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ORIGINAL ARTICLE

Novel mutations in a Japanese patient with CD19 deficiency

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Common variable immunodeficiency (CVID) is a heterogeneous disorder characterized by recurrent bacterial infections, hypogammaglobulinemia and low to normal numbers of circulating B cells. Mutations in the ICOS, TACI and CD19 genes have recently been identified in < 10% of CVID patients. We, herein, describe two novel CD19 gene disruptions in an 8-year-old Japanese boy, who had been clinically diagnosed as having CVID at the age of 5 years. Flow-cytometric analysis demonstrated absence of CD19 and reduced CD21 expression on CD20-postive peripheral blood B cells. Mutation analysis of CD19 revealed a mutation in the splice acceptor site of intron 5 (IVS5-1G> T) of the maternal allele, resulting in skipping of exon 6, and a truncated protein product. The paternal allele was disrupted by a gross deletion encompassing at least the ATP2A1, CD19 and NFATC2IP genes. The patient had a small number of IgD$^-$ CD27$^+$ memory B cells, in which somatic mutation were detected. His B cells showed substantial proliferation upon stimulation, but reduced IgG and IgA production in vitro. These findings extend the mutation spectrum of the CD19 deficiency to four, and confirm the homogeneity of the CD19 deficiency as a unique type of CVID.
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Keywords: *CD19; common variable immunodeficiency; hypogammaglobulinemia*

Introduction

Common variable immunodeficiency (CVID) is the most common primary immunodeficiency that requires clinical attention with an incidence of 1:10 000 to 1:50 000.[1] It is a type of primary antibody–deficiency syndrome, which is characterized by susceptibility to bacterial infections, hypogammaglobulinemia and low to normal numbers of circulating B cells.[2] CVID is a heterogeneous group of disorders, and diagnosis is mainly made by means of exclusion from other immunodeficiency diseases, such as X-linked agammaglobulinemia,[3,4] hyper-IgM syndrome[5] or X-linked lymphoproliferative syndrome.[6,7] However, in several CVID patients, mutations in genes involved in B-cell differentiation are thought to be affected. Recent studies demonstrated that in some CVID patients genetic defects in the *ICOS*, *TACI* and *CD19* genes underlie the antibody deficiency.[8–11] Whereas *TACI* is affected in ~8% of CVID patients,[8,9] mutations in *ICOS* and *CD19* are thought to be very rare.[10,11] CD19 is a member of the Ig superfamily expressed on the surface of B lymphocytes, and may play a pivotal role in B-cell differentiation and activation.[12] On mature B cells, the protein complex consisting of CD19, CD21, CD81 and CD225, signals in conjunction with the B-cell antigen receptor upon antigen encounter, and is necessary to decrease the threshold for receptor-dependent signaling.[12,13]

Recently, four hypogammaglobulinemic patients from two unrelated families were shown to have defective CD19, resulting from mutations in the *CD19* gene.[11] Due to an impaired antibody response and memory B-cell formation, these patients developed an immunodeficiency in the first decade of life. We, herein, describe a new case of CD19 deficiency with two novel mutations in the *CD19* gene. In spite of two novel mutations, the immunological and clinical phenotypes are in line with previous observations. The CD19 deficiency appears to be homogeneous and well-defined antibody deficiency.

Results

Identification of the CD19 defects

The exclusion of known genetically identifiable immunodeficiencies is necessary for diagnosis of CVID.[14] In light of this current notion, we attempted to immunologically and genetically re-evaluate a young male CVID patient. The patient had normal numbers of CD20-positive peripheral blood B cells, but two-color flow-cytometric analysis revealed the lack of CD19 membrane

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expression (Figure 1a). This raised the possibility that the patient had a CD19 deficiency. Furthermore, the level of CD19 expression in the mother was lower than that in the control, suggesting that the mother might be a carrier. In addition, expression of CD19-complex member CD21 was also slightly reduced on the patient's B cells as compared with that of a healthy control (Figure 1b). Mutation analysis of the patient's *CD19* gene disclosed a seemingly homozygous mutation at the splice acceptor site of intron 5 (IVS5-1G > T) (Figure 2a). CD19 transcript analysis showed that the mutation caused skipping of exon 6, and coupling of exons 5–7 (Figure 2b). As a result, the frame of exon 7 is shifted, leading to a truncated protein with four altered amino acids and a premature stop codon at position 320, prior to all conserved intracellular tyrosine residues (Figure 2c).

As expected, the mother was heterozygous for mutated allele (Figure 2a). The father, however, did not carry a mutated allele (Figure 2a). This raised the question whether the second allele of the patient was disrupted by a *de novo* mutation, or it contained a gross deletion including the mutation site. In order to confirm the latter hypothesis, DNA fluorescence *in situ* hybridization analysis using a CD19-specific probe was performed. Although two signals for the *CD19* gene were detected in the metaphases from a healthy control (Figure 3a), only one signal was detected in cells from the patient (Figure 3b). In addition, fluorescence *in situ* hybridization analysis was performed for the neighboring genes *ATP2A1* and *NFATC2IP*. The control samples revealed two signals for *ATP2A1* and *NFATC2IP* genes (Figures 3c and e), whereas the patient samples revealed only one signal for *ATP2A1* and *NFATC2IP* genes (Figures 3d and f). Thus, the patient carries an allele that contains a gross deletion of at least 68.5 kbp including the *ATP2A1*, *CD19* and *NFATC2IP* genes. Because no additional material could be obtained from the father, it remains unclear whether the gross genomic deletion in the patient was a *de novo* event or inherited from his father.

Memory B-cell sub-population

Similar to most CVID patients, the previously described CD19-deficient patients show decreased numbers of IgD^-CD27^+ Ig class-switched memory B cells.[11,15,16] Substantial numbers of Ig class-switched memory B cells were detectable, though reduced as compared to healthy controls, in peripheral blood of our patient, suggesting some potential to undergo class-switch recombination (Figure 4a).

To examine whether B cells of the patient were capable of undergoing somatic hypermutation, V_H5-Cμ transcripts were studied. Six out of 13 individual clones revealed somatic hypermutations (Figure 4b). The frequency of somatic hypermutations was 0.94% (normal adults 1.8% ± 0.4, and cord blood 0.07% ± 0.01), and was indicative of the potential to form an antigen-selected B-cell receptor repertoire.

B-cell antigen response

In agreement with previous observations,[11] our clinical and immunological findings of the patient suggested that the CD19 deficiency appeared to result in an impaired antigen response by B cells. To quantify the B-cell response in our patient, we employed two *in vitro* assays. First, proliferation of isolated B cells was studied

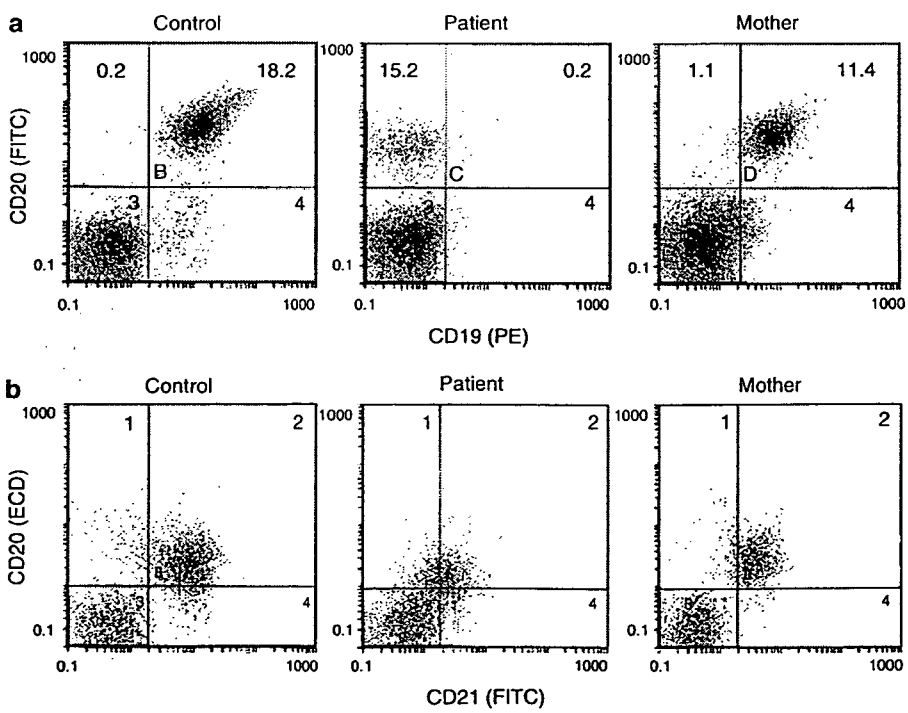

Figure 1 Flow-cytometric analysis of B cells. The peripheral blood B cells of a healthy control, the patient and his mother were evaluated by two-color flow cytometry employing FITC-conjugated anti-CD20 and PE-conjugated anti-CD19 monoclonal Abs (a), and ECD-conjugated anti-CD20 and FITC-conjugated anti-CD21 monoclonal Abs (b). Abs, antibodies; FITC, fluorescein isothiocyanate.

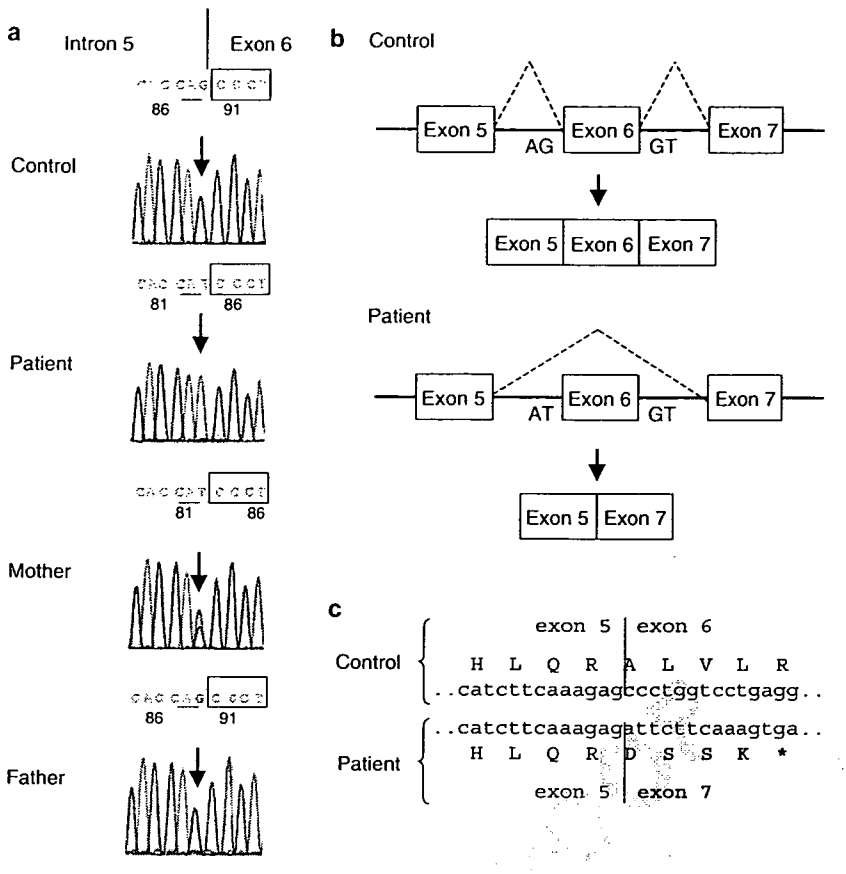

Figure 2 Mutation analysis of the *CD19* gene. (a) Electropherograms of the sequences representing the region around the splice acceptor site of exon 6 from a healthy control, the patient and his parents. The patient showed a seemingly homozygous mutation at an invariant position of the splice acceptor site of exon 6 (IVS5-1G>T; indicated by arrows). His mother was heterozygous for the mutation, whereas his father did not carry the mutation. (b) *CD19* transcript analysis showed the skipping of exon 6 in B cells of the patients. (c) The coupling of exons 5–exon 7 results in a frame shift and a premature stop codon at position 320 of the protein.

by [^{3}H]thymidine incorporation. The patient's B cells showed comparable proliferation upon stimulation with *Staphylococcus aureus* Cowan I + IL-2 or CpG to B cells from a healthy control (Figure 5a). *In vitro* Ig production showed reduced IgA and IgG production of patient's B cells after stimulation with CD40L and CD40L + IL-10, whereas IgM production was similar to a healthy control.

Discussion

In this study we describe a CD19-deficient patient, who was compound heterozygous for two novel mutations. Similarly to two previously identified disrupted *CD19* alleles,[11] these mutations lead to loss of CD19 membrane expression, and result in an antibody–deficiency syndrome, characterized by recurrent (mainly) bacterial infections.

The mutation in the splice acceptor site of exon 6 (IVS5-1G>T) results in skipping of exon 6, placing exon 7 out-of frame after exon 5, which leads to a premature stop codon at the intracellular amino acid 320. The resulting protein product is almost similar to the product resulting from the 972insA mutation identified before.[11] In line with this, the patient's B cells completely lack membrane CD19 expression. Furthermore, the lack of all intracellular domains responsible for signal transduction makes any expressed protein non-functional.[17]

The other mutated allele contained a gross deletion that involved *ATP2A1, CD19, NFATC2IP* and potentially more genes. In addition to the CD19 gene deletion, the patient lacks one allele of the *ATP2A1* and *NFATC2IP* genes. Mutations in the *ATP2A1* gene that encodes SERCA1 are associated with Brody disease, an inherited disorder of skeletal muscle function.[18] The patient did not show clinical symptoms suggestive of Brody disease. Furthermore, mutations in *ATP2A1* are associated with an autosomal recessive inheritance, making it likely that the other allele compensates for the loss. The exact function of the protein encoded by *NFAT2CIP* is not known. However, similar to *ATP2A1*, it is likely that the loss of one allele does not have clinical consequences.

The clinical presentation of the patient was very similar to the other four reported CD19-deficient patients,[11] making it a rather homogeneous disorder. All patients presented with clinical symptoms of antibody deficiency in the first decade of life and showed reduced serum Ig levels. In addition, all patients show an impaired antibody response after vaccination. Whereas normal numbers of peripheral blood B cells are found, they all show a marked decrease in memory B cells. In spite of decreased number, these memory B cells do

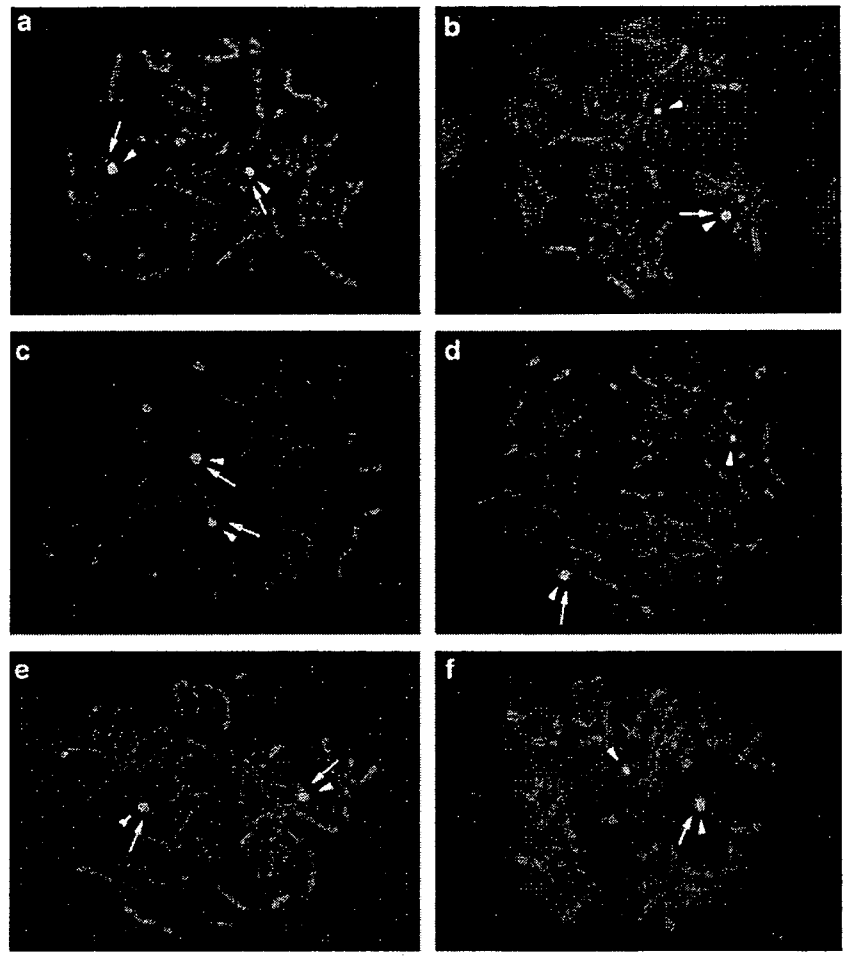

Figure 3 The patient is heterozygous for a gross deletion involving the *CD19*, *ATP2A1* and *NFATC2IP* gene. FISH analysis with *CD19*- (a and b), *ATP2A1*- (c and d) and *NFATC2IP*-specific probes (e and f) and the chromosome 16-specific D16Z3 probe in a healthy control (a, c and e) and the patient (b, e and f). In both a healthy control and the patient, two signals per cell were found with D16Z3 (green signals; indicated by arrowheads). Whereas the control also showed two signals for the *CD19*, *ATP2A1* and *NFATC2IP* probes, only one signal was found for each of these in the patient (red signals; indicated by arrows). FISH, fluorescence *in situ* hybridization.

show molecular signs of antigen-dependent differentiation. Finally, considering the thrombocytopenia in our patient, the CD19 deficiency might be associated with the development of autoimmune disease.

We, herein, described the fifth case in the world of a patient with CD19 deficiency, resulting from two novel mutations in the *CD19* gene. The clinical and immunological characteristics of the patient were similar to four recently identified cases of *CD19* deficiency. These observations support the idea that *CD19* deficiency is a distinct and unique subset of CVID. Our finding and the recent observation of a CD21 deficiency[19] suggest that defects in antigen recognition, resulting from mutations in CD19-complex members (CD19, CD21, CD81 and CD225) might be identified in more patients that suffer from an antibody deficiency.

Patients, materials and methods

Patient's report

The patient was an 8-year-old Japanese boy born to non-consanguineous healthy parents who had no family history of immunodeficiency. At 5 years of age, he presented with pyelonephritis, bronchitis and gastritis, and was admitted to the Takamatsu Red Cross Hospital. He showed hypogammaglobulinemia (IgG 249 mg dl^{-1}, IgA 10 mg dl^{-1}, IgM 18 mg dl^{-1}, IgD $<$0.6 mg dl^{-1} and IgE 9.0 U ml^{-1}), and the immunological tests demonstrated CD3 62.7% (1775 μl^{-1}), CD4 37.6% (1064 μl^{-1}), CD8 27.6% (781 μl^{-1}) and CD20 19.0% (538 μl^{-1}) in peripheral blood lymphocytes. Antibodies against measles, rubella, tetanus and pertussis toxin were not detectable, although the patient had been vaccinated. Since the patient had normal numbers of B cells in his blood, he was clinically diagnosed to have CVID, and thus was treated with intravenous immunoglobulin-replacement therapy. In addition, he had also been recently diagnosed with a mild thrombocytopenia that did not require medical attention. The patient is now doing well with intravenous immunoglobulin-replacement therapy.

Flow-cytometric analysis of B cells

Heparinized venous blood was obtained from the patient and his parents after obtaining the written informed consent. Peripheral blood B cells were assessed by two-

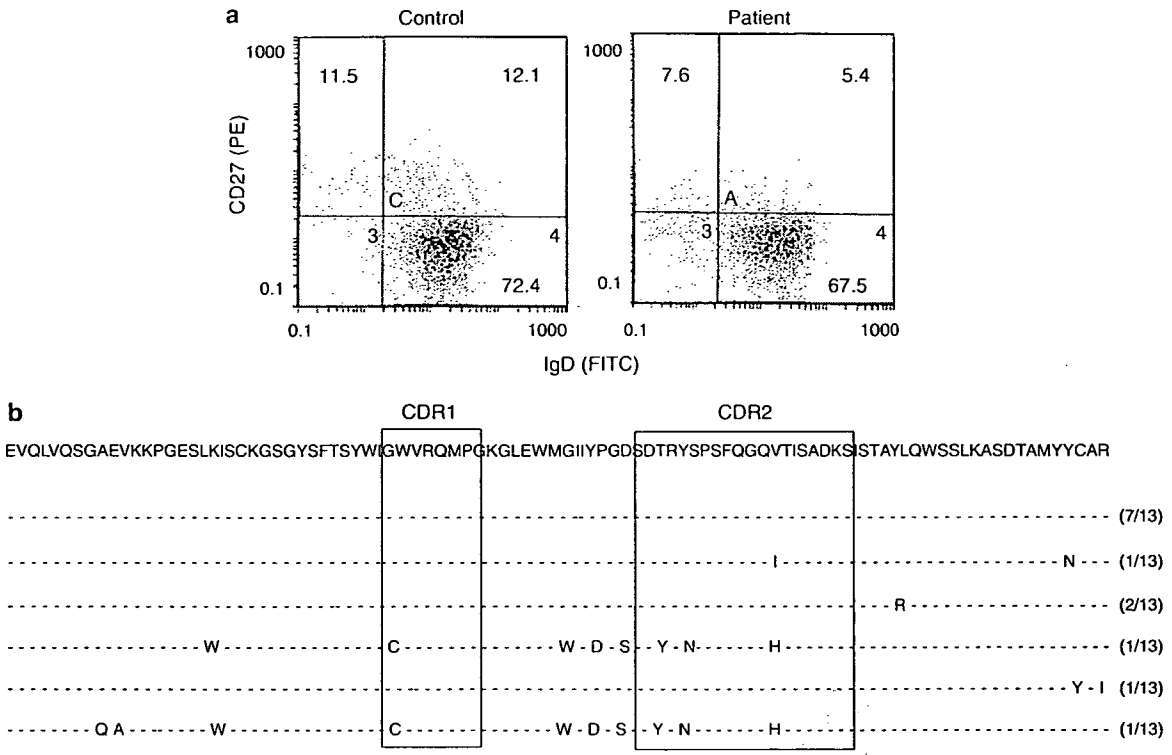

Figure 4 Analysis of memory B cells. (a) B-cell sub-populations. A three-color analysis was carried to analyze the relative distribution of naive and memory B cells in peripheral blood using CD27 and IgD within the CD20-positive B-cell compartments of a healthy control and the patient. (b) Analysis of the somatic hypermutations in the patient's B cells. The upper sequence is the germline gene of V_H5 with the amino-acid translation.

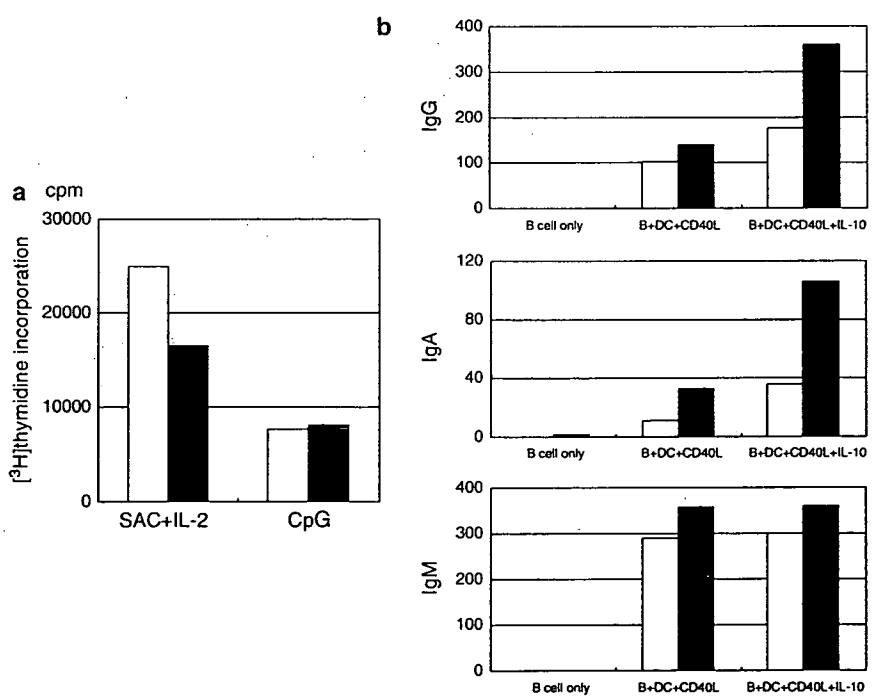

Figure 5 Functional characteristics of the patient's B cells. (a) Proliferation of B cells after stimulation with SAC and IL-2 or CpG, as assessed by [^{3}H]thymidine incorporation. The results are expressed as the mean of triplicate experiments. Open and closed bars indicate the patient and the normal control, respectively. (b) IgG, IgA and IgM production by B cells after stimulation with various stimuli. The spots were enumerated in each experiment. Open and closed bars indicate the patient and the normal control, respectively. SAC, *Staphylococcus aureus* Cowan I.

color flow-cytometric analysis employing CD19-PE, CD20-ECD (phycoerythrin-Texas Red), and CD21-fluorescein isothiocyanate mAbs (all from Beckman Coulter, Marseille, France) and CD20-fluorescein isothiocyanate mAb (DAKO Japan, Kyoto, Japan). In addition, the naive and memory B-cell sub-populations were evaluated by three-color analysis with a combination of fluorescein isothiocyanate-conjugated goat anti-IgD Ab (Southern Biotechnology Associates Inc., Birmingham, AL, USA), CD27-PE mAb (Becton Dickinson, Mountain View, CA, USA), and CD20-ECD mAb as described previously.[20]

Mutation analysis of the CD19 gene

PBMC were collected after Ficoll–Hypaque gradient centrifugation of total blood. DNA was extracted from PBMC using a QIAamp Blood Kit (Qiagen GMBH, Hilden, Germany), and was subjected to PCR. All of the coding exons and splice sites of the *CD19* gene were amplified, and a sequencing reaction was carried out with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an automated ABI PRISM 310 DNA Sequencer (Applied Biosystems). The PCR primers used for amplification of the *CD19* gene are listed in Table 1. RNA was extracted from PBMC using a TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), and was subjected to reverse transcription PCR. A fragment of the *CD19* cDNA was amplified from the first-strand cDNA with primer pairs encompassing exon 6 (5′-AGGGGCCTAAGTCATTGCTG-3′ and 5′-TGCCCAAGGTTGGAGTCGTT-3′) and sequenced to verify the mutation found in the genomic DNA.

Fluorescence in situ *hybridization analysis of the CD19 gene*

PBMC stimulated in culture with PHA for 72 h were treated with a 0.075 M KCl solution and fixed with Carnoy's solution (3:1 methanol and acetic acid) for metaphase preparation. A *CD19*-specific genomic probe (11 517 bp) was prepared by LA-PCR Kit (Takara, Kyoto, Japan) with primer pairs encompassing exons 1 and 15 (5′-CTGTTCTCAAAGACATTCTGGTGCAGG-3′ and 5′-TGCTTAGAACATAGTAAGGTCGGGCACT-3′). In addition, probes were designed to label the *ATP2A1* gene, which is located 30 kbp upstream of *CD19*, and the *NFATC2IP* gene, which is located 14 kbp downstream of *CD19*. The *ATP2A1*- and *NFATC2IP*-specific genomic probes (7045 and 6017 bp) were also prepared by LA-PCR Kit with primers encompassing exons 10 and 14 (5′-GGTTCAAGTGGCTCACCTTGTTACCAAC-3′ and 5′-GGTTCAAGTGGCTCACCTTGTTACCAAC-3′) and exons 3 and 7 (5′-CTTGCAGCTGCCATAGTTAGTTAGGACA-3′ and 5′-CAATTCAGACTTCTAGGCTCCAGAAGTG-3′), respectively. For fluorescence *in situ* hybridization analysis, denatured metaphase spreads were hybridized with the probes labeled with digoxigenin-11-dUTP using nick translation kit (Roche Diagnostics KK, Tokyo, Japan). The Spectrum Green-labeled chromosome 16 probe (D16Z3, Vysis, Tokyo, Japan) was used as a control. The CD19-, ATP2A1- and NFATC2IP-specific probes were detected with anti-digoxigenin rhodamine (Roche Diagnostics KK) providing a red signal, whereas D16Z3 was detected by a green signal. Metaphases were counterstained with 4′,6-diamino-2-phenylindole dihydrochloride, and the images of the hybridization were captured under fluorescence microscopy (Olympus Optical Co., Tokyo, Japan). At least 20 metaphases were observed in each setting.

B-cell proliferation assay

B cells were isolated from PBMC using a magnetic cell sorter (MACS; Miltenyi Biotech GMBH, Bergisch Gladbach, Germany) by negative selection according to the experiment procedure. Re-analysis showed that more than 97% of the purified cells were CD20 positive. The purified B cells were cultured in the presence of 0.01% *Staphylococcus aureus* Cowan I (Sigma-Aldrich Inc., St Louis, MO, USA) and 50 U ml^{-1} recombinant human (rh) IL-2 (Takeda Co., Osaka, Japan), or 5 μg ml^{-1} CpG (InvivoGen, San Diego, CA, USA) at a final cell density of 2.5×10^5 ml^{-1} in a volume of 200 μl per well. The cells were cultured in 96-well round-bottomed plates for 3 days at 37°C in a humidified atmosphere with 5% CO_2. The cells were pulsed with 0.5 μCi (18.5 Bq) [^{3}H]thymidine. After 18 h of incubation, the cells were harvested by an automatic cell harvester (Packard, Meriden, CT, USA), and [^{3}H]TdR incorporation was measured on a liquid scintillation analyzer (Packard).

Ig production assay

Ig production was assessed by an ELISPOT assay as described elsewhere.[21,22] First, the dendritic cells were derived from *in vitro* cultures of the human cord blood $CD34^+$ cells. Briefly, the $CD34^+$ cells were isolated from the human cord blood cells through positive cell

Table 1 PCR primers for amplification of the *CD19* gene

Exon(s)	*Sense primer sequence*	*Antisense primer sequence*
1	AGGAGGCAAGTGTTGTGAGT	GCCACAACTCAAGTTTCCAG
2	TGCTCTATAACCTGGTGTGA	TGCCCACCTAGCTCCGAGTT
3	TTTCACCTCTTCAGTTTCCC	TTTCACCTCTTCAGTTTCCC
4	GGGGAAACTGAAGAGGTGAA	TTCCACTTCTTCCCAGTACC
5	TCTCAGGATTGGGACGTAAG	TGGGAAGATGGAGTAGGGT
6	CCTGTCTCCTTTGTTGAACC	CCAAGGAGTTCTTCACAGAG
7	CTTCTGAAGGCCTGGACAGA	ACAGCGGATTGGAGGGATGA
8	AGACCCAGAGTCCATTCGCA	CCACTATTCGGGCCACAGAC
9–10	TCTGTGGCCCGAATAGTGGA	GGATCCCCTTCTAGGAGTGT
11–12	AATTGCAGCGCTGTGACACT	TGAGAGGAGTCACAGGATTG
13	CCTCAGTCTTCCTCCTTACC	AGGCTGGAAGGCAAATGTCAG
14	ACTTTGCCTTCCAGCCTACT	GTGTGAATCTTGGGGACTTG

selection using MACS. The $CD34^+$ cells were then cultured in the presence of 100 ng ml^{-1} rhGM-CSF (R&D Systems Inc., Minneapolis, MN, USA), 2.5 ng ml^{-1} rhTNF-α (R&D Systems Inc.), and 25 ng ml^{-1} rh stem cell factor (R&D Systems Inc.) in a RPMI-1640 medium supplemented with 10% fetal calf serum, gentamicin and penicillin G sodium. The dendritic cells were harvested after 12–14 days of culture. B cells isolated from peripheral blood of the patient and a healthy control were cultured at 1×10^5 ml^{-1} alone or in the presence of 1×10^5 ml^{-1} irradiated (3400 rad) dendritic cells and 3.75×10^4 ml^{-1} irradiated (7000 rad) CD40 ligand-transfected L cells (a gift from Dr Yong-Jun Liu, DNAX, Palo Alto, CA, USA) in a final volume of 200 µl. To each well, rhIL-10 (R&D Systems Inc.) was added at a concentration of 200 ng ml^{-1}. The cells were used on the sixth day for the ELISPOT assay.

IgG, IgA and IgM secretion of the B cells was detected by an ELISPOT assay after 6 days of culture using the Millipore Multiscreen Assay System (Millipore Corp., Billerica, MA, USA). The plates were precoated by 100 µl per well of a goat anti-human capture antibody (Southern Biotechnology Associates Inc.) at a concentration of 10 µg ml^{-1} in phosphate-buffered saline overnight at 4°C Cultured B cells were added to the plate after washing in phosphate-buffered saline–Tween and were re-suspended in RPMI-1640, and incubated at 37°C for 20 h. After discarding the cells, the plate was washed and 100 µl per well biotinylated detection antibody in phosphate-buffered saline with 0.5% bovine serum albumin was added. The plate was then incubated at 37°C for 2 h. Next, the plate was washed and 100 µl per well of avidin–enzyme complex in phosphate-buffered saline (VECTASTATIN Elite ABC Kit, Vector Laboratories Inc., Burlingame, CA, USA) was added, and incubated for 1 h at room temperature. After washing, 100 µl avidin–enzyme complex substrate (Sigma) was added per well for 4 min. Spot development was stopped under tap water. The plate was left to dry overnight in a dark place. The spots were counted using a computer-aided ELISPOT manual counting system (ELIPHOTO, Minerva Biolabs GMBH, Berlin, Germany).

Analysis of somatic hypermutations

Total RNA was isolated from purified patient B cells, and reverse-transcribed into cDNA using the oligo dT primer and Superscript II Reverse Transcriptase (Invitrogen). V_H5-Cµ transcripts were amplified using a V_H5 leader forward primer (5′-ATGGGGTCAACCGCCATCCT-3′) and a Cµ constant region reverse primer (5′-GTCCTGTGCGAGGCAGCCAA-3′).[20] The PCR products were ligated into the pCR2.1 vector (Invitrogen) and transformed into TOP10F′ bacteria (Invitrogen). Individual clones were selected, and plasmid DNA was purified. Next, DNA sequencing was performed with a dideoxy termination technique using an ABI 377 sequencer (Applied Biosytems).

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Workshop summary

Primary immunodeficiency diseases: An update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee

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Primary immunodeficiency diseases (PIDs) are a genetically heterogeneous group of disorders that affect distinct components of the innate and adaptive immune system, such as neutrophils, macrophages, dendritic cells, complement proteins, natural killer cells, and T and B lymphocytes. The study of these diseases has provided essential insights into the functioning of the immune system. More than 120 distinct genes have been identified, whose abnormalities account for more than 150 different forms of PID. The complexity of the genetic, immunologic, and clinical features of PID has prompted the need for their classification, with the ultimate goal of facilitating diagnosis and treatment. To serve this goal, an international committee of experts has met every 2 years since 1970. In its last meeting in Jackson Hole, Wyo, after 3 days of intense scientific presentations and discussions, the committee has updated the classification of PID, as reported in this article. (J Allergy Clin Immunol 2007;120:776-94.)

Key words: Primary immunodeficiency diseases, T cells, B cells, phagocytes, complement, immune dysregulation syndromes, innate immunity

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After the original invitation by the World Health Organization in 1970, a committee of experts in the field of primary immunodeficiency diseases (PIDs) has met every 2 years with the goal of classifying and defining this group of disorders. The most recent meeting, organized under the aegis of the International Union of Immunological Societies, with support from the Jeffrey Modell Foundation and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, took place in Jackson Hole, Wyo, in June 2007. In addition to members of the Experts Committee, the meeting gathered more than 30 speakers and more than 150 participants from 6 continents. Recent updates in the molecular and cellular pathophysiology of PID were reviewed and provided the basis for updating the classification of PID.

After an opening lecture in which Tom Waldmann, a founding member of the committee, highlighted some of his most remarkable achievements in the fields of PID and tumor immunology, Kenneth Murphy reviewed the signals that govern T_H cell development and differentiation into T_H1, T_H2, and T_H17 cells. This paved the way to presentations by Bill Paul and Anna Villa, who illustrated how 2 different mechanisms (ie, homeostatic proliferation of $CD4^+$ T cells in a lymphopenic host, and impaired

Abbreviations used
NK: Natural killer
PID: Primary immunodeficiency disease
STAT: Signal transducer and activator of transcription
TRAPS: TNF receptor–associated periodic syndrome

central and peripheral tolerance in mice with hypomorphic defects of V[D]J recombination) may lead to similar phenotypic manifestations that mimic Omenn syndrome.[1,2] The expanding field of genes involved in V(D)J recombination, class switch recombination, and DNA repair was reviewed by Jean Pierre de Villartay (who has reported on Cernunnos deficiency)[3] and Dick van Gent (DNA ligase 4 deficiency),[4] while Fred Alt illustrated how these and other defects may lead to generalized genomic instability[5] and contribute to tumor development. Later in the meeting, Qiang Pan-Hammarström expanded on chromosome instability syndromes, and in particular on the role played by *ATM*, the gene mutated in Ataxia-Telangiectasia, in DNA repair.[6]

John Ziegler reported on a recently identified form of PID, familial hepatic veno-occlusive disease and immunodeficiency, a combined immunodeficiency caused by mutations of the *SP110* gene, a component of PML nuclear bodies.[7] Stefan Feske presented his work on cloning of the *ORAI1* gene, which encodes for an integral component of calcium channels, whose mutations lead to a severe combined immune deficiency in which T-cell development is not arrested but peripheral T cells are unresponsive to proliferative signals.[8] Genevieve de Saint Basile discussed the basic mechanisms involved in cell-mediated cytotoxicity, and especially generation and trafficking of exocytic vesicles and cytolytic granules, as unraveled through the study of human models of impaired cytotoxicity.[9] Dale Umetsu reviewed the biology of natural killer (NK) T cells, and Sylvain Latour described a novel form of X-linked lymphoproliferative disease caused by mutations of the X-linked inhibitor of apoptosis gene, in which impaired apoptosis is associated with a severe decrease in NK T cells in the periphery.[10]

Amos Etzioni reported on leukocyte adhesion deficiency type 3, a disease characterized by impaired inside-out integrin signaling in leukocytes and platelets caused by mutations of the *CALDAG-GEF1* gene.[11] The different requirements for T-cell and B-cell immunologic memory by cytopathic versus noncytopathic viruses, and the possible need for persistence/boosting with antigen in this process, were reviewed by Rolf Zinkernagel.

In the last year, major advances have been achieved in the molecular and cellular characterization of hyper-IgE syndrome. Hajime Karasuyama gave an update on mutations of the *TYK2* gene and abnormal cytokine-mediated signaling in an autosomal-recessive form of the disease.[12] Steven Holland reported that heterozygous mutations of signal transducer and activator of transcription (STAT)–3 account for the more common autosomal-dominant form of the disease, a previously unknown finding also confirmed by the group of Karasuyama.[13] Two young investigators, Lilit Garibyan and Lalit Kumar, discussed the molecular mechanisms of transmembrane activator and CAML interactor (TACI) deficiency (providing evidence for intracellular preassembly of high-order multimers of the protein)[14] and the phenotype of *LRRC8* knockout mice, respectively.

Exciting results have recently appeared on the molecular and cellular characterization of severe congenital neutropenia. Cristoph Klein reported on the identification of 2 such defects: mutations of p14,[15] an endosomal scaffold protein, and of HCLS1-associated protein x1 (HAX1),[16] involved in control of apoptosis. The inflammasome was reviewed by Nunez, who showed that both gain-of-function and loss-of-function mutations of nucleotide-binding oligomerization domain (NOD)-like receptors may cause disease in human beings. Nunez especially focused on the interplay between pathogens and molecules of the innate immunity system.[17] Jean-Laurent Casanova reported on an unusual phenotype associated with mutations of the *CYBB* gene (which usually cause chronic granulomatous disease), further illustrating the importance of studying human patients to unravel novel molecules and functions within the immune system. The interplay between molecules of the immune system and pathogens was also discussed by Cox Terhorst, who reported on the role played by signaling lymphocyte activation molecule (SLAM) and SLAM family members in controlling bacterial infections. Michael Carroll illustrated the role played by complement in governing memory B-cell responses, whereas Peter Zipfel discussed how defects of the alternative pathway may lead to kidney disease.[18]

Immunodysregulatory disorders were introduced by Sasha Rudensky, who discussed the development and biology of regulatory T cells. Scott Snapper showed how mutations in Wiskott-Aldrich syndrome protein (WASP) lead to inflammatory bowel disease in mice. Alberto Bosque presented novel data on Fas ligand mutations in a subgroup of patients with autoimmune lymphoproliferative syndrome that result in impaired Bcl2-interacting protein (Bim) expression and hence in decreased apoptosis.[19] Richard Siegel discussed the molecular mechanisms involved in TNF receptor–associated periodic syndrome (TRAPS) and showed that retention of TRAPS-associated mutant TNF receptor 1 molecules in the endoplasmic reticulum results in ligand-independent signaling.[20]

In his concluding remarks, Alain Fischer summarized the heuristic value of PID. He pointed out that a substantial number of immune genes have been discovered (even in recent years) through the study of patients with PID, whereas for many others, the function has been clarified (or revealed) through the careful study of human patients. Although PIDs have been traditionally viewed as predisposing to a broad range of infectious pathogens, more and more examples are being identified in which they cause selective susceptibility to single pathogens. Furthermore, PIDs have illustrated the multiple pathways (impaired negative selection, defective development/function of

regulatory T cells, perturbed apoptosis of self-reactive lymphocytes in the periphery) that may cause autoimmunity. Much more than generation of artificial models in mice, the study of human beings with PID has demonstrated the variability of phenotypes that may associate with distinct mutations in the same gene. As Fischer emphasized, it is now time to look at novel approaches to therapy for PID based on the study of disease mechanisms. This is not restricted to gene therapy but also includes bypassing biochemical and/or cellular defects (as shown by the use of IFN-γ in familial mycobacteriosis) and exploiting the use of chemical compounds to allow reading-through nonsense mutations or correction of splice-site mutations.

At the end of the meeting, the International Union of Immunological Societies Expert Committee met to update the classification of PID, as presented in Tables I through VIII.

The manuscript that reports on *STAT3* mutations in patients with hyper-IgE syndrome, presented by Dr Holland at the meeting, is now in press.[21]

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TABLE I. Combined T-cell and B-cell immunodeficiencies

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Gene defects/ presumed pathogenesis
1. T^-B^+ SCID*						
(a) γc deficiency	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells	XL	Defect in γ chain of receptors for IL-2, -4, -7, -9, -15, -21
(b) JAK3 deficiency	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells	AR	Defect in JAK3 signaling kinase
(c) IL7Rα deficiency	Markedly decreased	Normal or increased	Decreased	Normal NK cells	AR	Defect in IL-7 receptor α chain
(d) CD45 deficiency	Markedly decreased	Normal	Decreased	Normal γ/δ T cells	AR	Defect in CD45
(e) CD3δ/CD3ε/ CD3ζ deficiency	Markedly decreased	Normal	Decreased	Normal NK cells	AR	Defect in CD3δ CD3ε or CD3ζ chains of T-cell antigen receptor
2. T^-B^- SCID*						
(a) RAG 1/2 deficiency	Markedly decreased	Markedly decreased	Decreased	Defective VDJ recombination	AR	Complete defect of RAG 1 or 2
(b) DCLRE1C (Artemis) deficiency	Markedly decreased	Markedly decreased	Decreased	Defective VDJ recombination, radiation sensitivity	AR	Defect in Artemis DNA recombinase-repair protein
(c) Adenosine deaminase deficiency	Absent from birth (null mutations) or progressive decrease	Absent from birth or progressive decrease	Progressive decrease	Costochondral junction flaring	AR	Absent ADA, elevated lymphotoxic metabolites (dATP, S-adenosyl homocysteine)
(d) Reticular dysgenesis	Markedly decreased	Decreased or normal	Decreased	Granulocytopenia, thrombocytopenia (deafness)	AR	Defective maturation of T, B, and myeloid cells (stem cell defect)
3. Omenn syndrome	Present; restricted heterogeneity	Normal or decreased	Decreased, except increased IgE	Erythroderma, eosinophilia, adenopathy, hepatosplenomegaly	AR	Missense mutations allowing residual activity, usually in RAG1 or 2 genes but also in Artemis , IL-7Rα, and RMRP genes
4. DNA ligase IV	Decreased	Decreased	Decreased	Microcephaly, facial dystrophy, radiation sensitivity	AR	DNA ligase IV defect, impaired NHEJ
5. Cernunnos/ XLF deficiency	Decreased	Decreased	Decreased	Microcephaly, *in utero* growth retardation, radiation sensitivity	AR	Cernunnos defect, impaired NHEJ
6. CD40 ligand deficiency	Normal	IgM^+ and IgD^+ B cells present, but others absent	IgM increased or normal, other isotypes decreased	Neutropenia, thrombocytopenia; hemolytic anemia, (biliary tract and liver disease, opportunistic infections)	XL	Defects in CD40 ligand (CD40L), defective B-cell and dendritic cell signaling
7. CD40 deficiency	Normal	IgM^+ and IgD^+ B cells present, other isotypes absent	IgM increased or normal, other isotypes decreased	Neutropenia, gastrointestinal and liver disease, opportunistic infections	AR	Defects in CD40, defective B-cell and dendritic cell signaling
8. PNP deficiency	Progressive decrease	Normal	Normal or decreased	Autoimmune hemolytic anemia, neurological impairment	AR	Absent PNP, T-cell and neurologic defects from elevated toxic metabolites (eg, dGTP)
9. CD3γ deficiency	Normal (reduced TCR expression)	Normal	Normal		AR	Defect in CD3γ chain
10. CD8 deficiency	Absent CD8, normal CD4 cells	Normal	Normal		AR	Defects of CD8 α chain

TABLE I. *(Continued)*

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Gene defects/ presumed pathogenesis
11. ZAP-70 deficiency	Decreased CD8, normal CD4 cells	Normal	Normal		AR	Defects in ZAP-70 signaling kinase
12. Ca^{++} channel deficiency	Normal counts, defective TCR mediated activation	Normal counts	Normal	Autoimmunity, anhydrotic ectodermic dysplasia, nonprogressive myopathy	AR	Defect in Orai-1, a Ca^{++} channel component
13. MHC class I deficiency	Decreased CD8, normal CD4	Normal	Normal	Vasculitis	AR	Mutations in *TAP1*, *TAP2* or *TAPBP* (tapasin) genes giving MHC class I deficiency
14. MHC class II deficiency	Normal number, decreased CD4 cells	Normal	Normal or decreased		AR	Mutation in transcription factors for MHC class II proteins (*C2TA*, *RFX5*, *RFXAP*, *RFXANK* genes)
15. Winged helix deficiency (nude)	Markedly decreased	Normal	Decreased	Alopecia, abnormal thymic epithelium (resembles nude mouse)	AR	Defects in forkhead box N1 transcription factor encoded by *FOXN1*, the gene mutated in nude mice
16. CD25 deficiency	Normal to modestly decreased	Normal	Normal	Lymphoproliferation (lymphadenopathy, hepatosplenomegaly), autoimmunity (may resemble IPEX syndrome), impaired T-cell proliferation	AR	Defects in IL-2R α chain
17. STAT5b deficiency	Modestly decreased	Normal	Normal	Growth hormone–insensitive dwarfism, dysmorphic features, eczema, lymphocytic interstitial pneumonitis	AR	Defects of *STAT5B* gene, impaired development and function of γδ T cells, T-regulatory and NK cells, impaired T-cell proliferation

ADA, Adenosine deaminase; *DCLRE*, DNA cross-link repair protein 1C; *dATP*, deoxyadenosine triphosphate; *dGTP*, deoxyguanosin triphosphate; *IPEX*, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; *AR*, autosomal recessive inheritance; *JAK3*, Janus kinase 3; *NHEJ*, nonhomologous end joining; *PNP*, purine nucleoside phosphorylase; *RAG*, recombinase activating gene; *RMRP*, RNA of mitochondrial RNA-processing endoribonuclease; *SCID*, severe combined immune deficiency; *TAP*, transporter associated with antigen processing; *TAPBP*, TAP binding protein; *TCR*, T-cell receptor; *XL*, X-linked inheritance; *XLF*, XRCC4-like factor.

*Atypical cases of SCID may present with T cells because of hypomorphic mutations or somatic mutations in T-cell precursors.

TABLE II. Predominantly antibody deficiencies

Disease	Serum immunoglobulin	Associated features	Inheritance	Gene defects/ presumed pathogenesis
1. Severe reduction in all serum immunoglobulin isotypes with profoundly decreased or absent B cells				
(a) Btk deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	XL	Mutations in Burton tyrosine kinase
(b) μ Heavy chain deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in μ heavy chain
(c) λ5 Deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in λ5
(d) Igα deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in Igα
(e) Igβ deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in Igβ
(f) BLNK deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in *BLNK*
(g) Thymoma with immunodeficiency	All isotypes decreased	Infections; decreased numbers of pro-B cells	None	Unknown
(h) Myelodysplasia	All isotypes decreased	Infections; decreased numbers of pro-B cells	Variable	May have monosomy 7, trisomy 8 or dyskeratosis congenita
2. Severe reduction in serum IgG and IgA with normal, low or very low numbers of B cells				
Common variable immunodeficiency disorders*	Low IgG and IgA; variable IgM	All have recurrent bacterial infections. Clinical phenotypes vary: autoimmune, lymphoproliferative and/or granulomatous disease	Approximately 10% have a positive family history (AR or autosomal-dominant)	Alterations in TACI, BAFFR, Msh5 may act as contributing polymorphisms†
(a) ICOS deficiency	Low IgG and IgA; normal IgM	—	AR	Mutations in *ICOS*
(b) CD19 deficiency	Low IgG, IgA and IgM	—	AR	Mutations in *CD19*
(c) X-linked lymphoproliferative syndrome 1‡	All isotypes may be low	Some patients have antibody deficiency, although most present with fulminant EBV infection or lymphoma	XL	Mutations in *SH2D1A*
3. Severe reduction in serum IgG and IgA with normal/ elevated IgM and normal numbers of B cells				
(a) CD40L deficiency§	IgG and IgA decreased; IgM may be normal or increased; B cell numbers may be normal or increased	Opportunistic infections, neutropenia, autoimmune disease	XL	Mutations in *CD40L* (also called *TNFSF5* or *CD154*)

TABLE II. *(Continued)*

Disease	Serum immunoglobulin	Associated features	Inheritance	Gene defects/ presumed pathogenesis
(b) CD40 deficiency§	Low IgG and IgA; normal or raised IgM	Opportunistic infections, neutropenia	AR	Mutations in *CD40* (also called *TNFRSF5*)
(c) Activation-induced cytidine deaminase deficiency	IgG and IgA decreased; IgM increased	Enlarged lymph nodes and germinal centres	AR	Mutations in *AICDA* gene
(d) UNG deficiency	IgG and IgA decreased; IgM increased	Enlarged lymph nodes and germinal centers	AR	Mutations in *UNG* gene
4. Isotype or light chain deficiencies with normal numbers of B cells				
(a) Ig heavy chain deletions	One or more IgG and/ or IgA subclasses as well as IgE may be absent	May be asymptomatic	AR	Chromosomal deletion at 14q32
(b) κ chain deficiency	All immunoglobulins have λ light chain	Asymptomatic	AR	Mutations in κ constant gene
(c) Isolated IgG subclass deficiency	Reduction in 1 or more IgG subclass	Usually asymptomatic; may have recurrent viral/bacterial infections	Variable	Unknown
(d) IgA deficiency associated with IgG subclass deficiency	Reduced IgA with decrease in 1 or more IgG subclass	Recurrent bacterial infections in majority	Variable	Unknown
(e) Selective IgA deficiency	IgA decreased/absent	Usually asymptomatic; may have recurrent infections with poor antibody responses to carbohydrate antigens; may have allergies or autoimmune diseases; a few cases progress to CVID; others coexist with CVID in the same family	Variable	Unknown
5. Specific antibody deficiency with normal Ig concentrations and normal numbers of B cells	Normal	Inability to make antibodies to specific antigens	Variable	Unknown
6. Transient hypogammaglobulinemia of infancy with normal numbers of B cells	IgG and IgA decreased	Recurrent moderate bacterial infections	Variable	Unknown

AR, Autosomal-recessive inheritance; *BAFFR*, B-cell–activating factor receptor; *BLNK*, B-cell linker protein; *CVID*, common variable immune deficiency; *ICOS*, inducible costimulator; *Msh5*, homolog of *E. coli* MutS; *UNG*, uracil-DNA glycosylase; *XL*, X-linked inheritance.

*There are several different clinical phenotypes, probably representing distinguishable diseases with differing immunopathogeneses; alterations in *TACI*, *BAFFR* and Msh5 sequences may represent contributing polymorphisms or disease-modifying alterations.

†A disease-causing effect has been identified for homozygous C140R, S144X, and A181E *TACI* mutations.

‡XLP1 (X-linked lymphoproliferative syndrome) is also included in Table IV.

§CD40L deficiency (X-linked hyper IgM syndrome) and CD40 deficiency are also included in Table I.

TABLE III. Other well defined immunodeficiency syndromes

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Gene defects/ presumed pathogenesis
1. WAS	Progressive decrease	Normal	Decreased IgM: antibody to polysaccharides particularly decreased; often increased IgA and IgE	Thrombocytopenia with small platelets; eczema; lymphomas; autoimmune disease; IgA nephropathy; bacterial and viral infections. XL thrombocytopenia is a mild form of WAS, and XL neutropenia is caused by missense mutations in the GTPase binding domain of *WASP*	XL	Mutations in *WASP*; cytoskeletal defect affecting hematopoietic stem cell derivatives
2. DNA repair defects (other than those in Table I)						
(a) Ataxia-telangiectasia	Progressive decrease	Normal	Often decreased IgA, IgE and IgG subclasses; increased IgM monomers; antibodies variably decreased	Ataxia; telangiectasia; increased α fetoprotein; lymphoreticular and other malignancies; increased X-ray sensitivity; chromosomal instability	AR	Mutation in *ATM*; disorder of cell cycle check-point and of DNA double-strand break repair
(b) Ataxia-telangiectasia-like disease	Progressive decrease	Normal	Often decreased IgA, IgE and IgG subclasses; increased IgM monomers; antibodies variably decreased	Moderate ataxia; severely increased radiosensitivity	AR	Hypomorphic mutation in *MRE11*; disorder of cell cycle checkpoint and of DNA double-strand break repair
(c) Nijmegen breakage syndrome	Progressive decrease	Normal	Often decreased IgA, IgE and IgG subclasses; increased IgM monomers; antibodies variably decreased	Microcephaly; birdlike face; lymphomas; ionizing radiation sensitivity; chromosomal instability	AR	Hypomorphic mutation in *NBS1* (*Nibrin*); disorder of cell cycle checkpoint and of DNA double-strand break repair
(d) Bloom syndrome	Normal	Normal	Reduced	Chromosomal instability; marrow failure; leukemia; lymphoma; short stature; birdlike face; sensitivity to the sun telangiectasias	AR	Mutation in *BLM*, a RecQ-like helicase
3. Thymic defects DiGeorge anomaly	Decreased or normal; often progressive normalization	Normal	Normal or decreased	Hypoparathyroidism; conotruncal heart defects; abnormal facies; interstitial deletion of 22q11-pter (or 10p) in some patients	*De novo* defect or AD	Contiguous gene defect in 90% affecting thymic development; mutation in transcription factor *TBX1*
4. Immuno-osseous dysplasias						

TABLE III. (*Continued*)

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Gene defects/ presumed pathogenesis
(a) Cartilage hair hypoplasia	Decreased or normal*	Normal	Normal or reduced; antibodies variably decreased	Short-limbed dwarfism with metaphyseal dysostosis; sparse hair; anemia; neutropenia; susceptibility to lymphoma and other cancers; impaired spermatogenesis; neuronal dysplasia of the intestine	AR	Mutation in *RMRP* (RNase MRP RNA)
(b) Schimke syndrome	Decreased	Normal	Normal	Short stature; spondyloepiphyseal dysplasia; intrauterine growth retardation; nephropathy	AR	Mutation in *SMARCAL1*
5. Hyper-IgE syndromes (HIES)						
(a) Job syndrome (AD HIES)	Normal	Normal	Elevated IgE	Recurrent skin boils and pneumonia often caused by *Staphylococcus aureus*; pneumatoceles; eczema, nail candidiasis; distinctive facial features (thickened skin, broad nasal tip); failure/delay of shedding primary teeth; hyperextensible joints	AD, many *de novo* mutations	Mutation in *STAT3*
(b) AR HIES with mycobacterial and viral infections	Normal	Normal	Elevated IgE	Susceptibility to intracellular bacteria (mycobacteria, *Salmonella*), fungi, and viruses; eczema No skeletal or connective tissue abnormalities i) CNS hemorrhage, fungal and viral infections	AR	Mutation in *TYK2*; Unknown
(c) AR HIES with viral infections and CNS vasculitis/ hemorrhage	Normal	Normal	Elevated IgE	Susceptibility to bacterial, viral and fungal infections; eczema; vasculitis; CNS hemorrhage; no skeletal or connective tissue abnormalities	AR	Unknown
6. Chronic mucocutaneous candidiasis	Normal	Normal	Normal	Chronic mucocutaneous candidiasis; impaired delayed-type hypersensitivity to *Candida* antigens; autoimmunity; no ectodermal dysplasia	AD, AR, sporadic	Unknown

TABLE III. (*Continued*)

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Gene defects/ presumed pathogenesis
7. Hepatic veno-occlusive disease with immuno-deficiency	Normal (decreased memory T cells)	Normal (decreased memory B cells)	Decreased IgG, IgA, IgM	Hepatic veno-occlusive disease; *Pneumocystis jiroveci* pneumonia; thrombocytopenia, hepatosplenomegaly	AR	Mutation in *SP110*
8. Hoyerall-Hreidarsson syndrome	Progressive decrease	Progressive decrease	Variable	Intrauterine growth retardation, microcephaly, digestive tract involvement, pancytopenia, reduced number and function of NK cells	XL	Mutation in Dyskerin

AD, Autosomal-dominant inheritance; *AR*, autosomal-recessive inheritance; *BLM*, Bloom syndrome gene; *CNS*, central nervous system; *HIES*, hyper-IgE syndrome; *RMRP*, RNA of mitochondrial RNA-processing endoribonuclease; *WAS*, Wiskott-Aldrich syndrome; *XL*, X-linked inheritance.
*Patients with cartilage-hair hypoplasia can also present also with typical severe combined immune deficiency or with Omenn syndrome.

TABLE IV. Diseases of immune dysregulaton

Disease	Circulating T cells	Circulating B cells	Serum Ig	Associated features	Inheritance	Gene defects/ presumed pathogenesis
1. Immunodeficiency with hypopigmentation						
(a) Chediak-Higashi syndrome	Normal	Normal	Normal	Partial albinism, giant lysosomes, low NK and CTL activities, heightened acute-phase reaction, encephalopathic accelerated phase	AR	Defects in *LYST*, impaired lysosomal trafficking
(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
(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
2. Familial hemophagocytic lymphohistiocytosis syndromes						
(a) Perforin deficiency	Normal	Normal	Normal	Severe inflammation, fever, decreased NK and CTL activities	AR	Defects in *PRF1*; perforin, a major cytolytic protein
(b) Munc 13-D deficiency	Normal	Normal	Normal	Severe inflammation, fever, decreased NK and CTL activities	AR	Defects in *MUNC13D* required to prime vesicles for fusion
(c) Syntaxin 11 deficiency	Normal	Normal	Normal	Severe inflammation, fever, decreased NK and CTL activities	AR	Defects in *STX11*, involved in vesicle trafficking and fusion
3. X-linked lymphoproliferative syndrome						
(a) XLP1	Normal	Normal or reduced	Normal or low immuno-globulins	Clinical and immunologic abnormalities triggered by EBV infection, including hepatitis, aplastic anemia, lymphoma	XL	Defects in *SH2D1A* encoding an adaptor protein regulating intracellular signals
(b) XLP2	Normal	Normal or reduced	Normal or low immuno-globulins	Clinical and immunologic abnormalities triggered by EBV infection, including splenomegaly, hepatitis, hemophagocytic syndrome, lymphoma	XL	Defects in *XIAP* encoding an inhibitor of apoptosis
4. Syndromes with autoimmunity						
(a) ALPS						
(i) CD95 (Fas) defects, ALPS type 1a	Increased double-negative (CD4- CD8-) 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 similar phenotype, ALPS 1a (somatic)
(ii) CD95L (Fas ligand) defects, ALPS type 1b	Increased double-negative (CD4- CD8-) T cells	Normal	Normal	Splenomegaly, adenopathy, autoimmune blood cytopenias, defective lymphocyte apoptosis, lupus	AD AR	Defects in *TNFSF6*, ligand for CD95 apoptosis receptor
(iii) Caspase 10 defects, ALPS type 2a	Increased CD4- CD8- T cells	Normal	Normal	Adenopathy, splenomegaly, autoimmune disease, defective lymphocyte apoptosis	AD	Defects in *CASP10*, intracellular apoptosis pathway