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Mutations in the tyrosine phosphatase CD45 gene in a child with severe combined immunodeficiency disease

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The hematopoietic-specific transmembrane protein tyrosine phosphatase CD45 functions to regulate Src kinases required for T- and B-cell antigen receptor signal transduction[1,2]. So far, there have been no reports to our knowledge of a human deficiency in a tyrosine-specific phosphatase. Here, we identified a male patient with a deficiency in CD45 due to a large deletion at one allele and a point mutation at the other. The point mutation resulted in the alteration of intervening sequence 13 donor splice site. The patient presented at 2 months of age with severe combined immunodeficiency disease. The population of peripheral blood T lymphocytes was greatly diminished and unresponsive to mitogen stimulation. Despite normal B-lymphocyte numbers, serum immunoglobulin levels decreased with age. Thus, CD45 deficiency in humans results in T- and B-lymphocyte dysfunction.

Severe combined immunodeficiency disease (SCID) is characterized by a defect in function and/or development of B and T lymphocytes, considerable lymphopenia, and a deficiency in humoral and cell-mediated immunity[3]. We studied a male child who presented at 2 months of age with SCID and eventually succumbed to a B-cell lymphoma at 2 years of age. Lymph node biopsies from the patient showed a lack of histological organization and germinal center formation, and stained thin sections from the lymph node did not show expression of CD45 (data not shown). Indeed, CD45 expression was lacking on all leukocytes (Table 1). Although peripheral blood monocyte numbers were within the normal range, T-lymphocyte numbers were considerably decreased[4] (Table 1). The percentage of lymphocytes that expressed T-cell receptor γδ was normal; however, the proportion of T-cell receptor αβ-positive cells was substantially decreased (Table 1). Moreover, the few peripheral T cells were not activated, as shown by the percentage of CD25⁺ cells (Table 1). In proliferative studies, the patient cells failed to respond to phytohemagglutinin, concanavalin A and pokeweed mitogen (less than 1% of control proliferative responses) (data not shown). Although it is possible that the few T cells in the periphery were of maternal origin, the CD45-deficient mouse also has a small number of T cells in the periphery, indicating that T-cell development is not completely blocked in the absence of CD45. Although B-cell numbers were increased (Table I), serum immunoglobulin levels decreased considerably with age. At 2 months of age, the patient's immunoglobulin levels were mostly within normal range, with slightly increased IgM; however, by 4 months of age, the patient's IgG was 1.75 mg/ml. IgA was less than 0.008 mg/ml and IgM was 0.26 mg/ml. Immunoglobulin replacement therapy was begun. IgM and IgA antibodies, which are not present at substantial levels in immunoglobulin infusion, decreased to very low or undetectable levels with time. The proportion of natural killer cells, as defined by $CD3^-/CD16^+/CD56^+$ expression, was below the normal level (Table 1). As reported for CD45-deficient mice, the patient's T-cell numbers were decreased and B-cell numbers were increased, yet the cells were dysfunctional[5–9].

To identify the genomic defect, genomic DNA from the child and both parents was prepared from peripheral blood monocytes. With full-length CD45 cDNA as a probe, Southern blot analysis indicated a large deletion in the CD45 gene in one chromosome of the mother and of the child (Fig. 1). The intensity of hybridization for fragments after digestion of DNA with *Eco*RI was 200% more in the father's sample than in samples from the mother or child (Fig. 1*a*). Hybridization with various probes derived from the CD45 cDNA indicated that the deletion

Table 1 Peripheral Blood Lymphocyte Subpopulations

Antigen	% Gated Lymphocytes[a] Patient	Normal[b]
CD3	8.9	64± 5
CD4	0.9	37±10
CD8	4.9	29± 4
CD19	84.5	24± 3
$CD3^-/CD16^+/CD56^+$	3.2	11± 4
$HLA\text{-}DR^+$	86.2	11± 4
CD25	2.9	2
$CD3^+/CD25^+$	1.9	—
TCR $\gamma\delta^+$	6.7	5± 4
TCR $\alpha\beta^+$	2.2	62± 7
CD45	0	100

[a]Data represent peripheral blood lymphocytes at 1.5 years of age: patient, 3.1×10^3 cell/mm³; normal range, 2.9×10^3–5.1 cells/mm³; [a]ref 4.

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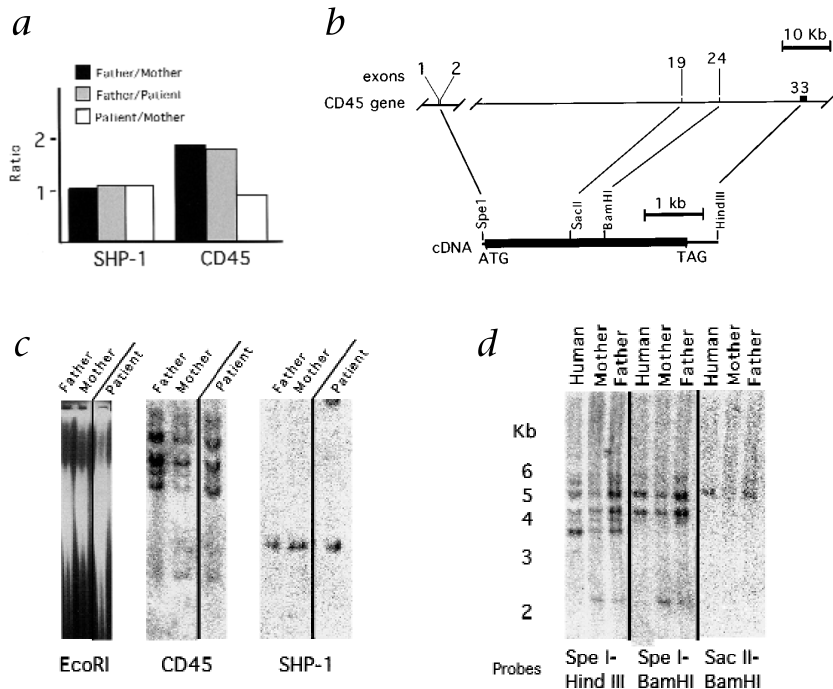

Fig. 1 Analysis of CD45 gene copy number. ***a***, Densitometric analysis of Southern blots. Genomic DNA from father, mother and patient were digested with *Eco*RI and hybridized to cDNAs for CD45 and SHP-1. Ratios normalized for amounts of loaded DNA compare the intensity of hybridization for a representative restriction fragment from either the CD45 or SHP-1 blots. ***b***, Southern blot analysis of *Eco*RI-digested genomic DNA from a non-symptomatic adult control (Human), mother and father. Blots were probed with either full-length CD45 cDNA (*Spe*I/*Hin*dIII fragment), a 5′ probe encompassing exons 2–24 (*Spe*I/*Bam*HI) or cDNA encompassing exons 19–24 (*Sac*II/*Bam*HI).

mapped to the 3′ end of the gene (Fig. 1*b*). This difference in hybridization indicates that the mother and child had lost a large region of the CD45 gene. There was no difference in hybridization for an unrelated tyrosine phosphatase, SHP-1, which maps to a different chromosome (CD45, chromosome 1; SHP-1, chromosome 12) (Fig. 1*a*).

To identify additional mutations that might account for the inactivation of the other allele, we sequenced the exons that encode the CD45 gene and intervening sequence boundaries in DNA from the father, mother, child and an unaffected sibling (Fig. 2*a* and data not shown). There were no mutations in the parents or unaffected sibling. However, we identified a unique G-to-A mutation in the patient at the donor splice site of intervening sequence 13. This mutation destroys the splice junction site and potentially allows abnormal processing at cryptic splice sites in other regions of the CD45 gene. To confirm pathogenicity of the identified mutation, we looked for evidence of aberrant CD45 mRNA splicing in an Epstein-Barr virus-transformed B-lymphoblastoid cell line derived from patient peripheral blood lymphocytes (Fig. 2*b*–*e*). Flow cytometric analysis of this cell line demonstrated negligible staining for CD45, and northern blot analysis showed no detectable CD45 mRNA (Fig. 2*b* and *c*). Both findings were consistent with the phenotype of the peripheral blood cells. We successfully amplified CD45 coding sequences spanning exons 7–11 and 7–15 from the patient's cell line by RT–PCR. The RT–PCR product from patient exon 7–15 but not exon 7–11 migrated slightly slower than the control sample because of the presence of a 48-base-pair insertion immediately 3′ of the sequence encoded by exon 13 (Fig. 2*d*). This insertion started at the mutant G-to-A substitution at intervening sequence-13 donor splice junction and extended 3′ into intervening sequence 13 before joining again with sequence encoded by exon 14 (Fig. 2*e*). These results are consistent with aberrant splicing induced by the observed mutation.

The findings in this child correlate well with the phenotype reported for CD45-deficient mice. In the patient, development of the few T cells in the periphery was impaired and these cells were unresponsive to mitogen stimulation. As for the B-cell population, the patient showed a

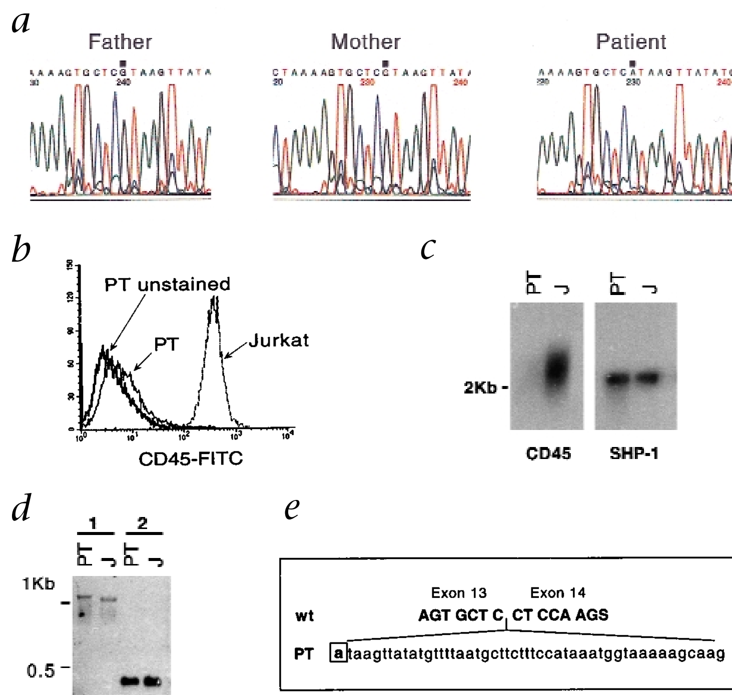

Fig. 2 Mutation at a donor splice site. ***a***, DNA sequence analysis of the donor splice site for exon 13 of CD45 in the parents and patient. Filled squares (top row), mutation. Sequence analysis was done of three or more independent PCR reactions from genomic DNA for each strand. ***b***–***e***, CD45 expression analyzed from an Epstein-Barr virus-transformed line derived from the patient's peripheral blood monoclonuclear cells. *b*, FACS analysis of patient cells (PT) and Jurkat cells (J) using FITC-conjugated antibody against CD45. PT unstained, negative control. *c*, Northern blot analysis with 20 µg total RNA per lane, probed with either human full-length CD45 cDNA (left) or mouse full-length SHP-1 cDNA (right). *d*, Oligo dT-primed RT–PCR analysis from patient (PT) or Jurkat (J) cell lines. PCR results using human CD45 primers for exons 7 and 15 (lane 1) or exons 7 and 11 (lane 2). Left margin (*c* and *d*), migration of molecular size markers. *e*, Insertion of intron sequence in patient (PT) at exon 13, compared with wild-type (wt) human CD45 cDNA. Sequence analysis was confirmed on independent PCR fragments.

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substantial increase in the number of B cells, but a considerable decrease in the serum immunoglobulin levels. This can be explained by a defect in B-cell maturation. In CD45-deficient mice, although B-cell numbers are normal, signaling through surface IgM cross-linking is absent. Furthermore, calcium influx into B cells is lacking, but intracellular calcium mobilization is normal in these cells. Most notably, immature B-cell selection is altered, increasing the normal threshold signal, thereby eliminating mature B cells.

Our results are consistent with essential involvement of CD45 in regulating antigen receptor signaling as well as T-cell and B-cell development and maturation. Indeed, during lymphocyte development, CD45 expression is upregulated in conjunction with antigen receptor expression[10]. Consequently, the decreased number of T-cell receptor αβ-positive cells in the patient correlates well with CD45 functioning as a regulator of lymphocyte development and maturation.

Analysis indicates that one of the alleles in the mother and child had a large deletion, most likely encompassing the CD45 gene. DNA sequence analysis showed that the patient had a unique G-to-A mutation in the donor splice site at intervening sequence 13, which resulted in aberrant splicing. These results indicate that it is most likely that the patient received a defective allele from the mother and that the allele from the father underwent spontaneous mutation (although, for the privacy of the family, paternity was not proven genetically).

This is the first report to our knowledge of a deficiency in a tyrosine phosphatase in humans. A deficiency in CD45 results in SCID. Our results further substantiate the involvement of CD45 in antigen receptor signaling and T-cell and B-cell development. These data demonstrate that like defects in tyrosine kinases, a defect in a tyrosine phosphatase can present with a severe immunological disorder[11–14].

Methods

Southern blot analysis. Patient and parental genomic DNA was prepared from peripheral blood lymphocytes and assessed by Southern blot analysis. DNA was digested overnight with the appropriate enzymes and separated by electrophoresis on a 0.8% agarose gel. The separated DNA fragments were transferred to nitrocellulose membrane for hybridization. cDNA probes were labeled with both α-^{32}P dGTP and α-^{32}P dCTP using random-primed labeling (Boehringer). Routinely, prehybridization, hybridization and washing of blots were done as described[15]. Washed blots were exposed to film or a fluorescence intensifying screen (Molecular Dynamics, Sunnyvale, California). Densitometry analysis of the Southern blots used ImageQuant software (Molecular Dynamics, Sunnyvale, California).

Sequence analysis. DNA sequencing of the exon–intron regions of the CD45 gene used dye-terminator cycle sequencing (Perkin Elmer, Norwalk, Connecticut). Oligonucleotides were derived for sequences within the intron and exon such that after PCR sequencing the entire exon and flanking sequences would be obtained. Sequences were obtained for the mother, father and patient.

mRNA analysis. B-lymphoblastoid cell lines were derived by infection of a cryo-preserved sample of patient peripheral blood mononuclear cells with Epstein-Barr viral stock B95.8. cDNAs were synthesized from total RNA samples by reverse transcription. CD45 PCR analysis used sense primer 5′–TCAACCACAACAATAGCTACT–3′ (derived from exon 7) and anti-sense primer 5′–GTCTCCATTGTGAAAATAGGC–3′ (derived from exon 15) or anti-sense primer 5′–GGGTTCAAGGTTTTCTAATTT–3′ (derived from exon 11) oligos. Northern blot analysis used full-length human CD45 cDNA and full-length mouse SHP-1 cDNA.

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