T Cell Target 1 (TCT.1): a Novel Target Molecule for Human Non-Major Histocompatibility Complex-restricted T Lymphocytes

By Fathia Mami-Chouaib, Christine Miossec, Paola Del Porto, Caroline Flament, Frédéric Triebel, and Thierry Hercend

From the Laboratoire d’Immunologie Cellulaire, INSERM U333, Institut Gustave-Roussy, 94805 Villejuif, France

Summary
We have studied two γ/δ T cell clones, E102 and E117, generated in a mixed lymphocyte culture using an allogeneic Epstein-Barr virus-transformed B cell line, E418. These clones were both found to express a molecular form of T cell receptor (TCR) infrequent in human peripheral blood, associating a V1-J1-δ chain and a V3-JP2-γ chain. Functionally, they appeared as cytotoxic T lymphocytes (CTL) with non-major histocompatibility complex (MHC) (class I and II) requiring cytotoxicity, able to kill both the immunizing (i.e., E418) and unrelated (e.g., K562, REX, F601, and KAS) target cells. A monoclonal antibody, anti-10H3, able to selectively inhibit the cytotoxic activity of the clones has been produced. This reagent defines a 43-kD molecule, designated TCT.1, with broad distribution in the hematopoietic system, that appears to be distinct from class I MHC gene products. A series of functional experiments using various effector/target cell combinations strongly suggested that TCT.1 may represent a unique TCR ligand involved in the interaction between these particular CTL clones and certain of the target cells tested, while others were likely to be recognized and killed through a TCR-independent natural killer-like pathway. Although further experimentation will be needed to strengthen our interpretation of the present data, this study provides additional evidence that some T lymphocytes, in particular of the γ/δ type, may interact specifically with target cells in a non-MHC class I/II-requiring fashion.
Material and Methods

Generation of Closed Cell Lines. Nonadherent PBMC were obtained from a healthy individual using Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient centrifugation followed by plastic adherences. CD3+, TCR-γ/δ+, and TCR-γ/δ- lymphocytes were purified by the immuno-rosetting technique (immuno-depletion), using anti-CD4 (OKT4), -CD8 (OKT8), -CD8 (MY4), -CD20 (B4), -NK1 (N901), and BMA031 mAbs as described previously (19). The CD3+, TCR-γ/δ+, enriched fraction was plated on a feeder layer (104 cells/well) of irradiated EBV-transformed B cell line (E418) in a U-bottomed 96-well plate at 2 x 104 cells/well. The cultures were restimulated weekly with irradiated E418 cells, and rIL-2 was added every 3 d starting from day 12. Limiting dilution cloning of the cell line was performed in V-bottomed 96-well plates at 0.5 cells/well on a feeder layer containing irradiated allogeneic PBL plus E418 cells (6 x 105 PBL + 4 x 105 E418/well).

JT9 and AB12 (α/β and γ/δ T cell clones, respectively) used as controls were described previously (8, 34–37). The NK cell line CD3-1 was has been developed from the CD3- PBL.

Previously Described mAbs and Phenotypic Analysis of the T Cell Clones. Anti-TryA mAb (38) recognizes a Vγ9-encoded epitope and recognizes approximately two-thirds of human γ/δ PBL. Anti-TCR-δ1, kindly provided by M.B. Brenner (Dana-Farber Institute, Boston, MA), reacts with a constant determinant of the TCR δ chain (39). δTCS1 mAb (40) reacts specifically with a structure encoded by Vδ1-Vδ6 gene products (37, 41). A13 and TiV62 (42) mAbs recognize approximately two-thirds of human y/b PBL. Anti-CD3-.1) has been developed from the CD3- PBL.

Generation of the Cloned Cell Lines. Anti-TCR-δ1 has been developed from the CD3- PBL.

Limiting dilution cloning of the cell line was measured by a conventional 3-h ³Cr-release assay using triplicate cultures in V-bottomed plates. E/T ratios were 10:1, 3:1, 1:1, and 0:3:1 on 5,000 target cells/well. Percent specific cytotoxicity was calculated conventionally; SD were <5%.

W6/32 (anti-class I), 9-49 (anti-class II), OKT3, δTCS1, as well as 10H3 mAbs were used in functional assays. The 2F3 mAb (anti-TNKTα) was used as a control. Functional effects of the antibodies, either on effector (OKT3, δTCS1, 10H3, and 2F3) or on target (10H3, W6/32, 9-49, and 2F3) cells, were tested by incubating each of them for 2 h at 37°C before the assay at the predetermined saturating concentration.

Immuno precipitation of the TCR-γ/δ and the TCT.1 Molecule. The cytoxic activity of the cloned cell lines was measured by a conventional 3-h ³Cr-release assay using triplicate cultures in V-bottomed plates. E/T ratios were 10:1, 3:1, 1:1, and 0:3:1 on 5,000 target cells/well. Percent specific cytotoxicity was calculated conventionally; SD were <5%.

W6/32 (anti-class I), 9-49 (anti-class II), OKT3, δTCS1, as well as 10H3 mAbs were used in functional assays. The 2F3 mAb (anti-TNKTα) was used as a control. Functional effects of the antibodies, either on effector (OKT3, δTCS1, 10H3, and 2F3) or on target (10H3, W6/32, 9-49, and 2F3) cells, were tested by incubating each of them for 2 h at 37°C before the assay at the predetermined saturating concentration.

Immuno precipitation of the TCR-γ/δ expressed by E102 and E117 was carried out overnight at 4°C by anti-TCR-δ1 and δTCS1 mAbs followed by protein A-Sepharose beads as described previously (42). Immuno precipitation of the TCT.1 molecule from the E418 B cell line was performed using anti-IOH3 mAb coupled to protein A. K562 cells were used as negative control. SDS-PAGE analysis was carried out using 10% polyacrylamide gels electrophoresis followed by protein A-Sepharose beads as described previously (42).

Generation of the α/β and γ/δ T Cell Clones. The K562 (derived from a patient with chronic myelogenous leukemia) and REX (α/β leukemia) cell lines were used in NK assays. B cell lines, homozygous for HLA-DR antigens KAS 116 (DR1), E418 1324 (DR2), RSH (DR3), JHAF (DR4), BM16 (DR5), Daudi (DR6), MOU (DR7), MADURA (DR8), ARBO (DR9), and F601 (DR10), kindly provided by Dr. J. Colombani (St. Louis Hospital, Paris), were used as targets in cytotoxicity assays.

Results

Generation of the E102 and E117 γ/δ T Cell Clones. PBMC were extracted from Ficoll-Hypaque centrifugation, and adherent cells were removed by two-steps adherence on plastic dishes. The γ/δ T cells were purified by an immuno-rosetting technique (19) using BMA031 (anti-TCR-α/β), anti-CD4, anti-CD8, anti-CD20 (B cell-specific), anti-CD14 (monocytes-specific), and anti-CD56/NKH1 (anti-N901) mAbs. Nonrosetting lymphocytes were cultured at 2 x 104 cells/well in the presence of irradiated (104 cells/well) EBV-transformed B cells, termed E418. Further stimulations by E418 cells were performed weekly over a 4-mo period. rIL-2 was added every 3 d, starting from day 12. The polyclonal cell line generated under these conditions was used as a source of γ/δ T cell clones.
conditions was found to display a stable CD3+, CD4-, CD8-, TCR-δ1+, δTC51+, A13+, TIV62-, and TryA- surface phenotype (data not shown). Note here that anti-TCR-δ1 mAb is specific for a constant determinant of the TCR-δ chain (39), anti-δTC51 mAb for an epitope encoded by Vδ1-J61 (and/or possibly Vδ1-J62; see reference 49)-rearranged gene segments (37), anti-A13 and anti-TIV62 for peptides encoded by Vδ1 and Vδ2 gene segments, respectively (42), and anti-TryA for the Vγ9 gene product (38, 50).

This cell line was then cloned by limiting dilution at 0.5 cells/well on a feeder layer containing both allogeneic PBh and the sensitizing E418 B cell line. A series of clones with cytolytic activity against the E418 cells were generated. Two of them, termed E102 and E117, were studied in detail. Fig. 1 shows the reactivity of both clones with relevant mAbs. In line with the phenotype of the originating cell line, they were found to be CD3+, BMA031- (TCR-δ1(TCR-δ1)), TCR-δ1+, δTC51+, A13+, TIV62-, TryA-, CD4-. The NKH1 molecule was present on a fraction of the cells, as well as CD8, which was expressed with very low density.

Together, this analysis indicated that the two clones posses a Vγ9+/Vδ1+ receptor. Such a TCR can only be found in a very small γ/δ peripheral cell fraction of the individual studied here. Indeed, phenotypic analysis of his PBL showed that he had slightly more than 5% circulating γ/δ lymphocytes with almost 5% TryA+, 5% TIV62+ cells, and <0.5% δTC51+ cells (data not shown). More generally, note that the δTC51+/TryA- phenotype corresponds to a minority of γ/δ T cells in the peripheral blood of most adult donors (8, 36, 51).

Molecular Characterization of the TCR Expressed by E102 and E117 T Cell Clones. A series of Southern blots were performed to further characterize the organization of the TCR-γ and TCR-δ genes in the E102 and E117 clones. Large molecular weight DNA from both cells was digested with either EcoRI, BamHI, HindIII, or KpnI, fractionated on agarose gel, blotted, and hybridized to a variety of relevant probes.

Regarding the δ chain rearrangements, Southern blot analysis of both clone DNAs with a Vδ1 probe showed a 3-kb EcoRI restriction fragment corresponding to the Vδ1 germ-line configuration, and a 3.3-kb EcoRI band known to include (5, 8) the Vδ1-J61 rearrangement (data not shown). This result is in line with the surface reactivity of the anti-δTC51 mAb.

To assess the γ chain rearrangements, we used the pH60 probe (a Jy1 fragment), which hybridizes to both Jy1 and Jy2 gene segments (3). There was no detectable rearrangement when HindIII- and EcoRI-digested DNAs were digested with this probe (data not shown), indicating that E102 and E117 do not use either the Jy1 or the Jy2 gene segments (4). When DNAs were digested with the KpnI restriction enzyme, hybridizations with pH60 led to the detection of the 16-kb Jy2 germ-line fragment plus two additional bands at 4.7 and 8.5 kb (Fig. 2 A). It has been previously shown that such fragments correspond to rearrangements of a member of either the Vγ1 or the VγIII gene subfamily to JP2 and JP1, respectively (6).
Figure 2. Southern blot analyses of E102 and E117 γ/β T cell clone DNAs. DNA samples (10 μg) were digested with EcoRI, HindIII, BamHI, or KpnI restriction enzymes, blotted, and hybridized with pH60 (A) or Vγ1 (B) probes. G corresponds to germline DNA used as controls.
Additional experiments were performed to define the actual V segments used by the cloned T cells. In BamHI digests, the pH60 probe detected a rearranged band of >40 kb (Fig. 2 A). Thus, it is appeared likely that the V segments belong to the Vγ1 family, because the Vγ10 (VγIII) rearrangements to JP1 and JP2 are known to correspond to 26- and 22-kb BamHI bands, respectively. This point was confirmed by the hybridization of the EcoR1 and the HindIII digests to the VγIII probe (4) showing a deletion of this gene segment in both E102 and E117 clones (data not shown). Hybridizations were then performed (Fig. 2 B) with a Vγ probe including the Vγ3 segment (47). After EcoR1 digestion, this probe detected in both clones a 5-kb rearranged fragment corresponding to a Vγ3-JP2 recombination (6). With HindIII, one rearranged fragment was seen at 2.2 kb corresponding to a Vγ8-JP1 recombination. Digestion with BamHI led to the detection of two rearranged bands at 19 and 47 kb corresponding to the Vγ3-JP2 and the Vγ8-JP1 recombinations, respectively. The former was not detected clearly with HindIII, nor the latter with EcoR1, because the corresponding rearranged fragments have approximately the same size as germline bands present in the digests (i.e., 4.2 and 5.4 kb, respectively). Note that such rearrangements on both E102 and E117 chromosomes have led to the deletion of the 3.8-kb EcoR1 band (Fig. 2 B) corresponding to the germline form of the Vγ8 gene segment (4, 6).

Together, these data indicated that the two clones have rearranged the TCRγ genes on both chromosomes. To identify the productive rearrangement, we studied the quaternary structure of the receptor. Indeed, it is well known that the use of JP1 leads to the production of disulfide-linked γ/δ dimers, while the use of JP2 results in the expression of non-disulfide-linked receptors (2). Immunoprecipitations performed with the anti-TCRδ1 and the anti-ÅTC51 mAbs led to the detection of two bands at ~48 and ~42 kd in SDS-PAGE analysis under nonreducing conditions (data not shown).

In conclusion, these data, which are in line with the phenotypic analysis, strongly suggested that both E102 and E117 cells express a Vδ1-J81-Cδ/Vγ3-JP2-Cγ2 heterodimer. They confirm that the cloned cell lines use a γ/δ receptor with a molecular structure infrequent in human peripheral blood. Because both clones were found to display the same γ and δ chain rearrangements, they are likely to be derived from the same cell. The complete sequence of their TCRγ/δ chains, particularly at the junctional regions, will have to be performed in future studies to conclude on this point.

Functional Activity of the E102 and E117 γ/δ T Cell Clones. E102 and E117 cells were assayed for cytotoxic activity against the E418-immunizing cells. The NK target cell line K562 was tested in parallel, as well as a panel of 10 EBV-transformed and tumor B cell lines, including Daudi, which is known for its susceptibility to lymphokine-activated killing. As shown in Fig. 3, both clones displayed a high level of toxicity against E418. In contrast, there was little if any activity against the Daudi target cell line. The cytotoxicity towards K562 varied from one experiment to another, while being generally weaker than that observed against E418. Among the B cell lines tested, only F601 and KAS (EBV-transformed B cells) were lysed by the two clones with a degree of efficiency (>15% lysis at 10:1 E/T ratio), allowing further investigation (shown in Fig. 4).

To assess whether the recognition of E418 cells by the clones involves conventional MHC molecules, we performed a series of blocking experiments using anti-class I and anti-class II antibodies. Neither W6/32 (anti-class I) nor 9-49 mAb (anti-class II) were able to inhibit E102 or E117 cytotoxic activity against E418 (Fig. 5, A-a and A-b).

Together, these data indicated that E102 and E117 are T lymphocytes with non-MHC class I/II-requiring cytotoxicity, able to kill both the immunizing (i.e., E418) and unrelated (e.g., K562, F601, and KAS) target cells.

Generation of Anti-10H3, a mAb that Specifically Blocks E102 and E117 Cytotoxicity. To identify molecules potentially recognized by the clones, we attempted to develop mAbs able to block their interaction with target cells. The E418 EBV-transformed B cell line was used to immunize 3-mo-old Bi-
ozzi mice. Cell fusions were performed, and antibodies were screened for their ability to alter the cytotoxicity of the E117 clone towards the E418 cells. Before testing the E117 lytic activity, ¹⁴C-labeled target cells were thus treated for 2 h with individual hybridoma supernatants. One hybridoma, termed 10H3 (IgGl), with strong inhibitory effects was selected for further analyses. Fig. 5, representative of multiple individual experiments, shows the virtual abrogation of the cytotoxicity against E418 obtained with 10H3 mAb using either E102 (A-a) or E117 (A-b) as effector cells.

Cellular Distribution and Characterization of the TCT1 Molecule. The expression of the molecule, designated TCT1, identified by the 10H3 mAb was assessed on both lymphoid and nonlymphoid cells. In a first series of experiments, the reactivity of anti-10H3 was tested on the E418, Daudi, REX, AB12, and K562 cell lines by indirect immunofluorescence analysis (Fig. 6). W6/32, B1.23.2 (anti-class I H chain), B2.G2.2 (anti-β2m), 9-49, B4 (anti-CD20), and OKT3 mAbs were used as positive and negative control reagents. Except for K562, all these cells were found to carry the TCT1 molecule. The intensity of expression varied, however, among the cell lines. Indeed, E418 (EBV-transformed B cell line) and AB12 (IL-2-dependent T cell clone) cells displayed much higher fluorescence density than Daudi (Burkitt lymphoma) and REX (T cell leukemia) cells. Results obtained with the REX (10H3⁺, W6/32⁺, B1.23.2⁺, B2.G2.2⁺, 9-49⁻) and the Daudi cell lines (10H3⁺, W6/32⁻, B1.23.2⁻, B2.G2.2⁻, 9-49⁺) are of particular interest because they suggest that the TCT1 protein is distinct from the classical MHC class I/II molecules and does not require the presence of the β2m for its expression.

More generally, resting PBL, monocytes, bone marrow cells, EBV-transformed B cells (including F601 and KAS), cloned T cell lines (including E102 and E117), and different leukemia cell types were found to be positive. The reactivity of anti-10H3 on HL60, KG1 (two myeloid cell lines), and polymorphonuclear cells was weak. Ramos (Burkitt lymphoma cell line), U937 (histiocytic cell line), and all the nonhematopoietic normal and tumoral tissues tested, including liver, kidney, breast, pancreas, placenta, colon, and ovary, were negative (results summarized in Table 1). All these cells were strongly positive with the W6/32 reagent, confirming that TCT1 expression does not correlate with that of the conventional class I molecules.

Immunoprecipitation experiments were performed with ¹²⁵I-labeled E418 cells to define biochemically the TCT1 structure. As shown in Fig. 7, the anti-10H3 mAb precipitated from the E418 cells a molecule resolving in SDS-PAGE analysis as a unique 43-kD band under both reducing and nonreducing conditions. The anti-W6/32 mAb tested in parallel, as a positive control reagent, immunoprecipitated from the same cell lysate two bands at 43 and 12 kD corresponding to the class I H chain and to the β2m, respectively (52). Note that the latter was not precipitated by the anti-10H3 mAb. Because the TCT1 molecule resolved at a molecular mass identical to that of the class I molecules, sequential immunoprecipitations were done using anti-10H3 and anti-W6/32. There were no crossalterations of the specific bands, confirming that the two reagents recognize distinct proteins (data not shown). Control experiments included anti-10H3 immunoprecipitations from the 10H3⁻ K562 cells where no signal was detected (data not shown).
Specificity of the Anti-10H3 mAb Inhibitory Effects. Because TCT1 was found to be expressed by CTL themselves, experiments were performed to assess whether the 10H3 anti-body inhibits cytotoxicity through interaction with the target cell membrane. It was found that anti-10H3 had no effect at all regardless of the target tested (i.e., E418, F601, and KAS) when incubated with E102 and E117 effector cells. In contrast, either treatment of target cells followed by subsequent washing or direct addition of the mAb in the microtiter wells revealed constantly the biological activity (Fig. 8).

In light of the broad distribution of the TCT1 molecule in the hematopoietic system, we tested the blocking activity of anti-10H3 in E/T cell combinations distinct from E102 (or E117)/E418. The selected killer cells included AB12 (a γδ T cell clone with the predominant peripheral TryA+/ TIVδ2+ phenotype [8, 36, 37]), JT9 (an αβ T cell clone defined through the expression of the NKTα clonotypic determinant [34, 35]), and CD3−, a polyclonal NK (OKT3−, NKH1+) cell line. The 10H3+ Daudi cells were used as a target because of their known susceptibility to the three types (i.e., α/β, γ/δ and NK cells) of effectors. As shown in Fig. 5 B, anti-10H3 had no effect at all in the cytotoxic reactions. Controls included W6/32 and 9-49 antibodies that were also inactive, while, as described previously (45), anti-TNKtar blocked specifically the cytotoxicity mediated by JT9 cells (Fig. 5 B-e). Note, in addition, that anti-10H3 was unable to inhibit the cytotoxicity mediated by the CD3− NK cells against the E418 cell line (Fig. 5 A-c). The activity of JT9 and AB12 against E418 was too weak to test the blocking effect of anti-10H3 in the corresponding combinations.

Together, these data strongly suggested that TCT1 is not involved in a generally operating pathway of cell-cell interaction. Further experiments were performed to assess whether anti-10H3 would alter the cytotoxic interaction between either E102 or E117 and all TCT1+ -susceptible target cells. Three cell lines F601, KAS (EBV-transformed B cells), and REX (a T cell leukemia commonly used in NK assays), were tested in addition to E418. The representative experiment presented in Fig. 4 shows that the activity of anti-10H3 was variable from one target cell to another. The antibody virtually abrogated the cytotoxicity against F601; its effect was moderate with KAS, while it was totally inactive with REX.
Discussion

To further investigate the antigenic specificity of γ/δ human T lymphocytes, we have developed clones able to recognize and kill an allogeneic EBV-transformed B cell line, termed E418. Two of them (E102 and E117), displaying a strong cytotoxic activity against the E418-immunizing cells, were found to express an infrequent γ/δ heterodimer encoded by the Vδ1-Jδ1-Çδ- and the Vγ3-JP2-Çγ2-rearranged genes.

The lytic activity of the E102 and E117 cells was tested against a series of additional target cells, including a panel of eight EBV-transformed B cell lines carrying various MHC class I and class II gene products, as well as conventional NK/lymphokine-activated killer (LAK) target cells (K562, REX, and Daudi). There was little if any cytotoxicity against six of the B cell lines, while two (F601 and KAS) were lysed more efficiently. The Daudi LAK target cell was not killed, and varying levels of lysis were found against K562 and REX. The cytotoxicity of both clones against the E418 cell line has not altered by either anti-W6/32 (anti-class I) or 9-49 (anti-class II) mAbs. Therefore, E102 and E117 appeared to display a non-MHC class I/II-requiring cytotoxic activity.

To further study target cell recognition by the E102 and E117 clones, we have generated a mAb, termed anti-10H3, initially selected for its ability to block their cytotoxic interaction with the E418-immunizing cells. The corresponding antigen, designated TCT.1, has been characterized as a 43-kD molecule. It was found to be broadly distributed in the hematopoietic system, while cells from various other origins appeared to be negative. Results obtained with the Daudi cell line indicated that the TCT.1 protein does not require the β2m for its expression, and is therefore distinct from the class I-like surface antigens.

Experiments performed with appropriate preincubation of either effector or target cells with anti-10H3 indicated that its inhibitory effect resulted from its binding to the membrane of target cells. Further investigations showed that anti-10H3 had no blocking activity when a variety of T and NK cells distinct from E102 and E117 were used as effectors. In addition to E418, the functional activity of anti-10H3 was assessed against NK target cells (REX) and EBV-transformed B cell lines (F601 and KAS), susceptible to the cytotoxic activity of E102 and E117. It was found to strongly inhibit the cytotoxicity against the F601 cells while being active, although less efficient, against the KAS cell line. In contrast, the antibody did not alter at all the interaction of E102 or E117 with the REX cell line.

Together, the present results support the view that the E102 and E117 lymphocytes "see" the TCT.1 molecule on the surface of target cells. It is now generally agreed that CTL can recognize and kill cells through either a TCR-dependent or a TCR-independent pathway, the latter corresponding to the so-called NK-like activity (53). We have recently postulated that this NK activity observed with CTL may represent an evolutionary conserved function (53). Such a conversion could allow for a broader in situ spectrum of target cell interaction at the effector step of the cytolytic reaction. It may thus contribute to destroy transformed cells that have lost, through mutations, the antigen that originally initiated the development of the T cell response.

Our data clearly indicate that the E102 and E117 clones display NK-like function. The variability of the activity found against K562 and REX probably corresponds to the known dependence of NK/LAK (i.e., IL-2-augmented TCR-independent NK activity mediated by either NK or T cells)
Table 1. Screening of the Anti-10H3 mAB Reactivity and its Comparison with that of the Anti-W6/32

| A. Cells and cell lines* | W6/32 reactivity | 10H3 reactivity |
|--------------------------|------------------|-----------------|
|                          | %                | %               |
| E418 T3- /9-49+/B4+     | 90 (205)         | 90 (179)        |
| Daudi T3- /9-49+/B4+    | 0                | 90 (105)        |
| Ramos T3- /9-49+/B4+    | 96 (172)         | 14              |
| REX T3+ /9-49+/B4-      | 95 (162)         | 95 (113)        |
| AB12 T3+ /9-49+/B4-     | 94 (185)         | 94 (192)        |
| E117 T3+ / ND /B4-      | ND               | 97 (173)        |
| HL60 T3- /9-49+/B4-     | 96 (159)         | 40 (79)         |
| KGI T3- /9-49+/B4-      | 93 (167)         | 75 (94)         |
| U937 T3- /9-49+/B4+     | 99 (193)         | 1               |
| K562 T3- /9-49+/B4-     | 68 (88)          | 0               |
| LAL (Lannibe) T3- /9-49+/B4+ | 99 (216) | 88 (87) |
| LLC (Helias) T3- /9-49+/B4+ | 99 (190) | 99 (152) |
| LAM (Tanguy) ND / ND /B4- | 90 (184) | 3               |

B. Cell fractions†

|                | %    | %    |
|----------------|------|------|
| PBL            | 97 (159) | 93 (125) |
| Monocytes      | 98 (187) | 89 (126) |
| Bone marrow    | 91 (189) | 82 (139) |
| Polymorphonuclear | 95 (127) | 46 (75) |

C. Allogeneic tissues§

| Tissue         | %    | %    |
|----------------|------|------|
| Liver          | +    | –    |
| Kidney         | +    | –    |
| Ovary          | +    | –    |
| Placenta       | +    | –    |
| Pancreas       | +    | –    |
| Breast         | ±    | –    |

Cells were analyzed by indirect immunofluorescence (A and B) or by radiisotope assay on microfolds (C). Data are percentage of positive cells. Numbers in parentheses correspond to fluorescence intensity mean.

* In vitro established tumor, viral-transformed cell lines, and cloned cytotoxic cells. B cells: E418, Daudi, Ramos. T cells: AB12, E117 (γ/δ), REX (α/β).

Nonlymphoid cell lines: K562, HL60, KG1, U937.

LAL: acute lymphoblastic leukemia (two LAL were tested).

LLC: chronic lymphoblastic leukemia (two LLC were tested).

LAM: acute myeloid leukemia (three LAM were tested).

† Three normal individuals were tested.

§ Normal and tumor tissues were tested. +, Radioactivity binding ratio, B/BO (B-specific radioactivity fixation, BO = nonspecific radioactivity fixation) >26% and <37%. ±, B/BO >6% and <18%. –, B/BO <3%.

lysis upon the effector cell status for IL-2-induced activation (24, 34, 53–55). The absence of cytotoxicity against the Daudi cell line, which represents one of the conventional LAK targets, reflects the heterogeneity at the clonal level regarding the IL-2-augmented NK function. This phenomenon, which is still poorly understood, has been documented extensively (24, 34, 53, 56).

The recognition of the TCT1 molecule may allow to distinguish the TCR-dependent and the TCR-independent target cell recognition by the E102 and E117 lymphocytes. That TCT1 is likely to be recognized via the γ/δ heterodimer is supported by several observations: (a) it has to be mentioned that the interaction between the clones and the E418 cells are inhibited by anti-TCR antibodies (data not shown). It is known, however, that such data may reflect the transduction of a negative signal in effector cells even when the TCR is not involved in target cell recognition (18, 24, 57); (b) the blocking activity of the antibody is dependent upon the use of unique effector CTL, named here E102 and E117. This implies that the latter cells carry either a unique determinant within a polymorphic molecule (i.e., their TCR) or alternatively express a novel monomorphic receptor (i.e., an unknown...
With the identification of the γ/δ lymphocytes, it is now better accepted that CTL/target cell interactions may occur through specific TCR-mediated recognition of molecules distinct from the class I or class II MHC gene products. For example, it is strongly suggested that certain lymphocytes recognize class I-like molecules such as Qa (22), TL (16), or CD1c (23, 24). These unconventional specificities may not be uniquely restricted to γ/δ T cells, while being more apparent with the latter lymphocytes where the role of the usual MHC molecules appears to be less predominant. Indeed, α/β receptors may also be able to interact with structures such as CD1a (23), and even with other types of surface molecules (45). Note, for example, that experimental observations quite similar to those presented here, where unique clones were able to recognize a broadly distributed molecule such as TNKtar/4F2 (45, 58), have been reported previously. In each individual case, including the one described here, the T cells involved have appeared to be relatively infrequent. This may reflect in part the multiplicity of these potential "novel" TCR ligands. Further characterization of the still poorly defined "non-MHC requiring specific interactions" may eventually lead to a better understanding of the role of T cells in immune responses.

References

1. Lefranc, M.P., and T.H. Rabbitts. 1985. Two tandemly organized human genes encoding the T-cell γ constant-region sequences show multiple rearrangement in different T-cell types. Nature (Lond.). 316:464.
2. Lefranc, M.P., A. Forster, and T.H. Rabbitts. 1986. Genetic polymorphism and exon changes of the constant regions of the human T-cell rearranging γ gene. Proc. Natl. Acad. Sci. USA. 83:9596.
3. Lefranc, M.P.A. Forster, and T.H. Rabbitts. 1986. Rearrangement of two tandem T-cell γ-chain variable-region genes in human DNA. Nature (Lond.). 319:420.
4. Forster, A., S. Huck, N. Ghanem, M.P. Lefranc, and T.H. Rabbitts. 1987. New subgroups in the human T-cell rearranging Vγ gene locus. EMBO (Eur. Mol. Biol. Organ.) J. 6:1945.
5. Hata, S., M.B. Brenner, and M.S. Krangel. 1987. Identification of putative human T cell receptor δ complementary DNA clones. Science (Wash. DC). 238:678.
6. Huck, S., and M.P. Lefranc. 1987. Rearrangement to the JPI, JP and JP2 segments in the human T-cell rearranging γ gene (TRG γ) locus. FEBS (Fed. Eur. Biochem. Soc.) Lett. 224:291.
7. Quertermous, T., W.M. Strauss, J.J.M. Van Dongen, and J.G. Seidman. 1987. Human T-cell γ chain joining regions and T-cell development. J. Immunol. 138:2687.
8. Triebel, F., F. Faure, F. Mami-Chouaib, S. Jitsukawa, A.L. Griselli, C. Genevee, S. Roman-Roman, and T. Hercend. 1988. A novel human Vδ gene expressed predominantly in the TγA' fraction of γ/δ+ peripheral lymphocytes. Eur. J. Immunol. 18:2021.
9. Takihara, Y., D. Trachuk, E. Michalopoulos, E. Champagne, J. Reinmann, M. Minden, and T.W. Mak. 1988. Sequence and organization of the diversity, joining and constant region genes of the human T-cell γ-chain locus. Proc. Natl. Acad. Sci. USA. 85:6097.
10. Takihara, Y., J. Reinmann, E. Michalopoulos, E. Ciccone, L. Moretta, and T.W. Mak. 1989. Diversity and structure of human T cell receptor δ chain genes in peripheral blood γ/δ.
bearing T lymphocytes. *J. Exp. Med.* 169:393.

11. Bank, I., R.A. Depinho, M.B. Brenner, J. Cassimeris, F.W. Alt, and L. Chess. 1986. A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes. *Nature (Lond.)* 322:179.

12. Brenner, M.B., J. McLean, H. Scheft, J. Riberdy, S.L. Ang, J.G. Seidman, P. Devlin, and M.S. Krangel. 1987. Two forms of the T-cell receptor γ protein found on peripheral blood cytotoxic T lymphocytes. *Nature (Lond.)* 325:689.

13. Borst, J., R.J. Van de Griend, J.W. Oostveen, S.L. Ang, C.J. Melief, J.G. Seidman, and R.L.H. Bolhuis. 1987. A T-cell receptor/CD3 complex found on cloned functional lymphocytes. *Nature (Lond.)* 325:683.

14. Moingeon, P., S. Jitsukawa, F. Faure, F. Troalen, F. Triebel, M. Graziani, F. Forestier, D. Bellet, C. Bohuon, and T. Hercend. 1987. A γ-chain complex forms a functional receptor on cloned human lymphocytes with natural killer-like activity. *Nature (Lond.)* 325:723.

15. Matis, L.A., R. Cron, and J.A. Bluestone. 1987. Major histocompatibility complex-linked specificity of γ/δ receptor-bearing T lymphocytes. *Nature (Lond.)* 330:262.

16. Bluestone, J.A., R.Q. Cron, M. Cotterman, B.A. Houlden, and L.A. Matis. 1988. Structure and specificity of T cell receptor γ/δ on major histocompatibility complex antigen-specific CD3+, CD4-, CD8- T lymphocytes. *J. Exp. Med.* 168:1899.

17. Rivas, A., J. Koid, M.L. Cleary, and E.G. Engleman. 1989. Evidence for involvement of the γ/δ T cell antigen receptor in cytotoxicity mediated by human alloantigen-specific T cell clones. *J. Immunol.* 142:3840.

18. Viccione, E., O. Viale, C. Bottino, D. Pende, N. Migone, G. Casorati, G. Tumbussi, A. Moretta, and L. Moretta. 1988. Antigen recognition by human T cell receptor γ-positive lymphocytes: specific lysis of allogeneic cells after activation in mixed lymphocyte culture. *J. Exp. Med.* 167:1517.

19. Jitsukawa, S., F. Triebel, F. Faure, C. Miossec, and T. Hercend. 1988. Cloned CD3+ TCRα/β+, TCRδ- peripheral blood lymphocytes compared to the TγA+ counterparts: structural differences of the γ/δ receptor and functional heterogeneity. *Eur. J. Immunol.* 18:1671.

20. Matis, L.A., A.M. Fry, R.Q. Cron, M.M. Cotterman, R.F. Dick, and J.A. Bluestone. 1989. Structure and specificity of a class II MHC alloreactive γδ T cell receptor heterodimer. *Science (Wash. DC)* 245:746.

21. Strominger, J.L. 1989. The γδ T cell receptor and class I MHC-related proteins: enigmatic molecules of immune recognition. *Cell.* 57:895.

22. Vidovic, D., M. Roglic, K. McKune, S. Guderer, C. MacKay, and Z. Dembic. 1989. QA-1 restricted recognition of foreign antigen by a γδ T-cell hybridoma. *Nature (Lond.)* 340:646.

23. Porcelli, S., M.B. Brenner, J.L. Greenstein, S.P. Balk, C. Terhorst, and P.A. Bleicher. 1989. Recognition of cluster of differentiation 1 antigens by human CD4+ CD8- T lymphocytes: specific lysis with peripheral blood γδ T cells. *Eur. J. Immunol.* 20:703.

24. Rauen, D.H. 1989. Antigens for γδ T cells. *Nature (Lond.)* 339:342.

25. O’Brien, R.L., M.P. Happ, A. Dallas, E. Palmer, R. Kudo, and W.K. Born. 1989. Stimulation of a major subset of lymphocytes expressing T cell receptor γδ by an antigen derived from Mycobacterium tuberculosis. *Cell.* 57:667.

26. Augustin, A., R.T. Kubo, and G.K. Sim. 1989. Resident pol-
44. Todd, R.F., S.C. Meuer, P.L. Romain, and S.F. Schlossman. 1984. A monoclonal antibody that blocks class II histocompatibility related immune interactions. *Hum. Immunology.* 10:23.

45. Hercend, T, R. Schmidt, A. Brennan, M.A. Edson, E.L. Reinherz, S.F. Schlossman, and J. Ritz. 1984. Identification of a 140-kDa activation antigen as a target structure for a series of human cloned natural killer cell lines. *Eur. J. Immunol.* 14:844.

46. Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503.

47. Lefranc, M.P., A. Forster, R. Baer, M.A. Stinson, and T.H. Rabbitts. 1986. Diversity and rearrangement of the human T-cell rearranging γ genes: nine germ-line variable genes belonging to two subgroups. *Cell.* 45:237.

48. Feinberg, A.P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6.

49. Koning, F., M. Knot, F. Wassenaar, and P.V. den Elsen. 1989. Phenotypical heterogeneity among human T-cell receptor γ/δ-expressing clones derived from peripheral blood. *Eur. J. Immunol.* 19:2099.

50. Triebel, F., F. Faure, M. Graziani, S. Jitsukawa, M.P. Lefranc., and T. Hercend. 1988. A unique V-J-C-rearranged gene encodes a γ protein expressed on the majority of CD3+ γ TCR-α/β+ circulating lymphocytes. *J. Exp. Med.* 167:694.

51. Triebel, F., and T. Hercend. 1989. Subpopulations of human peripheral T γ δ lymphocytes. *Immunol. Today.* 10:186.

52. Brodsky, F.M., P. Barham. C.J. Barnstable, M.J. Crumpton., and W.F. Bodmer. 1979. Monoclonal antibodies for analysis of the HLA system. *Immunol. Rev.* 47:3.

53. Faure, F., F. Triebel., and Th. Hercend. 1990. MHC-unrestricted cytotoxicity. *Immunol. Today.* 11:108.

54. Seeley, J.K., G. Masuci, A. Poros, E. Klein., and S.H. Golub. 1989. Studies on cytotoxicity generated in human mixed lymphocyte cultures. II. Anti-K562 effectors are distinct from allospecific CTL and can be generated from NK-depleted T cells. *J. Immunol.* 123:1303.

55. Rimm., I.J., S.F. Schlossman., and E.L. Reinherz. 1981. Antibody-dependent cellular cytotoxicity and natural-killer-like activity are mediated by subsets of activated T cells. *Clin. Immunol. Immunopathol.* 21:134.

56. Fisch, P., M. Malkovsky, E. Braakman, E. Sturm, R.H. Bolhuis, A. Prieeve, J.A. Sosman, V.A. Lam, and P.M. Sondel. 1990. T cell clones and natural killer cell clones mediate distinct patterns of non-major histocompatibility-restricted cytolysis. *J. Exp. Med.* 170:1567.

57. Lanier, L.L., and J.H. Phillips. 1986. Evidence for three types of human cytotoxic lymphocyte. *Immunol. Today.* 7:132.

58. Haynes., B.F., M.E. Hemler, D.L . Mann., G.S. Eisenhart, J. Shelhamer, H.S. Mostowski, C.A. Thomas., J. L. Strominger, and A.S. Fauci. 1981. Characterization of a monoclonal antibody (4F2) that binds to human monocytes and to a subset of activated lymphocytes. *J. Immunol.* 126:1409.