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T-cell receptor gene rearrangement and expression in human natural killer cells: natural killer activity is not dependent on the rearrangement and expression of T-cell receptor α, β, or γ genes

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Abstract. To test the hypothesis that the T-cell receptor (*Tcr*) γ gene encodes a natural killer (NK) cell receptor molecule, three human NK clones and fresh peripheral blood lymphocytes with NK activity from two patients with a $CD16^+$ lymphocytosis were analyzed for rearrangements and expression of the human *Tcr* α, β, and γ genes. Two of the clones displayed distinct rearrangements of their *Tcr* β and γ genes and expressed mature *Tcr* α, β, and γ RNA. However, one of the clones and both patient samples displayed marked NK activity but failed to rearrange or express any of their *Tcr* genes. These findings demonstrate that human natural killer activity is not dependent on *Tcr* γ gene rearrangement and expression. In addition, they confirm previous findings concerning the lack of *Tcr* α and β gene expression in some natural killer cells. Thus, they suggest the existence of additional NK-specific recognition molecules.

Introduction

Natural killer (NK) cells are a subset of lymphocytes that have been defined by their ability to recognize and kill a variety of tumor cell lines without prior sensitization (Herberman and Ortaldo 1981). Previous studies have clearly demonstrated that NK activity is mediated by a heterogeneous population of cells which display different constellations of cell surface antigens and vary in their ability to recognize and kill specific tumor cell lines (Zarling et al. 1981, Lanier et al. 1983, Pawelec et al. 1982, Lopez-Botet et al. 1983, Hercend et al. 1983a, Ortaldo et al. 1981). The majority of NK cells present in normal human peripheral blood express the E-rosette receptor CD2 as well as the more NK-specific CD16 and Leu-19 surface markers. However, most of these cells do not express the T-cell surface antigens CD3 or CD4 and display variable levels of expression of CD8. In contrast, a minority of peripheral blood cells which are capable of non-major histocompatibility complex (MHC) restricted killing display the $CD3^+$, $CD4^+$, and/or $CD8^+$ phenotype characteristic of more mature peripheral blood T lymphocytes. The majority of NK cells appear to differ from cytotoxic T lymphocytes (CTL) in that their killing does not require recognition of target cell MHC antigens (Herberman and Ortaldo 1981). However, NK clones have been identified which are able to mediate both MHC-independent NK killing and MHC-dependent CTL activity (Kornbluth et al. 1981, Kornbluth 1985, David et al. 1987).

The observed heterogeneity of NK cell surface phenotype and cytotoxic activity has raised a number of fundamental questions concerning both the lineage(s) of NK cells and the mechanisms used by these cells to recognize their tumor cell targets. Thus, it is unclear whether some or all NK cells are developmentally related to cells of the T lymphocyte lineage. In addition, the structures utilized by NK cells to recognize tumor cell antigens remain obscure. The in vitro growth of normal human NK clones (Hercend et al. 1983a, Kornbluth et al. 1981, Dennert 1980, Kornbluth et al. 1982) and the molecular cloning of the genes encoding the human T-lymphocyte receptor for antigen (Hedrick et al. 1984a, b, Saito et al. 1984a, b, Yanagi et al. 1984, Sim et al. 1984, Chien et al. 1984b) have made possible studies which begin to address these questions.

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Human helper and cytotoxic T cells recognize antigen via a heterodimeric, idiotypic T-cell receptor molecule which has been shown to be composed of acidic (α) and basic (β) glycoproteins each of relative mass 40 000–50 000 (Allison et al. 1982, Haskins et al. 1983, Meuer et al. 1983). The genes encoding both chains of the receptor have been cloned and shown to be composed of germ line variable (V), diversity (D) (at least in the case of the Tcr_β genes), joining (J), and constant (C) gene segments which undergo specific rearrangements during T-cell ontogeny to produce mature *Tcr* α and β genes (Hedrick et al. 1984a, b, Saito et al. 1984a, b, Yanagi et al. 1984, Sim et al. 1984, Chien et al. 1984a, Clark et al. 1984, Gascoigne et al. 1984, Siu et al. 1984a, b). In addition, a third T-cell specific gene, γ, has been described which is also composed of rearranged variable, joining, and constant gene segments (Saito et al. 1984a, Hayday et al. 1985, Quertermous et al. 1986). The product of the human Tcr_γ gene (in conjunction with a second chain termed δ) has recently been shown to be expressed on the surface of $CD3^+$ T cells from an immunodeficiency patient as well as on the surface of several T-cell tumor lines and a small subset of normal adult and fetal peripheral blood T cells (Brenner et al. 1986, 1987, Weiss et al. 1986, Moingeon et al. 1987, Borst et al. 1987). The function of the γ gene product remains unknown, but several Tcr_γ expressing clones have been shown to be capable of mediating the non-MHC restricted killing of a variety of tumor cell targets (Moingeon et al. 1987, Brenner et al. 1987, Borst et al. 1987). These findings have raised the possibility that the Tcr_γ gene encodes an NK antigen receptor molecule.

In the studies described in this report, we have examined the rearrangement and expression of *Tcr* α, β, and γ genes in three human NK clones which display different cell surface phenotypes, as well as in fresh peripheral blood lymphocytes (PBL) with NK activity from two patients with a $CD16^+$ lymphocytosis. The results demonstrate that NK activity is not dependent on the rearrangement and expression of the Tcr_γ gene complex. In addition, they confirm previous reports of the absence of *Tcr* α and β gene expression in some NK cells (Ritz et al. 1985, Yanagi et al. 1985, Reynolds et al. 1985, Lanier et al. 1986) and suggest a molecular mechanism which could explain the previously described dual NK and CTL function of certain NK clones.

Materials and methods

Isolation and characterization of human NK clones. NK clones 3.3, 1S, and 37 were derived from primary mixed lymphocyte culture (MLC)-activated PBL plated in soft agarose as previously described (Kornbluth et al. 1982). Cells were recloned twice in agarose and then expanded and maintained in medium consisting of RPMI 1640 supplemented with hydroxyethylpiperazine ethanesulfonic acid (HEPES; 25 m*M*), glutamine (2 m*M*), penicillin (100 units/ml), streptomycin (100 µg/ml), 15% heat-inactivated pooled human serum (Bio-Bee, Boston, Massachusetts) and 15% interleukin-2 medium (Lymphocult-T, Biotest Diagnostics Corp., Fairfield, New Jersey). NK clone 3.3 was derived from the MLC responder-stimulator combination DMx, clone 1S from the combination BDx, and 37 from the MLC EJx. All of these clones have strong and exclusive NK activity (Kornbluth et al. 1982, and Table 1). NK function was assessed in 4-h ^{51}Cr-release assays (Kornbluth et al. 1982) using the NK sensitive target cell lines K562, MOLT-4, and HSB-2. Control target cell lines included phytohemagglutinin-activated PBL derived from the MLC responder (designated auto) and stimulator (designated stim) donors used to generate the clones. Clones were phenotyped by indirect immunofluorescence flow cytometry using the monoclonal antibodies 9.6 (anti-CD2), 64.1 (anti-CD3), 66.1 (anti-CD4), and 51.1 (anti-CD8) generously provided by Dr. John Hansen. The B73.1 (anti-CD16) monoclonal antibody was kindly provided by Dr. Giorgio Trinchieri.

Patient samples. Both of the patients in this study were seen on an outpatient basis at Rush-Presbyterian-St. Luke's Medical Center. Patient 1 is a 56-year-old, otherwise healthy and asymptomatic female suspected of having chronic lymphocytic leukemia. Her absolute white blood cell count at the time of the study was 15 600/mm^3, with 66% lymphocytes and 34% polymorphonuclear lymphocytes (PMN). Patient 2 is a 35-year-old asymptomatic male suspected of having chronic lymphocytic leukemia. At the time of the study, his absolute white blood cell count was 25 400/mm^3 with 57% lymphocytes, 24% PMN, 9% monocytes, and 10% eosinophils. Peripheral blood from both patients was sterilely collected into preservative-free heparin and subjected to Ficoll gradient separation, and the mononuclear population was then divided into aliquots for direct immunofluorescence analysis and the preparation of DNA and RNA as described below. Cells were phenotyped by direct immunofluorescence flow cytometry on an EPICS C flow cytometer using the following monoclonal reagents: Leu-5 (anti-CD2), Leu-4 (anti-CD3), Leu-3 (anti-CD4), Leu-2 (anti-CD8), Leu-11 (anti-CD16), IL-2R (anti-CD25), Leu-7, DR (anti-HLA-DR), and Leu-19 (Becton-Dickinson, Mountain View, California). The surface phenotypes of the patients' cells were determined at least three times over a 3-month period, and those of both patients remained stable. Cells from patient 2 were fractionated into $CD3^+$ and $CD3^-$ populations by indirect panning with Leu-4 and Leu-12 on a goat anti-mouse antibody-coated plastic dish (Wysocki and Sato 1978) prior to the preparation of DNA and RNA. After panning, the nonadherent cells were 99% $CD3^-$ (data not shown). NK activity of the patient's cells was determined by a standard 3-h ^{51}Cr-release assay (Bray and Brahmi 1986) using the NK-sensitive target cell line K562. Cells were assayed both with and without 18 h of pretreatment with highly purified recombinant interleukin-2 (rIL-2) (Cetus, Emeryville, California) (100 units/ml).

Southern blot analysis. High relative mass DNA was prepared according to the method of Bell and co-workers (1981) except that the spooling step was replaced by ethanol precipitation at −20 °C. Southern blotting was performed on a minigel system using from 1 to 5 µg high relative mass DNA per lane. Alkaline blotting to Zeta Probe nylon membranes (Biorad, Richmond, California) and hybridizations were performed following the manufacturer's instructions except that 5% dextran sulfate (Oncor, Gaithersburg, Maryland) was included in some hybridizations. Probes were Tcr_β, the 790 bp Xho I/Eco RI, or the 389 bp Bgl II constant region fragments from the Tcr_β cDNA 12A1 (Leiden et al. 1986a) from the T-cell tumor line HPB-MLT, and Tcr_γ, the 0.8 kb Hind III/Eco RI genomic DNA fragment containing the $J_\gamma 1.3$ gene segment (Quertermous et al. 1986).

Northern blot analysis. Whole NK cell RNA (1–10 µg) was prepared from 5×10^5 to 5×10^6 NK cells and from the human T-cell tumor cell line HPB-ALL according to the urea/$LiCl_2$ method of Auffray and Rougeon (1980). Northern blotting was performed using a formaldehyde

Table 1. Phenotypic and functional characterization of three human NK clones

Clone	Phenotype	Antibody	Cytolytic activity* (% lysis)				
			K562	MOLT-4	HSB2	Auto†	Stim†
NK 3.3	$CD2^+$, $CD16^+$, $CD3^-$, $CD4^-$, $CD8^-$	—	48	50	47	ND#	ND
		+CD3‖	46	50	48	ND	ND
NK 1S	$CD2^+$, $CD3^+$, $CD4^+$, $CD8^-$	—	61	40	ND	5	7
NK 37	$CD2^+$, $CD3^+$, $CD4^+$, $CD8^-$	—	62	57	38	2	2
		+CD3	61	55	ND	ND	6

* Clones were assayed at effector-to-target cell ratios of 3 : 1
† See Materials and methods for a description of auto and stim target cells
# ND, not determined
‖ CD3-specific antibody 64.1 was present as indicated throughout the cytotoxicity assays at a final ascites dilution of 1 : 500

minigel system and Biotrans nylon membranes (ICN Biochemical, Plainview, New York) according to standard techniques (Maniatis et al. 1982). Prehybridization was for 2 h at 42 °C in 3× standard sodium citrate (SSC), 50% formamide, 50 m*M* HEPES (pH 7.0), 5× Denhardt's solution, 0.15 mg/ml denatured salmon sperm DNA, and 0.1% sodium dodecyl sulfate (SDS). ^{32}P-labeled, nick-translated probe (2×10^8 cpm/μg) was added to the prehybridization mixture and hybridization was carried out for 16–20 h at 42 °C. Washing was performed for 2 × 15 min in 1 × SSC, 0.5% SDS, at room temperature followed by four 15-min washes in 0.1 × SSC, at 55 °C. Autoradiography was performed for 1–10 days at −70 °C using Dupont intensifying screens. Probes were Tcr_α, the full-length Tcr_α cDNA from the human T-cell clone L17 (Leiden et al. 1986b) in pUC18; Tcr_β, the 12A1 Tcr_β cDNA from the human T-cell tumor line HPB-MLT (Leiden et al. 1986a) in pUC18; and Tcr_γ, the Tcr_γ cDNA from the human T-cell tumor line HPB-MLT (Dialynas et al. 1986) in pUC13.

Results

Characterization of NK cells. The three human NK clones used in these studies were derived from MLC as described above. Clone 3.3 displays the surface phenotype ($CD2^+$, $CD16^+$, $CD3^-$, $CD4^-$, $CD8^-$) characteristic of the majority of peripheral blood NK cells (Table 1). In contrast, clones 1S and 37 display a surface phenotype more characteristic of mature peripheral blood T lymphocytes ($CD2^+$, $CD3^+$, $CD4^+$, $CD8^-$) (Table 1). All three clones possess NK activity as defined by their ability to kill K562 human tumor cells (Table 1). This killing represents NK as opposed to CTL activity because all three cell lines fail to kill either their stimulator cells or cells expressing the same HLA antigens as their stimulator cells (Kornbluth et al. 1982 and Table 1). Moreover, neither clone 37 nor clone 3.3 killing of K562 targets is blocked by the addition of a monoclonal CD3-specific antibody (Table 1) which has been shown to block the CTL activity of a wide range of cytotoxic T-cell clones (Meuer et al. 1982).

The peripheral blood from both patients contained a predominance of lymphocytes with a cell-surface phenotype ($CD2^+$, $CD16^+$, $CD3^-$, $CD4^-$, $CD8^-$) similar to that of NK clone 3.3 (Table 2) and the majority of peripheral blood NK cells. As previously described (Pistoia et al. 1986), the $CD16^+$ cells from both patients are uniformly $CD3^-$. However, the $CD16^+$ cells from these two patients differ in their surface expression of the Leu-7 antigen in that the majority of the $CD16^+$ lymphocytes from patient 2 were strongly Leu-7 positive while only 19% of the $CD16^+$ cells from patient 1 coexpressed this antigen. Fresh peripheral blood cells from both of the patients displayed spontaneous NK activity as defined by MHC-independent cytotoxicity against the K562 target cell line (Table 3). rIL-2 treatment increased the NK activity of cells from both of the patients.

Rearrangement of Tcr genes in the NK cells. Southern blot analyses of Eco RI-, Hind III-, and Bam HI-digested NK-cell and control B-cell DNAs revealed that the NK clones

Table 2. Phenotypic characterization of freshly isolated peripheral blood mononuclear cells from two patients with a $CD16^+$ lymphocytosis*

Antigen	(Normal range)	Patient 1	Patient 2
CD2	(58–86)	98	96
CD3	(53–81)	14	32
CD4	(23–58)	5	16
CD8	(13–23)	8	18
CD16	(5–20)	83	53
CD25	(2–4)	<2	8
Leu-7	(5–20)	19	69
HLA-DR	(<2)	79	27
Leu-19	(5–20)	80	8

* Direct immunofluorescence was performed on Ficoll gradient-purified mononuclear cells on an EPICS C flow cytometer as described in Materials and methods. Results represent the percentage of PBL positive for a given marker. Note that both patients have very few circulating peripheral blood B cells as evidenced by the high percentage of $CD2^+$ cells

Table 3. NK cytolytic activity of peripheral blood mononuclear cells from two patients with a $CD16^+$ lymphocytosis

Patient	rIL-2*	Cytolytic activity†
1	—	185
	+	295
2	—	42
	+	116

* Cells were incubated in RPMI 1640, 10% FCS, and recombinant IL-2 (100 units/ml) for 18 h at 37 °C prior to the cytotoxicity assay

† Cytotoxicity is expressed as lytic units/10^7 cells. One lytic unit equals the number of effector cells required to produce 20% specific lysis of ^{51}Cr-labeled K562 target cells (see Materials and methods). Cytotoxicity assays were performed at effector-to-target ratios of 40, 20, 10, and 5 : 1, and results represent the mean lytic units of all four ratios. The mean values for healthy laboratory control PBLs are 30–90

and patient isolates can be divided into two groups based on their Tcr gene rearrangements. The $CD3^+$ 1S and 37 clones both displayed distinct Tcr β (Fig. 1A) and γ (Fig. 1B) gene rearrangements, while the $CD3^-$ 3.3 clone (Fig. 1) and the two patient samples (Fig. 2; patient 1, A and B; patient 2, C and D) displayed a germ-line configuration of *Tcr* β and γ genes which was identical with that of the control B-cell DNAs.

Although the fresh patient isolates did not appear to display a clonal pattern of Tcr gene rearrangement by Southern blot analysis, it could be argued that, as is true of normal PBL, the patient samples are composed of a large number of heterogeneous clones which have undergone multiple, distinct Tcr_β gene rearrangements (which would not be detected as distinct rearranged bands in this Southern blotting system). This is unlikely for three reasons. (i) The intensities of the Hind III 3.5 kb $C_\beta 1$-containing bands (Fig. 2, lanes A3 and C3) are almost identical with those of the 6.7 kb Hind III bands from these samples, as well as with those of the analogous control bands (Fig. 2, lanes A4 and C4). This pattern is quite distinct from that seen in polyclonal Tcr_β gene rearranging T-cell populations (such as normal PBL) which reveal significant decreases in the intensity of the 3.5 kb band, often accompanied by smearing in the region of the 10.0 kb germ-line Eco RI-$C_\beta 1$ containing band (data not shown). (ii) As shown in Figure 3, at least patient sample 2 does not show evidence of Tcr_β RNA expression which always appears to accompany Tcr_β gene rearrangement. (iii) In all cases previously studied, Tcr_γ gene rearrangement has preceded Tcr_β gene rearrangement. These γ

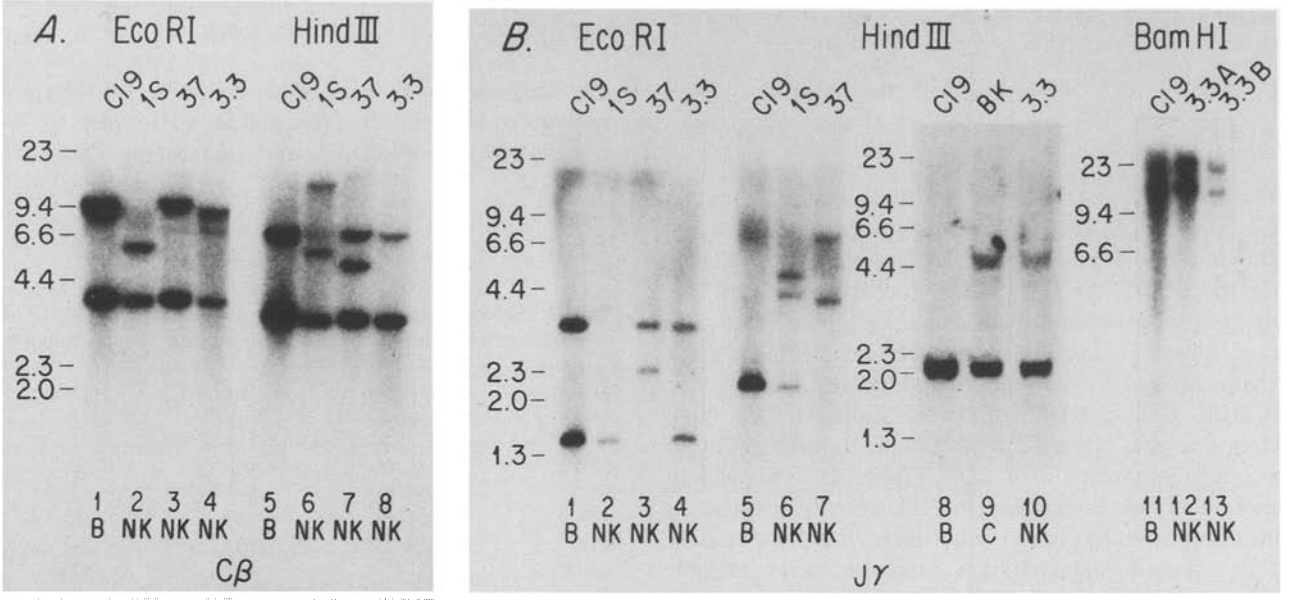

Fig. 1A and B. Southern blot analysis of Tcr gene rearrangements in NK clones. Clone 9 (Cl9) is an Epstein-Barr virus (EBV) transformed B lymphoblastoid cell line with germ line *Tcr* β and γ genes (Leiden et al. 1986). 3.3, 1S, and 37 are NK clones (see Table 1). BK is DNA isolated from the whole blood of a healthy volunteer. Three to five micrograms of DNA was digested with the appropriate restriction enzyme and loaded into each well. Size markers in kilobases are shown to the *left* of each set of blots. **A** Analysis of Tcr_β gene rearrangements in human NK clones. The 10.5 kb Eco RI band from the 37 clone (lane A3) is reproducibly larger than the germ-line 10.0 kb Eco RI band and therefore represents a Tcr_β gene rearrangement in this clone. The 9.0 kb C_β-containing Eco RI bands in the 37 and 3.3 cell lines (lanes A3 and 4) are due to a partially resistant Eco RI site near the $C_\beta 2$ gene segment (Furley et al. 1986) and therefore do not represent Tcr_β gene rearrangements. **B** Analysis of Tcr_γ gene rearrangements in human NK clones. The 5.0 kb J_γ-containing Hind III band in the 3.3 lane (lane B10) does not represent a Tcr_γ gene rearrangement but is due to the absence of a previously reported polymorphic Hind III site (Giuseppe et al. 1987) from one allele of this clone. This site is also absent from one allele of the DNA isolated from a healthy volunteer (BK; lane B9)

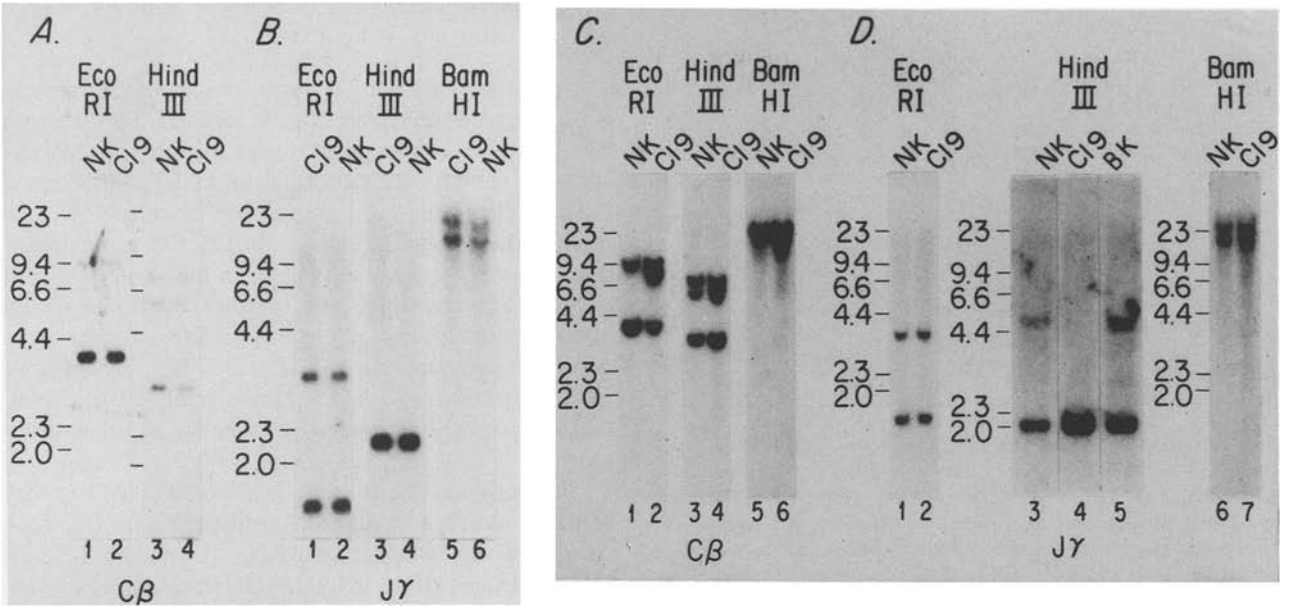

Fig. 2A–D. Southern blot analysis of Tcr gene rearrangements in PBL from two patients with a $CD16^+$ lymphocytosis. One to five micrograms of DNA from either the clone 9 (Cl9) B lymphoblastoid control cell line (see legend to Fig. 1), a healthy volunteer (BK), or from the PBL from patient 1 (NK; **A** and **B**) or patient 2 (NK; **C** and **D**) was digested with the appropriate restriction enzyme and loaded in each lane. Blots were probed with either Tcr β (**A** and **C**) or γ (**B** and **D**) specific probes (see Materials and methods). Size markers in kilobases are shown to the *left* of each set of blots. The 9.0 kb C_β-containing Eco RI band in the Cl9 sample (lane C2) reflects a partially resistant Eco RI site near the $C_\beta 2$ gene (see legend to Fig. 1). The 5.0 kb J_γ-containing Hind III band in patient sample 2 (lane D3) represents the absence of a polymorphic Hind III site in one allele from this patient (see legend to Fig. 1) and is also absent from one unrearranged Tcr_γ allele from a normal volunteer (BK; lane D5)

gene rearrangements are visible even on Southern blots of polyclonal T-cell populations (Quertermous et al. 1986). Thus, the finding that the 3.3 clone and the patient samples display a germ-line pattern of Tcr_γ genes is consistent with the finding that their Tcr_β genes also remain in a germ-line configuration.

Expression of Tcr genes in the NK cells. To determine whether the NK cells described in this report express *Tcr* genes, RNA from the three clones and one of the patient samples was subjected to Northern blot analyses using Tcr α, β, and γ specific probes (Figs. 3 and 4). Prior studies have shown that there are two forms of poly(A)$^+$ Tcr_β RNA: a mature 1.3 kb transcript, representing a functional V-D-JC rearrangement and a 1.0 kb transcript, representing a D-JC rearrangement which lacks V region sequences (Clark et al. 1984). Full-length Tcr α and γ transcripts are both 1.6 kb in size (Sim et al. 1984, Dialy-

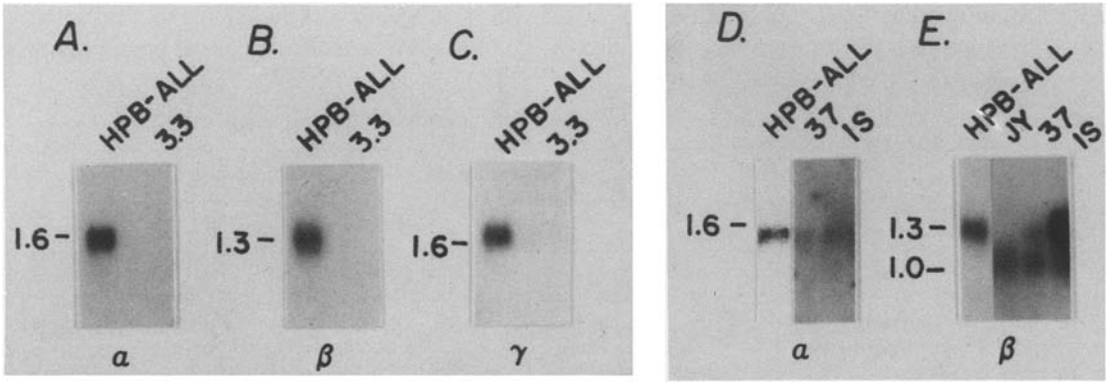

Fig. 3A–E. Northern blot analysis of Tcr gene expression in human NK clones. One to five micrograms of whole RNA from NK clones 3.3, 37, and 1S, the human T-cell tumor cell line HPB-ALL, or the B-lymphoblastoid cell line JY was loaded into each lane and subjected to Northern blot analysis as described in Materials and methods. The sizes of the previously characterized Tcr α (**A** and **D**), β (**B** and **E**), and γ (**C**) mRNAs are shown in kilobases to the *left* of each blot. The relatively light intensities of the Tcr_α bands in **D** are due to the fact that this blot had been previously hybridized to a Tcr_β probe, stripped by treatment with 80% formamide, 0.1 × SSC, at 68 °C, and reprobed with the Tcr_α probe

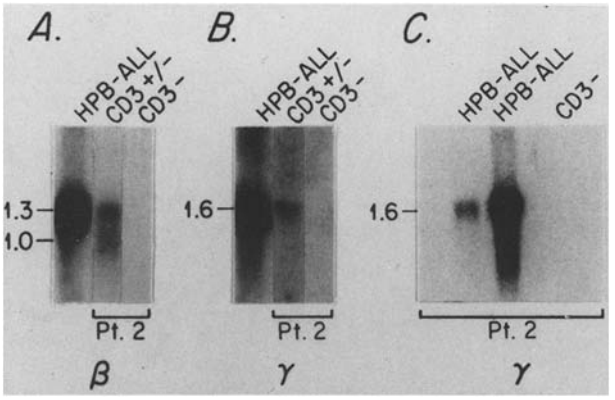

Fig. 4A–C. Northern blot analysis of Tcr gene expression in the PBL from one patient with a $CD16^+$ lymphocytosis. One to five micrograms of whole RNA from the human T-cell tumor line HPB-ALL or from the unfractionated PBL ($CD3^{+/-}$) or the $CD3^-$ cells from this patient was loaded in each lane and subjected to Northern blot analysis as described in Materials and methods. The sizes of the previously characterized Tcr β (**A**) and γ (**B** and **C**) mRNAs are shown in kilobases to the *left* of each set of blots. Two different RNA samples are shown in **B** and **C**. In **C**, 1 μg and 10 μg of HPB-ALL RNA are compared with 1 μg of RNA from the $CD3^-$ cells from patient 2. Hybridization of a similar blot to a Tcr_α probe failed to detect any Tcr_α RNA in the $CD3^-$ cells from this patient (data not shown). Autoradiograms have been intentionally overexposed to rule out low level Tcr gene expression by these cells

nas et al. 1986). Examples of these full-length Tcr transcripts are shown from the well-characterized human T-cell tumor cell line HPB-ALL (Figs. 3 and 4).

As seen in Figures 3 and 4 and summarized in Table 4, the NK clones can be divided into two subsets based on their patterns of Tcr gene expression. The $CD3^+$ clones 37 and 1S express full-length Tcr α and β (Fig. 3D and E) RNAs, while the $CD3^-$ 3.3 clone and the $CD3^-$, $CD16^+$ cells from patient 2 do not express detectable levels of full-length Tcr RNA. By loading five times more RNA and overexposing the autoradiograms, clone 3.3 can be shown to express very low levels of short Tcr β (1.0 kb) and γ (1.3 kb) RNAs (data not shown). These RNA species presumably reflect low level transcription of the unrearranged *Tcr* genes present in this clone.

Discussion

The present studies of Tcr gene rearrangement and expression in normal human NK clones and fresh PBL with NK activity from two patients with a $CD16^+$ lymphocytosis provide information concerning both the lineage and function of human peripheral blood NK cells. First, the results demonstrate that human NK function is not dependent upon Tcr_γ gene rearrangement and expression. In addition, they confirm previous studies which have shown that NK activity is not dependent on Tcr α/β gene rearrangement and expression (Ritz et al. 1985, Yanagi et al. 1985, Reynolds et al. 1985, Lanier et al. 1986). Thus, they suggest the existence of alternate NK receptor structures.

Furthermore, the present data, when taken together with previous reports, allow the delineation of at least four subsets of cells which are capable of mediating the non-MHC restricted killing of tumor cell targets. The first subset, which is represented in this study by the $CD16^+$, $CD3^-$, $CD4^-$, $CD8^-$ 3.3 clone and both fresh patient isolates, is probably representative of the majority of peripheral blood NK cells, at least by cell-surface phenotype. This subset of NK cells does not rearrange or express any of the known *Tcr* genes. The finding that the $CD3^-$ 3.3 clone and the $CD3^-$ cells from patient 2 do not express mature Tcr α, β, or γ transcripts and, therefore, presumably cannot express a mature Tcr molecule on their surfaces is in agreement with the previous observation that CD3 and Tcr cell-surface expression are tightly coupled (Weiss et al. 1984).

The 1S and 37 clones represent a second class of T cells which are capable of mediating the non-MHC restricted killing of NK targets. These clones, which display a cell-surface phenotype similar to that of mature peripheral blood T cells, rearrange their *Tcr* β and γ genes and express full-length Tcr α and β transcripts. Therefore, they probably express functional Tcr molecules on their surface. This is in accord with the finding that both clones are $CD3^+$. While we cannot rule out the possibility that

Table 4. Summary of Tcr gene rearrangement and expression in human NK clones and PBL from two patients with a $CD16^+$ lymphocytosis

Clone	Phenotype	Tcr gene rearrangement		Tcr gene expression		
		β	γ	α	β	γ
NK 3.3	$CD2^+$, $CD16^+$, $CD3^-$, $CD4^-$, $CD8^-$	*2gl**	*2gl*	—	—	—
NK 1S	$CD2^+$, $CD3^+$, $CD4^+$, $CD8^-$	*2r*†	*2r*	+	1.3/1.0‡	ND
NK 37	$CD2^+$, $CD3^+$, $CD4^+$, $CD8^-$	*2r*	*2r*	+	1.3/1.0	ND
Pt. 1	$CD2^+$, $CD16^+$, $CD3^-$, $CD4^-$, $CD8^-$	*2gl*	*2gl*	ND‖	ND	ND
Pt. 2	$CD2^+$, $CD16^+$, $CD3^-$, $CD4^-$, $CD8^-$	*2gl*	*2gl*	—	—	—

* gl, germ-line. The number of alleles in each configuration is shown
† r, rearranged
‡ The size of the Tcr_β RNA is shown in kilobases
‖ ND, not determined

these cells express a Tcr γ/δ receptor in association with CD3, it is far more likely that they express a CD3 associated α/β Tcr. All previously described Tcr γ/δ expressing cells display the $CD4^-/CD8^-$ surface phenotype and fail to coexpress full-length Tcr α and β transcripts. In contrast, both the 1S and 37 NK clones express the CD4 antigen, and both express full-length Tcr α and β transcripts. Because clone 37 killing of K562 targets is not inhibited by the addition of CD3-specific antibody, we would argue that the NK activity of this clone is not mediated by the Tcr/CD3 complex and would postulate that Tcr gene expression in this clone (and perhaps others with a similar cell-surface phenotype) may represent a coincident finding which is not related to its ability to recognize and kill NK targets. In fact, it is interesting to note that a subset of NK clones has been identified which express both NK and CTL activity (Kornbluth et al. 1981, Kornbluth 1985). These results could be explained if such cells utilize Tcr molecules for CTL function and a second, putative NK receptor for natural killing activity.

Two additional subsets of cells have been described which both appear to be capable of mediating the non-MHC restricted killing of NK targets via cell-surface Tcr/CD3 complexes. First, several $CD3^+$/Tcr α/β^+ NK clones have been described (Hercend et al. 1983b, David et al. 1987). Unlike clone 37, the killing of K562 targets by these $CD3^+$ clones is blocked by both monoclonal anti-CD3 reagents and by a monoclonal idiotype-specific antibody directed against the Tcr α/β molecule present on the clones. Moreover, several of these clones have also been shown to mediate allo-CTL activity via their CD3/Tcr receptors (David et al. 1987). In addition, several Tcr γ/δ^+ clones have been described which mediate non-MHC restricted cytotoxic activity which is also blocked by idiotype-specific and CD3-specific monoclonal antibodies (Moingeon et al. 1987, Brenner et al. 1987, Borst et al. 1987). Whether the non-MHC restricted killing mediated by these two subsets of Tcr expressing cells represents an artifact of prolonged in vitro culture in IL-2 or alternatively is representative of their bona fide in vivo function requires further study.

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Note added in proof:
During the preparation of this manuscript, Lanier and co-workers (*J. Immunol. 137:* 3375–3377, 1986) reported that freshly isolated $CD16^+$ human peripheral blood NK cells display a germ-line pattern of Tcr_γ genes.

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