A UNIQUE V-J-C-REARRANGED GENE ENCODES A γ PROTEIN EXPRESSED ON THE MAJORITY OF CD3+ T CELL RECEPTOR-α/β- CIRCULATING LYMPHOCYTES

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We have recently described (1) an mAb, anti-TiyA, that delineates a subset representing ~3% of human circulating lymphocytes. Using double-color immunofluorescence analysis, it was found that the great majority of TiyA+ cells are CD2+, CD3+, TCR-α/β−, CD4−, CD8+/−, CD5+, NK-H1−, and HLA class II−. Immunoprecipitations performed with anti-TiyA on an extensively studied (2) cloned cell line (F6C7) from fetal origin demonstrated that the protein recognized by the antibody is encoded by a T cell γ rearranging gene (1). Together, these data indicated that a majority of CD3+ TCR-α/β− circulating lymphocytes surface express a TCR γ chain carrying the TiyA epitope.

The present study was performed to characterize the gene encoding this determinant. Like α and β, the human T cell rearranging gene γ has variable (V), joining (J), and constant (C) region segments. 14 variable γ genes belonging to four subgroups have been described that are located upstream of two Cγ segments (see reference 4). Nine Vγ genes belong to subgroup I, whereas subgroups II, III, and IV each include a single gene designated V9, V10, and V11, respectively. Three joining gene segments were identified in early studies: JγP and Jγ1 upstream of Cγ1, and Jγ2 upstream of Cγ2. More recently, two additional Jγ genes (3), namely JγP1 and JγP2, have been found in the Cγ1 and Cγ2 loci, respectively. Based on this previously described organization, we have selected here appropriate J and Vγ probes to analyze a series of cells reacting with anti-TiyA mAbs; each lymphocyte surface expressing the corresponding epitope was found to transcribe a unique Vγ9—JγP—Cγ1—rearranged gene.

Materials and Methods

Isolation of Peripheral Blood CD3+ TCR-α/β- TiyA+ Cells. Nonadherent mononuclear cells were treated for 30 min with FITC-conjugated anti-TiyA and purified by cell sorting.
using an Epics C instrument (Coulter Electronics, Inc., Hialeah, FL). Sorted cells were cultured after PHA plus IL-2 stimulation to generate a polyclonal cell line termed B2-2. In addition, a fraction of the lymphocytes were seeded immediately after sorting at 0.3 cells/well to generate a series of clones.

**Southern and Northern Blotting.** DNA samples (8 μg) were digested with the indicated restriction enzyme, subjected to electrophoresis through 0.7% agarose gels, and transferred in 0.4 N NaOH, 0.6 M NaCl to nylon membranes. Total cellular RNA (4 μg) was denatured in glyoxal-DMSO, subjected to electrophoresis in 1% agarose, and transferred in 10× SSC to nylon membranes. Hybridizations were performed as described previously (4).

**Preparation of ssRNA Probes.** The probes were derived by subcloning in reverse orientation in the pBS plasmid, the indicated restriction fragments: pH60 (a 600-bp Eco RI–Hind III fragment) which contains Jy1, Vγ1 (a 500-bp Sac I–Kpn I fragment), VγII (a 410-bp Eco RI–Acc I fragment), Cγ (a 550-bp Bam HI–Acc I fragment), downstream from the T3 promoter for pH60, Vγ1, Cγ and downstream from the T7 promoter for VγII. The pH60 probe was isolated from a 2.1-kb Hind III M13H60 insert, the Vγ1 probe from a 1.18-kb Sac I Vγ3 insert from λSH4, and the VγII probe from a 1-kb Pst I–Eco RI V9 Jγ2 insert from K20PR (5). The Cγ fragment was isolated from a cDNA library derived from pH28, a diphtheria toxoid-specific T cell clone (4). 32P-labeled ssRNA probes complementary to T cell receptor mRNAs were transcribed in vitro from the linearized pBS plasmid using either T3 or T7 polymerase in the presence of 10 μM 32P-UTP (800 Ci/mmol) and excess cold ATP, GTP, and CTP.

**Results and Discussion**

To define the genomic organization of the T cell rearranging γ genes encoding for the TrγA antigenic determinant on F6C7 cells, we used the Jγ1 probe, pH60. This probe has been used to assign V rearrangements occurring to J1 or J2 (5); it also detects rearrangements to the other Jγ segments JP1, JP, and JP2, when hybridized to Kpn I digests (3). In F6C7 clone, both chromosomes are rearranged (see schematic representation of F6C7 cell rearrangements shown in Fig. 1). Vγ3 is rearranged to Jγ1 on one chromosome (Eco RI: 5.4 kb; Hind III: 3.7 kb; Bam HI: 16 kb; Kpn I: 1.8 kb; Fig. 2, a–d, assignment according to reference 5) and Vγ9 is rearranged to JγP on the other (Fig. 2d). Note that after hybridization with pH60, this latter rearrangement cannot be detected in DNA digested by either Bam HI or Eco RI or Hind III (Fig. 2, a–c). Indeed, with Bam HI (Fig. 2c) the rearranged band (19.5 kb) has virtually the same size as the Jγ1 germline band (20 kb). With Eco RI and Hind III (Fig. 2, a and b), bands are in the germline configuration as it is observed for all the rearrangements involving JP1, JP, and JP2 segments (5, 7). It is only after Kpn I digestion that the Vγ9–JγP rearrangement is visualized as a 12-kb restriction fragment (Fig. 2d).

The presence of this particular rearrangement was confirmed by hybridization...
FIGURE 2. (a–e) Rearrangements of the T cell rearranging \( \gamma \) genes in F6C7 cells. F6C7 DNA was digested with Eco RI (a, e), Hind III (b), Bam HI (c), or Kpn I (d). Digested F6C7 or germline (G) DNAs were hybridized with pH60, a \( \gamma I \) probe (a–d) or with a \( \gamma II \) probe (e).

of the \( \gamma II \) probe to the F6C7 Eco RI–digested DNA (Fig. 2e) that shows an expected 2-kb rearranged band (size deduced from cloned DNA segments; references 3 and 5). Note that the 5.2-kb \( \gamma 9 \) germline fragment (5) is absent in F6C7 cells because it is also deleted on the chromosome that displays the \( \gamma 3 \) rearrangement. Transcription analyses with probes specific for the \( \gamma 1 \) family (\( \gamma 1 \) to \( \gamma 8 \)) or for the \( \gamma II \) family (a unique \( \gamma 9 \) gene; reference 5) were carried out. No transcript was seen with the \( \gamma I \) probe (Fig. 3, lane 2) whereas a 1.6-kb messenger RNA was detected with either the \( \gamma I \) probe (Fig. 3, lane 1) or the \( \gamma II \) probe (Fig. 3, lane 3), indicating that the \( \gamma 9—\gamma P \)–rearranged gene is the only one to be transcribed in these cloned cells. These data strongly suggested that the \( \gamma A \) protein is encoded by a \( \gamma 9—\gamma P—\gamma 1 \) gene. Indeed, the \( \gamma A \) chain is expressed as part of a disulfide-linked dimer (1, 6) while \( \gamma 1 \) is known to possess (as opposed to \( \gamma 2 \)) a cystein involved in interchain disulfide bonds (3). Yet, anti-\( \gamma A \) mAb does not recognize the constant region of the protein because we have found (6), using anti-CD3 immunoprecipitations under appropriate conditions, that two distinct CD3\(^+\) TCR-\( \alpha/\beta^-\) clones carry a disulfide-
linked (that is Cγ1 encoded) dimer while they do not express the TiyA epitope. Thus, the anti-TiyA mAb should be specific for either a Vγ9 or a Vγ9-JγP peptide.

To confirm this point, we studied PBLs in a healthy adult individual. TiyA+ cells were purified by cell sorting procedures and a polyclonal cell line (termed B2-2) was generated as well as 11 CD3+ TCR-α/β+ TiyA+ clones (note that the cell line and the clones were developed in parallel immediately after sorting and not sequentially). A CD3+ TCR-α/β+ clone designated C8 (6), which is unreactive with anti-TiyA mAb while expressing a disulfide-linked CD3-associated complex, was used as a control in these experiments. It was found that the B2-2 cell line and the 11 TiyA+ clones have rearranged the Vγ9 segment to JγP, as shown by the 12-kb Kpn I band with pH60 probe (Fig. 4a) and the 2-kb Eco R I band with the VγII probe (Fig. 4b). Note that all clones except one (clone 11 which surface expresses the TiyA epitope while possessing the Vγ9—JγP rearrangement exclusively) have lost the 9-kb Kpn I germline band (Fig. 4a) indicating that the other chromosome is also rearranged as it was the case for the F6C7 cells. The Kpn I bands corresponding to the rearrangement on the second chromosome are respectively at 12 kb (for clone 3 where both alleles have rearranged Vγ9 to JγP), 8.7 kb (clones 2, 4, 5, 7, 8, and 10), 7.5 kb (clone 9), 6.2 kb (clone 1), or 1.8 kb (clone 6). Evidently, these data indicate that the TiyA+ subset is polyclonal. Northern blot analysis performed with both the B2-2 cell line and the 11 clones using a probe specific for the VγII family confirmed that the Vγ9 segment was actually transcribed in all these cells (data not shown). Finally, two TiyA+ clones from two additional donors that had been obtained independently in other series of experiments were also tested and found to transcribe the Vγ9—JγP-rearranged gene (data not shown).

Together, the present data show that anti-TiyA detects a Vγ9—JγP gene product. This has important biological implications because the TiyA epitope is expressed on approximately two-thirds of the circulating CD3+ TCR-α/β+ frac-

**FIGURE 3.** Northern blot analysis of total cellular RNA from F6C7 cells. The probes are (1) Cγ, (2) VγI, and (3) VγII.
FIGURE 4. (a and b) Rearrangements of the T cell–rearranging \( \gamma \) genes in Ti\( \gamma \)A\(^+\) cell lines. The probes are (a) pH60 and (b) V\( \gamma \)H1. DNA samples from C8, a Ti\( \gamma \)A\(^-\)CD3\(^+\) WT31\(^-\) clone, B2-2, a Ti\( \gamma \)A\(^+\) cell line, and 11 independently derived Ti\( \gamma \)A\(^+\) clones (lanes 1–11) were digested with Kpn I (a) or Eco R1 (b).

The protein diversity arising from this unique V-J rearrangement can theoretically come from only two mechanisms, namely generation of an N region and somatic mutations. N regions have been found in \( \gamma \) transcripts from TCR-\( \alpha/\beta \)\(^+\) lymphocytes but no somatic mutations (7). In cell surface–expressing \( \gamma \) chains that may undergo a selection pressure, these questions have not been studied. Thus, it will be of particular interest to sequence \( \gamma \)y9–J\( \gamma \)P–Cy1 messages from a series of relevant Ti\( \gamma \)A\(^+\) clones. In any case, a limited variability will be found in this broadly distributed \( \gamma \) protein.

Summary

We have recently described an mAb, anti-Ti\( \gamma \)A, that recognizes an antigenic determinant carried by a TCR \( \gamma \) chain. This antibody binds to \(~3\%\) of human PBLs and delineates a CD2\(^+\), CD3\(^+\), TCR-\( \alpha/\beta \)\(^-\), CD4\(^-\), CD8\(^-\), CD5\(^+\), NKH1\(^-\), and HLA class II\(^-\) subset. The present study was designed to identify the gene encoding the Ti\( \gamma \)A epitope. A first analysis was carried out on a previously characterized TCR \( \gamma \)\(^+\) fetal-clone cell line termed F6C7. It was found that F6C7 cells have one \( \gamma \) rearrangement on each chromosome: one joins Vy3 to Jy1, and the second joins Vy9 to JyP. Because only the latter allele appeared to be transcribed in the F6C7 lymphocytes, these data strongly suggested that anti-Ti\( \gamma \)A mAb is specific for either a Vy9 or a Vy9–JyP–encoded peptide. To confirm this point, we studied an additional series of 13 randomly selected Ti\( \gamma \)A\(^+\)
cloned cells derived from peripheral blood of three distinct adult individuals. Each one of these lymphocytes was shown to both possess and transcribe a Vγ9—JyP—Cy1-rearranged gene. It is therefore concluded that a predominant subpopulation of CD3+ TCR-α/β human circulating T lymphocytes (namely, the subset defined by anti-TiγA mAb) surface expresses a γ protein with a limited potential of variability from one cell to another.

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