T Cell Receptor Complexes Containing FcεRIγ Homodimers in Lieu of CD3ξ and CD3η Components: A Novel Isoform Expressed on Large Granular Lymphocytes

By Shigeo Koyasu,* Luciano D'Adamio,* Antonio R. N. Arulanandam,* Sheena Abraham, Linda K. Clayton,* and Ellis L. Reinherz†

From the Laboratory of Immunobiology, Dana-Farber Cancer Institute and Departments of *Pathology and †Medicine, Harvard Medical School, Boston, Massachusetts 02115

Summary
CD3ξ and CD3η form disulfide-linked homo- or heterodimers important in targeting partially assembled Tio-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/Tio-ε, CD4-, CD8-, CD16+, NKI.1+ mouse large granular lymphocytes (LGL), which are devoid of CD3ξ and CD3η proteins. The TCRs of this subset contain homodimers of the γ subunit of the high affinity receptor for IgE (FceRIγ) in lieu of CD3ξ and/or CD3η proteins. The LGL display natural killer-like activity and are cytotoxic for B cell hybridomas producing anti-CD3ε 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.

The TCR has been described as a multimolecular complex formed by three groups of transmembrane proteins: (a) the clonotype antigen/MHC recognition unit, termed Tio-β (or Tγ-δ) heterodimer (1–3); (b) the highly homologous CD3γ, CD3δ, and CD3ε subunits (4–8); and (c) the structurally distinct CD3ξ and CD3η subunits, alternatively spliced products of a common genetic locus (9–12). FceRIγ, an essential component of FceRIγ and the transmembrane type FcγRII (CD16) (13), has significant structural homology to CD3ξ and CD3η (9, 10, 14, 15) and is encoded on the same chromosome (mouse chromosome 1), suggesting that CD3ξ/η and FceRIγ are derived from a common ancestral gene (11, 12, 16, 17). In addition, CD3ξ can substitute for FceRIγ to form a high affinity IgE receptor on Xenopus oocytes injected with mRNAs for FceRIα, FceRIβ, and CD3ξ in the absence of FceRIγ (18). Moreover, in the CTLL cell line, CD3ξ, CD3η, and FceRIγ 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 CD3ξ/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 FceRIγ homodimer in lieu of CD3ξ and η subunits.

Materials and Methods

Flow Cytometric Analysis. LGL cells (2–3 × 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-Tl3,-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 (NK1.1; 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 Tla-13, 0% for Tl3,-δ and CD4, 7% for CD8, 83% for FcγRII/III, and 88% for NK1.1.

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'GGTGGCATAAGCTTGAGGAAC3' located at base pairs 470–488 of FcγRII and the
antisense amplimer 5'GGAGGCACATCACTAGGGAGY at base pairs 738-714 in the transmembrane region of Fc-κIII (numbers are according to reference 20). The PCR product of FcRκIII is a 269-bp fragment. To identify FcRγIb2 and FcRγIIb, the same sense amplimer was used with the antisense amplimer 5'GCA-GCTTCTCCAGATCAGG3', which lies at base pairs 1232-1213 of FcRγIIb2. 3' to the 138-bp insertion found in FcRγIIb2, as compared with FcRγIIb2. Amplification of FcRγIIb2 and FcRγIIb2 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 thermostate 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 were exposed to Kodak X-Omat AR x-ray film.

**RNA Analysis.** Total RNA was isolated from cell lines using guanidine isothiocyanate (21). RNA concentrations were measured spectrophotometrically and 10 µg of total RNA was run per lane on a 1% agarose gel containing 2.2 M formaldehyde and 1× MOPS buffer (21). The RNA was then transferred to nitrocellulose in 20 × SSC, 5 × Denhardt's, 10 µg/ml denatured salmon sperm DNA, and 0.1% SDS at 54°C. The blot was then washed for 20 min in 6 × SSC-0.1% SDS at the hybridization temperature and exposed at -70°C to Kodak X-Omat AR x-ray film.

**Results and Discussion**

Splencocytes from B10.BR and C3H/HeJ mice cultured for several weeks in the presence of rIL-2 as described (24) express both Tcr-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+, Tcr-β+, Tγ-δ+, CD4−, CD8−, FcγR+, 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 isotype, the mouse homologue of CD16 (Fig. 1 B). In contrast, S49 thymoma cells express both FcγRII and FcγRIII (Fig. 1 B) while Cl.MC/57.1 mast cells express readily detectable FcγRIII (Fig. 1 B) and, on longer exposures of the autoradiogram, FcγRII (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 amplimers 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

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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 FcγR isotype by PCR. Reverse PCR was performed with RNAs from Cl.MC/57.1 (mast cell line; FcγRIIα, FcγRIIIα), B10.BR LGL, and S49 (T lymphoma; FcγRIIα, FcγRIIIα) using specific oligo primers to FcγRIII (CD16, previously denoted as FcγRIIa) or FcγRIIb (previously denoted as FcγRIIb1 and FcγRIIb2). 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 FcεRIα expression. 10 μg of total RNA from a T cell hybridoma (2B4.11), a mast cell line (Cl.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 FcεRIα. The positions of 18S and 28S ribosomal RNAs are indicated. (D) RNase protection analysis of CD3γ/ζ mRNA. 15 μg of total RNA from thymocytes and B10.BR LGL were analyzed for CD3γ/ζ mRNAs by RNase protection analysis. 173 and 100 bp signals represent CD3γ and CD3ζ, 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

| LGL           | Vβ8 | Vβ6 | Vβ3 |
|---------------|-----|-----|-----|
|               | %   |     |     |
| B10.BR no. 1  | 77  | <1  | 3   |
| B10.BR no. 2  | 16  | 41  | <1  |
| C3H/HeJ       | 43  | <1  | <1  |

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 Vβ was determined by flow cytometry using an irrelevant IgG mAb as a control.

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 Tix-β+, CD4−, CD8−, NK1.1+ subpopulation than in other T cells (28). Although not tested, it is likely that the previously described Tix-β+, NK1.1+ population (28) expresses CD16 and represents <1% of total splenic T cells (28).

Because CD16 expression requires the FceRIγ subunit (29), we examined LGL for FceRIγ mRNA. As shown in Fig. 1 C, the amount of steady-state 0.7-kb FceRIγ mRNA in the LGL is equivalent to or greater than that of the mast cell line C1.MC/57.1. To specifically address whether FceRIγ 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 Tix-β), (b and g) 2C11 (hamster mAb against CD3ε), (c) normal rabbit serum, (d and h) rabbit antiserum no. 386 against CD3γεγ (30), or (e and f) rabbit antibody against human FceRIγ (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 Tix-β and FceRIγ, respectively.
metabolically labeled with 35S-methionine/cysteine and immunoprecipitation was performed with either the anti-CD3ε mAb, 2C11, anti-CD3ζ/η 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 CD3ζ/η dimers (CD3ζ/ζ, CD3ζ/η, or CD3η/η) are observed in 2C11 or anti-CD3ζ/η immunoprecipitates (Fig. 2 b and d, respectively). This contrasts with the results from T cells expressing "conventional" TCR subunits containing CD3ζ/ζ homodimers (Fig. 2 g and h). Instead of CD3ζ/η, 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 CD3ζ/ζ and/or CD3ζ/η dimers. The absence of CD3ζ/η proteins is not a consequence of a weak association between the TCR and CD3ζ/η dimers in these cells since direct immunoprecipitation with rabbit anti-CD3ζ/η antibody also failed to identify CD3ζ/η dimers (Fig. 2 d).

Of particular interest, RNase protection analysis identifies the presence of CD3ζ and CD3η 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 CD3ζ/η mRNA and protein expression is striking but not without precedent. We have previously shown that the level of CD3ζ/η 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 CD3ζ/η protein in LGL expressing CD3ζ/η mRNAs defines yet another likely posttranscriptional control mechanism. Given that CD3ζ-FceRIγ and CD3η-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, MAζ-η301 (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 MAζ-η301, 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 CD3ζ and CD3η homo- or heterodimers in targeting partially assembled Tiα-β/CD3γε 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 CD3ζ/η-FceRIγ family (19, 32, 35, 36). Although it is not known whether the signal transduction properties of FceRIγ-containing TCRs are distinct from conventional CD3ζ- and CD3ζ-containing TCRs, this is a likely possibility. It is also noteworthy that members of CD3ζ/η-FceRIγ family can dimerize differentially in other receptor complexes. For example, human NK cells express CD3ζ 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 CD3ζ/η-FceRIγ dimers.
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Address correspondence to Shigeo Koyasu, Laboratory of Immunobiology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.

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References

1. Meuer, S.C., O. Acuto, T. Hercend, S.F. Schlossman, and E.L. Reinherz. 1984. The human T cell receptor. Annu. Rev. Immunol. 2:23.

2. Davis, M.M., and P.J. Bjorkman. 1988. T cell antigen receptor genes and T cell recognition. Nature (Lond.). 334:395.

3. Brenner, M.B., J.L. Strominger, and M.S. Krangel. 1988. The γδ T cell receptor. Adv. Immunol. 43:133.

4. Clevers, H., B. Alarcon, T. Wileman, and C. Terhorst. 1988. The T cell receptor/CD3 complex: a dynamic protein ensemble. Annu. Rev. Immunol. 6:629.

5. Ashwell, J.D., and L.D. Klausner. 1990. Genetic and mutational analysis of the T cell antigen receptor. Annu. Rev. Immunol. 8:139.

6. van den Elsen, P., G. Bruns, D.S. Gerhard, D. Pravtcheva, C. Jones, D. Housman, F.H. Ruddle, S. Orkin, and C. Terhorst. 1985. Assignment of the gene coding for the T3 delta subunit to the T3/T cell receptor complex to the long arm of the human chromosome 11 and to mouse chromosome 9. Proc. Natl. Acad. Sci. USA. 82:2920.

7. Krissansen, G.W., M.J. Owen, W. Verbi, and M.J. Crumpton. 1986. Primary structure of the T3 γ subunit of the T3/T cell antigen receptor complex deduced from cDNA sequences: Evolution of the T3 γ and δ subunits. EMBO (Eur. Mol. Biol. Organ.) J. 5:1799.

8. Gold, D.P., I.J.M. van Dongen, C.C. Morton, G.A.P. Bruns, P. van den Elsen, A.H.M. van Kessel Geurts, and C. Terhorst. 1987. The gene encoding the epsilon subunit of the T3/T cell receptor complex maps to chromosome 11 in humans and to chromosome 9 in mice. Proc. Natl. Acad. Sci. USA. 84:1664.

9. Weissman, A.M., M. Baniyash, D. Hou, L.E. Samelson, W.H. Burgess, and R.D. Klausner. 1988. Molecular cloning of the zeta chain of the T cell antigen receptor. Science (Wash. DC). 239:1018.

10. Jin, Y.-J., L.K. Clayton, F.D. Howard, S. Koyasu, M. Sieh, R. Steinbrich, G.E. Tarr, and E.L. Reinherz. 1990. Molecular cloning of the CD3γ subunit identifies a CD3γ-related product in thymus-derived cells. Proc. Natl. Acad. Sci. USA. 87:3319.

11. Baniyash, M., V.W. Hau, M.F. Seldin, and R.D. Klausner. 1989. The isolation and characterization of the murine T cell antigen receptor ζ chain gene. J. Biol. Chem. 264:13252.

12. Clayton, L.K., L. D'Adamo, F.D. Howard, M. Sieh, R.E. Hussey, S. Koyasu, and E.L. Reinherz. 1991. CD3γ and CD3ζ are alternatively spliced products of a common genetic locus and are transcriptionally and/or post-transcriptionally regulated during T cell development. Proc. Natl. Acad. Sci. USA. 88:5202.

13. Ra, C., M.-H.E. Jouvin, U. Blank, and J.-P. Kinet. 1989. A macrophage Fcγ receptor and the mast cell receptor for IgE share an identical subunit. Nature (Lond.). 341:752.

14. Blank, U., C. Ra, L. Miller, K. White, H. Metzger, and J.-P. Kinet. 1989. Complete structure and expression in transfected cells of high affinity IgE receptor. Nature (Lond.). 337:187.

15. Ra, C., M.-H.E. Jouvin, and J.-P. Kinet. 1989. Complete structure of the mouse mast cell receptor for IgE (FcεRI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. J. Biol. Chem. 264:15323.

16. Huppi, K., D. Siwarski, B.A. Mock, and J.-P. Kinet. 1989. Gene mapping of the three subunits of the high affinity FcR for IgE to mouse chromosomes 1 and 19. J. Immunol. 143:3787.

17. Küster, H., B. Thompson, and J.-P. Kinet. 1990. Characterization and expression of the gene for the human Fc receptor γ subunit: definition of a new gene family. J. Biol. Chem. 265:6448.

18. Howard, F.D., H.-R. Rodewald, J.-P. Kinet, and E.L. Reinherz. 1990. CD3ζ substitutes for FcεRI-3, in assembly and functional expression of the high affinity IgE receptor: evidence for inter-receptor complementation. Proc. Natl. Acad. Sci. USA. 87:7015.

19. Orloff, D.G., C. Ra, S.J. Frank, R.D. Klausner, and J.-P. Kinet. 1990. Family of disulphide-linked dimers containing the ζ and η chains of the T cell receptor and the γ chain of Fc receptors. Nature (Lond.). 347:189.

20. Ravetch, J.V., A.D. Luster, R. Weinshank, J. Kochan, A. Pawloues, D.A. Portnoy, J. Huuley, Y.-C.E. Pan, and J.C. Unkeless. 1986. Structural heterogeneity and functional domains of murine immunoglobulin G Fc receptors. Science (Wash. DC). 234:718.

21. Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

22. Melton, D.A., P.A. Krieg, M.R. Rebagliati, T. Maniatis, K. Zinn, and M.R. Green. 1984. Efficient synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. 12:7035.

23. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.). 227:680.

24. Koyasu, S., Y. Tagaya, K. Sugiie, S. Yonehara, I. Yodoi, and I. Yahara. 1991. The expression of IL-2R α-chain is enhanced by activation of adenylate cyclase in large granular lymphocytes and natural killer cells. J. Immunol. 146:233.

25. Perussia, B., G. Trinchieri, A. Jackson, N.L. Warner, J. Faust,
H. Rumpold, D. Kraft, and L.L. Lanier. 1984. The Fc receptor for IgG on human natural killer cells: Phenotypic, functional and comparative studies with monoclonal antibodies. *J. Immunol.* 133:180.

26. Lanier, L.L., T.J. Kipps, and J.H. Phillips. 1985. Functional properties of a unique subset of cytotoxic CD3⁺ T lymphocytes that express Fc receptors for IgG (CD16/Leu-11 antigen). *J. Exp. Med.* 162:2089.

27. Sentman, C.L., V. Kumar, G. Koo, and M. Bennett. 1989. Effector cell expression of NK1.1, a murine natural killer cell-specific molecule, and ability of mice to reject bone marrow allografts. *J. Immunol.* 142:1847.

28. Levitsky, H.L., PT. Golumbek, and D.M. Pardoll. 1991. The fate of CD4⁻8⁻ T cell receptor-αβ⁺ thymocytes. *J. Immunol.* 146:1113.

29. Hibbs, M.L., P. Selvaraj, O. Carpen, T.A. Springer, H. Kuster, M.-H.E. Jouvin, and J.-P. Kinet. 1989. Mechanisms for regulating expression of membrane isoforms of FcγRIII (CD16). *Science (Wash. DC).* 246:1608.

30. Orloff, D.G., S.J. Frank, F.A. Robey, A.M. Weissman, and R.D. Klausner. 1989. Biochemical characterization of the η chain of the T cell receptor: a unique subunit related to ζ. *J. Biol. Chem.* 264:14812.

31. Bauer, A., D.J. McConkey, F.D. Howard, L.K. Clayton, D. Novick, S. Koyasu, and E.L. Reinherz. 1991. Differential signal transduction via T cell receptor CD3ζ, CD3ζ-η, and CD3η2 isoforms. *Proc. Natl. Acad. Sci. USA.* 88:3842.

32. Baniyash, M., P. Garcia-Morales, J.S. Bonifacino, L.E. Samelson, and R.D. Klausner. 1988. Disulfide linkage of the ζ and η chains of the T cell receptor: possible identification of two structural classes of receptors. *J. Biol. Chem.* 263:9874.

33. Weissman, A.M., S.J. Frank, D.G. Orloff, M. Mercep, J.D. Ashwell, and R.D. Klausner. 1989. Role of the zeta chain in the expression of the T cell antigen receptor: genetic reconstitution studies. *EMBO J.* 8:3651.

34. Frank, S.J., B.B. Niklinskia, D.G. Orloff, M. Mercep, J.D. Ashwell, and R.D. Klausner. 1990. Structural mutations of the T cell receptor ζ chain and its role in T cell activation. *Science (Wash. DC).* 249:174.

35. Clayton, L.K., A. Bauer, Y.-J. Jin, L. D'Adamio, S. Koyasu, and E.L. Reinherz. 1990. Characterization of thymus-derived lymphocytes expressing ΤιζβCD3γδεζχζ, ΤιζβCD3γδεζχζ or ΤιζβCD3γδεζχζα η antigen receptor isoforms: Analysis by gene transfection. *J. Exp. Med.* 172:1243.

36. Koyasu, S., L. D'Adamio, L.K. Clayton, and E.L. Reinherz. 1991. T cell receptor isoforms and signal transduction. *Curr. Opin. Immunol.* 3:32.

37. Anderson, P., M. Caligiuri, J. Ritz, and S.F. Schlossman. 1989. CD3-negative natural killer cells express ζ TCR as part of a novel molecular complex. *Nature (Lond.)* 341:159.

38. Lanier, L.L., G. Yu, and J.H. Phillips. 1989. Co-association of CD3ζ with a receptor (CD16) for IgG Fc on human natural killer cells. *Nature (Lond.)* 342:803.

39. Anderson, P., M. Caligiuri, C. O'Brien, T. Manley, J. Ritz, and S.F. Schlossman. 1990. Fc receptor type III (CD16) is included in the ζ NK receptor complex expressed by human natural killer cells. *Proc. Natl. Acad. Sci. USA.* 87:2274.