*	2·05	1·41	0·58	0·59	Tetraspanin 4
Transcription						
1566989_at	*ARID1B*	1·42	1·27	0·09	0·73	AT-rich interactive domain 1B (SWIl-like)
203973_s_at	*CEBPD*	3·06	1·51	0·33	0·49	CCAAT/enhancer binding protein (C/EBP), delta
221598_s_at	*CRSP8*	1·60	1·29	0·71	0·68	Cofactor required for Spl transcriptional activation, subunit 8, 34 kDa
205249_at	*EGR2*	1·33	4·27	0·67	0·60	Early growth response 2 (Krox-20 homologue, *Drosophila*)
206115_at	*EGR3*	1·31	6·15	0·69	0·48	Early growth response 3
201328_at	*ETS2*	1·57	1·72	0·43	0·40	V-ets erythroblastosis virus E26 oncogene homologue 2 (avian)
218810_at	*FLJ23231*	2·13	1·37	0·63	0·63	Zinc finger CCCH-type containing 12A
209189_at	*FOS*	21·56	1·31	0·13	0·69	V-fos FBJ murine osteosarcoma viral oncogene homologue
223408_s_at	*FOXK2*	2·26	1·22	0·48	0·78	Forkhead box K2
202723_s_at	*FOXO1A*	1·47	1·27	0·57	0·73	Forkhead box O1A (rhabdomyosarcoma)
224211_at	*FOXP3*	1·62	1·41	0·59	0·23	Forkhead box P3
207156_at	*HIST1H2AG*	1·73	1·30	0·41	0·70	Histone 1, H2ag
220042_x_at	*HIVEP3*	1·26	1·65	0·74	0·56	Human immunodeficiency virus type I enhancer binding protein 3
207826_s_at	*ID3*	1·34	8·64	0·60	0·66	Inhibitor of DNA binding 3, dominant negative helix-loop-hetix protein
204549_at	*IKBKE*	2·33	1·29	0·71	0·66	Inhibitor of kappa light polypeptide gene enhancer in B cells
219878_s_at	*KLF13*	1·89	1·26	0·34	0·74	Kruppel-like factor 13
207667_s_at	*MAP2K3*	1·33	1·28	0·72	0·57	Mitogen-activated protein kinase kinase 3
201502_s_at	*NFKBIA*	2·31	1·29	0·71	0·57	Nuclear factor of κ light polypeptide gene enhancer in B cells inhibitor
222105_s_at	*NKIRAS2*	1·84	1·21	0·69	0·79	NFKB inhibitor interacting Ras-like 2
204622_x_at	*NR4A2*	1·35	4·31	0·65	0·63	Nuclear receptor subfamily 4, group A, member 2
207978_s_at	*NR4A3*	1·33	3·53	0·62	0·67	Nuclear receptor subfamily 4, group A, member 3
202600_s_at	*NRIP1*	1·86	1·39	0·26	0·61	Nuclear receptor interacting protein 1
216841_s_at	*SOD2*	1·25	1·73	0·36	0·75	Superoxide dismutase 2, mitochondrial
201416_at	*SOX4*	1·53	2·21	0·47	0·38	SRY (sex determining region Y)-box 4
223635_s_at	*SSBP3*	2·12	1·25	0·75	0·62	Single-stranded DNA binding protein 3
206506_s_at	*SUPT3H*	1·47	1·31	0·57	0·69	Suppressor of Ty 3 homologue (*S. cerevisiae*)
221618_s_at	*TAF9L*	1·25	1·49	0·47	0·75	TAF9-like RNA polymerase II
203177_x_at	*TFAM*	1·63	1·23	0·77	0·57	Transcription factor A, mitochondrial
213943_at	*TWIST1*	1·89	3·14	0·04	0·11	Twist homologue 1 (acrocephalosyndactyly 3; Saethre-Chotzen syndrome)
219836_at	*ZBED2*	1·33	4·76	0·67	0·21	Zinc finger, BED-type containing 2
211965_at	*ZFP36L1*	2·02	1·47	0·29	0·53	Zinc finger protein 36, C3H type-like 1
230760_at	*ZFY*	1·41	1·25	0·75	0·02	Zinc finger protein, Y-linked
228854_at	*ZNF145*	3·26	1·21	0·40	0·79	Transcribed locus
235121_at	*ZNF542*	2·68	1·33	0·63	0·67	Zinc finger protein 542

To investigate whether increased expression of the *IL-17* gene is a general feature of PB-derived $CD4^+$ T cells, we also tested *IL-17* gene expression in the above-described additional samples by real-time RT-PCR analysis. As shown in Fig. 6, all of four PB-derived $CD4^+$ T-cell samples revealed significantly increased gene expression of *IL-17* when compared with the CB-derived samples at 1 week. At 2 weeks, however, *IL-17* gene expression in PB-derived $CD4^+$ T cells was diminished while some of the CB-derived $CD4^+$ T cells (such as sample CB 4) exhibited increased *IL-17* gene expression. When the data were analysed statistically, expression of the *IL-17* gene was found to be

Table 4. Genes up-regulated in $CD4^+$ T cells from peripheral blood (PB)

Affi ID	Gene abbreviation	Fold change CB 1	CB 2	PB 1	PB 2	Gene name
Apoptosis						
1553681_a_at	*PRF1*	0·66	0·51	1·41	1·34	Perforin 1 (pore-forming protein)
B- and T-cell development						
224499_s_at	*AICDA*	0·06	0·44	1·56	3·47	Activation-induced cytidine deaminase
205495_s_at	*GNLY*	0·40	0·51	1·49	6·34	Granulysin
217478_s_at	*HLA-DMA*	0·67	0·39	1·33	1·35	Major histocompatibility complex, class II, DM alpha
203932_at	*HLA-DMB*	0·64	0·31	2·02	1·36	Major histocompatibility complex, class II, DM beta
211991_s_at	*HLA-DPA1*	0·50	0·14	1·54	1·50	Major histocompatibility complex, class II, DP alpha 1
212671_s_at	*HLA-DQA1*	0·44	0·23	1·56	2·56	Major histocompatibility complex, class II, DQ alpha 1
211656_x_at	*HLA-DQB1*	0·63	0·48	1·37	7·07	Major histocompatibility complex, class II, DQ beta 1
210982_s_at	*HLA-DRA*	0·58	0·37	1·50	1·42	Major histocompatibility complex, class II, DR alpha
208306_x_at	*HLA-DRB1*	0·51	0·24	1·49	1·61	Major histocompatibility complex, class II, DR beta 3
204670_x_at	*HLA-DRB5*	0·63	0·22	1·47	1·37	Major histocompatibility complex, class II, DR beta 5
211634_x_at	*IGHV1-69*	0·69	0·77	1·23	1·99	Immunoglobulin heavy variable 1–69
211645_x_at	*IgK*	0·15	0·49	1·51	6·62	Immunoglobulin kappa light chain (IGKV)
221651_x_at	*IGKC*	0·46	0·68	1·32	5·57	Immunoglobulin kappa constant
215379_x_at	*IGLC2*	0·62	0·41	1·38	4·26	Immunoglobulin lambda joining 2
209031_at	*IGSF4*	0·50	0·03	2·33	1·50	Immunoglobulin superfamily, member 4
205686_s_at	*CD86*	0·70	0·23	1·30	1·39	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)
204698_at	*ISG20*	0·68	0·49	1·32	1·64	Interferon stimulated exonuclease gene, 20 kDa
213915_at	*NKG7*	0·72	0·42	1·28	2·31	Natural killer cell group 7 sequence
Cell growth and maintenance						
201334_s_at	*ARHGEF12*	0·74	0·50	1·26	1·96	Rho guanine nucleotide exchange factor (GEF) 12
230292_at	*CHC1L*	0·70	0·56	1·30	2·02	Regulator of chromosome condensation (RCC1)
205081_at	*CRIP1*	0·56	0·73	1·27	1·75	Cysteine-rich protein 1 (intestinal)
31874_at	*GAS2L1*	0·77	0·52	1·23	2·35	Growth arrest-specific 2 like 1
202364_at	*MXI1*	0·43	0·73	1·27	1·44	MAX interactor 1
219304_s_at	*PDGFD*	0·65	0·71	1·29	3·68	Platelet-derived growth factor D
213397_x_at	*RNASE4*	0·64	0·46	1·36	2·21	Ribonuclease, RNase A family, 4
213566_at	*RNASE6*	0·69	0·39	1·49	1·31	Ribonuclease, RNase A family, k6
219077_s_at	*WWOX*	0·40	0·78	1·25	1·22	WW domain containing oxidoreductase
Cytokine and chemokine						
207861_at	*CCL22*	0·76	0·52	1·24	2·47	Chemokine (C–C motif) ligand 22
238750_at	*CCL28*	0·74	0·45	1·26	1·41	Chemokine (C–C motif) ligand 28
1555759_a_at	*CCL5*	0·71	0·23	1·29	1·92	Chemokine (C–C motif) ligand 5
208304_at	*CCR3*	0·50	0·12	1·50	2·35	Chemokine (C–C motif) receptor 3
205898_at	*CX3CR1*	0·30	0·20	1·70	4·16	Chemokine (C–X3–C motif) receptor 1
204533_at	*CXCL10*	0·80	0·16	1·20	2·53	Chemokine (C–X–C motif) ligand 10
219255_x_at	*IL-17RB*	0·73	0·04	1·27	1·29	Interleukin 17 receptor B
206148_at	*IL-3RA*	0·60	0·54	2·46	1·40	Interleukin 3 receptor, alpha (low affnity)
226333_at	*IL-6R*	0·22	0·79	1·21	2·43	Interleukin-6 receptor
206693_at	*IL-7*	0·09	0·54	1·46	5·86	Interleukin-7
Signal transduction						
204497_at	*ADCY9*	0·76	0·40	1·24	2·40	Adenylate cyclase 9
206170_at	*ADRB2*	0·58	0·35	1·42	3·97	Adrenergic, beta-2-, receptor, surface
202096_s_at	*BZRP*	0·50	0·54	1·59	1·46	Benzodiazapine receptor (peripheral)
230464_at	*EDG8*	0·04	0·09	1·91	2·42	Endothelial differentiation, sphingolipid G-protein-coupled receptor 8
223423_at	*GPR160*	0·54	0·68	1·40	1·32	G protein-coupled receptor 160
227769_at	*GPR27*	0·07	0·08	1·92	244	G protein in-coupled receptor 27
210095_s_at	*IGFBP3*	0·27	0·20	1·73	5·25	Insulin-like growth factor binding protein 3
38671_at	*PLXND1*	0·08	0·65	1·35	2·57	Plexin D1
226101_at	*PRKCE*	0·56	0·43	1·72	1·44	Protein kinase C. epsilon
232629_at	*PROK2*	0·01	0·13	1·87	2·09	Prokineticin 2