EXPRESSION AND REARRANGEMENT OF THE \( \alpha, \beta, \) AND \( \gamma \) CHAIN GENES OF THE T CELL RECEPTOR IN CLONED MURINE LARGE GRANULAR LYMPHOCYTE LINES

No Correlation with the Cytotoxic Spectrum

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NK cells have been originally described as “null” cells (1, 2), or more recently “the third population of lymphocytes,” distinguishing them from conventional T and B cells, and the cellular lineage of these cells has been a matter of arguments for over 10 years. Since NK activity exists in athymic nude mice (3), they obviously do not require the thymus for their functional differentiation. On the other hand, several cytological characteristics of NK cells have been considered to imply their relatedness to T cells, including expression of certain surface antigens such as Thy-1 and Qa 5 (4, 5), proliferative responsiveness to IL-2 (6), and even production of several lymphokines such as IL-2 and IFN-\( \gamma \) (7, 8).

Recent work on the isolation and characterization of the T cell receptor (TCR)\(^1\) genes have revealed that they have striking similarities with the Ig genes not only in the structure but also in the mode of rearrangement (9–18). The TCR is composed of two polypeptide chains, \( \alpha \) and \( \beta \) (19–21), both with a V and a C region. The V regions of the \( \alpha, \beta, \) and \( \gamma \) chains are encoded by separate germline DNA segments, V, D (only for \( \beta \) chain), and J, which are brought together to complete a V-region exon in T lymphocytes. Such advances have made direct analysis of TCR genes possible in a variety of cells. Recently, the expression and rearrangement of some of the TCR genes have been directly examined using several continuous cell lines with NK activity both in humans and animals (22–24). The results, however, are somewhat controversial. One of the reasons seems to be the heterogeneity of the lines used in these studies. Some of these, for instance, had surface phenotype of mature T cells (22, 23), or even original functional features as CTL (22), and others were leukemic tumor lines (24). Among cell lines with features close to normal NK cells, the \( \beta \) chain gene

\(^1\)Abbreviations used in this paper: BMC, bone marrow cells; HeLa-Ms, measles virus-infected HeLa cells; LAK, lymphokine-activated killers; LGL, large granular lymphocytes; PEC, peritoneal exudate cells; TCR, T cell receptor.
of TCR was reported to be rearranged in the murine system (23), whereas no
evidence of the rearrangement was obtained in the human system (22).

We have already reported a number of cloned murine large granular lymphocyte
(LGL) lines from normal mice with essentially the same characteristics as
endogenous NK cells in terms of cytotoxic repertoires, surface phenotypes, and
morphology (25). More recently, we could also establish similar LGL lines from
athymic nude mouse. To determine whether TCR is involved in the target
recognition by NK cells or not, we performed a systematic examination of the
TCR genes in these LGL lines using complete sets of genetic probes for the α,
β, and γ chains of TCR with reference to cytotoxic spectrum.

Materials and Methods

Mice. Female BALB/c, +/+ , and nu/nu, mice were purchased from Shizuoka Experi-
mental Animal Center, Hamamatsu, Japan.

Cell Lines. Murine lymphoma cell lines, YAC-1, RLd1, P815, EL4, were all main-
tained in RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine, 1 mM sodium
pyruvate, 10 mM Hepes, 100 U/ml penicillin, 100 μg/ml streptomycin, and nonessential
amino acids (complete RPMI). HeLa cells persistently infected with measles virus (HeLa-
Ms) were cultured in Eagle's MEM supplemented with 5% FCS and antibiotics. Kirstein
murine sarcoma virus (Ki-MSV)-transformed NIH 3T3 cell line, 12NY-c165, was provided
by Dr. Y. Ikawa of the Institute of Physical and Chemical Research, and maintained in
McCoy's medium supplemented with 5% FCS. An x-ray-induced murine T cell lymphoma
C6XL was kindly provided by Dr. J. P. Allison of the University of Texas and was cultured
as previously described (26). A murine tumor-specific CTL line CTLL-2 (27) was main-
tained in complete RPMI 1640 supplemented with human rIL-2 and 5 μM of 2-ME. A
mouse myeloma QUPC52 was expanded subcutaneously in BALB/c mice.

Cloned LGL Lines. Continuous, IL-2-dependent LGL lines, PEC-1, PN, and SN, were
established from either conventional or nude mice as described before (25). Briefly, spleen
cells from mice that had been injected intraperitoneally with 10^6 HeLa-Ms cells 4 d before
were passed through a nylon wool column and then cocultured with normal resident
peritoneal adherent cells that had been irradiated with 3,000 rad gamma ray using a
Gamma Cell (37Cs; Atomic Energy of Canada, Ltd., Ottawa, Canada) in the presence of
100 U/ml rIL-2. Peritoneal exudate cells (PEC) were also similarly cultured. The cultures
were fed with rIL-2 every other day. In a few weeks, the growing cells became apparent,
and such nonadherent cells were serially passed on fresh irradiated adherent cells with
rIL-2. Meanwhile, cells were cloned by means of limiting dilutions (0.5–0.5 cell/well) in
the presence of adherent cells and IL-2. The continuous growth of the clones was
dependent on the presence of both IL-2 and macrophages (25). PEC-1 cl.1H5.13 and
PEC-1 cl.1H5.23, PN-14 and PN-31, and SN-13 and SN-14 lines are clones derived from
PEC-1, PN, and SN lines, respectively.

The SPB line was established in a somewhat different way. Mice were subcutaneously
injected with Ki-MSV-transformed NIH 3T3 cell line (12NY-c165). Ki-MSV proviruses
were integrated into the chromosomal DNA of 12NY-c165 cells, as judged by Southern
blot hybridization analysis using v-ras fragment as probe. Furthermore, 12NY-c165 was
found to be a potent virus producer and the culture supernatants could transform Rat-2
cells quite efficiently. Mice injected subcutaneously with the cells developed massive
splenomegaly in a few weeks. Cells obtained from such enlarged spleens were cultured in
the presence of rIL-2 (100 U/ml) alone without any additive feeder cells. In a few weeks,
the growing cells became apparent and such cells could now successfully be expanded in
the presence of rIL-2 alone. Unlike former LGL lines, no additive feeder cells were
required for continuous growth of the SPB line. In contrast to 12NY-c165, no proviral
genome of Ki-MSV could be detected in the SPB line. Morphological examination
indicated that the SPB line consisted of homogenous cells with typical morphology of
LGL. Such a procedure has quite reproducibly induced continuous IL-2-dependent LGL
lines from a variety of strains of mice with rIL-2 alone (M. Hattori and N. Minato, manuscript in preparation).

**Phenotypical Analysis.** Surface phenotypes of the clones were analyzed using a FACS 302 (Becton Dickinson & Co., Mountain View, CA) as described before (25). Antibodies used included monoclonal anti-Thy-1.2 (IgM), anti-Lyt-1.2 (IgG2b), anti-Lyt-2.2 (IgM), anti-L3T4 (rat, IgG1), anti-T200 (IgG1), rabbit anti-asialo GM1 (AsGM1) (IgG fraction), and monoclonal anti-mouse IL-2-R antibody (AMT13). Fc receptor for IgG was examined by rosette-forming assay using sheep erythrocytes sensitized with rabbit anti-sheep erythrocyte.

**Cytotoxicity Assay.** Cytotoxic activity of the clones was assayed using a 51Cr release method, as described (28). Assay time was 5 h for lymphoma and normal bone marrow cell (BMC) targets and 12 h for adherent cells such as HeLa-Ms and 12NY-c165. Calculation of specific 51Cr release was described before.

**Electron Microscopy.** The pelleted clones (10^8) were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) overnight at 4°C. Subsequently, the pellets were cut into pieces and postfixed in 2% OsO_4 in phosphate buffer (pH 7.4) at 4°C for 2 h. The blocks were dehydrated and embedded in Epon. Ultrathin sections were stained with saturated uranyl acetate and lead citrate, and observed under a 100 CX electron microscope (JEOL USA, Peabody, MA) at 100 kV.

**Preparation of DNA and RNA.** High molecular weight DNAs were prepared from BALB/c liver and LGL lines as previously described (29). Total cellular RNAs were extracted from various cell lines as described elsewhere (30). Poly(A)^+ RNAs were purified by oligo(dT) cellulose column chromatography as described (31).

**Northern Blot Hybridization.** Poly(A)^+ RNAs (5 μg each) were treated with glyoxal and DMSO, electrophoresed on a 1% agarose gel in 10 mM sodium phosphate buffer, pH 7, and transferred to a nitrocellulose filter as described (32). The filters were hybridized as described (33) using DNA fragments labeled with 32P by nick translation as probes (34). The filters were washed at 50°C in 15 mM NaCl/1.5 mM sodium citrate/0.1% SDS.

**Southern Blot Hybridization.** DNAs (2 μg each) were digested with Eco RI, electrophoresed on a 0.7% agarose gel, and transferred to a nitrocellulose filter as described (35). The filters were hybridized as above. The filters were washed at 65°C in 15 mM NaCl/1.5 mM sodium citrate/0.1% SDS.

**Probes.** T cell receptor α and β chain cDNA clones, T7II (15) and 86T5 (10), respectively, were kindly provided by Dr. M. M. Davis of Stanford University, Stanford, CA, γ chain cDNA clone Tγ5 (36) and Jγ cosmid clone TA28.1 (16) were generous gifts from Dr. H. von Boehmer of the Basel Institute for Immunology (Basel, Switzerland) and Dr. L. Hood of the California Institute of Technology (Pasadena, CA), respectively. The Cα probe is 540-bp Sau3AI fragment of T7II and contains most of Cα segment. The Jα probe is a 3.5-kb Eco RI/Hind III fragment of TA28.1 and contains the Jα segment, which is supposed to be located in the most 5′ region of the Jα gene cluster (16). The Cγ probe is a 730-bp Eco RI fragment of 86T5 and contains a part of a Vγ, Jγ, and Cγ segment (10). The Dα1, Jα1, Jα2 probes are 3.2-kb Hind III/Bam HI, 2.5-kb Xba 1/Sac I, 2.3-kb Eco RI fragments, respectively (12, 13), of genomic β chain gene clones, which were isolated with the Cα probe by screening mouse genomic libraries. The Dα1, Jβ1, and Jβ2 probes contain the Dα1,1, Jβ1,1, Jβ1,1,6, and Jβ2,1,6 segments, respectively. The Cγ probe is a 1.4-kb Eco RI fragment of Tγ5. The ranges of the Jα, Dα1, Jβ1, and Jβ2 probes are illustrated in Fig. 4 (F).

Results

**Cloned LGL Lines Derived from Euthymic and Athymic Nude Mice.** The cytological characteristics of LGL lines derived from either spleen cells or PEC of BALB/c mice are summarized in Table 1. PFC-1 and SPB lines were derived from conventional BALB/c (+/+) mice, whereas PN and SN lines originated from athymic nude mice. As indicated, all of the lines showed the same surface phenotype. Although all of the lines expressed Thy-1 antigen, none of them
showed any other detectable T cell–associated antigens such as Lyt-2 and L3T4. The expression of AsGM1 was extremely intense in all lines. IL-2R was positive on all lines, and was especially intense on the SPB line. Cloned lines from each of parental lines exhibited the same features. Ultrastructural examination of the cell lines revealed characteristic morphology identical to that of normal NK cells, as reported by Grossi et al. (37). As shown in Fig. 1, A and C, the abundant cytoplasm contained characteristic electron dense granules and well-developed Golgi apparatus. The granules contained numerous vesicles, as well as dense homogeneous material, and was considered to have derived from multivesicular bodies of lysosomes (Fig. 1 B). Small vesicles were frequently found near the Golgi apparatus and in some cases contained dense materials inside, resembling the “rod-cored” structure reported as the most distinct feature of pit cells (Fig. 1 D) (38).

**NK Activity of the Cloned LGL Lines.** The striking functional feature of NK cells in normal animals is a sort of a priori spectrum of the cytotoxicity against target cells, including certain tumor cell lines, various virus-infected normal or tumor cells, and some of normal cells such as BMC and fibroblasts. Thus the spectra of the cytotoxic activity of cloned LGL lines were examined using representative target cells in comparison with those of NK cells in freshly isolated, nylon wool–passed spleen cells from normal mice. The results are summarized in Fig. 2. Most of the clones exhibited basically the same pattern of cytotoxic spectrum as normal endogenous NK cells, except that the cytotoxicity against normal BMC was usually hard to detect in fresh NK cells, most likely due to the coexisting macrophages (25). However, some of the clones, such as IH5.23 and SN-13, showed selective deletion of the cytotoxicity against RL6I cells. The SPB line, on the other hand, consistently exhibited rather weak cytotoxic activity to YAC-1 cells. SPB cells were completely unreactive to 12NY-c65 cells used for the in vitro induction. The results thus confirmed the minor clonal heterogeneity of cytotoxic activity among NK clones (25, 39), and also provided the basis for the comparison of the cytotoxic spectrum with the rearrangement patterns of TCR genes described below. Culture of normal spleen cells with a variety of conditions is known to generate various “anomalous” NK-like killer activity. A typical example is the culture of normal spleen cells with IL-2; as shown in Fig. 2, this leads to the generation of “pan-cytotoxicity” against a number of cell lines (termed lymphokine-activated killer [LAK] activity [40]). It was already reported

### Table I

**Murine Large Granular Lymphocyte Lines**

| Parental lines | Strains of mice | Organs derived | In vitro stimulation | Surface phenotypes | Growth requirements |
|----------------|-----------------|----------------|----------------------|-------------------|---------------------|
| PEC-1 BALB/c, +/+ | PEC | RL6I | + | + | + | + | IL-2 and Mφ |
| PN BALB/c, nu/nu | PEG | HeLa-Ms | + | + | + | + | IL-2 and Mφ |
| SN BALB/c, nu/nu | Spleen | HeLa-Ms | + | + | + | + | IL-2 and Mφ |
| SPB BALB/c, +/+ | Spleen | 12NY-c65 | + | + | + | + | IL-2 |

Mice were injected intraperitoneally with $10^6$ of either syngeneic RL6I or HeLa-Ms cells. 4 d later, peritoneal exudate cells or spleen cells were recovered and cultured, as described in Materials and Methods, to obtain the cell lines. 12NY-c65 (Kirsten virus–transformed NIH 3T3 line) were injected subcutaneously ($10^6$). In a few weeks, the mice developed massive splenomegaly, and the cells from such spleens were cultured in the presence of rIL-2 (100 U/ml) without any additive feeder cells. The cultures were fed with rIL-2 twice a week to develop SPB cell line.
that most of such LAK activity was mediated by the effector cells with phenotypes of mature T cells (41), rather than NK cells. The cytotoxic spectrum, as well as morphological and phenotypical characteristics of these clones, thus indicated that the clones used here represented endogenous NK cells rather than anomalous killer cells. It was also noted that no functional difference whatsoever was observed between the clones derived from euthymic and athymic mice.

Expression of TCR Genes in LGL Lines. To determine whether the TCR genes are transcribed in LGL lines, Northern blot hybridization was performed. As shown in Fig. 3, a T cell lymphoma C6XL and a CTL line CTLL-2 had all the transcripts of the α, β, and γ chain genes, whereas a myeloma QUPC52 showed
none of the transcripts of TCR genes. It has been reported that the transcripts of the α, β, and γ chain genes are classified in two types, one of larger size which is assumed to be transcribed from complete V-(D)-J complexes and the other of smaller size from D-J complexes or germline J segments (14, 36). Because the C6XL line has been shown to express both the α and β chain polypeptides on the cell surface (26), the transcripts of the larger size of the α and β chain genes in the C6XL line are considered to be active transcripts derived from complete V-(D)-J complexes. Since the RNA of the C6XL line was extracted from splenic tumors expanded in C57BL/6 mice, the transcripts of the γ chain gene in the C6XL line could be derived from CTL of host origin. PEC-1, PN, and SN lines, as well as clones thereof, were all found to have the transcripts of the α, β, and γ chain genes. The SPB line showed the transcripts of the α and β chain genes, but no transcript of the γ chain gene. Comparison with the two T cell lines revealed that the transcripts of the α, β, and γ chain genes expressed in the LGL lines were full-length mRNAs, most likely derived from complete V-(D)-J complexes.

Rearrangement of TCR Genes in LGL Lines. To know the rearrangement profile of the T cell receptor genes in LGL, Southern blot hybridization analysis was done in LGL lines. As shown in Fig. 4, the α, β, and γ chain genes were rearranged in all the LGL lines, including PN and SN lines derived from nude mice. In case of the murine α chain gene, more than 18 Jα segments are located over 60 kb of DNA 5' to the Cα gene (16). The Jα probe used contains the Jα1 segment which is located in the most 5' region of the Jα gene cluster (16). By using the Jα probe, the rearrangement or the deletion of the Jα1 segment can be
detected by Southern blot hybridization analysis. As shown in Fig. 4 A, the \( J_{\alpha 1} \) segment was completely deleted in all the LGL lines. The result indicates that the \( \alpha \) chain gene is rearranged on both chromosomes and that the rearrangements involve the \( J_{\alpha} \) segments located in the region 3' to the \( J_{\alpha 1} \) segment.

The rearrangement of the \( \beta \) chain gene in the LGL lines showed the characteristic profile (Fig. 4, B–D). PEC-1, PN, and SN lines exhibited the identical pattern of rearrangement, although, the pattern of rearrangement in SPB line was different from the other three lines. In PEC-1 PN, and SN lines, the \( J_{\beta 1} \) and \( J_{\beta 2} \) probes hybridized with 3.0- and 7.3-kb Eco RI fragments, respectively, except for the germline fragments. The 7.3-kb Eco RI fragment was hybridized also with the \( D_{\beta 1} \) probe. Because the \( D_{\beta 1} \) probe also contains a DNA fragment 5' to the \( D_{\beta 1} \) segment, the result indicates that the 7.3-kb Eco RI fragment contains a
FIGURE 4. Rearrangement of TCR genes in LGL lines. Southern blots were hybridized with (A) Jα, (B) Dα, (C) Jβ, (D) Jβ2, and (E) Cγ, probes. Origins of DNA are: (1) BALB/c mouse liver; (2) PEC-1 Cl.1H5.13; (3) PEC-1 Cl.1H5.25; (4) PN-14; (5) PN-31; (6) SN-13; (7) SN-14; (8) SPB4.2. Arrows and arrow heads indicate the 7.3- and 8.7-kb Eco RI fragments, respectively, which were hybridized with both the D and J probes. The genomic fragments used as probes are shown as horizontal bars in partial maps of the murine α and β chain genes (F) and abbreviated as: a, Jα; b, Dα; c, Jβ; and d, Jβ2. Restriction sites are abbreviated as: E, Eco RI; H, Hind III; and B, Bam HI.
Dβ1·Jα2 complex but no Vα gene. Thus, because the 7.3-kb Eco RI fragment was derived from the inactive Dβ1·Jα2 complex, the 3.0-kb one is considered to contain an active V-(D)-J complex. The 3.0-kb Eco RI fragments showed no minor size differences among the cell lines. The results suggest that the same Vα gene is rearranged to the same Jα2 segment in the fragment in PEC-1, PN, and SN lines. Since the Dβ1,1 and Dβ2,1 segments were 12 and 14 bp long (14), respectively, the difference between the Dβ segments would not be detected by Southern blot hybridization analysis. In the SPB line, the Jα1 fragment was completely deleted from the genome and the Jα2 probe hybridized with 8.7- and 1.5-kb Eco RI fragments. Since the 8.7-kb fragment was also hybridized with the Dβ1 probe, it is expected to contain a Dβ1·Jα2 complex. Therefore, the 1.5-kb Eco RI fragment is considered to have an active V-(D)-J complex.

It has been reported that the γ chain gene is expressed only in the CTL recognizing class I MHC determinants (42). Th and CTL specific for the class II MHC determinants are reported to have no transcript of the γ chain gene. The rearrangement pattern of the γ chain gene is very restricted in mice (18). Only one mode of productive rearrangement has been reported in the γ chain gene in mouse T cell lines and hybridomas, and CTL specific for class I MHC antigens have the productively rearranged γ chain genes (17-kb Eco RI fragment hybridized with the Cγ3 probe) (17, 18, 42); Th have the nonproductively rearranged ones. Southern blot hybridization analysis showed that in PEC-1, PN, and SN lines, the Cγ3 probe hybridized with the rearranged Eco RI fragment that was 17 kb long, which is considered to be specific for the productive rearrangement of the γ chain gene (Fig. 4 E). In the SPB line the Cγ3 probe hybridized with the 22- kb Eco RI fragment, indicating that the rearrangement was a nonproductive one. These results are compatible with the data of Northern blot hybridization analysis, which show that the transcripts of the γ-chain gene are present in PEC-1, PN, and SN lines but not in the SPB line. The clones derived from PEC-1, PN, and SN lines showed the identical pattern of rearrangement of the α, β, and γ chain genes with each other (Fig. 4), as well as with parental lines (data not shown), ensuring the clonal continuity between the parental lines and clones.

Discussion

The cellular lineage of NK cells has been a matter of great debate for years. One of the major complications in terms of cellular lineage of NK cells stems from the heterogeneity of effector cells that can mediate NK-like activity (5, 39). By using various culture conditions, a variety of NK-like cytotoxic effector cells could be generated, each of which exhibited phenotypical and functional characteristics distinct from endogeneous NK cells in normal animals. Thus, for instance, effector cells with phenotypical features indistinguishable from conventional CTL, (5) as well as those with monocytic features (43), have been reported to exert NK-like activity. In certain situations, even well-defined, cloned CTL lines could show NK-like activity in addition to original antigen-specific cytotoxicity (44).

In the present study, we used a complete set of the α, β, and γ chain gene probes to examine the TCR genes of a number of murine cloned LGL lines. Phenotypical and morphological features of all the cloned lines corresponded
exactly to the endogenous NK cells (NK_T, reference 5) in normal mice (Table
1). Furthermore, functional study using a panel of target cells indicated that the
cloned lines exhibited essentially the same cytotoxic repertoire as those of
endogenous NK cells, functionally distinguishing them from, for instance, IL-2-
induced LAK activity (Fig. 2). The results indicated that in all the LGL lines
(PEC-1, PN, and SN) except for one line, SPB, the full-length transcripts of the
\(\alpha\), \(\beta\), and \(\gamma\) chain genes were detected. In the SPB line, no transcript of the \(\gamma\)
chain gene could be detected, although the transcripts of the \(\alpha\) and \(\beta\) chain genes
were clearly detected (Fig. 3). In the LGL lines studied, all of the \(\alpha\), \(\beta\), and \(\gamma\)
chain were rearranged (Fig. 4). Surprisingly, independent LGL lines, PEC-1, PN,
and SN, showed the same rearrangement pattern of the \(\beta\) chain gene. This
result strongly suggests that PEC-1, PN, and SN lines use the same \(V_\beta\) and \(J_\beta\)
segments. The \(\gamma\) chain gene was productively rearranged in PEC-1, PN, and SN
lines and aberrantly in the SPB line. The result was compatible with that of
Northern blot hybridization analysis which showed that only the SPB line had
no transcript of the \(\gamma\) chain gene.

It was particularly noted that NK clones derived from spleen and PEC of
athymic nude mice were indistinguishable, in these genetic analyses, from those
derived from euthymic mice, and all of their TCR genes were functionally
rearranged and transcribed. We believe that this is the first indication that cell
lines derived from nude mice showed complete rearrangement and transcription
of the \(\alpha\), \(\beta\), and \(\gamma\) chain genes of TCR. At present, we can not completely
eliminate the possibility that the lines underwent the gene rearrangement and
transcription during the culture process. There is, however, evidence that the
Thy-1* population of nude mouse spleen cells, which is quite enriched with NK
cells, showed rearrangements of the \(\beta\) chain gene of TCR (Y. Yaoita and T.
Honjo, unpublished observation). It is thus suggested that NK cells might
undergo the rearrangement and transcription of the TCR genes in the absence
of thymus in vivo, although we cannot completely exclude the possibility that
the thymus rudiment in the early stage of development of nude mice might be
involved in the rearrangement and expression of the TCR genes in the LGL
lines. There is accumulating evidence that NK cells reside in a variety of tissues
of normal animals in addition to the recirculation pool, including liver as pit cells
(38), lung (45), epithelial tissues like intestine and epididymis as intraepithelial
lymphocytes (46, 47), and epidermis (47). It is thus tempting to speculate that
NK cells represent a special category of lymphocytes of T cell lineage, which
migrate directly from bone marrow to the above peripheral tissues, skipping
thymus, and differentiate extrathymically.

Another major question to be addressed then would be whether NK cells use
such TCR gene products for manifestation of cytotoxic activity, as conventional
T cells do. In terms of the \(\gamma\) chain, the SPB line that lacked transcript of the \(\gamma\)
chain gene was found to be functionally indistinguishable from other NK lines,
as well as normal NK cells, and no specific deletion of cytotoxic activity was
observed. It thus seems unlikely that the \(\gamma\) chain is directly involved in the
recognition of the NK cells. Since all of the clones examined showed gene
rearrangement, as well as full-length transcripts of the \(\alpha\) and \(\beta\) chain genes of
TCR, it seemed possible that NK cells used TCR consisting of the \(\alpha\) and \(\beta\) chains.
as has been implied by Yanagi et al. (23). However, detailed functional comparison with the patterns of such gene rearrangement made such a possibility rather unlikely. Thus, for instance, PFC-I cl.1H5.23 and SN-13, which selectively lacked the cytotoxic activity against RLβ1 while fully maintaining the activity against other susceptible targets, showed β chain gene rearrangement patterns indistinguishable from other clones reactive to RLβ1 target, as judged by a number of the β chain gene probes. On the other hand, the SPB line that was highly cytotoxic to RLβ1 exhibited a rearrangement pattern of the β chain gene that was very distinct from those in PEC-1, PN, and SN lines; however, in this case, there was the possibility that the same Vβ gene in the SPB line as in the others was rearranged to different Dβ and Jβ segments. However, if this be the case, the antigen specificity of the TCR of the SPB line should become different from that of the PEC-1, PN, and SN lines. It thus seems very unlikely that the characteristic cytotoxic spectrum of endogenous NK cells is mediated by the specificity defined by the single entity of the TCR on NK clones. This would be compatible with the findings that some of the LGL lines without the β chain gene rearrangement could still exhibit NK activity in rats (24) and humans (22). It should be noted that all of the present clones were primarily induced by IL-2, and we have already indicated that normal NK cells were heterogeneous in terms of IL-2 responsiveness, namely that NK1 (Thy-1\(^{-}\)) was unresponsive, and NK1 (Thy-1\(^{+}\)) was responsive (5). Indeed, all of the clones were NK\(1\) type. In terms of NK1, no genetic information of TCR is available, simply because no cell lines with this category have been established yet. In this regard, the recent observation by Dorshkind et al. (48) that severe combined immunodeficiency mice did show normal NK activity and the effector was totally NK1 type is very suggestive. Suppose NK1 and NK\(1\) belong to the same cellular lineage as we implied before (5); that would be a case in which the major transitional event could be the rearrangement of TCR genes.

At present, we have no idea which kinds of specificity TCRs on NK clones define. In this aspect, it was of particular interest that most of the NK clones established from different organs from independent mice showed indistinguishable pattern of the β chain gene rearrangement. Similar observation was also made by Yanagi et al. (23). Three basic explanations could be considered: (a) The cloning, as well as expansion procedure, using macrophages and IL-2 somehow selected the NK clones with particular TCR. The finding that SPB and other NK clones established by the different procedures, using a retrovirus-producer cell line as an inducer, showed quite different patterns of rearrangement (data not shown) supports this notion; (b) NK cells in normal animals might have some preference for particular Vβ genes irrespective of the specificity. For instance, it is known that, in murine pre-B cell lines established from fetal liver by using Abelson murine leukemia virus, the V\(\text{H}\) genes that are located in the most 3' region of the V\(\text{H}\) gene cluster are preferentially rearranged to the D\(\text{J}_{\text{H}}\) complexes (49); (c) finally, the common TCRs of the lines might recognize some ubiquitous structures among their targets, which may not be directly linked to cytotoxic activity, but is associated with some other functions of the cells. Most recently, it was reported that NK cell-mediated cytotoxicity was negatively affected by the presence of the class I MHC antigens on target cells (50). One
possibility would be then that TCRs on NK cells are directed toward MHC antigens and involved in the negative regulation of the intrinsic cytotoxic manifestation of them against MHC-expressing targets. In any case, the specificity, as well as the possible functional role, of TCR on NK cells remains to be elucidated.

Summary

Using cloned murine large granular lymphocyte (LGL) lines, the expression and the rearrangement of the α, β, and γ chain genes of the T cell receptor (TCR) were analyzed. Morphological, phenotypical, as well as functional studies indicated that the LGL lines were identical to normal, endogenous NK cells. Northern blot hybridization analysis indicated that the full-length transcripts of all the α, β, and γ chain genes were expressed in most of the LGL lines, including two lines derived from athymic nude mice. In one line, SPB, however, no transcript of the γ chain gene was detected, whereas the α and β chain genes were clearly expressed. In every LGL line studied, all of the α, β, and γ chain genes were rearranged. Conforming to the results of Northern blot hybridization study, the γ chain gene of the SPB line was aberrantly rearranged, whereas those of all the other lines were productively rearranged. The results clearly revealed that NK cells represented a population of lymphocytes genetically committed to the T cell lineage. It was also suggested that the expression and rearrangement of the TCR genes of NK cells might occur in a thymus-independent fashion.

An SPB line without expression of the γ chain gene could exhibit NK activity indistinguishable from other NK lines. Furthermore, the rearrangement patterns of the β chain gene did not correlate with the specificity of the cytotoxic activity. These results strongly suggested that the cytotoxic activity in NK cells was not directly mediated by TCR on them. We particularly noted that the β chain gene of most independently established LGL lines showed identical patterns of rearrangement, indicating that they used the same Vβ and Jβ gene segments. The significance of the restricted pattern of rearrangement of the β chain gene in LGL lines, as well as the possible functional roles of TCR on NK cells, was discussed.

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