T Cell Receptor Complexes Containing FcEREIγ Homodimers in Lieu of CD3ζ and CD3η Components: A Novel Isoform Expressed on Large Granular Lymphocytes

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Summary

CD3ζ and CD3η form disulfide-linked homo- or heterodimers important in targeting partially assembled Tiα-β/CD3γδε T cell receptor (TCR) complexes to the cell surface and transducing stimulatory signals after antigen recognition. Here we identify a new TCR isoform expressed on splenic CD2⁺, CD3-/Tiα-β⁺, CD4⁻, CD8⁺, CD16⁺, NKI.1⁺ mouse large granular lymphocytes (LGL), which are devoid of CD3ζ and CD3η proteins. The TCRs of this subset contain homodimers of the γ subunit of the high affinity receptor for IgE (FcEREIγ) in lieu of CD3ζ and/or CD3η proteins. The LGL display natural killer-like activity and are cytotoxic for B cell hybridomas producing anti-CD3e and anti-CD16 monoclonal antibodies, demonstrating the signaling capacity of both TCR and CD16 in this cell type. These findings provide evidence for an additional level of complexity of TCR signal transduction isoforms in naturally occurring T cell subsets.

T he TCR has been described as a multimolecular complex formed by three groups of transmembrane proteins: (a) the clonotype antigen/MHC recognition unit, termed Tiα-β (or Trγ-δ) heterodimer (1-3); (b) the highly homologous CD3γ, CD3δ, and CD3ε subunits (4-8); and (c) the structurally distinct CD3ζ and CD3η subunits, alternatively spliced products of a common genetic locus (9-12). FcEREIγ, an essential component of FcEREI and the transmembrane type FcγRIII (CD16) (13), has significant structural homology to CD3ζ and CD3η (9, 10, 14, 15) and is encoded on the same chromosome (mouse chromosome 1), suggesting that CD3ζ/η and FcEREIγ are derived from a common ancestral gene (11, 12, 16, 17). In addition, CD3ζ can substitute for FcEREIγ to form a high affinity IgE receptor on Xenopus oocytes injected with mRNAs for FcEREIα, FcEREIβ, and CD3ζ in the absence of FcEREIγ (18). Moreover, in the CTLL cell line, CD3ζ, CD3η, and FcEREIγ genes are coexpressed and their proteins form atypical disulfide-linked dimers in the TCR complex of that cell (19). These in vitro results suggested to us that subunits other than CD3ζ/η might be incorporated into a functional TCR. To investigate this possibility and determine whether heterogeneity in TCR signal transduction subunits exists within physiologic cell populations, we focused our attention on a subset of T lymphocytes with unusual phenotypic and functional attributes and the morphology of LGL. The results identify among splenocytes a population of IL-2-responsive T cells expressing a “nonconventional” functional TCR isoform containing an FcEREIγ homodimer in lieu of CD3ζ and η subunits.

Materials and Methods

Flow Cytometric Analysis. LGL cells (2-3 x 10⁶ cells/ml) were stained with RM2-1 (anti-CD2; 10 μg/ml), 2C11 (anti-CD3ε; 10 μg/ml), H57-597 (anti-Tia-13; 10 μg/ml), 3A10 (anti-Ti3,-8; 10 μg/ml), GK1.5 (anti-CD4; 10 μg/ml), ADH4 (anti-CD8; culture supernatant), 2.4G2 (FcγRII/III; 10 μg/ml), and PK136 (NKI.1; culture supernatant) followed by FITC-conjugated second antibodies and analyzed on an Epics V cell sorter. Percent reactivities were 17% for CD2, 92% for CD3, 88% for Tia-13, 0% for Ti3,-8 and CD4, 7% for CD8, 83% for FcγRII/III, and 88% for NKI.1. Note that the percent CD2 reactivity is misleading since essentially all LGL express low levels of CD2. Although most LGL are CD8⁻, a small fraction of the cells (5-15%) were found to be CD8⁺.

Polymerase Chain Reaction Analysis. For PCR analysis, a cDNA copy was produced from 15 μg of total cellular RNA using an oligo(dT) primer and AMV reverse transcriptase (Molecular Genetics Resources, Tampa, FL). 10% of the product was used as a template for PCR using the sense amplimer 5’GTTGCAATAGCTGGAGGAAC3’ located at base pairs 470-488 of FcγRIII and the
cDNAs produce DNA fragments of 484 and 345 bp, respectively. The PCR product of FcγRIII is a 269-bp fragment. To identify FcγRIIb and FcγRIIa, the sense amplimer was used with the antisense amplimer 5'GGAGGCACATCACTAGGGAGY at base pairs 738-714 in the transmembrane region of FcγRIII (numbers based on reference 20). Amplification of FcγRIIb and FcγRIIa cDNAs produce DNA fragments of 484 and 345 bp, respectively. For PCR, the denaturing, annealing, and extension were performed at 94°C for 1 min, 60°C for 1 min, and 72°C for 0.5 min, respectively, on a Techne thermocycler using the Gene Amp Kit reagents (Perkin Elmer Cetus, Norwalk, CT) for 35 cycles. The products were run on a 2% agarose gel, blotted to Zeta-Probe membrane, and exposed at -70°C for the indicated time.

**Results and Discussion**

Splenocytes from B10.BR and C3H/HeJ mice cultured for several weeks in the presence of rIL-2 as described (24) express both Tac-β and CD16. This result is of note since these two structures are mutually exclusive in cellular distribution with rare exception (25, 26). As shown in Fig. 1 A, IL-2-dependent LGL from B10.BR are CD2⁺, CD3⁺, Tac-β⁺, Thy-1⁺, CD4⁺, CD8⁺, FcγRI⁺, NK1.1⁺. The phenotype of LGL from C3H/HeJ is identical except for the absence of the allelic NK1.1 marker, which is not expressed in the C3H/HeJ strain (27). Because antibody 2.4G2 does not distinguish between FcγRII and FcγRIII, PCR analysis was used. PCR with specific oligonucleotide amplimers shows that FcγR on the LGL are exclusively of the FcγRIII isoform, the mouse homologue of CD16 (Fig. 1 B) while C1.MC/57.1 mast cells express readily detectable FcγRI (data not shown). Flow cytometric analysis using mAbs against three different Vβ gene products showed that the LGL are polyclonal (Table 1). Consistent with this finding, we detected usage of multiple Vβ segments in both Vβ6⁺ and Vβ8⁺ populations using PCR analysis with amplifiers for Vβ and Jβ regions (L. D'Adamio, manuscript submitted for publication) (data not shown). These results indicated that the LGL populations are not restricted to a specific Vβ segment.

1 Abbreviation used in this paper: 2-D, two-dimensional.
Figure 1. Characterization of LGL from B10.BR mice. (A) Flow cytometric analysis of surface antigens on B10.BR LGL. (B) Analysis of FcyR isotype by PCR. Reverse PCR was performed with RNAs from C1.MC/57.1 (mast cell line; FcyRII=, FcyRIIb=), B10.BR LGL, and S49 (T lymphoma; FcyRII=, FcyRIIb=) using specific oligo primers to FcyRIII (CD16; previously denoted as FcyRIla) or FcyRIIb (previously denoted as FcyRIIb1 and FcyRIIb2). PCR products were blotted and probed with specific internal oligonucleotides. Numbers on the left side indicate the molecular weight markers in base pairs. (C) Northern blotting analysis of FceRI expression. 10 μg of total RNA from a T cell hybridoma (2B4.11), a mast cell line (C1.MC/57.1), and B10.BR LGL were size fractionated and transferred onto a nitrocellulose filter. The filter was hybridized with a specific probe for FceRIγ. The positions of 18S and 28S ribosomal RNAs are indicated. (D) RNase protection analysis of CD3ζ/η mRNA. 15 μg of total RNA from thymocytes and B10.BR LGL were analyzed for CD3ζ/η mRNAs by RNase protection analysis. 173 and 100 bp signals represent CD3ζ and CD3η, respectively. Exposure times for RNAs from thymus and LGL are 6 and 15 h, respectively.
Table 1. Vβ Usage in LGL Determined by Flow Cytometry

<table>
<thead>
<tr>
<th>LGL</th>
<th>Vβ8</th>
<th>Vβ6</th>
<th>Vβ3</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10.BR no. 1</td>
<td>77</td>
<td>&lt;1</td>
<td>3</td>
</tr>
<tr>
<td>B10.BR no. 2</td>
<td>16</td>
<td>41</td>
<td>&lt;1</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>43</td>
<td>&lt;1</td>
<td>&lt;1</td>
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</table>

Cells were stained with mAbs F23.1 (anti-Vβ8), 44.22.1 (anti-Vβ6), and KJ25 (anti-Vβ3), and FITC-conjugated second antibody. The fraction of cells specifically expressing each VB was determined by flow cytometry using an irrelevant IgG mAb as a control.

unique clonotype. The LGL herein have a similar phenotype to a population of thymus-dependent cells distinct from NK cells described by Pardoll and colleagues (28) in both thymus and spleen. The high percentage of Vβ8 usage in the above LGL is consistent with the finding that the Vβ8 family is used at a greater frequency in the Tα-β+, CD4−, CD8+, NK1.1+ subpopulation than in other T cells (28). Although not tested, it is likely that the previously described Tα-β+, NK1.1+ population (28) expresses CD16 and represents <1% of total splenic T cells (28).

Because CD16 expression requires the FcεRIγ subunit (29), we examined LGL for FcεRIγ mRNA. As shown in Fig. 1 C, the amount of steady-state 0.7-kb FcεRIγ mRNA in the LGL is equivalent to or greater than that of the mast cell line Cl.MC/57.1. To specifically address whether FcεRIγ might also be a component of the TCR on LGL, cells were

Figure 2. Characterization of the TCR complex expressed on LGL. Metabolically labeled (a−e) and surface iodinated (f) LGL and metabolically labeled MAγγ301 (g and h) were lysed in digitonin lysis buffer solution and immunoprecipitated with (a) 3A10 (hamster mAb against Tγ-δ), (b and g) 2C11 (hamster mAb against CD3ε), (c) normal rabbit serum, (d and h) rabbit antiserum no. 386 against CD3γε (30), or (e and f) rabbit antibody against human FcεRIγ (19). Proteins were resolved in 2-D non-reducing/reducing SDS-PAGE followed by autoradiography (a−e, g, and h) or fluorography (f). Closed and open arrowheads indicate positions of Tγ-δ and FcεRIγ, respectively.
metabolically labeled with $^{35}$S-methionine/cysteine and immunoprecipitation was performed with either the anti-CD3ε mAb, 2C11, anti-CD3γ/δ antibody (30), or anti-FceRIγ antibody (19). As shown in Fig. 2, 2C11 precipitates the Tiα-β heterodimer as an off-diagonal spot in a nonreducing/reducing 2-D-diagonal gel. Surprisingly, however, no CD3γ/δ dimers (CD3ε/γ, CD3ε/δ, or CD3γ/δ) are observed in 2C11 or anti-CD3γ/δ immunoprecipitates (Fig. 2, b and d, respectively). This contrasts with the results from T cells expressing "conventional" TCR subunits containing CD3γ/δ homo- or heterodimers (Fig. 2, g and h). Instead of CD3γ/δ, we observe a disulfide-linked homodimer of molecular weight ~9,000 associated with TCR (Fig. 2 b). This low molecular weight structure represents FceRIγ dimers as shown by the fact that rabbit anti-FceRIγ antibody precipitated both the FceRIγ homodimer and Tiα-β heterodimer (Fig. 2 e). Furthermore, the same dimers are precipitated from surface-iodinated LGL cells by the anti-FceRIγ antibody (Fig. 2 f). Identical results are obtained with each of the LGL in Table 1. Thus, we conclude that LGL express on their cell surface a novel type of TCR complex in which an FceRIγ homodimer substitutes for CD3γ/δ and/or CD3δ dimers. The absence of CD3γ/δ proteins is not a consequence of a weak association between the TCR and CD3γ/δ dimers in these cells since direct immunoprecipitation with rabbit anti-CD3γ/δ antibody also failed to identify CD3γ/δ dimers (Fig. 2 d).

Of particular interest, RNase protection analysis identifies the presence of CD3γ and CD3δ mRNAs in the LGL (Fig. 1 D), and these mRNAs are of the appropriate size as judged by RNA blots (data not shown). This discordance between CD3γ/δ mRNA and protein expression is striking but not without precedent. We have previously shown that the level of CD3γ/δ proteins increases during T cell differentiation despite a decrease in the steady-state level of their mRNAs, demonstrating that expression is controlled, at least in part, by a posttranscriptional mechanism (12). The lack of detectable CD3γ/δ protein in LGL expressing CD3γ/δ mRNAs defines yet another likely posttranscriptional control mechanism. Given that CD3γ-FceRIγ and CD3δ-FceRIγ heterodimers but not FceRIγ homodimers have been described in CTLL (19), the regulation of dimer expression among these subunits is likely to be complex.

To examine whether this novel TCR is functional in LGL, cells were analyzed for cytotoxic activity and IL-2 production after receptor crosslinking. As shown in Fig. 3, LGL show a strong cytotoxic activity against two B cell hybridomas, one expressing an anti-CD3ε mAb (2C11) and a second expressing anti-CD16 mAb (2.4G2). Unlike with 2C11 or 2.4G2 hybridomas, no significant killing of NS1 or PC61 producing an anti-IL-2Rα (p55) mAb was observed (data not shown). LGL also show spontaneous cytotoxic activity against the NK-sensitive target YAC-1 cells but failed to lyse L cells. In contrast, none of the targets are killed by a helper T cell hybridoma, MA7301 (31). These results indicate that CD16 as well as the novel TCR containing FceRIγ can transmit signals leading to cytotoxic activity of LGL. However, unlike MA7301, LGL produce no significant level of IL-2 when incubated in wells precoated with anti-CD3ε mAb (data not shown).

In conclusion, FceRIγ homodimers can substitute for CD3γ and CD3δ homo- or heterodimers in targeting partially assembled Tiα-β/CD3γδε TCR complexes to the cell surface and transducing stimulatory signals after TCR triggering (31-36). Thus, TCRs can exist in multiple isoforms being comprised of various disulfide-linked dimers of the CD3γ/δ-FceRIγ family (19, 32, 35, 36). Although it is not known whether the signal transduction properties of FceRIγ-containing TCRs are distinct from conventional CD3γ- and CD3δ-containing TCRs, this is a likely possibility. It is also noteworthy that members of CD3γ/δ-FceRIγ family can dimerize differentially in other receptor complexes. For example, human NK cells express CD3γ as well as FceRIγ in association with CD16 in the absence of other TCR components (Tiα, Tiβ, CD3γ, CD3δ, CD3ε) (37-39). It is now critical to ascertain the functional attributes of the various CD3γ/δ-FceRIγ dimers.

<table>
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<th>Target Cells</th>
<th>Yac-1</th>
<th>2C11</th>
<th>2.4G2</th>
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</tr>
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<tbody>
<tr>
<td>Specific Killing (%)</td>
<td>100</td>
<td>50</td>
<td>30</td>
<td>10</td>
</tr>
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Figure 3. Cytotoxic activity of LGL. Cytotoxic activity of B10.BR LGL no. 2 (O), C3H/HeJ LGL (●), and MA7301 (△) were analyzed by standard 4-h $^{51}$Cr release assay with the indicated E/T ratios.

<table>
<thead>
<tr>
<th>E : T Ratio</th>
<th>30</th>
<th>10</th>
<th>5</th>
<th>1</th>
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<tbody>
<tr>
<td>Yac-1</td>
<td>C3H/HeJ</td>
<td>B10.BR</td>
<td>MAC-7301</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>O</td>
<td>O</td>
<td>O</td>
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<tr>
<td>2.4G2</td>
<td>O</td>
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<td>1.0</td>
<td>O</td>
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