

## SPONTANEOUS LOSS AND ALTERATION OF ANTIGEN RECEPTOR EXPRESSION IN MATURE CD4<sup>+</sup> T CELLS

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The CD3-associated TCR- $\alpha/\beta$  heterodimer is expressed on the surface of the vast majority of mature CD4<sup>+</sup> or CD8<sup>+</sup> T cells in the peripheral blood (PB)<sup>1</sup> and lymphoid organs (1-3). A second class of TCRs consisting of  $\gamma$  and  $\delta$  chains (TCR- $\gamma/\delta$ ), also CD3-associated, is found mainly in the double-negative (CD4<sup>-</sup>8<sup>-</sup>) T cell population (4-6). The TCR and CD3 are thought to form a functional unit in antigen recognition and signal transduction, and the association of all the components appears to be essential for cell surface expression of the molecular complex (for a review, see reference 7). Thus, inactivation of a gene encoding a protein of the TCR/CD3 complex should lead to loss of surface TCR/CD3 expression and thereby result in defective antigen recognition and cell activation. Many mutant cell lines lacking surface expression of the TCR/CD3 complex have been obtained from human T lymphoma cells exposed to radiation or chemical mutagens (8-17). These cells failed to respond to various mitogen and antibody stimuli (9, 12-15). Most of these mutant lines were found to have defects in TCR expression (mostly TCR- $\beta$ ) rather than CD3 expression and intracellular accumulation of the incompletely assembled complexes. This suggests that TCR genes are more susceptible to mutagenesis than CD3 genes.

These four TCR genes are all located on chromosomes 7 ( $\beta$ ,  $\gamma$ ) and 14 ( $\alpha$ ,  $\delta$ ) (18). Similar to Ig genes in B cells, but unlike ordinary autosomal genes, only one of two TCR alleles in T cells is thought to be active in protein expression (19). Although the mechanism of allelic exclusion in TCR expression is not fully understood at the molecular level, such a mechanism may be essential in order for a given T cell clone to have only one idiotype on its TCR, which is necessary for the maintenance of an ordered immune network. As a result of allelic exclusion, it is expected that TCR expression-loss variants are frequently generated by a single inactivation event even though TCR genes are autosomally located. In fact, many mutant cell lines lacking the TCR have been established in vitro from T lymphoma cell lines (8-17), as men-

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<sup>1</sup> *Abbreviations used in this paper:* AT, ataxia telangiectasia; DS, Down's syndrome; FA, Fanconi's anemia; FL, fluorescein-labeled; PB, peripheral blood; PE, phycoerythrin-labeled; PI, propidium iodide, <sup>35</sup>S-met, <sup>35</sup>S-methionine.

tioned above, although the mutation rates have not been determined yet. In this report, we will address the question of whether such variant cells with alterations of TCR/CD3 expression exist in detectable numbers among in vivo mature T cell populations. Aberrant, immature T cells with nonproductive rearrangements of both TCR alleles are assumed to die in the thymus (19, 20). It is unknown whether a similar selection mechanism depletes such aberrant mature T cells induced in periphery by somatic mutations. If such variant T cells do exist, by what molecular alterations and at what frequencies have they been generated?

For this study, we detected and isolated variants with loss or alteration of TCR/CD3 expression from a human PB CD4<sup>+</sup> T cell population using a cell sorter. Most of these variants had abnormalities in the expression of either the TCR- $\alpha$  or - $\beta$  chain. The TCR abnormalities probably were caused by inactivation or alteration of the TCR genes, which resulted in defect or decrease in membrane transport of the incomplete TCR/CD3 complex. The variant frequency was measured for normal donors and patients with genetic diseases showing somatic gene instability. Implications for the T cell immunodeficiency and malignancy will also be discussed.

### Materials and Methods

*mAbs.* mAbs used were anti-Leu-4 (CD3), NU-T3 (CD3) (21), anti-Leu-3a (CD4), anti-Leu-6 (CD1), anti-Leu-5b (CD2), anti-Leu-1 (CD5), anti-Leu-2a (CD8), anti-Leu-7 (CD57), anti-Leu-11a (CD16), anti-Leu-16 (CD20), anti-Leu-M3 (CD14), anti-IL-2-R (CD25), WT31 (TCR $\alpha\beta$ ) (22),  $\beta$ F1 (TCR- $\beta$ ) (23),  $\alpha$ F1 (TCR- $\alpha$ ), anti-TCR- $\delta$ 1 (TCR- $\delta$ ) (24), and anti-TCR- $\gamma/\delta$ 1 (TCR- $\gamma$  or - $\gamma/\delta$  complex) (25). Anti-Leu and WT31 (TCR-I) mAbs were purchased from Becton Dickinson Immunocytometry Systems (San Jose, CA). NU-T3 mAb was obtained from Seikagaku Kogyo Co. (Tokyo, Japan). Anti-TCR- $\delta$ 1 mAb was purchased from T Cell Science, Inc. (Cambridge, MA), and anti-TCR- $\gamma/\delta$ 1 mAb was kindly provided by Dr. J. Borst, The Netherlands Cancer Institute (Amsterdam, The Netherlands). Preparation and characterization of  $\alpha$ F1 antibody is described in a technical data sheet provided by T Cell Science, Inc.

*Sorting and Culture of Variant T Cells.* PB samples were derived from healthy volunteers and from children with genetic diseases such as ataxia telangiectasia (AT), Fanconi's anemia (FA), and Down's syndrome (DS). PBMC were isolated from blood by density separation using Ficoll/Hypaque. PBMCs were stained with fluorescein-labeled anti-Leu-3a (FL-anti-Leu-3a) and phycoerythrin-labeled anti-Leu-4 (PE-anti-Leu-4) antibodies, as described by the supplier. Before flow cytometry, propidium iodide (PI) was added at a final concentration of 10  $\mu$ g/ml to gate out dead cells. For sorting variant CD4<sup>+</sup> T cells with loss or decreased expression of CD3, a sorting window was set to the region where the surface CD3 level was <4% of that of normal CD4<sup>+</sup> cells. Approximately 1,000 cells were sorted and cultured with GIT medium (Wako Pure Chemical Industry, Osaka, Japan) containing 10% FCS (M. A. Bioproducts, Walkersville, MD), PHA (1:1600, Difco Laboratories, Detroit, MI), rIL-2 (2 ng/ml; Takeda Chemical Industries, Osaka, Japan), and feeder cells for 2 wk. A mixture of allogeneic PBMCs from three normal donors and from an allogeneic B cell line, OKIB, established by us was used as feeder cells after irradiation with 50 Gy. For cloning variant T cells, immediately after sorting, cells were distributed into 96-well plastic plates at a mean frequency of  $\sim$ 1 cell per well, and were cultured for 4 wk under the same condition described above. Normal CD4<sup>+</sup> clones were also established from normal donors and AT patients. Clones derived from these cultures were expanded with feeder cells for further analysis.

*Cytoplasmic Immunofluorescence.* Variant and normal T cell clones were cytocentrifuged onto a glass slide and fixed with acetone for 10 min at  $-20^{\circ}\text{C}$ . The fixed cell specimens were incubated with the first antibodies (anti-Leu-4, WT31,  $\alpha$ F1,  $\beta$ F1, TCR- $\delta$ 1, and TCR- $\gamma/\delta$ 1) or control myeloma proteins (MOPC21 and UPC10) for 1 h at room temperature. After being washed with PBS three times, the specimens were stained with F(ab)<sub>2</sub> fragments of anti-

mouse Igs (Tago Inc., Burlingame, CA) for 45 min. After extensive washing, the samples were fixed with 1% paraformalin for observation under a fluorescence microscope (Nikon, Tokyo, Japan).

**Immunoprecipitation Analysis.** Radiolabeling and immunoprecipitation were performed using the following methods: T cell clones were biosynthetically labeled with 37 MBq  $^{35}\text{S}$ -methionine ( $^{35}\text{S}$ -Met) (New England Nuclear, Boston, MA) in RPMI 1640 Met-free medium at 37°C for 6 h. For  $^{125}\text{I}$  surface labeling, cells were incubated with 74 MBq Na  $^{125}\text{I}$  (New England Nuclear) at 30°C for 4 min in 150  $\mu\text{l}$  PBS containing 100  $\mu\text{g}$  lactoperoxidase (Sigma Chemical Co., St. Louis, MO) and 20  $\mu\text{l}$  of 0.03%  $\text{H}_2\text{O}_2$ , and the mixture was further incubated at 25°C for 10 min (26). After radiolabeling, cells were washed with PBS five times and lysed in the extraction buffer (10 mM triethanolamine, 0.15 M NaCl, pH 7.8 [containing as inhibitors], 10 mM iodacetamide, 1 mM EDTA, 1 mM PMSF, and 1  $\mu\text{g}/\text{ml}$  each of leupeptin, antipain, pepstatin, and chymostatin) containing 1% digitonin or 0.5% NP-40, as described in a previous report (27). Immunoprecipitations were performed by incubating the detergent extracts with 2  $\mu\text{g}$  of antibody on ice for 3–4 h, then adding a 20  $\mu\text{l}$ -packed volume of protein A-Sepharose (Pharmacia LKB Biotechnology AB, Uppsala, Sweden), and further incubating the mixture for 1 h. For anti-TCR- $\delta 1$  and MOPC 21 antibodies, protein A-Sepharose was precoated with rabbit anti-mouse Igs. Immunoprecipitates were washed five times in the same buffer used for cell extraction and were solubilized in reducing or nonreducing Laemmli sample buffer subjected to SDS-PAGE.

**Southern Blotting.** High-molecular weight genomic DNA was extracted from variant T cell clones and a B cell line, as described in a previous report (28). DNA samples (10  $\mu\text{g}$ ) were digested with Bam HI, subjected to electrophoresis through 0.7% agarose, transferred to nitrocellulose filters, and probed with cDNA of the TCR- $\beta$  C region gene (29).  $^{32}\text{P}$  labeling was performed using a random primer method (Amersham International, Amersham, UK). Filters were washed with  $0.1 \times \text{SSC}$  and 0.1% SDS at 65°C.

**Chromosome Analysis.** Variant T cell clones were examined for karyotype analysis using the G-banding method (30). Briefly, after exposure of T cells to colcemid (0.05  $\mu\text{g}/\text{ml}$ ) for 4 h, chromosome specimens were prepared by an air-dried method. The slides were treated with a 0.1% trypsin solution (Gibco Laboratories, Grand Island, NY) for 15–20 s at 30°C and then stained with 2% Giemsa solution for 15 min. In each clone, >10 metaphases were analyzed.

## Results

**Detection of Variant T Cells.** Flow cytometry is useful for detecting and isolating cells with rare phenotypes in vitro (31) and in vivo (32–34) at frequencies of  $>10^{-6}$ . To detect variant  $\text{CD4}^+$  T cells with altered TCR/CD3 expression, two-color fluorescence analysis of normal PBL stained with FL-anti-CD4 and PE-anti-CD3 antibodies was performed using a cell sorter. Using WT31 antibody to react with the TCR- $\alpha/\beta$  complex did not work for detecting variants; the resolution between positive and negative cells was not great enough. The sorting window was set in the region for variant  $\text{CD4}^+$  cells, as shown in Fig. 1 A. Variant cells showed a discrete cluster in the window and were observed in normal donors at a frequency of several cells per 10,000  $\text{CD4}^+$  cells (Fig. 1 A). All events occurring in the variant window were sorted onto a glass slide and were observed under a fluorescence microscope to determine whether they exhibited an FL green fluorescence and not PE orange fluorescence. Sorted cells were also stained with PI to examine the shapes of their nuclei. Such experiments revealed that >95% of events appearing in the variant window were apparently mononuclear cells with only FL fluorescence, not polymorphonuclear cells or debris. Other surface markers expressed in the variant cells were examined by two-color analysis using FL-labeled or PE-labeled anti-CD4 and anti-CD3 with or without a third fluorescence-labeled antibody. Calculation of the differ-

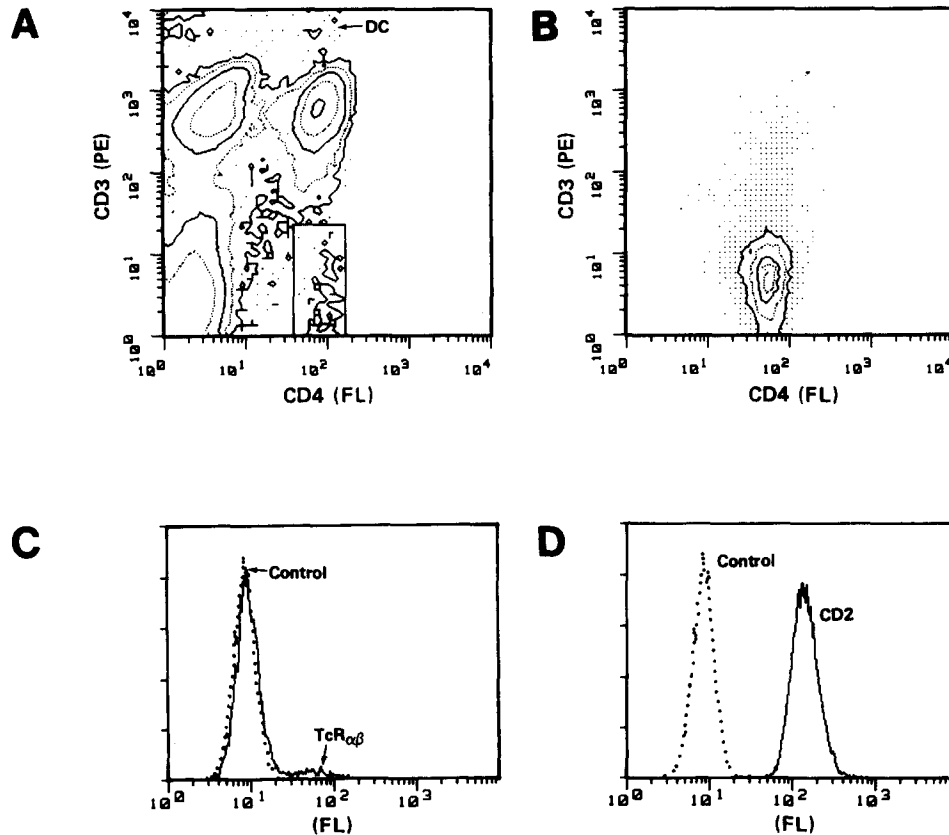


FIGURE 1. Detection and isolation of variant CD4<sup>+</sup> T cells with defective or decreased CD3 expression. (A) Flow distribution of 10<sup>6</sup> PBL stained with FL-anti-CD4 (Leu-3a) and PE-anti-CD3 (Leu-4) antibodies. Before flow cytometry, propidium iodide was added to gate out dead cells (DC). For two-color analysis, the lymphocyte fraction was gated by forward and right angle light scatter. Contours differ by a factor of 10 in events per channel, with the lowest contour representing one event per channel. To sort variant T cells, a sorting window was set in the region shown in this figure. Approximately 10<sup>3</sup> cells were sorted and cultured with PHA, r-IL-2, and feeder cells for 2 wk. Cultured T cells were analyzed for their CD3 and CD4 expression with FL-Leu-3a and PE-Leu-4 antibodies (B), TCR- $\alpha/\beta$  expression with FL-WT31 antibody (C), and CD2 expression by indirect immunofluorescence with anti-Leu-5b (D). Broken lines represent control staining with myeloma IgG antibody.

ence between the frequencies of cells in the window with and without the third antibody staining showed that >90% of the variants expressed pan-T cell markers (CD2 and CD5), but not TCR- $\alpha/\beta$ , monocyte marker (CD14), or pan-B cell marker (CD20). These results strongly suggested that most events in the variant window are mature CD4<sup>+</sup> T cells with altered TCR- $\alpha/\beta$  CD3 expression.

To confirm the presence of CD4<sup>+</sup> T cells with altered TCR/CD3 expression, ~1,000 cells were sorted from the window and expanded by bulk culture for 2 wk with feeder cells, PHA, and rIL-2. Most of the cells grown in this culture were found to lack or have decreased surface expression of the TCR- $\alpha/\beta$ -CD3 complex with minor contamination by normal CD4<sup>+</sup> cells (Fig. 1, B and C). These cultured cells

expressed CD2 antigens (Fig. 1 D) and IL-2-Rs (data not shown), suggesting that PHA may activate some populations of TCR/CD3<sup>-</sup> T cells via CD2 molecules to induce the expression of the IL-2-R, as discussed below. Other surface marker characteristics of these cells were CD1<sup>-</sup>, 5<sup>+</sup>, 8<sup>-</sup>, 11<sup>-</sup>, 16<sup>-</sup>, 20<sup>-</sup>, and 57<sup>-</sup>, indicating that these variant cells have the surface phenotype of typically mature CD4<sup>+</sup> T cells, except for altered TCR/CD3 expression. Previous studies using a TCR-defective T lymphoma cell line have demonstrated the accumulation of CD3 molecules in cytoplasm (7-17). Similar cytoplasmic expression of CD3 was observed in these *in vivo* derived variant T cells by immunofluorescence staining of fixed cells (Fig. 2).

*Isolation and Characterization of Variant Clones.* Next, we established cloned variant CD4<sup>+</sup> T cell lines from PB for further molecular analysis of TCR/CD3 expression. Although the cloning efficiency for variant cells was ~5-10% (much lower than the 50-80% for normal CD4<sup>+</sup> T cells), 37 variant T cell clones could be established from four normal healthy donors by limiting dilution of sorted cells. These variant clones were classified into three types, according to the surface staining characteristics with anti-CD3 (Leu-4) and anti-TCR- $\alpha/\beta$  (WT31) antibodies (Fig. 3, Table I). Type I clones lacked membrane expression of both CD3 and TCR- $\alpha/\beta$ . Type II showed low expression of CD3, but the reactivity with WT31 antibody was faint or almost negative. Type III demonstrated a low level of expression of both CD3 and TCR- $\alpha/\beta$ . These variant phenotypes have been stable in culture for >6 mo.

Expression of CD3, TCR- $\alpha$ , and TCR- $\beta$  of the variant clones was analyzed by cytoplasmic immunofluorescence and immunoprecipitation using various antibodies against CD3 (Leu-4 or NU-T3 antibody), TCR- $\alpha/\beta$  dimer (WT31), TCR- $\alpha$  ( $\alpha$ F1), TCR- $\beta$  ( $\beta$ F1), TCR- $\delta$  (anti-TCR- $\delta$ 1), and TCR- $\gamma/\delta$  complex (anti-TCR- $\gamma/\delta$ 1). As shown in Table I, strong expression of CD3 in the cytoplasm was observed for all of the variant clones. On the other hand, the expression of TCR- $\alpha$  and - $\beta$  chains was variable. All of the type I clones were found to lack cytoplasmic expression of either TCR- $\alpha$  or - $\beta$  chains as detected by  $\alpha$ F1 or  $\beta$ F1 antibodies, respectively. SDS-PAGE analysis of <sup>35</sup>S-Met-labeled polypeptides immunoprecipitated with anti-CD3 anti-

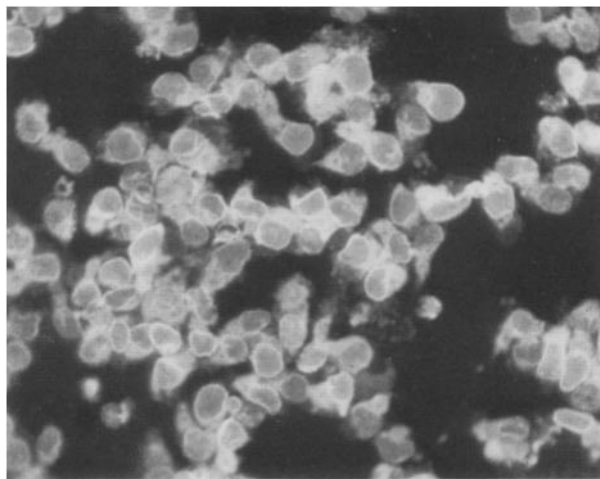


FIGURE 2. Cytoplasmic expression of CD3 antigens in CD4<sup>+</sup> variant T cells by indirect immunofluorescence staining.

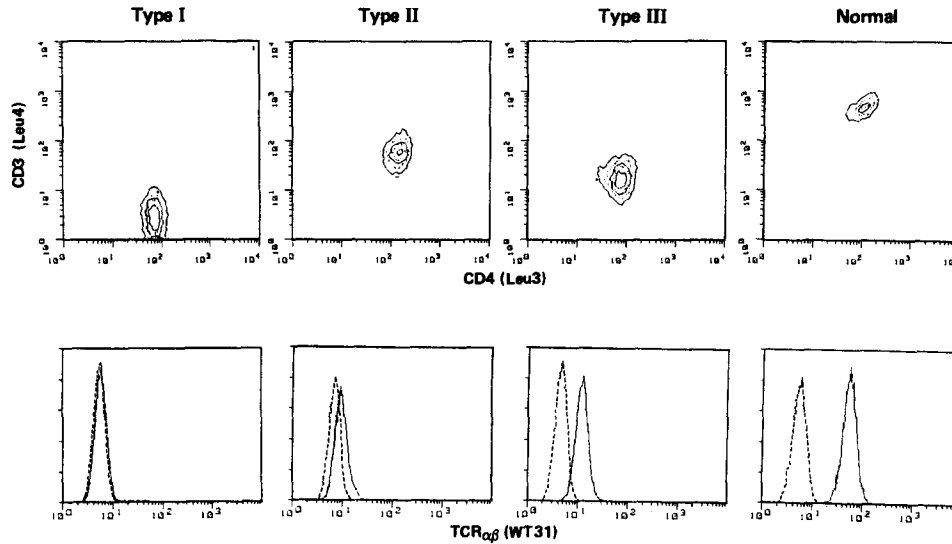


FIGURE 3. Surface expression of the TCR/CD3 complex in variant T cell clones. Variant T cell clones were stained with either PE-anti-CD3 (Leu-4) and FL-anti-CD4 (Leu-3a) (*top*) or FL-WT31 antibodies (*bottom*). Broken lines represent control staining using FL-labeled myeloma IgG protein.

body (NU-T3) under nonreduced conditions also demonstrated that these clones expressed only monomeric (40 kD), not dimeric, TCRs (Fig. 4 A). No abnormalities in the expression of CD3- $\delta$  and - $\epsilon$  were detected for these variants by SDS-PAGE, although other CD3 subunits could not be labeled with <sup>35</sup>S-Met in this labeling condition (35). These TCR- $\alpha$  or - $\beta$  chain monomers could be associated with CD3 mol-

TABLE I  
Surface and Cytoplasmic Expression of the TCR/CD3 Complex in  
Variant CD4<sup>+</sup> T Cell Clones

Variant type	Antigens: Antibodies:	Surface		Cytoplasm				No. of clones
		CD3 (Leu-4)	TCR- $\alpha/\beta$ (WT31)	CD3 (Leu-4)	TCR- $\alpha/\beta$ (WT31)	TCR- $\alpha$ ( $\alpha$ F1)	TCR- $\beta$ ( $\beta$ F1)	
I				++*	-	+	-	22
		-	-	++	-	-	+	6
II		L†	-	++	-	+	+	3 <sup>§</sup>
				++	-	-	-	1 <sup>  </sup>
III		L	L	++	+	+	+	5
Total								37

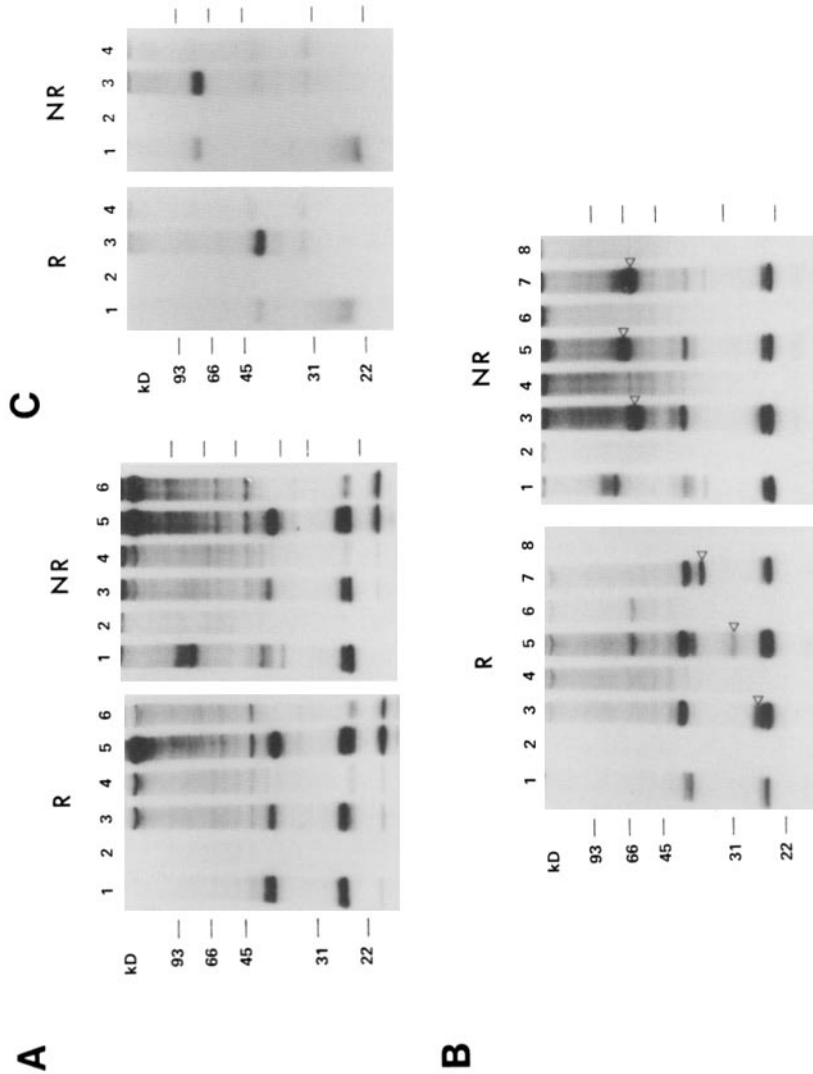
Surface and cytoplasmic immunofluorescence was performed as described in Materials and Methods.

\* The cytoplasmic staining was scored as follows: -, negative; +, positive; ++, strong.

† Low expression (see Fig. 3).

§ Partial deletions of either TCR- $\alpha$  or - $\beta$  chain were detected by immunoprecipitation analysis using anti-CD3 antibody (see Fig. 4).

|| This variant clone was found to be reactive with TCR- $\delta$ 1 antibody, but not with anti-TCR- $\gamma/\delta$ 1 antibody.



**FIGURE 4.** SDS-PAGE analyses of the TCR/CD3 complex in variant T cell clones. (A) Anti-CD3 immunoprecipitate from type I variant clones. After labeling with <sup>35</sup>S-Met, a normal CD4<sup>+</sup> T cell clone (lanes 1 and 2), a TCR- $\alpha$ -defective variant clone (lanes 3 and 4), and a TCR- $\beta$ -defective variant clone (lanes 5 and 6) were solubilized with digitonin buffer. Immunoprecipitates were prepared with either anti-CD3 antibody, NUT3 (IgG2a) (lanes 1, 3, and 5), or control IgG2a antibody, UPC10 (lanes 2, 4, and 6), and analyzed by SDS-PAGE on 10–20% gradient gels under reduced (R) or nonreduced (NR) conditions. (B) Immunoprecipitation of digitonin lysates from <sup>35</sup>S-labeled normal CD4<sup>+</sup> clone (lanes 1 and 2) and three type II variants (lanes 3–6) with either anti-CD3 (lanes 1, 3, 5, and 7) or control (lanes 2, 4, 6, and 8) antibodies. Arrowheads indicate shortened TCR monomers (R) or dimers (NR). (C) Immunoprecipitation of digitonin (lanes 1 and 2) or NP-dimers (lanes 3 and 4) extracts from <sup>35</sup>S-surface labeled TCR- $\beta$ <sup>+</sup> type II variant. Immunoprecipitations were obtained with anti-CD3 (lane 1), anti-TCR- $\delta$ 1 (IgG1) (lane 3), control IgG2a (lane 2), and control MOPC 21 (IgG1) (lane 4) antibodies.

ecules, but the molecular complex was not successfully transported to the membrane. Thus, lack of surface TCR/CD3 complex expression in type I clones can be explained simply by a defect of either TCR- $\alpha$  or - $\beta$  chain expression, as previously reported in variant T cell lines (7-15). Preliminary experiments using Northern blotting analysis of several type I variants also confirmed the defect of full-length mRNA synthesis from either the TCR- $\alpha$  or - $\beta$  gene.

Type II variants, which expressed CD3 on their cell surfaces at low levels, were rather complicated, having various TCR abnormalities. In type II variants, significant reactivity with WT31 antibody could not be detected either on the cell surface or in the cytoplasm (Fig. 3, Table I). But protein bands corresponding to TCR chains were detected by SDS-PAGE analysis of immunoprecipitates of <sup>125</sup>I-labeled surface protein with anti-CD3 antibody (data not shown). However, since TCR protein bands in SDS-PAGE were very weak for three of four type II clones listed in Table I, these three clones were labeled internally with <sup>35</sup>S-Met for further immunoprecipitation experiments (Fig. 4 B). Those clones, which were reactive with both  $\alpha$ F1 and  $\beta$ F1 antibodies, showed two TCR protein bands under reduced conditions; one was normal in size, but the other TCR chain had a relatively low molecular mass (indicated by the arrowheads in Fig. 4 B). These TCR chains could form TCR heterodimers, with lower molecular mass (60-65 kD) than normal TCR dimers (80 kD), as shown by nonreduced SDS-PAGE analysis. Using Northern blot analysis, it was demonstrated that these variants expressed either full-length TCR- $\alpha$  or - $\beta$  mRNA and a relatively short length of TCR- $\beta$  or - $\alpha$  mRNA, respectively, suggesting that the small size of the TCR chains observed by SDS-PAGE was due to partially deleted forms of the TCR- $\alpha$  or - $\beta$  proteins. It is possible that some part of the TCR framework recognized by WT31 antibody is truncated in this type of clone, so that the association with CD3 required for surface expression of the complex cannot occur normally.

Another type II clone (Table I), which was not reactive with either  $\alpha$ F1 or  $\beta$ F1 antibodies, was found to express anti-TCR- $\delta$ 1-reactive antigens on its cell surface. However, this clone was found nonreactive both on the cell surface and in the cytoplasm with anti-TCR- $\gamma/\delta$ 1 antibody recognizing the TCR- $\gamma$  or TCR- $\gamma/\delta$  complex (25). SDS-PAGE patterns of <sup>125</sup>I-labeled immunoprecipitates with either anti-CD3 or anti-TCR- $\delta$ 1 antibodies revealed a TCR band (40 kD) under reduced conditions and a disulphide-linked dimer (80 kD) detected under nonreduced conditions (Fig. 4 C). Northern blotting analysis showed the production of full-length mRNA for TCR- $\gamma$  in this clone, suggesting that this variant synthesized both TCR- $\gamma$  and - $\delta$  chains; therefore, the 40-kD band under reduced conditions was most likely an overlap of the TCR- $\gamma$  and - $\delta$  chains as described by Hochstenbach et al. (36). All these data suggested that this CD4<sup>+</sup> variant expressed disulphide-linked TCR- $\gamma/\delta$  heterodimer and that its TCR- $\gamma$  or - $\delta$  chain (most likely  $\gamma$  chain) had a partial deletion or mutation in a region that may be recognized by anti-TCR- $\gamma/\delta$ 1 antibody.

Type III variants were found to express both TCR- $\alpha$  and - $\beta$  chains in the cytoplasm by using immunofluorescence, but surface expression of the TCR/CD3 complex was much lower than that of normal clones (Table I, Fig. 3). TCR expression of these type III clones was also examined by immunoprecipitation of <sup>35</sup>S-Met-labeled proteins with anti-CD3 antibodies. Although these clones appeared to produce both TCR- $\alpha$  and - $\beta$  chains at normal levels, the protein band corresponding to the TCR dimer was very weak by SDS-PAGE analysis under nonreduced conditions (data



not shown). We speculate that these variants have some mutations in the framework of either the TCR- $\alpha$  or - $\beta$  protein, which did not alter the epitope structure recognized by  $\alpha$ F1,  $\beta$ F1, or WT31 antibodies. It is likely that such abnormalities in the TCR chains consequently inhibit either the formation of the TCR dimer or its association with CD3 molecules, so that surface expression of TCR/CD3 complex is considerably diminished.

*Rearrangement of TCR Genes in Variant Clones.* To examine the possibility that the defect of TCR- $\alpha$  or - $\beta$  chain expression of type I variants is due to the lack of TCR gene rearrangement, we analyzed the TCR genes of the variant clones by Southern blot. Fig. 5 reveals the restriction patterns of the TCR- $\beta$  genes from TCR- $\beta$ -defective type I variants, demonstrating that TCR- $\beta$  genes were rearranged in all these variants. No evidence for loss of the entire TCR- $\beta$  constant (C $\beta$ ) gene could be observed by Southern analysis using the C $\beta$  cDNA probe.

Among the 37 variant clones examined here, 11 clones derived from one of the donors showed identical TCR- $\beta$  gene rearrangement patterns (Fig. 5 shows four of these clones), using three different restriction enzymes (Eco RI, Hind III, and Bam HI). The TCR- $\gamma$  gene for these variants also revealed identical rearrangements (data not shown). This reflects *in vivo* clonality, since T cell cloning had been done immediately after cell sorting without preculture. The TCR- $\beta$  gene restriction patterns by Bam HI digestion of these clones exhibited three rearranged bands (Fig. 5). This suggested the occurrence of gross alterations of the TCR- $\beta$  gene induced by partial gene duplication or unequal sister chromatid exchange (19). It can be presumed that such inactivation occurred in a TCR- $\beta$  gene of a proliferating T cell and the variant was clonally expanded *in vivo*.

*Chromosome Aberration in Variants.* It has long been observed that translocations between chromosomes 7 and 14 are frequent in T cells from normal donors (37). The breakpoints on these chromosomes are also highly nonrandom, involving three regions, i.e., 7(p13-15), 7(q32-35), and 14(q11-12), where TCR- $\gamma$ , TCR- $\beta$ , and TCR- $\alpha/\delta$  genes, respectively, are located, according to recent studies (18). The frequency of t(7:14) has been reported to be  $4 \times 10^{-4}$  (37), which is very similar to the frequency of TCR variants as described below. It is possible that t(7:14) is accompanied by inactivation of TCR genes. If so, the TCR variants cloned here might frequently bear aberrations of chromosomes 7 and 14.

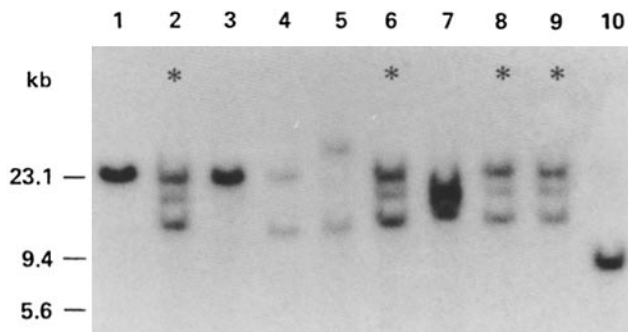


FIGURE 5. Southern blot analysis of TCR- $\beta$  gene rearrangements in type I variant clones (lanes 2-10) with defective TCR- $\beta$  expression. EB virus-transformed B cells from a normal donor served as a control for the germ-line configuration (lane 1). For one clone (lane 3), rearrangement was not apparent with Bam HI digestion, but clear rearranged bands were detected in Eco RI restriction pattern. Four variant clones, as indicated by asterisks, represent identically rearranged bands.

When we analyzed the chromosomes of 14 type I and five type II variant clones, no t(7:14) were observed. One type I clone showed a translocation between 14(q11) and (13 q32) (Fig. 6). Since this variant was found to be defective in TCR- $\alpha$  expression but not in TCR- $\beta$  expression, it is very possible that this chromosome translocation might have induced nonexpression in an active TCR- $\alpha$  gene allele that was located at the breakpoint. TCR gene inactivation, however, accompanied chromosome translocations less frequently than we had expected. It may be possible that variants bearing such chromosome aberrations cannot be cloned in culture due to their inability to grow. Intrachromosomal rearrangements, i.e., duplications, deletions, or insertions, also may be involved.

*Variant Frequency In Vivo and In Vitro.* As shown above, partial characterization of TCR/CD3 variants in PB exhibited a variety of TCR abnormalities. Next, we measured the frequency of variant cells in either PB or culture by flow cytometry in normal donors and in patients with hereditary disease showing instability of somatic genes.

The variant frequency was found to be almost constant in each donor, when five normal donors were examined three or more times during 1 yr (coefficients of variation for each donor were <20%). The mean frequency for 127 normal donors was  $\sim 2.5 \times 10^{-4}$  on average, and it significantly ( $p < 0.0001$ ) increased with age (Fig. 7). Since these variants should have a defect in antigen recognition and activation for specific immune responses, their increasing frequency may be one of the factors related to age-dependent decrease in T cell function (for a review, see reference 38).

FA patients showed higher variant frequencies than those of normal young donors, but a DS patient had a normal frequency (Fig. 7). Much higher variant frequencies were observed in patients with AT. On the other hand, the heterozygous parents of the AT patient no. 2 (in Fig. 7), were found to have frequencies of  $3.4$  and  $3.5 \times 10^{-4}$ , which are within the normal range. Thus, measurement of the TCR/CD3 variants in PB can be applied to the diagnosis of AT. AT is a recessive inherited disease characterized by defective DNA repair after ionizing radiation (39, 40), and it is known to have a high frequency of spontaneous somatic mutations of erythrocytes (41) and of lymphocyte chromosome aberrations (38), especially in chromosomes 7 and 14 (42, 43). High radiation sensitivity of T cells from these two AT patients has been confirmed. We also found an elevation of in vivo frequency of dele-

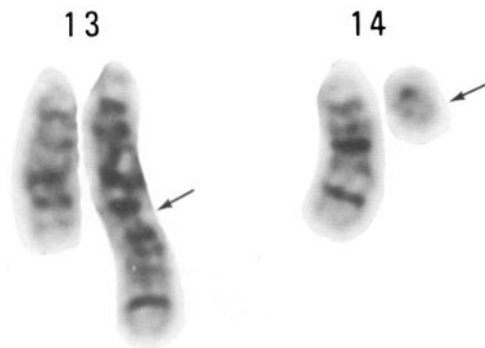


FIGURE 6. Translocation between chromosomes 13 and 14 seen in the type I variant clone lacking TCR- $\alpha$  expression. 18 metaphases were examined and all of them exhibited a reciprocal translocation t(13;14)(q32;q11). Arrows indicate the breakpoints.

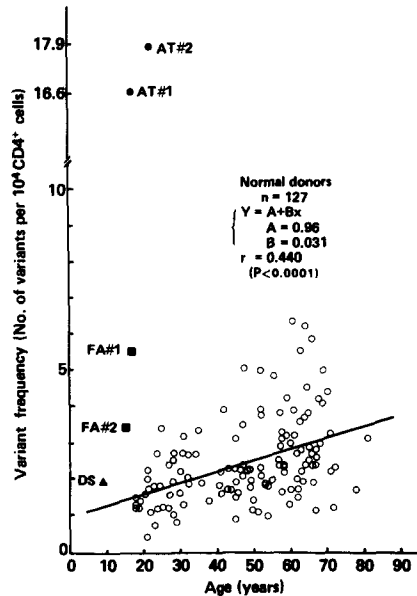


FIGURE 7. Relationship between donor age and variant T cell frequency in PB from 127 normal donors and five patients with genetic diseases such as AT, FA, and DS. Variant T cells are detected as shown in Fig. 1 A. Variant frequency equals the value of the number of variants occurring in the window divided by the total number of CD4<sup>+</sup> cells. The line corresponds to a linear regression calculated from the variant frequencies for the normal donors.

tion type mutants at the glycoprotein A locus of erythrocytes for AT patient no. 1 (unpublished observation).

It is also known that AT patients have a defect in thymus development (44) and various dysfunctions in T cell response (39, 45–48). Such abnormal thymus development might result in the defective maturation of T cells and provide a source of variant T cells with defective TCR/CD3 expression to the PB. However, as shown in Fig. 8, normal T cell clones (CD3<sup>+</sup>4<sup>+</sup>) derived from AT patients revealed high frequencies ( $1.5\text{--}5.0 \times 10^{-3}$ ) of spontaneous loss of surface TCR/CD3 expression in vitro, whereas clones from normal donors had low frequencies ( $1\text{--}5 \times 10^{-4}$ ). In addition, the normal CD8<sup>+</sup> T cell clone (CD3<sup>+</sup>8<sup>+</sup>) from AT patient 1 also exhibited a high frequency of spontaneous loss of surface TCR/CD3 in vitro (although the data is not shown). These results suggest that the high frequency of in vivo variants in AT patients is not attributable to abnormal differentiation of T cells in the thymus, but to the intrinsic instability of TCR genes in mature T cells.

### Discussion

In this paper, we have demonstrated the presence of variant CD4<sup>+</sup> T cells with alterations of TCR/CD3 expression in human PB. These variant clones were found to have abnormalities in the expression of one of the chains in the TCR heterodimer, and they expressed the incomplete TCR/CD3 complex in the cytoplasm or the cell surface at various levels. We assume that the in vivo variant is generated not by defects in productive rearrangements of TCR genes in the thymus (followed by failures to eliminate the cells), but by spontaneous somatic mutation, such as inactivation, partial deletion, and point mutation of productively rearranged TCR genes in the peripheral mature T cells. The first reason for this consideration is the increase of variant frequency with age (Fig. 7), which indicates the accumulation of abnormal clones

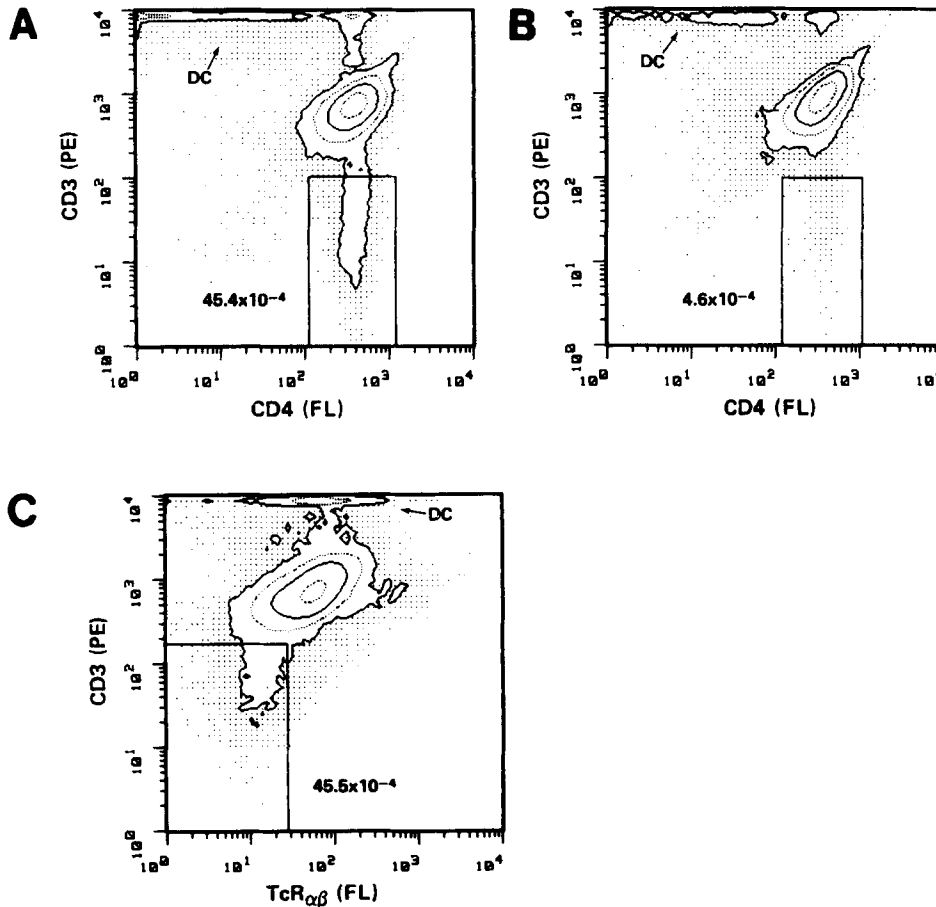


FIGURE 8. Spontaneous loss of TCR/CD3 expression in vitro in CD4<sup>+</sup> T cell clones derived from AT patients. CD4<sup>+</sup> T cell clones normally expressing the TCR/CD3 were established from AT patient no. 1 (A and C), listed in Fig. 7, and a normal donor (B). T cells logarithmically proliferating in culture were stained with either FL-anti-CD4 (Leu-3a) and PE-anti-CD3 (Leu-4) (A and B) or FL-WT31 and PE-Leu-4 (C). After staining dead cells (DC) with propidium iodide, 5 × 10<sup>5</sup> cells were analyzed, as described for Fig. 1. Contours differ by a factor of 10 in events per channel with a minimal dot indicating one event per channel. The cell frequency in the windows are shown in the figure.

in the PB after thymus involution. Second, a high variant frequency was observed in AT patients (Fig. 7), who also have a high frequency of erythrocyte mutation and lymphocyte chromosome aberrations. Third, spontaneous inactivation of TCR/CD3 expression occurred at a similar frequency in cloned mature T lymphocytes in vitro (Fig. 8). Fourth, ionizing radiation could induce TCR gene inactivation in vitro in a dose-dependent fashion in T lymphoma cells (unpublished observation), and significant elevation of the variant frequency was observed in radiation-exposed donors (Kyoizumi, S., et al., manuscript in preparation).

If TCR gene abnormalities are the results of somatic mutation, the variant frequency of  $2.5 \times 10^{-4}$ , which is equal to  $1.3 \times 10^{-4}$  per single TCR locus (a simple calculation made by considering the dimeric structure of TCR and an allelic exclusion in TCR genes), is  $\sim 10$ – $100$  times higher than that of other gene loci, such as T cell hypoxanthine guanine phosphoribosyltransferase (49–51), erythrocyte glycoporphin A (32–34), and T cell HLA class I antigen (52), but it is comparable with those of B cell Ig genes (53–57). It has been reported that inactivation of IgH or L chain genes in myeloma and hybridoma cell lines (53–55) and reversion of the amber mutation of the IgH gene in the pre-B cell line (56, 57) occurred at a high rate ( $10^{-3}$  to  $10^{-5}$  per cell per generation). These Ig mutational events include many types of genetic changes, such as deletions, point mutations, and frameshifts (58–61). Such a high rate of alteration of the active Ig genes is believed to be reflected by the instability of Ig genes in the normal process of B cell differentiation, including the rearrangements of V region gene, the class switch of IgH gene, and the induction of point mutations in the V region genes for the generation of diversity (62–64). The Ig recombination system in B cells may also participate in mediating translocation of the Burkitt's lymphoma chromosome at breaking points 2p11, 14q32, and 22q11, where the Ig $\kappa$ , IgH, and Ig $\lambda$  genes are located (65). Like the Ig genes of B cells, the TCR genes are programmed to dynamically rearrange in the thymus during T cell ontogenesis, so that the TCR genes of T cells are presumed to be inherently unstable and be prone to chromosome aberrations even after rearrangement. A recent report has shown that an immature T cell line can spontaneously undergo secondary rearrangements in vitro replacing the preexisting productive V $\alpha$ -J $\alpha$  rearrangements (66), although the frequency of such rearrangements is unknown. It is presumed that such secondary rearrangements of TCR genes might be occasionally or frequently nonproductive, and this would be the candidate for one of the TCR inactivation mechanisms.

Many studies using variant T cell lines lacking a chain of the TCR/CD3 complex have shown that incompletely assembled receptor complexes do not reach the plasma membrane (7–15). The present results also demonstrated that in vivo derived T cell variants lacking TCR- $\alpha$  or - $\beta$  protein (type I variants) did not express the TCR/CD3 complex on the surface. Further, it is noteworthy that three type II variants bearing a shortened TCR- $\alpha$  and - $\beta$  chain, probably a partial deletion, were able to express a low amount of the TCR/CD3 complex on the surface. The shortened TCR chain was able to form a disulfide-linked heterodimer with the other normal TCR chain, as shown in Fig. 4, indicating the retention of the cysteine residue in the C region proximal to transmembrane domain (7, 19). Since the transmembrane domain of TCR, especially the region including some basic amino acid residues, appears to be essential for the association with CD3 molecules (7, 67), the deletion of TCR should not include this region in membrane domain. In addition, this incomplete heterodimer was reactive with  $\alpha$ F1 or  $\beta$ F1 antibodies but not with WT31 antibody. All these results suggested that the shortened TCR chains possessed an internal deletion in the portion of the C region, which is recognized by WT31 antibody and probably is distal to the transmembrane domain. The small internal deletion in the C region might directly or indirectly induce conformational changes of the CD3-binding portion, including the transmembrane domain, resulting in a decreased affinity

for molecular assembly. Thus, most of the incomplete TCR chains would not form a stable molecular complex in the endoplasmic reticulum to be exported to the plasma membrane, and seem to enter the degradative pathway. Sequence analysis of the cDNA from the shortened TCR mRNA will localize the deletions and their role of the deleted portion in molecular assembly and transport.

The variants include the CD4<sup>+</sup> clone bearing altered TCR- $\gamma/\delta$  expression. The TCR- $\gamma/\delta$  dimer is mainly expressed in CD4<sup>-</sup>8<sup>-</sup> non-MHC-restricted cytotoxic T cells (4-6), and infrequently in intestinal cytotoxic CD8<sup>+</sup> T cells (68). Recent reports have suggested the presence of peripheral CD4<sup>+</sup> T lymphocytes bearing TCR- $\gamma/\delta$  at a low frequency (25, 69). We have also confirmed these observations and established 17 CD4<sup>+</sup> TCR- $\gamma/\delta$ <sup>+</sup> clones from PB. All were found to be strongly reactive with both anti-TCR- $\delta 1$  and anti-TCR- $\gamma/\delta 1$  antibodies and expressed CD3 antigen at a higher level than that of TCR- $\alpha/\beta$ -bearing CD4<sup>+</sup> T cells. SDS-PAGE analysis of <sup>125</sup>I-labeled immunoprecipitates from these clones showed TCR- $\gamma$  and - $\delta$  chains (37-44 kD) (Kyoizumi, S., M. Akiyama, Y. Hirai, and Y. Kusunoki, manuscript submitted for publication). Since the surface expression of CD3 antigens on the TCR- $\delta 1$ <sup>+</sup> variant established here was much lower than that of normal CD4<sup>+</sup> lymphocytes expressing the  $\gamma/\delta$  heterodimer, we presumed that the mutation that occurred in the TCR- $\gamma$  or - $\delta$  chain also affected the surface expression of the TCR- $\gamma/\delta$ -CD3 complex, an effect similar to that seen on the TCR- $\alpha/\beta$ -CD3 complex.

Only a minor population (5-10%) of the CD4<sup>+</sup> variants detected by flow cytometry could be cloned *in vitro* by culturing with PHA and IL-2. The majority of variants could not be cloned. This inability suggested that the surface expression of the TCR/CD3 complex affected the activation pathway initiated by PHA to enter the growth cycle from the G<sub>0</sub> state in association with the induction of IL-2-R expression. The CD2 molecule appears to be a PHA-binding protein (70), and may be involved in the growth activation pathway. Many studies using mutant T lymphoma cell lines have provided evidence for functional interaction between the CD2 antigen and the TCR/CD3 complex in stimulation of IL-2 production by anti-CD2 antibody (9, 12-15). A recent report has also shown the physical association between these two structures on the cell surface (71). We speculate that without TCR/CD3 expression, the CD2 molecule cannot transmit the growth signal after PHA binding for the majority of T cells. In contrast, ~10% of the sorted variants did respond to PHA and did grow in culture with IL-2. Possibly, these variants have an alternative pathway for growth stimulation after CD2-PHA binding. We presume that other hypothetical CD2-associated molecules (71) that can substitute for TCR/CD3 and function in CD2-mediated growth triggering might be expressed by a minor T cell population. The variant clones established here might be derived from this minor population. Similar results were obtained by Moretta et al. (12) using variants derived from the IL-2-producing Jurkat cell line. They showed that ~90% of the TCR/CD3<sup>-</sup> variant clones (eight of nine clones) did not respond to PHA in spite of their CD2 expression, while only one clone could be activated by PHA to produce IL-2. The latter appears to correspond to our clonable variants.

Of particular interest is the abnormally high frequency of variant T lymphocytes *in vivo* and *in vitro* in AT patients who show the high incidence of T lymphoma carrying translocations and inversions in chromosomes 7 and 14 (18, 39, 42, 43). Analyses of T cell leukemia and peripheral T cells derived from AT patients have

demonstrated the direct involvement of the TCR- $\alpha$  and - $\beta$  genes in the translocation of chromosomes 7 and 14 (72, 73), although causative relationship between the translocations and the developmental mechanism of T lymphoma is still unknown. Furthermore, many studies have shown abnormalities of T cell function in most AT patients (45–48), including decreased responses to mitogens, impaired production of lymphokines, and defects of the Th cell function in Ig production. It was also reported that AT patients have abnormal thymus development (44). Since the TCR/CD3 complex must be directly or indirectly involved in both T cell functions and development, spontaneous inactivation of the TCR gene might significantly contribute to the array of T cell-mediated defects. We presume that in AT patients there might be a causal relationship between the high frequency of the TCR gene inactivations observed here, the high rate of t(7:14), the high incidence of T lymphoma development, and the immunodeficiency of T cells. Similarly, in normal individuals, the observed increment of the variant frequency with aging might be related to the age-dependent increase in the incidence of chronic lymphocytic leukemia (74), including T lymphoma, which is frequently associated with t(7:14) translocations (18), and the age-dependent decrease of T cell functions for immune response (38).

### Summary

The TCR/CD3 complex plays a central role in antigen recognition and activation of mature T cells, and, therefore, abnormalities in the expression of the complex should induce unresponsiveness of T cells to antigen stimulus. Using flow cytometry, we detected and enumerated variant cells with loss or alteration of the surface TCR/CD3 expression among human mature CD4<sup>+</sup> T cells. The presence of variant CD4<sup>+</sup> T cells was demonstrated by isolating and cloning them from peripheral blood, and their abnormalities can be accounted for by alterations in TCR expression such as defects of protein expression and partial protein deletion. The variant frequency in peripheral blood increased with aging in normal donors and was highly elevated in patients with ataxia telangiectasia, an autosomal recessive inherited disease with defective DNA repair and variable T cell immunodeficiency. These findings suggest that such alterations in TCR expression are induced by somatic mutagenesis of TCR genes and can be important factors related to age-dependent and genetic disease-associated T cell dysfunction.

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### References

1. Allison, J. P., B. W. McIntyre, and D. Bloch. 1982. Tumor-specific antigen of murine T lymphoma defined with monoclonal antibody. *J. Immunol.* 129:2293.
2. Haskins, K., R. Kubo, J. White, M. Pigeon, J. Kappler, and P. Marrack. 1983. The

- major histocompatibility complex-restricted antigen receptor on T cells. I. Isolation with a monoclonal antibody. *J. Exp. Med.* 157:1149.
3. Meuer, S. C., K. A. Fitzgerald, R. E. Hussey, J. C. Hodgdon, S. F. Schlossman, and E. L. Reinherz. 1983. Clonotypic structures involved in antigen-specific human T cell function: relationship to the T3 molecular complex. *J. Exp. Med.* 157:705.
  4. Brenner, M. B., J. McLean, D. P. Dialynas, J. L. Strominger, J. A. Smith, F. L. Owen, J. G. Seidman, S. Ip, F. Rosen, and M. S. Krangel. 1986. Identification of a putative second T-cell receptor. *Nature (Lond.)* 332:145.
  5. Lew, A. M., D. M. Pardoll, W. L. Maloy, B. J. Fowlkers, A. Kruisbeek, S. F. Cheng, R. N. Germain, J. A. Bluestone, R. H. Schwartz, and J. E. Coligen. 1986. Characterization of T cell receptor gamma chain expression in a subset of murine thymocytes. *Science (Wash. DC)* 234:1401.
  6. Borst, J., R. J. Van de Griend, J. W. Van Oostveen, S. L. Ang, C. J. Melief, J. G. Seidman, and R. L. H. Bolhuis. 1987. The T cell receptor  $\gamma$ /CD3 complex found on cloned functional lymphocytes. *Nature (Lond.)* 325:683.
  7. Clevers, H., B. Alarcon, T. Wileman, and C. Terhorst. 1988. The T cell receptor/CD3 complex: A dynamic protein ensemble. *Annu. Rev. Immunol.* 6:629.
  8. Weiss, A., and J. Stobo. 1984. Requirement for the coexpression of T3 and the T cell antigen receptor on a malignant human T cell line. *J. Exp. Med.* 160:1284.
  9. Weiss, A., J. Imboden, D. Shoback, and J. Stobo. 1984. Role of T3 surface molecules in human T cell activation: T3 dependent activation results in a rise in cytoplasmic free calcium. *Proc. Natl. Acad. Sci. USA.* 81:4169.
  10. Ohashi, P., T. Mak, P. Van den Elsen, Y. Yanagi, Y. Yoshikai, A. Calman, C. Terhorst, J. Stobo, and A. Weiss. 1985. Reconstitution of an active surface T3/T cell antigen receptor by DNA transfer. *Nature (Lond.)* 316:606.
  11. Saito, T., A. Weiss, J. Miller, M. A. Norcross, and R. N. Germain. 1987. Specific antigen-Ia activation of transfected human T cell expression murine T $\alpha\beta$ -human T3 receptor complexes. *Nature (Lond.)* 325:125.
  12. Moretta, A., A. Poggi, D. Olive, C. Bottino, C. Fortis, G. Pantaleo, and L. Moretta. 1987. Selection and characterization of T-cell variants lacking molecules involved in T-cell activation (T3 T-cell receptor, T44, and T11): analysis of the functional relationship among different pathways of activation. *Proc. Natl. Acad. Sci. USA.* 84:1654.
  13. Weiss, A., R. Shields, N. Newton, B. Manger, and J. Imboden. 1987. Ligand-receptor interactions required for commitment of the activation of the interleukin 2 gene. *J. Immunol.* 138:2169.
  14. Alcover, A., C. Albertini, A. Oreste, L. K. Clayton, C. Transy, G. C. Spagnoli, P. Moinjeon, P. Lopez, and E. L. Reinherz. 1988. Interdependence of CD3-Ti and CD2 activation pathways in human T lymphocytes. *EMBO (Eur. Mol. Biol. Organ) J.* 7:1973.
  15. Bockenstedt, L. K., M. A. Goldsmith, M. Dustin, D. Olive, T. A. Springer, and A. Weiss. 1988. The CD2 ligand LFA-3 activates T cells but depend on the expression and function of the antigen receptor. *J. Immunol.* 141:1904.
  16. Uppenkamp, M., S. Pittaluga, R. Coupland, O. Colamonici, and J. Cossman. 1988. Stable T3<sup>-</sup> and T3<sup>+</sup> subclones derived from the mosaic human T cell leukemia cell line, CEM. *J. Immunol.* 140:2802.
  17. Maecker, H. T., and R. Levy. 1988. Spontaneous T cell antigen receptor variants of a human T leukemia cell line. *J. Immunol.* 141:2994.
  18. Kirsch, I. R., and G. F. Hollis. 1988. The involvement of the T-cell receptor in chromosome aberrations. In *The T-Cell Receptors*. T. W. Mak, editor. Plenum Publishing Company, New York. 175-94.
  19. Kronenberg, M., G. Siu, L. E. Hood, and N. Shastri. 1986. The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. *Annu. Rev. Immunol.* 4:529.



20. Kinnon, C., R. A. Diamond, and E. V. Rothenberg. 1986. Activation of T cell antigen receptor  $\alpha$ - and  $\beta$ -chain genes in the thymus: implications for the lineages of developing cortical thymocytes. *J. Immunol.* 137:4010.
21. Knapp, W. (Ed). 1989. Abstracts from 4 th International Conference on Human Leucocyte Differentiation Antigens. *Tissue Antigens.* 33:62.
22. Spits, H., J. Borst, W. Tax, P. J. A. Capel, C. Terhorst, and J. E. de Vries. 1985. Characteristics of a monoclonal antibody (WT31) that recognizes a common epitope on the human T cell receptor for antigen. *J. Immunol.* 135:1922.
23. Brenner, M. B., J. McLean, H. Scheft, R. A. Warnke, N. Jones, and J. L. Strominger. 1987. Characterization and expression of the human  $\alpha\beta$  T cell receptor by using a framework monoclonal antibody. *J. Immunol.* 138:1502.
24. Band, H., F. Hochstenbach, J. McLean, S. Hata, M. S. Krangel, and M. B. Brenner. 1987. Immunological proof that a novel rearranging gene encodes the T cell receptor  $\delta$  subunit. *Science (Wash. DC).* 238:682.
25. Borst, J., J. J. M. Van Dongen, B. L. H. Bolhuis, P. J. Peters, D. A. Hafler, E. de Vries, and R. J. Van de Griend. 1988. Distinct molecular forms of human T cell receptor  $\gamma/\delta$  detected on viable T cells by a monoclonal antibody. *J. Exp. Med.* 167:1625.
26. Kyoizumi, S., M. Akiyama, N. Kohno, K. Kobuke, M. Hakoda, S. L. Jones, and M. Yamakido. 1985. Monoclonal antibodies to human squamous cell carcinoma of the lung and their application to tumor diagnosis. *Cancer Res.* 45:3274.
27. Oettgen, H. C., C. L. Pettey, W. L. Maloy, and C. Terhorst. 1986. A T3-like protein complex associated with the antigen receptor on murine T cells. *Nature (Lond.).* 320:272.
28. Hakoda, M., Y. Hirai, H. Shimba, Y. Kusunoki, S. Kyoizumi, Y. Kodama, and M. Akiyama. 1989. Cloning of phenotypically different human lymphocytes originating from a single stem cell. *J. Exp. Med.* 169:1265.
29. Yanagi, Y., Y. Yoshikai, K. Leggett, S. P. Clark, I. Aleksander, and T. W. Mak. T. W. 1984. Human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. *Nature (Lond.).* 308:145.
30. Seebright, M. 1971. A rapid banding technique for human chromosomes. *Lancet.* ii:971.
31. Kipps, T. J., P. Parham, J. Punt, and L. A. Herzenberg. 1985. Importance of immunoglobulin isotype in human antibody-dependent cell-mediated cytotoxicity directed by murine monoclonal antibodies. *J. Exp. Med.* 161:1.
32. Langlois, R. G., W. L. Bigbee, and R. H. Jensen. 1986. Measurements of the frequency of human erythrocytes with gene expression loss phenotypes at the glycophorin A locus. *Hum. Genet.* 74:353.
33. Langlois, R. G., W. L. Bigbee, S. Kyoizumi, N. Nakamura, M. A. Bean, M. Akiyama, and R. H. Jensen. 1987. Evidence for increased somatic cell mutations at the glycophorin A locus in atomic bomb survivors. *Science (Wash. DC).* 236:445.
34. Kyoizumi, S., N. Nakamura, M. Hakoda, A. A. Awa, M. A. Bean, R. H. Jensen, and M. Akiyama. 1989. Detection of somatic mutations at the glycophorin A locus in erythrocytes of atomic bomb survivors using a single beam flow sorter. *Cancer Res.* 49:581.
35. Borst, J., S. Alexander, J. Elder, and C. Terhorst. 1983. The T3 complex on human T lymphocytes involves four structurally distinct glycoprotein. *J. Biol. Chem.* 258:5135.
36. Hochstenbach, F., C. Parker, J. McLean, V. Gieselmann, H. Band, I. Bank, L. Chess, H. Spits, J. L. Strominger, J. G. Seidman, and M. B. Brenner. 1988. Characterization of a third form of the human T cell receptor  $\gamma/\delta$ . *J. Exp. Med.* 168:761.
37. Hecht, F., B. K. McCaw, D. Peakman, and A. Robinson. 1975. Non-random occurrence of 7-14 translocations in human lymphocyte cultures. *Nature (Lond.).* 255:243.
38. Makinodan, T., and M. B. Kay. 1980. Age influence on the immune system. *Adv. Immunol.* 29:287.
39. Arlett, C. F., and A. R. Lehmann. 1978. Human disorders showing increased sensitivity

- to the induction of genetic damage. *Annu. Rev. Genet.* 12:95.
40. Taylor, A. M. R., D. G. Harnden, C. F. Arlett, A. S. Harcourt, A. R. Lehmann, S. Stevens, and B. A. Bridges. 1975. Ataxia telangiectasia: human mutation with abnormal radiation sensitivity. *Nature (Lond.)* 258:427.
  41. Bigbee, W. L., R. G. Langlois, M. Swift, and R. H. Jensen. 1989. Evidence for an elevated frequency of in vivo somatic cell mutations in ataxia telangiectasia. *Am. J. Hum. Genet.* 44:402.
  42. Hecht, F., K. McCaw, and R. D. Koller. 1973. Ataxia-telangiectasia: clonal growth of translocation lymphocytes. *N. Engl. J. Med.* 289:286.
  43. Aurias, A., B. Dutrillaux, D. Buriot, and J. Lejeune. 1980. High frequencies of inversions and translocations of chromosome 7 and 14 in ataxia telangiectasia. *Mutat. Res.* 69:369.
  44. Peterson, R. D. A., W. D. Kelly, and R. A. Good. 1964. Ataxia telangiectasia, its association with a defective thymus, immunological-deficiency disease and malignancy. *Lancet.* i:1189.
  45. McFarlin, D. E., and J. J. Oppenheim. 1969. Impaired lymphocyte transformation in ataxia-telangiectasia in part due to a plasma inhibitory factor. *J. Immunol.* 103:1212.
  46. Waldmann, T. A., S. Broder, C. K. Goldman, K. Frost, S. J. Korsmeyer, and M. A. Medici. 1983. Disorders of B cells and helper T cells in the pathogenesis of the immunoglobulin deficiency of patients with ataxia telangiectasia. *J. Clin. Invest.* 71:282.
  47. Nelson, D. L., W. E. Biddison, and S. Shaw. 1983. Defective in vitro production of influenza virus-specific cytotoxic T lymphocytes in ataxia-telangiectasia. *J. Immunol.* 130:2629.
  48. Boutin, B., D. K. Wagner, and D. L. Nelson. 1987. Analysis of CD3 antigen expression in patients with ataxia-telangiectasia. *Clin. Exp. Immunol.* 68:320.
  49. Albertini, R. J., K. L. Castle, and W. R. Borchering. 1982. T-cell cloning to detect the mutant 6-thioguanine-resistant lymphocytes present in human peripheral blood. *Proc. Natl. Acad. Sci. USA.* 79:6617.
  50. Morley, A. A., K. J. Trainor, R. Seshadri, and R. G. Ryall. 1983. Measurement of in vivo mutations in human lymphocytes. *Nature (Lond.)* 302:155.
  51. Hakoda, M., M. Akiyama, S. Kyoizumi, K. Kobuke, A. A. Awa, and M. Yamakido. 1988. Measurement of in vivo HGPRT-deficient mutant cell frequency using a modified method for cloning human peripheral blood T lymphocytes. *Mutat. Res.* 197:161.
  52. Janatipour, M., K. J. Trainor, R. Kutlaca, G. Bennett, J. Hay, D. R. Turner, and A. A. Morley. 1988. Mutations in human lymphocytes studies by an HLA selection system. *Mutat. Res.* 198:221.
  53. Coffino, P., and M. P. Scharff. 1971. Rate of somatic mutation in immunoglobulin production by mouse myeloma cells. *Proc. Natl. Acad. Sci. USA.* 68:219.
  54. Baumal, R., B. K. Birshtein, P. Coffino, and M. D. Scharff. 1973. Mutations in immunoglobulin-producing mouse myeloma cells. *Science (Wash. DC)* 182:164.
  55. Cotton, R. G. H., D. S. Secher, and C. Milstein. 1973. Somatic mutation and the origin of antibody diversity. Clonal variability of the immunoglobulin produced by MOPC 21 cells in culture. *Eur. J. Immunol.* 3:135.
  56. Wabl, M., P. D. Burrows, A. Von Gabain, and C. Steinberg. 1985. Hypermutation at the immunoglobulin heavy chain locus in a pre-B-cell line. *Proc. Natl. Acad. Sci. USA.* 82:479.
  57. Jäck, H. M., and M. Wabl. 1987. High rates of deletions in the constant region segment of the immunoglobulin  $\mu$  gene. *Proc. Natl. Acad. Sci. USA.* 84:4934.
  58. Adetugbo, K., C. Milstein, and D. S. Secher. 1977. Molecular analysis of spontaneous somatic mutants. *Nature (Lond.)* 265:299.
  59. Cook, W. D., and M. D. Scharff. 1977. Antigen-binding mutants of mouse myeloma cells. *Proc. Natl. Acad. Sci. USA.* 74:5687.
  60. Kenter, A. L., and B. K. Birshtein. 1979. Genetic mechanism according for precise im-

- munoglobulin domain deletion in a variant of MPC 11 myeloma cells. *Science (Wash. DC)*. 206:1307.
61. Dunnick, W., T. H. Robbitts, and C. Milstein. 1980. An immunoglobulin deletion mutant with implications for the heavy-chain switch and RNA splicing. *Nature (Lond.)*. 286:669.
  62. McKean, D., K. Huppi, M. Bell, L. Staudt, W. Gerhard, and M. Weigart. 1984. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. *Proc. Natl. Acad. Sci. USA*. 81:3180.
  63. Wysocki, L., T. Manser, and M. L. Gefter. 1986. Somatic evolution of variable region structures during an immune response. *Proc. Natl. Acad. Sci. USA*. 83:1847.
  64. Siekevitz, M., C. Kocks, K. Rajewsky, and R. Dildrop. 1987. Analysis of somatic mutation and class switching in native and memory B cells generating adoptive primary and secondary responses. *Cell*. 48:757.
  65. Leder, P., J. Battey, G. Lenoir, C. Moulding, W. Murphy, H. Potter, T. Stewart, and R. Taub. 1983. Translocations among antibody genes in human cancer. *Science (Wash. DC)*. 222:765.
  66. Marolleau, J. P., J. D. Fondel, M. Malissen, J. Trucy, E. Barbier, K. B. Marcu, P.-A. Cazenave, and D. Primi. 1983. The joining of germline V $\alpha$  to J $\alpha$  genes replaces the preexisting V $\alpha$ -J $\alpha$  complexes in a T cell receptor  $\alpha$ ,  $\beta$  positive T cell line. *Cell*. 55:291.
  67. Morley, B. J., M. E. Chin, M. E. Newton, and A. Weiss. 1988. The lysine residue in the membrane-spanning domain of the  $\beta$  chain is necessary for cell surface expression of the T cell antigen receptor. *J. Exp. Med.* 168:1971.
  68. Goodman, T., and L. Lefrancois. 1988. Expression of the  $\gamma$ - $\delta$  T-cell receptor on intestinal CD8<sup>+</sup> intraepithelial lymphocytes. *Nature (Lond.)*. 333:855.
  69. Groh, V., S. Porcelli, M. Fabbi, L. L. Lanier, L. J. Picker, T. Anderson, R. A. Warnke, A. K. Bhan, J. L. Strominger, and M. B. Brenner. 1989. Human lymphocytes bearing T cell receptor  $\gamma/\delta$  are phenotypically diverse and evenly distributed throughout the lymphoid system. *J. Exp. Med.* 169:1277.
  70. O'Flynn, K., A. M. Krensky, P. C. L. Beverley, S. J. Burakoff, and D. C. Linch. 1985. Phytohaemagglutinin activation of T cells through the sheep red blood cell receptor. *Nature (Lond.)*. 313:686.
  71. Brown, M. H., D. A. Cantrell, G. Brattsand, M. J. Crumpton, and M. Gullberg. 1989. The CD2 antigen associates with the T-cell antigen receptor CD3 antigen complex on the surface of human T lymphocytes. *Nature (Lond.)*. 339:551.
  72. Heppell, A., S. V. Butterworth, R. J. Hollis, A. A. Kennaugh, D. W. Beatty, and A. M. R. Taylor. 1988. Breakage of the T cell receptor  $\alpha$  chain locus in non malignant clones from patients with ataxia telangiectasia. *Hum. Genet.* 79:360.
  73. Russo, G., M. Isobe, L. Pegoraro, J. Finan, P. C. Nowell, and C. M. Croce. 1988. Molecular analysis of a t(7;14) (q35;q32) chromosome translocation in a T cell leukemia of a patient with ataxia telangiectasia. *Cell*. 53:137.
  74. Wintrobe, M. W., R. G. Lee, D. R. Boggs, T. C. Bithell, J. Foerster, J. W. Athens, and J. N. Lukens. 1981. *Clinical Hematology* (8th edition). Philadelphia Lee and Febiger, Inc., Philadelphia. 1455-1458.