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Phenotypic and Cytokine Analysis of Human Peripheral Blood γδ T Cells Expressing NK Cell Receptors[1]

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The presence of NK receptors (NKR) on populations of T cells has been proposed to play a regulatory role in T cell function, fine tuning the response to Ag, and influencing the nature of the immune response through rapid secretion of large amounts of cytokines. In this study, we assessed the nature and distribution of NKR on human peripheral blood γδ T cells and established clones to study cytokine release. In circulating γδ T cells, ~80% expressed CD94, ~25% expressed NKR-P1A, and ~20% expressed p58, values substantially higher than those found on αβ T cells from the same donors. When cloned for specific NKR expression, most cells in culture were NKR-P1A$^+$ whereas p58 expression was variable, suggesting that the NKR-P1A phenotype can be acquired in culture whereas expression of p58 is more stable. Some clones were triple positive for CD94, NKR-P1A, and p58. Vδ2$^+$ cells generally expressed a wider range of NKR than Vδ1$^+$ cells. Following activation through CD3, all γδ T cell clones released large amounts of IFN-γ, commencing as early as 4 h postactivation. Some clones also released TNF-α and IL-4, but no correlation with specific NKR expression was noted. Activation through NKR-P1A induced moderate levels of IFN-γ without inducing IL-4. The results suggest that activation of most γδ T cells is regulated by signaling events occurring via both the TCR and the NKR. They further show that peripheral blood γδ T cells may function as a source of the proinflammatory cytokines IFN-γ and TNF-α. *The Journal of Immunology,* **1997, 159: 3723–3730.**

It has been known for some time that both αβ TCR$^+$ and γδ TCR$^+$ T cells express NK cell receptors (NKR)[4] but the complexity and function of these receptors on T cells remains to be defined. With increasing awareness of the role that NKRs play in MHC class I recognition, it has been possible to show that they modulate various T cell functions, including cytotoxic activity and cytokine release, as well as activities dependent on MHC class II (1–6). NKR are grouped according to the MHC class I alleles they recognize and fall into two major structural families: type I membrane glycoproteins of the Ig superfamily and type II membrane glycoproteins containing a C-type lectin domain. Members of the Ig family can be divided into those expressing two (p58) or three (p70 and p140) extracellular Ig-like domains, as well as those with inhibitory or stimulatory activity for NK cell function. Inhibitory function has been shown to correlate with the presence of immunoreceptor tyrosine-based inhibitory motifs (ITIM) in the cytoplasmic tail that are lacking in NKR$^+$ cells with stimulatory activity (7). Members of the C-type lectin NKR consist of the Ly49 and NKR-P1 families in the mouse and the CD94 and NKR-P1A in humans. These NKR may also inhibit or stimulate NK cell function depending on interactions with other type II membrane glycoproteins of the NKG2 family (7–10).

Recently, it has also been suggested that certain populations of NKR$^+$ T cells function at the interface between the innate and acquired immune response by rapidly secreting large amounts of cytokines involved in the initiation of distinct effector cell functions in other populations of T cells (11). In mouse thymocytes, NK1.1 (a member of the NKR-P family) is present on a specialized subset of αβ TCR$^+$CD4$^+$ or CD4$^-$CD8$^-$ (DN) T cells that display a restricted Vα-Vβ pairing and an invariant Vα CDR3 (Vα14Jα281) sequence with a single N-region insertion. These cells rapidly release large amounts of IL-4 following activation through the CD3 complex (12–15). An equivalent subpopulation of γδ TCR$^+$ mouse thymocytes has also been detected that selectively secrete IL-4, albeit at levels lower than that found for TCRαβ$^+$NK1.1$^+$DN or CD4$^+$ T cells (16). It has also been proposed that peripheral blood Vα24JαQ Vβ11$^+$ DN T cells expressing a canonical TCR represent the human equivalent of the murine cells (17–19). Whether a comparable human γδ T cell population exists has not been determined.

γδ T cells form a unique population of cells within the immune system. In common with TCRαβ$^+$ cells they express a rearranged TCR, but the mechanisms involved in the acquisition of CDR3 region diversity, as well as the nature of the Ags recognized, and the restriction elements involved, are clearly different (20, 21). γδ T cells also share many features in common with NK cells, including high levels of spontaneous or induced cytotoxicity and a bias toward IFN-γ secretion (22, 23). Although NKR have been detected on γδ T cells, little information is available on the distribution and types of NKR present on these cells, or the relationship of NKR expression to cytokine release. In this study, we have analyzed NKR expression by human peripheral blood γδ T cells,

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[4] Abbreviations used in this paper: NKR, natural killer cell receptors; ITIM, immunoreceptor tyrosine-based inhibitory motifs.

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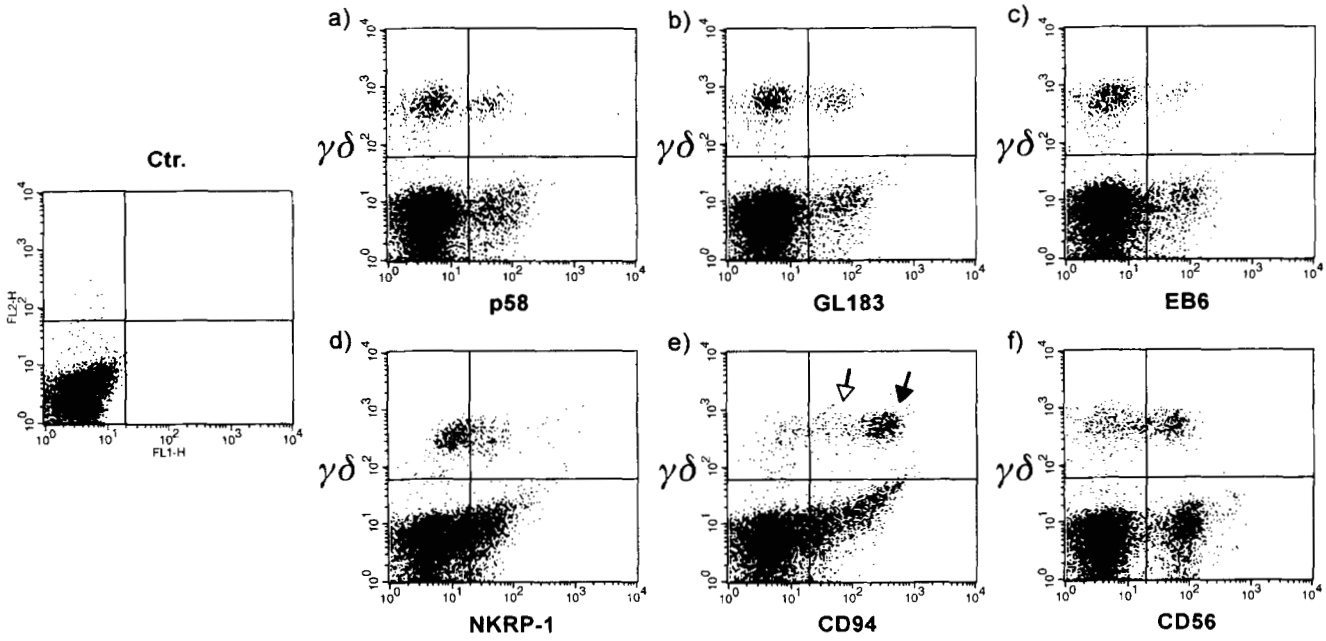

FIGURE 1. FACS analysis of PBLs stained for the γδ TCR and different NK cell receptors. A representative analysis from one healthy donor (corresponding to donor 2 in Table I) is shown. Staining was performed as described in *Materials and Methods*. Note the two distinct populations of $CD94^{dull}$ (open arrow) and $CD94^{+bright}$ (arrowhead) cell populations.

and have cloned them to determine cytokine expression following activation through either the CD3 complex or NKR.

Materials and Methods

Antibodies

Abs to CD3, αβTCR, γδTCR, CD56, CD4, and CD8 were purchased from Becton Dickinson Immunocytometry Systems (San Jose, CA). Anti-CD94 was purchased from PharMingen (San Diego, CA). FITC- or phycoerythrin-conjugated goat anti-mouse isotype-specific Abs were purchased from Coulter (Hialeah, FL). Abs to p58, p58.1 (GL183), and p58.2 (EB6) were purchased from Immunotech (Marseille, France). The Ab to human NKR-P1A (DX-1) was kindly supplied by Dr. L. Lanier (DNAX, Palo Alto, CA) and to the inhibitory form of CD94 (Z199), to Vδ2 (BB3) and to Vδ1 (A13) by Dr. A. Moretta (Dipartimento di Scienze Biomediche e Biotecnologie, Università di Brescia, Brescia, Italy) and Dr. L. Moretta (Istituto Scientifico Tumori e Centro di Biotecnologie Avanzate, Genoa, Italy).

Cells

PBMC were obtained from 11 healthy donors by discontinuous density centrifugation over Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) according to standardized procedures. γδ T cell lines and clones were obtained by sorting using a Becton Dickinson Vantage following staining with anti-γδ TCR and with anti-CD94 or anti-p58 or anti-NKRP-1A. Cells were sorted at one or 10 cells/well in 96-well plates (Costar, Cambridge, MA) in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% human serum (BioWhittaker, Walkersville, MD), 5% FCS (HyClone, Logan, UT), 200 mM L-glutamine (Life Technologies), 100 μM MEM nonessential amino acids (Life Technologies), 2-ME (Life Technologies), MEM sodium pyruvate (Life Technologies) pen/strep (Life Technologies), and PHA (1 μg/ml; Murex, Dartford, U.K.) and human recombinant IL-2 (100 U/ml; Boehringer Mannheim, Mannheim, Germany). Cells were expanded with IL-2 and restimulated every 2 wk with PHA and irradiated feeder cells (3000 rad) according to standard procedures.

FACS analysis

Immunofluorescence analysis was performed on a Becton Dickinson FACS Vantage. Data were analyzed using CellQuest software (Becton Dickinson).

Cytokine production

Cells were stimulated for 12 to 48 h on 96-well plates coated with anti-CD28 and anti-CD3 or with anti-CD94 or anti-NKRP-1A (all at 1 μg/ml). Supernatants were collected and the presence of cytokines determined by ELISA.

ELISA

The presence of IFN-γ, IL-4, and TNF-α in the supernatants was determined by a standard two-site sandwich ELISA. Abs to IFN-γ were purchased from Endogen (Woburn, MA). Abs for IL-4 and TNF-α were purchased from PharMingen. Enhanced protein-binding ELISA plates (Nunc Maxisorb; Nunc Maxi Corp., Roskilde, Denmark) were used.

Results

Phenotypic analysis of NKR expression on circulating γδ T cells

FACS analysis was performed on freshly isolated PBMCs stained for TCRγδ and CD56, p58.1 and p58.2, and NKR-P1A and CD94. Representative FACS analysis for one donor is shown in Figure 1, and results for six representative donors are shown in Table I and Figure 2. In agreement with previous studies, γδ T cells constituted between 1 to 10% of the total T cell population in all individuals analyzed. Expression of the NK-related cell surface molecule CD56 was highly variable ranging from 85% in one individual to 10% in another. In culture, expression of CD56 by γδ T cells has been shown to be induced by IL-2 plus IL-12. Thus, the expression of CD56 by γδ T cells in vivo may reflect the immune status of the donor at the time of tissue collection (24). In contrast, expression of CD94 was found on the majority of circulating γδ T cells in all individuals studied. This distribution of CD94 is considerably higher than that found in $\alpha\beta TCR^+$ cells (~4.2% from the same donors) and is closer to that found on NK cells, where virtually all express CD94 (9, 25, 26). Two forms of the CD94 receptor have been identified, one inhibitory and the other stimulatory for NK cell function. In general, the inhibitory form is found within the $CD94^{bright}$ population whereas the stimulatory receptor

Table I. *Phenotypic analysis of NK receptors on γδ T cells in peripheral blood*

Donor	%γδ	% γδ T Cells Expressing[a] CD94	p58	p58.1	p58.2	NKR-P1	CD56
1	4.6	80.0	10.4	11.2	9.0	10.8	13.2
2	7.5	78.1	16.9	8.6	20.3	27.7	40.6
3	6.3	93.0	10.0	6.2	9.9	18.1	9.5
4	1.3	86.8	28.0	8.0	14.4	30.0	21.1
5	10.3	98.2	25.8	10.0	17.5	35.4	85.6
6	0.9	51.1	23.7	22.0	12.3	26.0	35.0

[a] Phenotype was determined by FACS analysis as described in *Materials and Methods*.

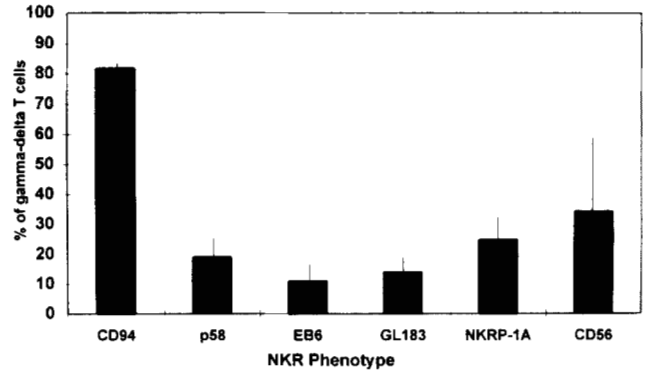

FIGURE 2. Phenotypic analysis of NK receptor expression on peripheral blood γδ T cells. Cumulative data (mean ± SD) for all donors tested are shown. Note that most circulating γδ T cells express CD94 whereas expression of other NKR is variable.

is found within the $CD94^{dull}$ population (9, 10). In γδ T cells, the majority of the $CD94^+$ cells expressed the $CD94^{bright}$ form of the receptor (Fig. 1*e*) (see also below). The NKR-P1A receptor is the human homologue of the NK1.1 receptor and is thus also a member of the disulfide-linked C-type lectin family of NKR (27). In PBMCs we found variable expression of NKR-P1A among donors with a mean value of ~25%, also considerably higher than that on $\alpha\beta TCR^+$ cells (~6.4% in the same donors). Approximately 20% of γδ T cells also expressed p58, a member of the Ig family of NKR with two Ig domains in the extracellular region. The p58 receptor exists as two different species p58.1 and p58.2 that are recognized by the EB6 and GL183 mAbs, respectively (28). As noted for NK cells, both p58.1 and p58.2 were found on partially overlapping subsets with no particular bias toward either molecular species. Analysis of other cell surface phenotypes showed that all γδ T cells expressed CD45RO, ~25% expressed CD8, and virtually none expressed CD4.

Phenotypic analysis of NKR-selected γδ T cell clones

To examine in greater detail potential correlations between the expression of different NKR families, we then established T cell lines and clones from these and other normal healthy donors. Lines (10 cells/well) or clones (one cell/well) were established by FACS sorting for cells that were $TCR\gamma\delta^+/CD94^+$, $TCR\gamma\delta^+/NKR\text{-}P1A^+$, $TCR\gamma\delta^+/p58^+$, or $CD3^+/CD56^+$, and assessed for cell surface expression of NKR and production of the cytokines IFN-γ, IL-4, and TNF-α following stimulation with immobilized Abs to CD3, CD94, or NKR-P1A. Data from a representative clone are shown in Figure 3 and cumulative data from 20 clones in Table II. The results clearly show that individual clones can coexpress more than one NKR, are of the memory phenotype, are mostly DN, and secrete large amounts of IFN-γ following activation with plate-bound anti-CD3. With respect to the cells cloned for expression of NKR-P1A (clones 1–5), all of them expressed CD94, none expressed p58, all were $CD4^-CD8^-$, and all used Vδ2 (the most prevalent γδ T cell subset in normal peripheral blood). Of the clones sorted for expression of CD94 (clones 6–15), all coexpressed NKR-P1A and four coexpressed p58. Since NKR-P1A was not detected on all γδ T cells in the freshly isolated PBMCs, these data suggest that either the NKR-P1A phenotype can be acquired in vitro or that these cells are inherently more viable in long-term cultures. To address this issue further, we examined NKR-P1A expression over time in culture in two of the clones, B7 and C1, that were initially NKR-P1A negative. Both of these clones became positive for NKR-P1A, indicating that γδ T cells can indeed acquire NKR-P1A in culture (but see also below). Expression of p58 by $TCR\gamma\delta^+CD94^+$ cells was variable from clone to clone, with the number of positive clones correlating well with the distribution of p58 expression in PBMCs. This suggests that p58 expression is not modulated following long-term culture, in agreement with data for $TCR\alpha\beta^+p58^+$ T cells (2). When cloned for p58 expression (clones 17–20), CD94 expression was absent on two, but all expressed NKR-P1A, further supporting the conclusion that expression of this receptor can be acquired in culture. Of the clones that were selected for CD3/CD56 expression, only one used the γδ TCR (D1). No distinctive features were noted in this clone.

As observed in PBMCs, most of the clones expressed the $CD94^{bright}$ phenotype, a population of cells known to contain the inhibitory form of CD94. To address this question further, we reacted some of the clones with the Z199 Ab that exclusively recognizes the inhibitory form of CD94 (9). Of the five clones and 11 lines studied, four clones reacted with the Z199 Ab (see for example Fig. 3*d*) and only one clone was negative for Z199. All the lines showed variable expression, even in those in which 100% were $CD94^{bright}$. Thus, as in NK cells, the $CD94^{bright}$ population is not homogeneous and contains populations of cells with differing activities, but most γδ T cells preferentially express the inhibitory form of CD94.

Correlation of NKR expression and Vδ usage

It has been noted that $TCR\alpha\beta^+$ cells expressing NK1.1 display a restricted V gene usage. γδ T cells differ from αβ T cells in having fewer V genes available for recombination. When tested for Vδ gene usage, the results showed that all of the lines and clones selected by sorting for CD94 or NKR-P1A used Vδ2 (Table II) and none expressed Vδ1. The observation that most Vδ2 cells express CD94 has been noted previously (1, 10). To address possible association of NKR with Vδ1, we prepared another set of cultures in which cells were positively selected for Vδ1 and the distribution of NKR examined as before. As shown in Table III, three of four expressed $CD94^{bright}$ (confirmed by the expression of Z199) and one expressed NKR-P1A. Thus, in contrast to the Vδ2 clones shown in Table II, none of the Vδ1-positive cells expressed more than one NKR.

Cytokine expression by NKR^+ γδ TCR^+ clones

The observation that $TCR\alpha\beta^+NK1.1^+$ mouse thymocytes expressing an invariant TCR rapidly release large amounts of IL-4 following cross-linking of CD3 has led to the suggestion that these cells play a pivotal role in defining the nature of the immune response that develops following Ag stimulation (11, 29). Interestingly, however, the nature of the cytokines produced has been

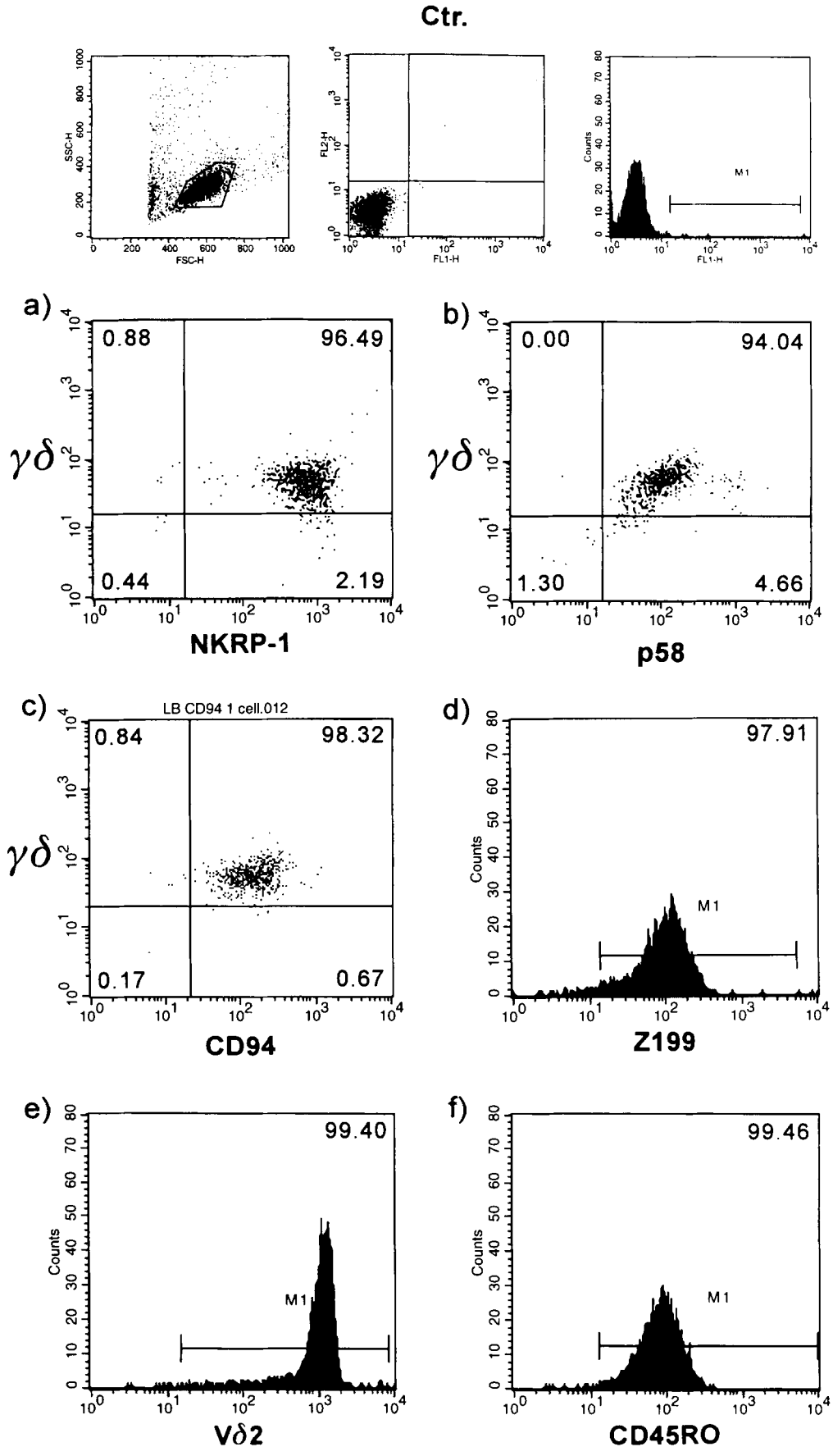

FIGURE 3. Phenotypic analysis of an NKR$^+$ T cell clone. Representative FACS scans of one clone (X5) are shown. This clone was triple positive for NKRP-1 (*a*), p58 (*b*), and CD94 (*c*). Positive immunoreactivity with Z199 (*d*) indicates that this clone expressed the inhibitory form of CD94. In common with all CD94$^+$ and NKR-P1A$^+$ clones, this clone also expressed the Vδ2 TCR (*e*) and was CD45RO$^+$ (*f*), indicating a memory phenotype.

Table II. *Phenotypic analysis and cytokine profiles of NKR+ γδ T cell clones*[a]

Clone	CD94	p58	NKR-P1A	CD45RO	Vδ2	CD4	CD8	IFNγ (CD3)[b]	IFNγ (NKR-P1A)[b]	IL-4 (CD3)[b]	IL-4 (NKR-P1A)[b]	TNFα (CD3)[b]	TNFα (NKR-P1A)[b]
H5	+	−	+	+	+	−	−	+++	++	−	−	ND	ND
E10	+	−	+	+	+	−	−	+++	++	−	−	ND	ND
A1	+	−	+	+	+	−	−	+++	++(+)	+	−	++	−
A9	+	−	+	+	+	−	−	+++	++	++	−	ND	ND
H2	+	−	+	+	+	−	−	+++	+	−	−	ND	ND
C1	+	−	+	+	+	−	−	+++	−	−	−	ND	ND
C5	+	−	+	+	+	−	−	+++	−	−	−	ND	ND
B7	+	−	+	+	+	−	−	+++	−	−	−	ND	ND
B12	+	−	+	+	+	−	−	+++	+	−	−	ND	ND
H6	+	−	+	+	+	−	+	+++	+(+)	++	−	+++	−
C5	+	−	+	+	+	−	−	++	−	+	−	+	−
X2	+	+	+	+	+	−	−	++	+	++	−	+	−
X3	+	+	+	+	+	−	−	++	−	+(+)	−	+	−
X4	+	+	+	+	+	−	−	+++	−	−	−	+++	−
X5	+	+	+	+	+	−	−	+++	−	+/−	−	+++	−
D1	+	−	+	+	+	−	−	++	+	−	−	+	−
C2	−	+	+	+	+	−	+	++(+)	−	++	−	+++	−
P8	−	+	+	+	+	−	−	+++	+	++(+)	−	+	−
Lx2	+	+	+	+	+	−	−	+++	ND	−	ND	−	ND
Lx6	+	+	+	+	+	−	−	+++	ND	−	ND	−	ND

[a] Phenotype was determined by FACS analysis and cytokine levels by ELISA as described in *Materials and Methods*. Cytokine data are presented as follows: +++=>1000 pg/ml, ++=300–1000 pg/ml, += <300 pg/ml, −=<100 pg/ml.
[b] Indicates the antibody used for activation.

Table III. *NKR expression by Vδ1-positive cells*[a]

Clone	Vδ	CD94	Z199	p58	NKR-P1A	CD8	CD45
B8	1	+	+	−	−	−	+
B12	1	+	+	−	−	+	+
B16	1	+	+	−	−	−	+
B17	1	−	−	−	+	−	+

[a] Phenotype was determined by FACS analysis as described in *Materials and Methods*.

found to be tissue specific and modulated by the activating procedure used (30, 31). To study cytokine production by these clones we activated them via plate-bound Abs to CD3, NKR-P1A, or CD94. All of the lines (data not shown) and clones (Table II and Fig. 4) produced large amounts of IFN-γ (>1.5 ng/ml) following activation with plate-bound anti-CD3. Some also released TNF-α and IL-4, but no clear correlation was noted between the expression of specific NKR and the cytokines released. However, no IL-4 was induced in clones that spontaneously secreted IFN-γ. Figure 4 shows cytokine levels expressed as pg/ml from two clones, X4 and H6, selected initially for CD94 expression and which in culture were either triple positive for CD94, NKR-P1A, and p58 (X4), or double positive for CD94 and NKR-P1A (H6). The results show that the levels of IL-4 that could be induced in these cells were considerably less than those for IFN-γ and TNF-α. The highest level of IL-4 induction was found in clone P8, which expressed 1 ng/ml IL-4 compared with 3 ng/ml IFN-γ.

We then examined the time course of IFN-γ release following activation with plate-bound CD3 in two lines (L1 and L9, initially selected for CD94 expression) and one clone (H6, also initially selected for CD94 expression). As shown in Figure 5, IFN-γ could first be detected in the supernatant at 4 h poststimulation, with clone H6 and line L9 secreting ~500 pg/ml and line L1 secreting 1.5 ng/ml. In each set of cultures, the levels then increased rapidly such that by 12 h, greater than 1.2 ng/ml of IFN-γ could be detected in the supernatants, with line L1 secreting ~5 ng/ml.

Cytokine production following stimulation through NKR

To determine whether cytokines could also be induced by cross-linking different NKR, we activated the clones with plate-bound CD94 and NKR-P1A. No cytokines were induced following signaling via CD94 (data not shown). However, when these same clones were activated through NKR-P1A, moderate levels of IFN-γ were detected without co-secretion of IL-4. In some of these supernatants, we also tested for TNF-α but none was detected (Table II).

Discussion

In this study we have shown that the majority of circulating γδ T cells in normal healthy adults express at least one type of NKR, with many expressing more than one. Although recent studies have examined the distribution of NKR on the total PBMC population (32, 33), no previous analyses have specifically addressed the distribution of these receptors on the γδ population. Taken together, the results of this study, and those of Mingari et al. (32) demonstrate that NKRs are more widely distributed on most γδ T cells than on αβ T cells. These data suggest that regulation through both the TCR and NKR occurs more frequently in γδ T cells than in αβ T cells. T cells and NK cells appear to have evolved complementary functions in the immune response, with T cells responding to Ag in the context of MHC and NK cells responding to cells that have lost MHC class I expression, the "missing self" hypothesis (34). Different NKR recognize different MHC class I alleles and form functionally heterogeneous subsets that either inhibit (killer inhibitory receptors) or activate (killer activatory receptors) NK cell function depending on the signals that they send following interaction with MHC class I. Recent studies strongly support the conclusion that the presence of ITIM-like sequences in the cytoplasmic domain of the receptor confer inhibitory activity following receptor cross-linking (7, 9).

On circulating γδ T cells, the most frequently expressed NKR was CD94, a member of the C-type lectin superfamily of NKR that

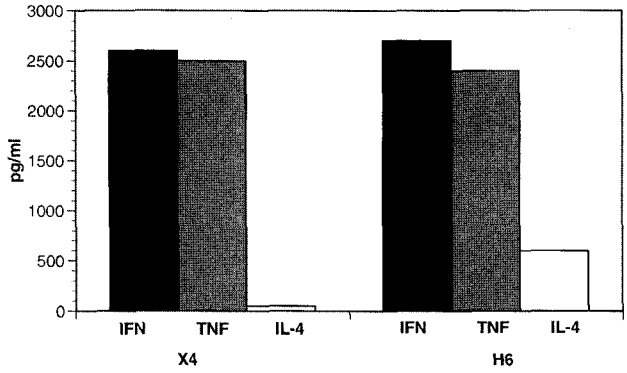

FIGURE 4. Cytokine production by NKR$^+$ γδ T cell clones. Representative data for two NKR$^+$ γδ T cell clones (X4 and H6) are shown. The cells were stimulated for 24 h by plate-bound anti-CD3, supernatants collected, and cytokine levels determined by ELISA. Concentrations of each cytokine are expressed in pg/ml. Note that both clones secreted large amounts of IFN-γ and TNF-α but considerably lower amounts of IL-4.

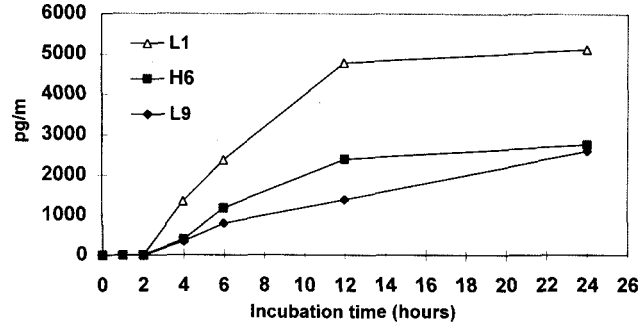

FIGURE 5. Kinetics of IFN-γ production from NKR$^+$ γδ T cells. Two γδ T cell lines (L1 and L9) and one clone (H6) were stimulated with plate-bound anti-CD3, supernatants collected at varying time-points for 24 h, and IFN-γ levels determined by ELISA. Note that production commenced ~4 h poststimulation and then increased rapidly such that peak levels were found ~12 h poststimulation.

forms a heterodimer on the cell surface with the NKG2 gene family and displays a broad specificity in MHC class I recognition involving different HLA-A, B, C (35–38), or G alleles (10). Most circulating γδ T cells were found to express the inhibitory form of the receptor, an observation that was further substantiated by staining with the Z199 Ab. In NK cells, inhibitory activity correlates with the use of the NKG2A gene that has been shown to be recognized by the kp43 Ab (39, 40). Earlier studies with this Ab had also detected the presence of this receptor on a fraction of γδ T cells (25), but in that study the total number of positive cells was considerably less than detected here, suggesting that the remaining CD94$^+$ are covalently linked with other NKG2 gene products. In addition to CD94, many circulating γδ T cells also expressed NKR-P1A, another type II membrane glycoprotein of the C-type lectin superfamily. This NKR is the homologue of the mouse NKR-P family, but the ligand for this receptor is not known and its physiologic role remains undefined (27). An unexpected finding was that almost all γδ T cell lines and clones that were Vδ2$^+$ were NKR-P1A$^+$, suggesting that expression of this receptor can be up-modulated in vitro. In this regard, it is interesting to note that whereas Lanier, Chang, and Phillips (27) found NKR-P1A expression to be stable on NK cells in culture, Eichelberger and Doherty found that the NK1.1 phenotype was up-regulated in culture on mouse γδ T cells, but not αβ T cells, isolated from influenza-infected mice (41). Chen, Huang, and Paul have also shown that in the mouse, NK1.1 expression on TCR-αβ$^+$ T cells is down-regulated following activation, again indicating that the expression of this receptor is not stable on cells in culture (42). Interestingly, in four clones that expressed the Vδ1 TCR, none coexpressed other NKR, and only one expressed NKR-P1A. γδ T cells that express the Vδ1 TCR are rare in adult peripheral blood. However, they are more common in fetal cord blood (43), and an increase in this subset of γδ T cells has been noted in patients with AIDS (44). Thus, studies of these populations should permit a more extensive analysis of NKR-P1A γδ T cells expressing different V region genes. Nevertheless, the results suggest that the regulation of NKR expression may differ in hematopoietic cells of different lineages and may fluctuate depending upon the activation state of the cell.

To study NKRs belonging to the Ig superfamily, we assessed the distribution of the p58/50 family of receptors corresponding to the inhibitory and stimulatory receptors, respectively. On NK cells these receptors are grouped according to their class I MHC specificity (5, 7, 9), which encompasses different alleles of HLA-C. As in NK cells, we found expression of p58.1 and p58.2 on partially overlapping populations. Interestingly, when clones were sorted for expression of CD94, some of these clones also expressed p58. Selective expression of p58 has been noted on subsets of αβ T cells, where it has been found that p58.2 is overexpressed on Vβ16$^+$ cells (32).

A pivotal question that arises is what role do NKR play in T cell function? Both CD94 and p58/50 have been detected on αβ and γδ T cells and have been shown to modulate both cytotoxic and cytokine activity regulated by MHC class I molecules, as well as responses to peptides presented by class II molecules (1–6). With respect to toxicity, in both αβ and γδ T cell clones, CD3-mediated triggering of cytotoxicity as determined by redirected killing assays, as well as by superantigen-induced killing, could be inhibited by cross-linking of p58 (2), p70 (45), or CD94 (4) NKR. In the superantigen studies, these results could be shown to be dependent on TCR engagement since only the relevant TCR-Vβs were involved (45). Although NK-like killing was also affected in some of these studies, it should be noted that killing of K562 cells is not universally observed in T cells expressing NKR (46), and none of the clones studied here showed significant toxicity (>30%) for K562 cells (G. Borsellino et al., manuscript in preparation). With respect to cytokine release, particular attention has been paid to the production of TNF-α following activation through the CD3 receptor (1, 3, 4). Both increased (1, 3) as well as decreased (4) TNF-α release has been observed in the presence of Abs to CD94 and p58. Since these effects were absolutely dependent on costimulation via the CD3 complex, the data indicate an important regulatory role for NKR on T cells. Additional studies have shown that in αβ T cells, the p50 receptor acts as a costimulatory molecule enhancing and prolonging T cell response to superantigens (6), suggesting that the presence or absence of ITIM-like sequences in the cytoplasmic domain has a similar function in T cells as in NK cells.

Another intriguing question is whether any of the NKR$^+$ cells detected here represent a population of γδ T cells that functions in a manner akin to, or perhaps reciprocal to, the TCRαβCD4$^+$NK1.1$^+$ T cells detected in the mouse (12–15). These T cells have been referred to as natural T cells, in part because of the invariant nature of the TCR expressed that would be expected to respond to highly conserved Ags, facilitating the rapid release of large amounts of cytokines, such as IL-4, at the initiation of an immune response (11, 29). We have previously detected the presence of conserved CDR3 motifs within the δ-chain (47), and

ongoing studies have identified several invariant γ- and δ-chains that are common amongst individuals (L. Battistini et al., manuscript in preparation). As expected, all of these sequences are short and are essentially germ line encoded with only a single N-region insertion. Thus, γδ T cells that are likely to be reactive with highly conserved Ags are present in the peripheral blood. However, in all of the γδ lines and clones studied, none secreted high levels of IL-4, but in $\alpha\beta^+$NK1.1$^+$ T cells it has been shown that the expression of CD4 is a critical correlate of high IL-4 expression (12, 13, 48, 49). Vanishingly few γδ T cells express CD4, thus determining potential correlations between CD4 expression, NKR-P1A, and high IL-4 release in circulating γδ T cells will be difficult. An alternative explanation is that peripheral blood γδ T cells represent a different population of NKR$^+$ cells specialized to secrete large amounts of IFN-γ. In this regard it is interesting to note that in the peritoneal cavity of mice, $\alpha\beta$TCR$^+$CD4$^-$CD8$^-$NK1.1$^+$ cells selectively secrete IFN-γ (30). In addition, Arase, Arase, and Saito (31) have shown that in mouse NK spleen cells and $\alpha\beta$TCR$^+$NK1.1$^+$ thymocytes, signaling via NKR-P1 induced high levels of IFN-γ, with levels equivalent to those found following activation with IL-12. Furthermore, NK1.1$^+$ T cells that produced both IFN-γ and IL-4 when stimulated via the CD3 complex secreted only IFN-γ following stimulation via NKR-P1. Identical results were found in this report in human γδ T cells particularly in the clones initially selected for NKR-P1A expression.

In conclusion, the results of these studies show that in contrast to most circulating $\alpha\beta$TCR$^+$ cells, in which NKR expression is relatively rare, most circulating γδ T cells in humans express at least one type of NKR and many express more than one. These data indicate that the regulation of γδ T cell function is likely to be different from that found in most αβ T cells, involving activation (or inhibition) by signaling through both the TCR and the NKR. Elucidating the interactions between these different receptors will clearly be complex and will require consideration of the relative contributions of both inhibitory and stimulatory forms of each of them. The fact that immune responses are closely regulated by both negative and positive signals has been well recognized, and it has been suggested that NKR on T cells function as costimulatory molecules that are exquisitely responsive to changes in cell surface expression of MHC modulated by infection or the activation state of the cell (7). Furthermore, the fact that these NKR$^+$ γδ T cells rapidly produced large amounts of IFN-γ following cross-linking of either the CD3 complex or NKR-P1A suggests that, like NK cells, they could function as a source of IFN-γ, tipping the balance toward a Th1-type response. These cells could, therefore, play a major role in regulating the immune response, both by the release of cytokines that play a pivotal role in defining the nature of the immune response and by regulating the activation of other immunocompetent cells.

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