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

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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-C δ chain and a V3-JP2-C2 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.

The molecular characterization of the human TCR-γ/δ has progressed very rapidly (1–10). However, the physiological contribution to immune responses of the minor T lymphocyte fractions expressing this recognition structure remains unclear. Multiple in vitro studies aimed at defining the antigenic repertoire of the γ/δ TCR are being performed that will eventually contribute to elucidate the biological functions of the corresponding cells. It has been shown initially that IL-2-dependent γ/δ T lymphocytes may display a non-MHC requiring cytotoxicity (11–14), suggesting their possible role in "immune surveillance" as effector lymphocytes able to kill virally infected or tumor cells. Allogeneic γ/δ CTL recognizing either class I (15–18) or class II (19, 20; F. Mami-Chouaib and T. Hercend unpublished data) MHC gene products have also been identified in murine and human systems. In addition, several reports have suggested that γ/δ lymphocytes may interact with target cells through recognition of MHC class I-like molecules (reviewed in reference 21), such as TL (16), Qa1 (22), and CD1c (23, 24). Other specific responses, either conventionally MHC restricted or not, have been described against various antigens such as mycobacterias, heat-shock proteins, tetanus toxoid, and Igs (25–33). Together, these data have indicated that γ/δ T lymphocytes can recognize a large variety of antigens while not using MHC class I or class II gene products as predominant restriction elements like the α/β counterparts.

With particular respect to CTL, the reported reactivities (e.g., class I, class II, TL, and CD1c) have appeared to involve very limited cell fractions within the γ/δ population (16–20, 23, 24). Thus, we have tried to further assess the specificity of human γ/δ T cells via the generation of alloreactive cloned cell lines. We describe here two clones, E102 and E117, generated in MLR against an EBV-transformed B cell line, designated E418. Both of these γ/δ lymphocytes were found to recognize and kill the E418-immunizing cells. A 43-kD surface molecule, termed T cell target 1 (TCT.1),[1] broadly distributed in the hematopoietic system, was shown to be recognized on the target cells in these unique cytotoxic interactions.

[1] *Abbreviations used in this paper:* LAK, lymphokine-activated killer; TCT.1, T cell target 1.

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Material and Methods

Generation of Cloned 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^+ lymphocytes were purified by the immuno-rosetting technique (immuno-depletion), using anti-CD4 (OKT4), -CD8 (OKT8), -CD14 (MY4), -CD20 (B4), -NKH1 (N901), and BMA031 mAbs as described previously (19). The $CD3^+$, TCR-γ/δ^+-enriched fraction was plated on a feeder layer (10^4 cells/well) of irradiated EBV-transformed B cell line (E418) in a U-bottomed 96-well plate at 2×10^4 cells/well. The cultured cells 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 (6×10^3 PBL + 4×10^3 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) has been developed from the $CD3^-$ PBL.

Previously Described mAbs and Phenotypic Analysis of the T Cell Clones. Anti-TiγA 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-Jδ1 gene products (37, 41). A13 and TiVδ2 (42) react with Vδ1 and Vδ2 gene products, respectively. BMA031, kindly provided by Dr. R. Kurrle (Behring Company, Marburg, FRG), reacts with a monomorphic determinant of the TCR-α/β receptor. The anti-NKTa mAb recognizes an α/β clonotypic determinant (35). OKT3, OKT4, and OKT8 (Ortho Diagnostics Systems Inc., Westwood, MA) react with CD3, CD4, and CD8 proteins, respectively. W6/32 (43) and 9-49 (44) mAbs recognize nonpolymorphic determinants of HLA class I and class II gene products, respectively. B1.23.2 (anti-class I H chain) and B2.G2.2 (anti-$\beta_2$microglobulin [β2m]) were kindly provided by Dr. F. Lemonier (Luminy, Marseille, France). 2F3 (anti-TNKTar) reacts with a 140-kD activation antigen described previously as a target structure for a series of human $NKTa^+$ T cell clones (45). Each mAb was used at a saturating concentration predetermined by titration curves on positive cloned cell lines.

Phenotypic analysis of the cloned cell lines was carried out by indirect immunofluorescence using an Epics C flow cytometer as described previously (34). Allogeneic tissues were analyzed by a radioisotope immunofiltration assay using disposable microfolds (V & P Scientific, Inc.). 10^4 cells/filter were incubated for 2 h at room temperature, washed, and then 10^5 cpm of ^{125}I-radiolabeled 10H3, W6/32, or irrelevant mAb were added. After a 2-h incubation and three-step washing, radioactivity on the filter disks was counted using a LKB gamma counter.

Generation of the 10H3 mAb. 12-wk-old Biozzi mice were immunized with E418 EBV-transformed B cells; 5×10^6 cells were intraperitoneally injected in CFA followed by three intraperitoneal injections of 5×10^6 cells emulsified in IFA at 2-wk intervals. 10 d later, 5×10^6 cells were injected intravenously in PBS, and splenocytes were fused after an additional 3 d to NS1 cells as previously described (34). Screening of the hybridomas was carried out by inhibition of the E117 cytotoxicity against the E418 target cells.

Target Cells. 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 (DRW6), MOU (DR7), MADURA (DRW8), ARBO (DRW9), and F601 (DRW10), kindly provided by Dr. J. Colombani (St. Louis Hospital, Paris), were used as targets in cytotoxicity assays.

Cytotoxicity Assays. The cytotoxic activity of the cloned cell lines was measured by a conventional 3-h ^{51}Cr-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-TNKTar) 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.

Immunoprecipitation of the TCR-γ/δ and the TCT.1 Molecule. E102, E117, E418, and K562 cells were surface labeled with ^{125}I using a lactoperoxidase method as described previously (14, 38). Labeled cells were then lysed with phosphate buffer containing 1% Triton X-100 (Sigma Chemical Co., St. Louis, MO). The lysates were precleared several times with Staphylococcus A suspension and irrelevant mAbs before specific immunoprecipitation.

Immunoprecipitation 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). Immunoprecipitation of the TCT.1 molecule from the E418 B cell line was performed using anti-10H3 mAb coupled to protein A beads. K562 cells were used as negative control. SDS-PAGE analysis was carried out using 10% polyacrylamide gels ether under nonreducing conditions or reducing conditions after adding 5% of 2-ME.

Southern Blot Analysis. High molecular weight genomic DNA samples (10 μg) were digested either by EcoRI, BamHI, HindIII, or KpnI restriction enzymes, fractionated in 0.7% agarose gel, and blotted on to Gene Screen Plus nylon membrane (46). Hybridization was carried out at 65°C in 6× SSC, 0.5% SDS, 5× Denhart's, 0.01 M EDTA, and 100 μg/ml denatured salmon sperm DNA. Blots were washed twice at 65°C for 30 min in 0.1× SSC, 0.01% SDS. Vδ1 (a 250-bp EcoRI-BamHI fragment cloned from F6C7 cells) (37), pH60 (a 700-bp EcoRI-HindIII fragment containing Jγ1 segment) (3), VγI (a 1,100 bp SacI fragment containing a Vγ3 segment) (47), and VγIII (a 600-bp PstI-EcoRI fragment containing a Vγ10 segment) (4) probes were labeled by the random priming method using α-[^{32}P]dCTP (48).

Results

Generation of the E102 and E117 γ/δ T Cell Clones. PBMC were extracted by 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 2×10^4 cells/well in the presence of irradiated (10^4 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 found to display a stable CD3$^+$, CD4$^-$, CD8$^-$, TCR-δ1$^+$, δTCS1$^+$, A13$^+$, TiVδ2$^-$, and TiγA$^-$ 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-δTCS1 mAb for an epitope encoded by Vδ1-Jδ1 (and/or possibly Vδ1-Jδ2; see reference 49)-rearranged gene segments (37), anti-A13 and anti-TiVδ2 for peptides encoded by Vδ1 and Vδ2 gene segments, respectively (42), and anti-TiγA 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 PBL 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-α/β$^-$), TCR-δ1$^+$, δTCS1$^+$, A13$^+$, TiVδ2$^-$, TiγA$^-$, 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% TiγA$^+$, 5% TiVδ2$^+$ cells, and <0.5% δTCS1$^+$ cells (data not shown). More generally, note that the δTCS1$^+$/TiγA$^-$ 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 germline configuration, and a 3.3-kb EcoRI band known to include (5, 8) the Vδ1-Jδ1 rearrangement (data not shown). This result is in line with the surface reactivity of the anti-δTCS1 mAb.

To assess the γ chain rearrangements, we used the pH60 probe (a Jγ1 fragment), which hybridizes to both Jγ1 and Jγ2 gene segments (3). There was no detectable rearrangement when HindIII- and EcoRI-digested DNAs were hybridized to this probe (data not shown), indicating that E102 and E117 do not use either the Jγ1 or the Jγ2 gene segments (4). When DNAs were digested with the KpnI restriction enzyme, hybridizations with pH60 led to the detection of the 16-kb Jγ2 germline 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 VγI or the VγIII gene subfamily to JP2 and JP1, respectively (6).

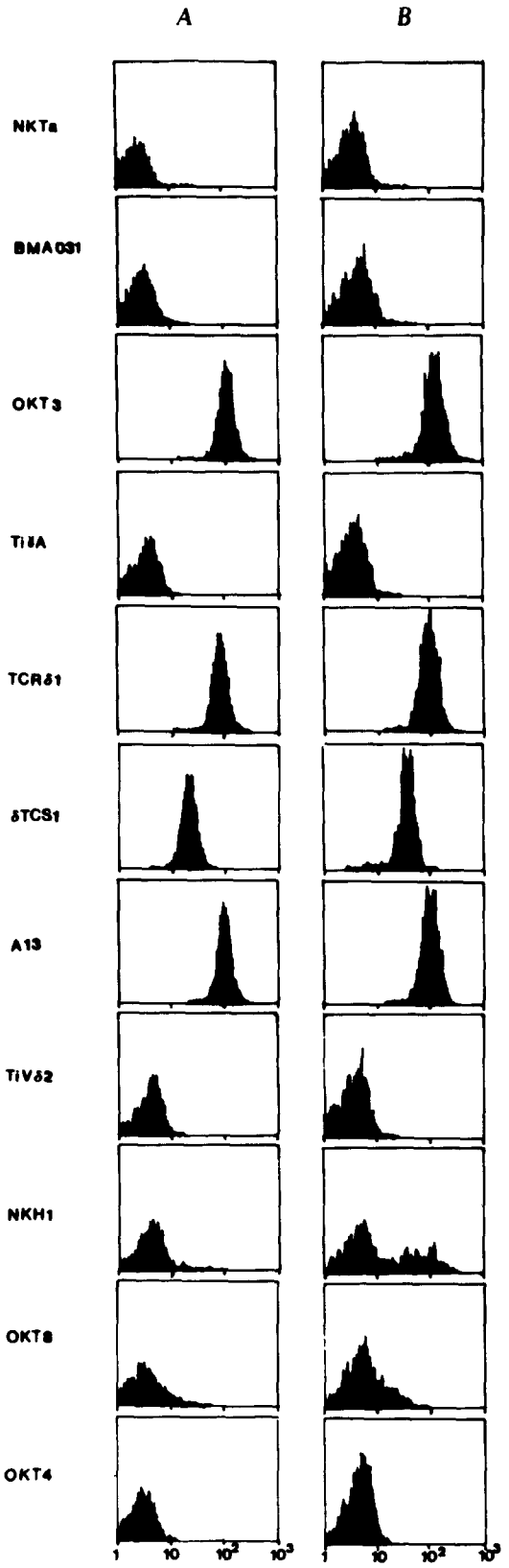

Figure 1. Phenotypic analysis of E102 (*A*) and E117 (*B*) γ/δ T cell clones performed by indirect immunofluorescence experiments.

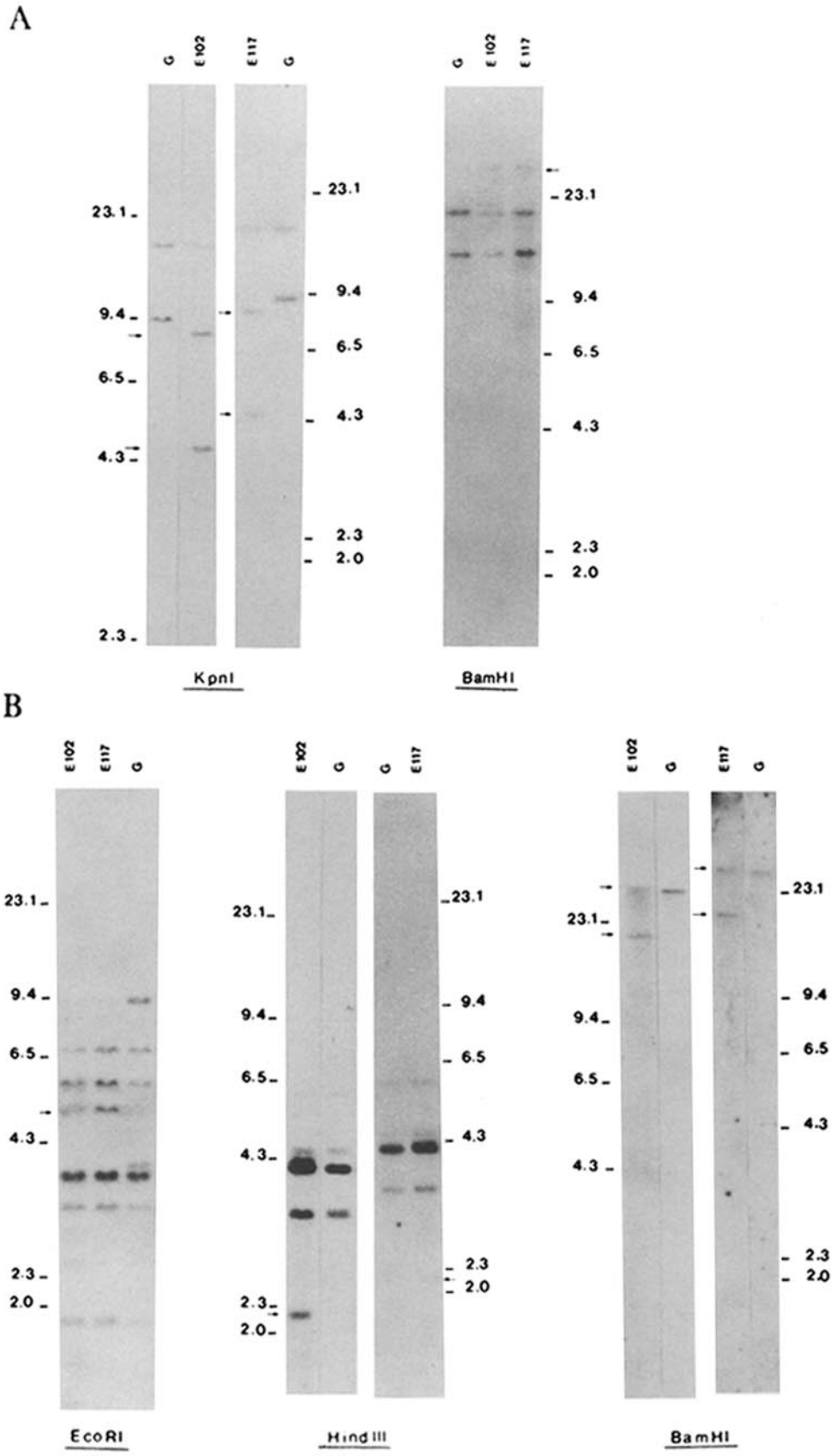

Figure 2. Southern blot analyses of E102 and E117 γ/δ T cell clone DNAs. DNA samples (10 μg) were digested with EcoRI, HindIII, BamHI, or KpnI restriction enzymes, blotted, and hybridized with pH60 (*A*) or VγI (*B*) probes. G corresponds to germline DNA used as controls.

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, is appeared likely that the V segments belong to the Vγ I 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 EcoRI 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 I probe including the Vγ3 segment (47). After EcoRI 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 EcoRI, 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 EcoRI 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 TCR γ 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-TCR-δ1 and the anti-δTCS1 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-Jδ1-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 TCR-γ/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-

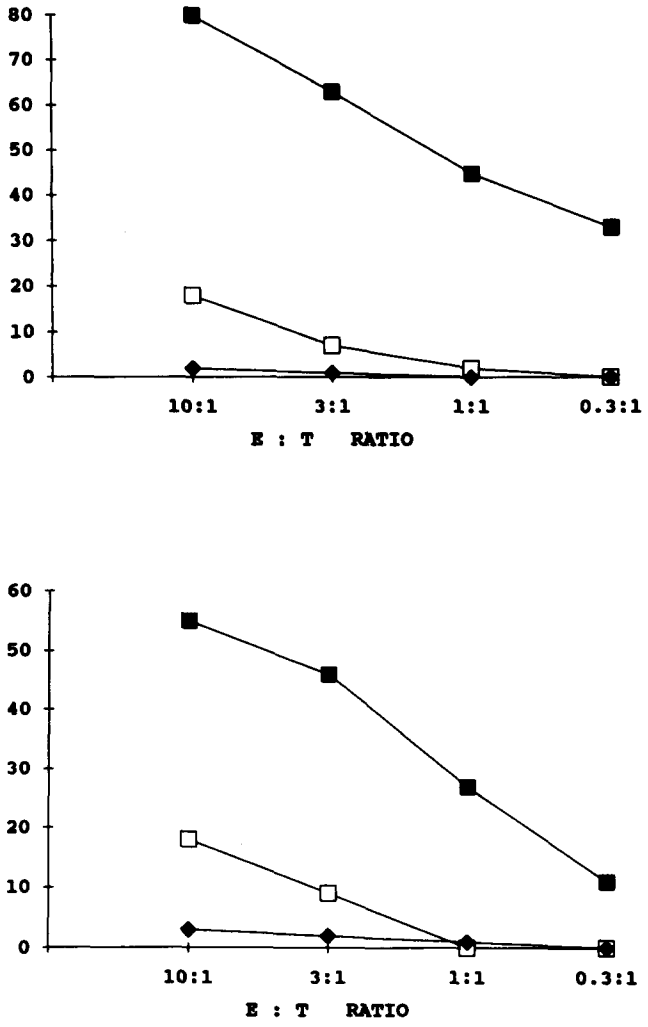

Figure 3. Cytotoxic activity of E102 (*A*) and E117 (*B*) γ/δ T cell clones against E418 EBV-transformed B cell line (■), Daudi (◆), and K562 (□) target cells. E/T ratios were 10:1, 3:1, 1:1, and 0.3:1.

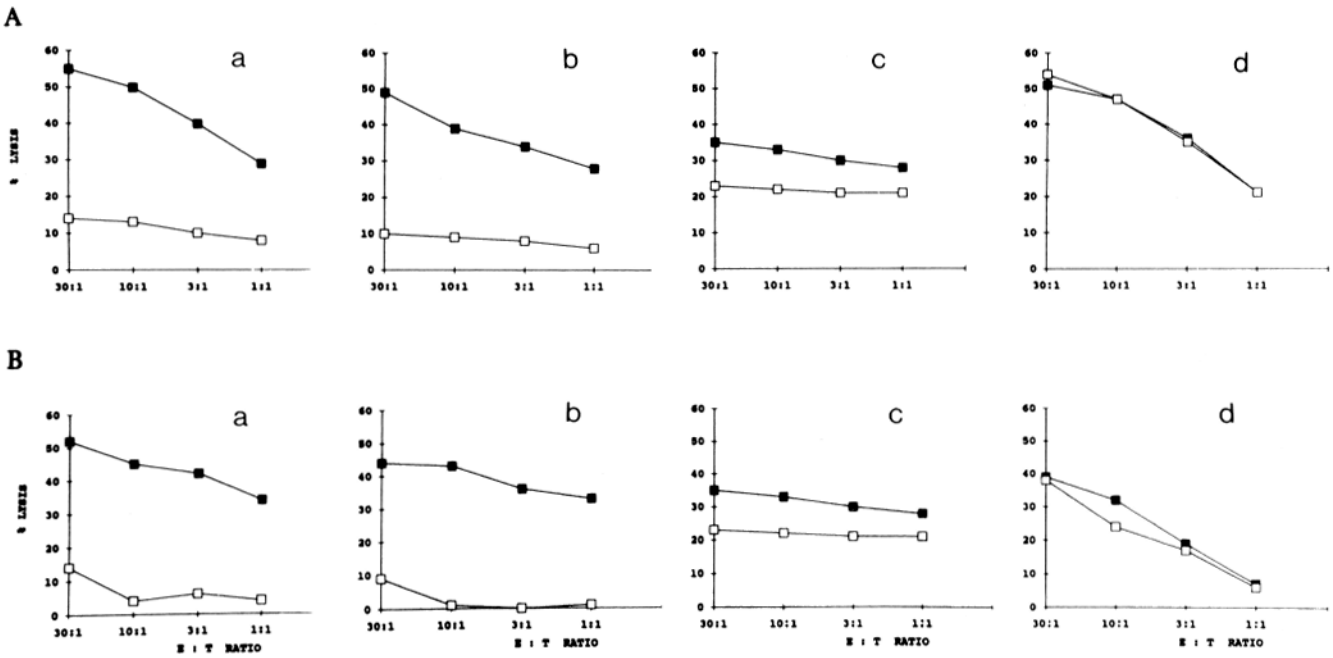

Figure 4. Cytotoxic activity of E102 (*A*) and E117 (*B*) T cell clones against E418 (*a*), F601 (*b*), KAS (*c*), and REX (*d*) target cells at 30:1, 10:1, 3:1, and 1:1 E/T ratios. Cytotoxicity experiments were performed after target incubation either in media (■) or in the presence of the 10H3 mAb (□).

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, ^{51}C-labeled target cells were thus treated for 2 h with individual hybridoma supernatants. One hybridoma, termed 10H3 (IgG1), 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 TCT.1 Molecule. The expression of the molecule, designated TCT.1, 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 TCT.1 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 TCT.1 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 (histocytic 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 TCT.1 expression does not correlate with that of the conventional class I molecules.

Immunoprecipitation experiments were performed with ^{125}I-labeled E418 cells to define biochemically the TCT.1 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 TCT.1 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).

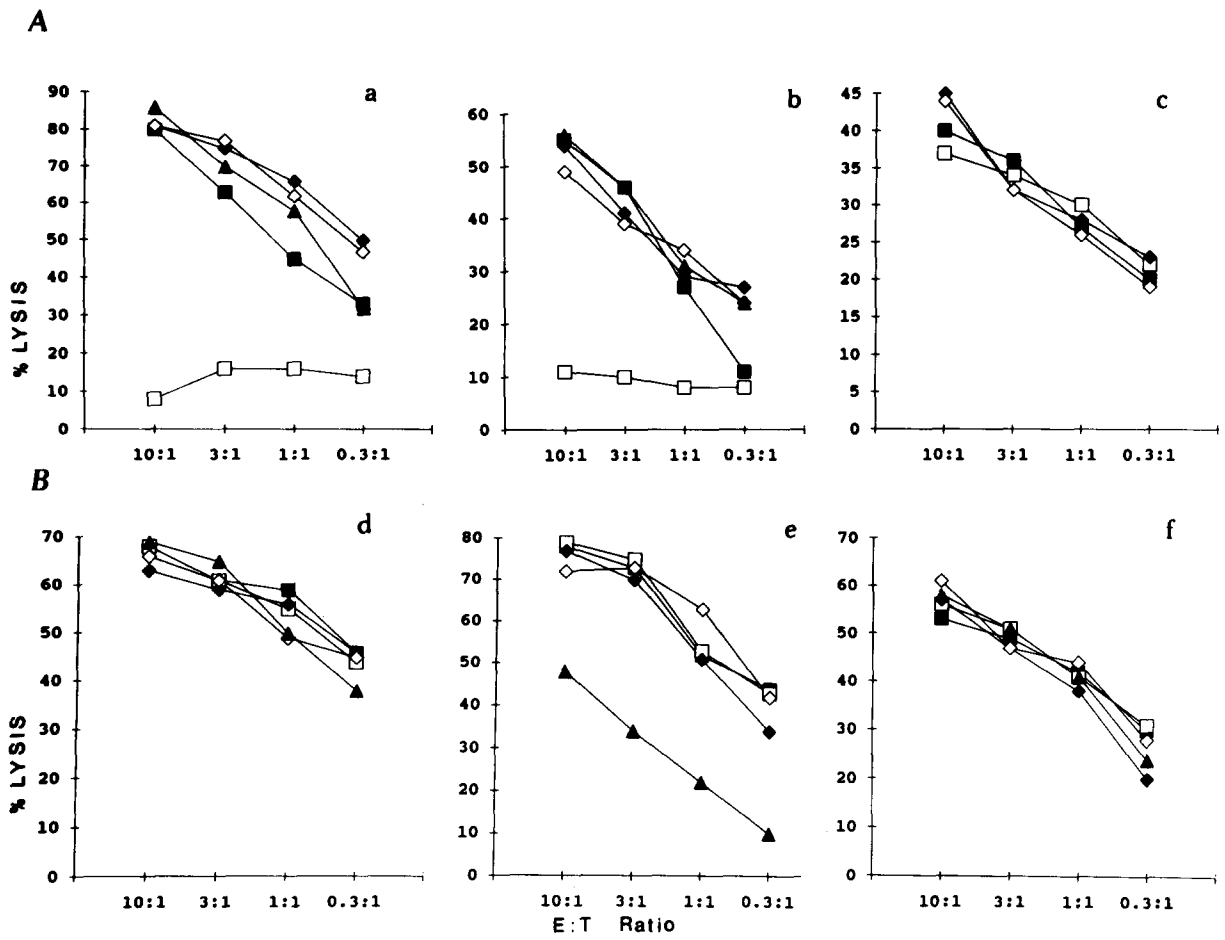

Figure 5. Cytotoxic activity of E102 (*a*), E117 (*b*), and CD3⁻.1 NK cell line (*c*) towards E418 target (*A*); and that of AB12 (*d*), JT9 (*e*), and the CD3⁻.1 NK cells (*f*) towards Daudi target (*B*). E/T ratios were 10:1, 3:1, 1:1, and 0.3:1. Cytolytic experiments were performed either in media (■) or in the presence of mAbs. E418 and Daudi target cells were preincubated for 2 h with saturating concentrations of the 10H3 (□), W6/32 (anti-class I) (◆), 9-49 (anti-class II) (◇), or 2F3 (anti-TNKTar) (▲) mAbs, and then effector cells were added.

Specificity of the Anti-10H3 mAb Inhibitory Effects. Because TCT.1 was found to be expressed by CTL themselves, experiments were performed to assess whether the 10H3 antibody 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 TCT.1 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 γ/δ T cell clone with the predominant peripheral TiγA$^+$/Ti$V\delta2^+$ phenotype [8, 36, 37]), JT9 (an α/β T cell clone defined through the expression of the NKTa clonotypic determinant [34, 35]), and CD3⁻.1, 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⁻.1 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 TCT.1 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 TCT.1$^+$-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.

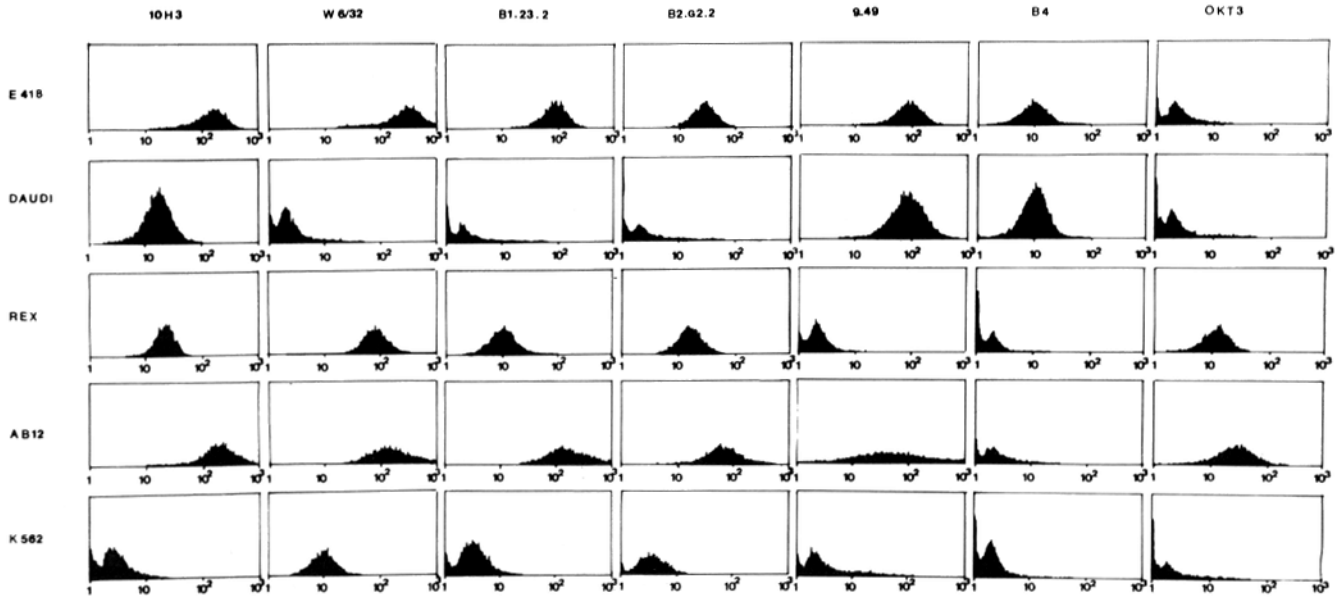

Figure 6. Immunofluorescence analysis of E418, Daudi (B cell lines), AB12 (γ/δ T cell clone), REX (α/β T cell line), and K562 cells with 10H3, W6/32, B1.23.2 (anti-class I H chain), B2.G2.2 (anti-β2m), 9-49 (anti-class II), and B4 (anti-CD20) mAbs. Indirect immunofluorescence studies were performed with saturating concentrations of each mAb and FITC-conjugated goat anti-mouse Ig serum.

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-Cδ- and the Vγ3-JP2-Cγ2-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 β_2m 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 KA 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*	Phenotype	W6/32 reactivity	10H3 reactivity
		%	%
E418	$T3^-/9\text{-}49^+/B4^+$	90 (205)	90 (179)
Daudi	$T3^-/9\text{-}49^+/B4^+$	0	90 (105)
Ramos	$T3^-/9\text{-}49^+/B4^+$	96 (172)	14
REX	$T3^+/9\text{-}49^-/B4^-$	95 (162)	95 (113)
AB12	$T3^+/9\text{-}49^+/B4^-$	94 (185)	94 (192)
E117	$T3^+$/ ND /$B4^-$	ND	97 (173)
HL60	$T3^-/9\text{-}49^-/B4^-$	96 (159)	40 (79)
KG1	$T3^-/9\text{-}49^+/B4^-$	93 (167)	75 (94)
U937	$T3^-/9\text{-}49^+/B4^-$	99 (193)	1
K562	$T3^-/9\text{-}49^-/B4^-$	68 (88)	0
LAL (Iannibe)	$T3^-/9\text{-}49^+/B4^+$	99 (216)	88 (87)
LLC (Helias)	$T3^+/9\text{-}49^-/B4^-$	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§			
Liver		+	−
Kidney		+	−
Ovary		+	−
Placenta		+	−
Pancreas		+	−
Breast		±	−

Cells were analyzed by indirect immunofluorescence (*A* and *B*) or by radioisotope 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).

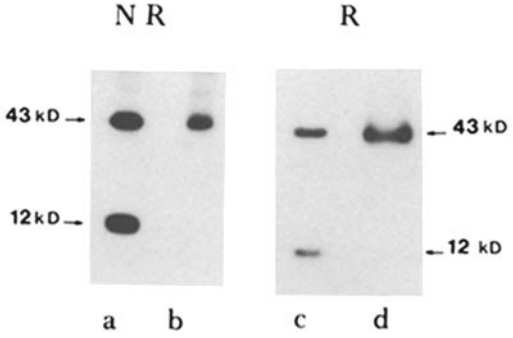

Figure 7. Immunoprecipitation of the class I and the TCT.1 molecules from E418 cell line by W6/32 (lanes *a* and *c*) and anti-10H3 (lanes *b* and *d*) mAbs, respectively. Immunoprecipitated material was analyzed on a minigel by SDS-PAGE under nonreducing (NR) or reducing (R) conditions.

The recognition of the TCT.1 molecule may allow to distinguish the TCR-dependent and the TCR-independent target cell recognition by the E102 and E117 lymphocytes. That TCT.1 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

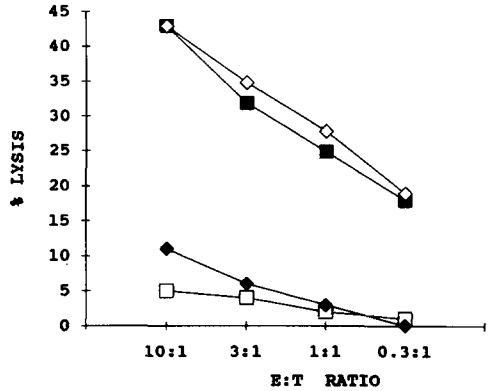

Figure 8. Cytotoxic activity of E102 T cell clone against E418 target cells at 10:1, 3:1, 1:1, and 0.3:1 E/T ratios. Cytotoxicity experiments were performed either in media (■), after incubation for 2 h in the microtiter wells of E418 with the 10H3 mAb before the assay (□), and after target (◆) or effector (◇) cells treatment for 2 h by the 10H3 mAb followed by subsequent washing.

TCT.1 ligand) with highly restricted cellular distribution that would be functionally critical in cell-cell interaction (given the virtual abrogation of cytotoxicity found with certain target cells); (*b*) the blocking activity is dependent upon the use of individual target cells. If the TCT.1/ligand system would operate through a simple model of bimolecular interaction, one would expect that the specific antibody alters the cytotoxic interactions of E102 and E117 cells against all TCT.1$^+$ targets. The data obtained here, particularly in the REX assays, where anti-10H3 has no effect at all, does not favor the latter possibility. A more likely hypothesis is that REX cells are recognized by a distinct mechanism than the EBV-transformed B cell lines, namely in an NK-like TCR/TCT.1-independent fashion. In addition, the varying levels of functional inhibition obtained with the three B cell lines tested further suggest the existence of a complex pathway of TCT.1 recognition. Future studies will have to assess more directly the potential TCR/TCT.1 interaction and to establish whether TCT.1 may serve as a peptide-presenting structure. This will require further experimentation, including extended correlations between relevant TCR structures and TCT.1 involvement, characterization of the TCT.1 molecule by gene cloning, and peptide-dependent induction of target cell recognition.

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.

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