The large granular lymphocyte (LGL) population, which effects a natural killer (NK) function and antibody-dependent cell-mediated cytotoxicity (ADCC, K cell activity). They function in vivo as effectors of natural resistance against foreign cells, certain infectious agents, and tumor cells. The LGL/NK population is heterogeneous and consists of cells sharing phenotypic and functional properties with both T cells and myelomonocytic cells. As a result, their precise lineage derivation and mechanisms of immune function have remained unresolved and their place in hematopoietic differentiation pathways unclear.

The relationship of LGL development and function to the T cell lineage can be approached by analyzing T cell receptor gene rearrangements and expression. Two genes, Tα34 and Tα58, code for the complete T cell antigen receptor molecule in thymocytes and in peripheral blood T cells. Tα gene rearrangements precede Tα gene rearrangements38 and occur in all functional T cell subsets early during thymic differentiation. A third gene, the Tγ gene, isolated from cytotoxic T cells is also a Tα-specific rearranging gene. The Tγ gene is believed to rearrange even earlier in T cell differentiation.

Seven of these cases were reactive with monoclonal antibody WT31, which suggested expression of an αβ heterodimer on the cell surface. The other group (17/17 cases) was CD3+ with unrearranged Tγ genes. These results indicate that the normal LGL/NK population is homogeneous and distinct from the normal T cell population because it does not express, and as a result, cannot effect its immune function through the T cell receptor molecules. Conversely, TγLPDs represent a heterogeneous group of lymphoproliferative diseases within which the TγLPD cases most likely represent the neoplastic counterpart of normal LGL cells. The more frequent CD3+ cases may be related to recently described NK-like T cells. The observations that normal LGLs maintain germline Tγ genes and that many CD3+ TγLPD display an αβ heterodimer suggest that a Tγ-containing receptor may not be necessary for NK or NK-like cytotoxicity. 

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MATERIALS AND METHODS

Isolation of Peripheral Blood LGLs, T Lymphocytes, and Thymocytes

Isolation of LGLs. Normal mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation of fresh heparinized peripheral blood obtained from healthy donors. The peripheral blood
mononuclear cells were depleted of monocytes by plastic adherence and passage over a nylon wool column and were then fractionated on a six-step Percoll gradient ranging from 35% to 47.5%.24 Cells from the low-density fractions (60% to 80% LGLs, 20 × 10^6 cell/mL) were collected and further depleted of mature T cells by repeated 30-minute incubations with monoclonal antibody (MoAb) OKT3 (diluted 1:100; gift of Dr E. Reinherz, Boston) or MoAb Leu-1 (Becton Dickinson, Mountain View, CA) and 60-minute incubations with complement (diluted 1:4, Pel-Freeze Laboratories, Rogers, AR). The purity of each LGL preparation was assessed by morphologic expression of MoAb-defined cell surface antigens, and in vitro cytotoxic activity (see Table 1). Purified fractions of phenotypically distinct LGL/NK subpopulations were then obtained by column immunoabsorption or cell sorting using MoAbs OKT8 and Mol, which identify phenotypically26 and functionally25,29 distinct LGL/NK subsets. MoAb OKT8 recognizes the CD8 antigen, which is present on the mature T cell subset commonly associated with suppressor/cytotoxic function3 and on approximately 30% of LGLs (Table I).26 Mol reacts with the Mol epitope of the C3bi receptor,31 which is present on monocytes, myeloid cells,32 and approximately 70% of LGLs (Table I).26,27,32

T lymphocytes. Peripheral blood T lymphocytes were isolated by fractionation of peripheral blood mononuclear cells on a Ficoll-Hypaque density gradient according to their capacity to form E rosettes with Vibrio cholerae neuraminidase–treated sheep erythrocytes.33 The E' fraction consisted of >95% T cells as determined by CD3 expression. The E' fraction was incubated with OKT8 and OKT4 MoAbs and complement at 37°C to obtain purified fractions of CD4+ and CD8+ cells, respectively. The resulting cell populations were 95% pure as determined by CD4 and CD8 expression.

Thymocytes. Thymocytes were isolated from portions of thymuses collected during the course of cardiac surgery. MoAb-defined cell surface markers and morphology. The surface immunophenotype of the isolated cells was determined by indirect immunofluorescence using a Zeiss microscope and/or a FACSVantage (Becton Dickinson). The following panel of MoAbs was used: OKT3, OKT6, OKT8, OKT11 (Ortho Diagnostic Systems, Inc, Raritan, NJ, or the gift of Dr R. Reinherz, Dana-Farber Cancer Institute, Boston); HNK-1 (courtesy of Dr A. Abol, Birmingham, AL), which is reactive with NK cells and a subset of T cells; B73.1 (courtesy of Dr G. Trinchieri, Philadelphia)3 and AB8.28 (courtesy of Dr Malavasi, Turin, Italy)39 both reactive with a structure closely associated with the Fc, receptor of LGLs; N901, reactive with LGLs and some myeloid precursors (courtesy of Dr J. Griffin, Boston);36 WT31, reactive with a common epitope of the T cell receptor α/β heterodimer (courtesy of Dr W.J.N. Tax, Amsterdam36); Leu-11, reactive with the CD16 antigen; and Leu-7 (Becton Dickinson Monoclonal Center, Inc, Mountain View, CA).

Table 1. Phenotypic and Functional Analysis of LGLs and LGL Subpopulations

<table>
<thead>
<tr>
<th>LGL Population</th>
<th>CD8</th>
<th>CD3</th>
<th>Mol</th>
<th>HNK-1</th>
<th>N901</th>
<th>NK</th>
<th>ADCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGL</td>
<td>30</td>
<td>0</td>
<td>70</td>
<td>65</td>
<td>60</td>
<td>42</td>
<td>22</td>
</tr>
<tr>
<td>LGL-CD8+</td>
<td>97</td>
<td>0</td>
<td>44</td>
<td>61</td>
<td>36</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>LGL-CD8−</td>
<td>&lt;5</td>
<td>0</td>
<td>44</td>
<td>38</td>
<td>ND</td>
<td>41</td>
<td>16</td>
</tr>
<tr>
<td>LGL-Mol+</td>
<td>ND</td>
<td>0</td>
<td>99</td>
<td>46</td>
<td>ND</td>
<td>46</td>
<td>23</td>
</tr>
<tr>
<td>LGL-Mol−</td>
<td>31</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>ND</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>45</td>
<td>98</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

*Numbers represent the percentage of positive cells for each of the MoAb stainings.
†Results are expressed as means and refer to an E:T ratio of 50:1.

Cytotoxicity assays. NK activity was assessed by using the K562 cell line as effector-target cell (E:T) ratios ranging from 25:1 to 100:1 in a four-hour test with 10°Cr release assay as previously described.24 For ADCC,5°Cr-labeled TLX9 murine lymphoma cells sensitized with 1:10,000 rabbit antibody were used as targets in a four-hour test.

DNA extraction and Southern blot analysis. DNA was prepared from cell lysis, proteinase K digestion, extraction with phenol, and precipitation with ethanol.39 Fifteen micrograms of DNA were digested with the appropriate restriction endonuclease, electrophoresed in a 0.8% agarose gel, denatured, neutralized, transferred to a nitrocellulose filter, and hybridized and washed according to Southern.39 Hybridized filters were boiled for five minutes in distilled H2O before hybridization to a second DNA probe.

RNA extraction and Northern blot analysis. Total cellular RNA was extracted according to the guanidine-isothiocyanate method.38 Five to 10 µg of purified total RNA were electrophoresed in a 1% formaldehyde–containing agarose gel, transferred to nitrocellulose, hybridized, and washed according to established procedures.39

DNA Probes

Tα probe. The Tα probe used in this study was derived from a human Tα cDNA clone (YTJ-2) isolated from the Jurkat-T2 T lymphoma cell line (courtesy of Dr Tak Mak).3 This clone hybridizes to both alleles (Cα, and Cα2) of the constant region and to one or more alleles of the variable regions.34

Tβ probes. Three different Tβ probes were used in this study. The Ca/Cβ and V2 probes were derived from two human Tα cDNAs. The F7 cDNA isolated from the frez2.2 T lymphoma cell line represents an aberrantly rearranged Tα allele.35 The V2 probe is representative of the variable region of F7 cDNA and belongs to the Vβ1 variable region family.36 The Tα, cDNA isolated from a peripheral T cell library represents a truncated Tα constant region. The Ca/Cβ probe represents the first exon of Tα cDNA (M. Subar et al, submitted for publication). The pJx2 probe is a genomic probe derived from the G2.0 T, genomic phage isolated from a Charon 4A human placental library. It represents a unique EcoR1-HindIII fragment containing the putative J region of the F7 cDNA clone.37 The positions of these three probes with respect to the F7,7 and Tα cDNA clones and the human Tα gene are schematically represented in Fig 1. DNA fragments were labeled by nick-translation for use as probes.

RESULTS

Analysis of T Cell Receptor (α,β) Gene Rearrangements and Expression in LGL/NK Cells and Subsets

Freshly isolated peripheral blood LGLs display the germ-line organization of the Tα gene but express a truncated version of Tα mRNA.36,37 However, the majority of clonal LGL/NK cell populations display Tα gene rearrangements.15,19 Because T cells invariably display Tα gene rearrangements and expression, these data do not permit an unequivocal understanding of the relationship between LGL/NK cells and T cells. To clarify this issue, we tested the hypothesis that minor LGL/NK subpopulations may display T cell receptor gene rearrangements. This hypothesis is particularly relevant in view of the fact that (a) a minor LGL/NK subpopulation (30%, see Table 1) is identifiable by the expression of the T cell–specific CD8 antigen and (b) a minor LGL/NK subpopulation carrying Tα gene rear-
rangement could escape analysis when the total LGL/NK population was examined. For these reasons, we analyzed the organization and expression of T cell receptor α and β genes in phenotypically distinct LGL/NK cell subpopulations as well as in unFractionated normal LGL/NK cells.

The genomic organization of the Tβ gene was investigated by Southern blot hybridization using a DNA probe specific for the human Tβ gene locus. We had previously shown that hybridization of this probe to EcoRI-digested DNAs allows the detection of Tβ gene rearrangements in both monoclonal and polyclonal T cell populations. The biallelic rearrangement of part of the constant region (TβC) can be recognized as a loss of the 12.0-kb EcoRI band corresponding to the Cβ1 region in normal, polyclonal human thymocytes and T lymphocytes (see Fig 2, lane T-L). When the total LGL/NK population was studied in this manner, the Tβ gene was found in its unrearranged germline configuration. Identical results were obtained in purified CD8+ and Mol+ LGL/NK subpopulations (Fig 2). In the experiment shown in Fig 2, LGLs were pretreated with anti-CD3 to remove possible conventional T-cell contaminants, but similar results were obtained with anti-Leu-1 (CD5)-treated cells or with the untreated Percoll-purified population. Comparative densitometric analysis of the EcoRI 12.0-kb band v the other bands in LGL/NK and control cells confirmed that there is no appreciable loss of the Cβ1 allele in the different LGL/NK populations analyzed (data not shown). Therefore, we conclude that the Tβ gene remains unrearranged in the vast majority of normal LGL/NK cells.

We next investigated Tα gene expression in freshly isolated LGL/NK cells because a molecular assay to detect Tα gene rearrangements in normal polyclonal cells is not yet available. RNA was extracted from purified LGLs and from the CD8+ LGL/NK subpopulation and analyzed by Northern blot hybridization using a Tα probe (clone PY1.4, a gift from T. Mak). Tα RNA was not detectable in either sample (see Fig 3, middle lane). The same RNA blots were used for hybridization with a human Tβ probe (Fig 3, first lane), and no specific hybridization signal was detected. The absence of the CD3 antigen and Tβ and Tα gene expression in LGL/NK cells proves that LGL/NK cells do not express an α/β heterodimer on their surface.

Analysis of Tγ Gene Rearrangements and Expression in Normal LGLs, LGL Subpopulations, and T Cells

The lack of Tβ gene rearrangements and Tα/Tα gene expression in the majority of LGL/NK cells suggests that the “conventional” T cell antigen receptor is not involved in NK target cell interaction. However, the ontogenic relationship between LGL/NK cells and T cells remains unresolved because Tα and Tα genes are neither rearranged nor expressed in early thymocytes and both early thymocytes...
and the majority of LGL/NK cells lack CD3 antigen expression and express the CD8 antigen. We then chose to analyze LGL/NK cells for rearrangement and expression of a third T cell receptor gene, the T- gene, which is believed to rearrange early during T cell differentiation before the T- and T- genes and may therefore represent a marker for the earliest stages of the thymic differentiation pathway. The T- gene has been reported to remain in its germline organization in the total LGL/NK population. However, this analysis was limited to the investigation of T- gene rearrangements involving the T-1 or T-2 regions. We have recently shown that T- rearrangements involving previously unidentified T- regions (Jx2; M. Subar et al, submitted for publication) may occur in 25% of T cells and up to 30% of pre-B cells.

Our experimental approach involved Southern blot hybridization analysis of T- gene organization in DNAs extracted from the total LGL/NK cell population as well as the CD8 and CD8 LGL/NK subpopulations. The results were compared with Southern blot analyses of the T- gene organization in DNAs extracted from normal polyclonal T cell populations. The assay for identification of different patterns of T- gene rearrangements was based upon results of the analysis of T- gene rearrangements in a large variety of clonal T cell populations (M. Subar et al, submitted for publication). A diminution in the intensity of the 5.5-kb EcoRI band hybridizing to a T first-exon probe (Ca/Cb) in all polyclonal T cells (see Fig 4A) corresponds to deletions of the T-C1 locus, which are constantly associated with rearrangements of the T-C2 locus (M. Subar et al, submitted for publication). The appearance of a 7.5-kb Stu1 band hybridizing to the T- variable region probe (see Fig 4B) reflects rearrangements involving a member of this variable region family and is seen in polyclonal T cells as in the majority of clonal T cell populations (M. Subar et al, submitted for publication). The appearance of a few faintly hybridizing, Jx2-rearranged bands corresponds to T-C1 rearrangements (see Fig 4C).

The T- gene was present in its germline configuration in all DNAs extracted from polyclonal LGL/NK cells and the CD8 and CD8 LGL/NK subpopulations. Representative Southern blots for the CD8 and CD8 LGL/NK DNAs are shown in Fig 4. We also analyzed the expression of the T- gene in the total LGL/NK population and in LGL/NK subsets. No specific hybridization signal was detected in any of the samples (for representative results, see Fig 3, right lane). We conclude that LGL/NK cells and CD8 and CD8 LGL/NK cell subpopulations are clearly distinct from both mature and immature T cells with respect to the organization of the T- gene and that the T- gene is not rearranged or expressed in the majority of, if not all, LGL/NK cells.

**Gene Rearrangements and Expression Analysis of T Cell Receptor Genes in Human LGL/NK-Derived Leukemias**

An approach commonly used to investigate properties of a normal cell population is to analyze its clonally expanded neoplastic counterpart. This is based on the concept that malignant transformation results in freezing the target cell at a given stage in its normal differentiation pathway, which
results in a uniform phenotype for the neoplastic cell population. For this reason, we analyzed the genomic organization of the T cell receptor genes $\beta$ and $\gamma$ in a collection of 17 LGL leukemias, so-called T$\gamma$ lymphoproliferative disorders (T$\gamma$LPD).42

The principal immunophenotypic, functional, and immunogenotypic properties of 12 of these 17 cases of T$\gamma$LPD have been reported in detail.16 The five additional cases of T$\gamma$LPD shown in this study displayed similar phenotypic and functional features including CD2, HNK-1, AB8.28, and B73.1 expression; Fc receptors identified by rosetting technique; and ADCC effector function (data not shown). Surface markers CD3, CD8, OKM1, and N901 were detected in the LGLs isolated from some but not all cases (data not shown). In particular, two cases did not express the CD3 antigen (Table 2). LGLs from all T$\gamma$LPD patients had high ADCC, whereas high NK activity was only detected in six cases (Table 3).16 Fifteen cases displayed clonal rearrangements of the T$\gamma$ gene, and two cases displayed the germline configuration of the T$\gamma$ gene upon EcoRI and/or BamHI digestion and hybridization to a T$\gamma$ probe (data not shown). The two cases displaying the T$\gamma$ gene germline organization were the same two cases that lacked the CD3 antigen.

All 17 cases of T$\gamma$LPD were analyzed for the organization of the T$\gamma$ gene. Representative Southern blots of this analysis are shown in Fig 5, and a synthesis of the results is reported in Table 2. We detected T$\gamma$ gene rearrangements in 15 of 17 T$\gamma$LPD cases tested using the same combination of restriction enzymes and probes described for the analysis of T$\beta$ gene

**Table 2. Phenotypic and Immunogenotypic Analysis of T$\gamma$LPDs**

<table>
<thead>
<tr>
<th>No. Cases</th>
<th>CD3 Antigen Expression†</th>
<th>T$\gamma$ Gene‡</th>
<th>T$\gamma$ Gene‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>–</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>R</td>
<td>R (T$\gamma$1)</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>R</td>
<td>R (T$\gamma$1)</td>
</tr>
</tbody>
</table>

† + represents >20% positive cells after staining with OKT3 MoAb.
‡ G, germline; R, rearranged.

**Table 3. Phenotypic and Functional Analysis of T$\gamma$LPD Mononuclear Cells**

<table>
<thead>
<tr>
<th>Phenotypic Markers*</th>
<th>Cytoxicity‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case No.</td>
<td>WT31</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>95</td>
</tr>
<tr>
<td>8</td>
<td>76</td>
</tr>
<tr>
<td>9</td>
<td>77</td>
</tr>
<tr>
<td>11</td>
<td>85</td>
</tr>
<tr>
<td>14</td>
<td>51</td>
</tr>
<tr>
<td>15</td>
<td>56</td>
</tr>
</tbody>
</table>

*Results are expressed as percentages of positive cells.
†Results are expressed as means and refer to an E:T ratio of 50:1.

**Fig 5.** T$\gamma$ gene rearrangement analysis of T$\gamma$LPDs. DNAs were extracted from leukemic LGLs of different T$\gamma$LPD patients, digested with the indicated restriction enzyme, and hybridized to the indicated probe. Arrows indicate rearranged bands. Lane C, human fibroblast DNA.
In 12 of 17 TγLPD cases tested, the Ca/Cb hybridizing 5.5-kb EcoR1 band corresponding to exon I of the Tγ1 gene appeared deleted or fainter than the respective control bands, which suggested a deletion of that DNA region on one or both alleles (see Fig. 5A, lanes 1 and 2, for representative results displaying the biallelic or monoallelic deletion of the Tγ1 gene, respectively). The same DNA bands were digested with the Ssrl restriction enzyme and hybridized to the Ca/Cb probe to detect rearrangements involving the J1 or J2 regions (for a map of the Tγ locus, see Fig. 1A). One or two rearranged bands were detected in each of these 12 cases (see Fig. 5B, lanes 1 and 2). Hybridization of the same blots to a Tγ variable region probe (V2) showed the appearance of one or two rearranged bands (Fig. 5D, lanes 1 and 2) with at least one rearranged band containing both V2 and Ca/Cb hybridizing sequences as indicated by arrows in Fig. 5B and D. We conclude that 12 TγLPD cases displayed rearrangements occurring in the Tγ2 5' flanking regions of the Tγ1 locus. A new Ssrl restriction fragment hybridizing to the pJx2 probe were detected in three TγLPD cases, which suggested the occurrence of Tγ1 gene rearrangements (Fig. 5C, lane 3). Hybridization of the same blots to a Tα variable region probe (V2) showed that the new bands also contained variable region sequences as indicated by arrows in Fig. 5C and D. Finally, in two cases (Table 2), the Tγ gene was found in its germline configuration using all of the combinations of restriction enzymes and probes described (see Fig. 5A, B, C, and D, lane 4 for a representative case). As in the case of the Tα gene, these two cases also lacked the CD3 antigen (Table 2). We conclude that the Tγ gene is rearranged in all CD3+ cases of TγLPD but it is retained in its germline configuration in the less frequently occurring CD3− cases of TγLPD. In addition, Tγ gene rearrangements using Tγ1/C1 or Tγ2/C2 are present in CD3+ TγLPD cases in similar proportions to those observed in T cell tumors.

Two and one representative cases of CD3− and CD3+ TγLPD respectively were also analyzed for T cell receptor gene expression. The two CD3− TγLPD cases displayed expression of full-length mRNAs from Tα, Tγ, and Tγ, whereas the CD3+ TγLPD case displayed no hybridization signal for all the T cell receptor gene probes (data not shown).

In an effort to elucidate whether the CD3+ TγLPD with rearranged and expressed T cell receptor genes produces a "conventional" cell surface α/β heterodimer or the recently described Tγ-containing T cell receptor,43-45 we investigated seven cases of CD3+ TγLPD with MoAb WT31, which reacts with a common epitope of the α/β heterodimer but not with Tγ, Tγ, Tγ, or Tγ, cells.7,43-45 In preliminary experiments we confirmed that CD3+ clones were WT31+.43-45 As summarized in Table 3, all seven cases were WT31+. In case 15, in spite of the monoclonal nature of the CD3+ expansion revealed by Southern blot analysis (see earlier), a fraction of CD3+ cases were not appreciably stained by the WT31 MoAb. It is of interest in this context that all TγLPD cases were CD8+ (as usually observed)16-42 and that T cell clones expressing a Tγ-containing receptor are CD8−, CD4+.43-45 Thus, the observation of WT31 reactivity together with the finding of Tγ and Tγ mRNA and CD8 expression suggests that the majority of TγLPD express a "conventional" α/β heterodimer on their surface.

**DISCUSSION**

LGL/NK cells constitute a heterogeneous population that cannot be clearly assigned to a specific lymphoid lineage. We sought to clarify the functional and ontogenetic relationship between LGL/NK cells and T cells by analyzing the organization and expression of the T cell receptor genes β, α, and γ in unfractioanted normal circulating LGL/NK cells, in phenotypically distinct normal LGL/NK cell subsets, and in neoplastic LGL/NK cell proliferations (TγLPD). The normal circulating LGL/NK cells and the phenotypically distinct normal LGL/NK subsets that we analyzed, including the T cell–like CD8+, CD2+ subset, displayed the germline configuration and/or lack of expression of the T cell receptor β, α, and γ genes. The lack of Tγ/Tγ rearrangement/expression together with the lack of CD3 antigen expression confirms the notion that LGL/NK cell immune function is clearly distinct from T cell immune function. The lack of Tγ, gene rearrangement/expression in LGL/NK cells and subsets strongly suggests that LGL/NK cells are ontogenetically distinct from T cells because Tγ gene rearrangements are believed to represent a very early event during thymic development.44 Taken together, these data favor the hypothesis that LGL/NK cells represent a cell lineage that is independent and distinct from the T cell lineage. In further support of this conclusion, it has recently been shown that C.B-17 scid mice that lack functional T and B cells and whose bone marrow fails to repopulate the thymus have a normal content of NK progenitor cells in the bone marrow.45 TγLPD are believed to represent the transformed counterparts of normal LGL/NK cells. Yet, we observed heterogeneity of the TγLPD cases analyzed with respect to their expression of the CD3 antigen and the organization of the Tγ and Tγ loci. The minority of cases (2/17) were CD3− and shared important immunogenotypic, phenotypic, and functional properties with normal circulating LGL/NK cells. They showed germline organization of the Tγ and Tγ genes, an absence of CD3 antigen expression, expression of the Fcγ receptor, and in vitro NK/ADCC activity. Thus, these cases may represent the neoplastic correlate to the major circulating LGL/NK cell population. The remaining 15 cases of TγLPD displayed a combination of the phenotypic, functional, and immunogenotypic properties seen in both T cells and LGL/NK cells but distinct from any single subset of LGL/NK cells that we have analyzed (Tables 1 and 2). Specifically, they all showed Tγ and Tγ gene rearrangements and CD3 antigen expression, typical of mature T cells. They also expressed the Fcγ receptor and exhibited NK and/or ADCC activity, typical of LGL/NK cells.
On the basis of the combined functional, phenotypic, and genotypic analysis of normal and neoplastic T lymphocytes and LGL/NK cells, three distinct populations can be identified: (a) major histocompatibility complex (MHC)-restricted T cells and peripheral T cell neoplasms, CD3⁺, FCₐ⁺, Tₐ⁺, T₄⁺, ADCC⁺; (b) MHC-nonrestricted LGL/NK cells and two of 17 T₄LPD, CD3⁺, FCₐ⁺, Tₐ⁺, T₄⁺, ADCC⁺; and (c) 15 of 17 T₄LPD, CD3⁺, FCₐ⁺, Tₐ⁺, T₄⁺, ADCC⁺. This raises the question of the relationship of the majority of T₄LPD populations, the CD3⁺ FCₐ⁺ Tₐ⁺ T₄⁺ Tₕ⁺, to normal T cells and/or LGL/NK cells. They may represent T⁺ cells or LGL/NK cells that have acquired atypical properties during the transformation process or the neoplastic expansion of a separate normal cellular counterpart. Although several authors have reported that FCₐ⁺ receptors are not present on the surface of CD3⁺ T lymphocytes isolated from peripheral blood, concanavalin A-activated cultures, or mixed lymphocyte response cultures, the coexistence of FCₐ⁺ receptors and CD3 antigen has been reported in selected conditions, eg, the peripheral blood of patients with lymphoproliferative disorders or in a variety of in vitro cultured T cell clones (NK-like clones). This functionally unique subset of cytotoxic mononuclear cells, identified by the coexpression of the CD3 antigen and the FCₐ⁺ receptor, may be the normal counterpart of the CD3⁺, FCₐ⁺ T₄LPD. In normal peripheral blood CD3⁺, FCₐ⁺ cells represent a minor, usually undetectable (<1%) lymphoid population, which, given its low frequency, was not amenable to analysis by the Southern and Northern blot techniques used in the present study.

The ontogenic relationship between the CD3⁺, FCₐ⁺ cell type (T₄LPD or NK-like T cells) and T cells or LGL/NK cells bears consideration. Conflicting data have been reported with respect to this issue. Hercend et al reported the isolation of NK-like T cell clones from peripheral T cells, which lent credence to the hypothesis of an ontogenic relationship between NK cells and NK-like T cells. Several recent papers have described a small population of CD3⁺, WT3¹⁺, CD4⁺, CD8⁻ T lymphocytes that have rearranged Tₐ genes, express T protein on the cell surface, and display non-MHC-restricted cytotoxic activity and ADCC. These findings raise the question of the role of the Tₐ gene product in NK or NK-like cytotoxicity. Our observations that LGLs, which account for virtually all peripheral blood NK activity, maintain the Tₐ gene in its germline configuration and that CD3⁺, CD8⁻, T₄LPD with non-MHC-restricted cytotoxicity express Tₐ and Tₕ mRNAs and display WT3¹ reactivity suggest that the expression of a Tₐ-containing antigen recognition structure is not an absolute requirement for non-MHC-restricted cytotoxicity.

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T cell receptor (alpha, beta, gamma) gene rearrangements and expression in normal and leukemic large granular lymphocytes/natural killer cells

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