The Journal of Immunology
RESEARCH ARTICLE | APRIL 15 1991

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L Di Fabrizio; ... et. al

J Immunol (1991) 146 (8): 2495–2503.
https://doi.org/10.4049/jimmunol.146.8.2495

0022-1767/91/1468-2495$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 146, 2495–2503, No. 8, April 15, 1991
Printed in U.S.A.

SPECIFIC TRIGGERING OF γ,δ T CELLS BY K562 ACTIVATES THE γ,δ T CELL RECEPTOR AND MAY REGULATE NATURAL KILLER-LIKE FUNCTION[1]

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Freshly isolated and resting γ/δ T cell lines, although capable of lysing a variety of MHC-unrestricted targets, fail to lyse K562. Yet, the killing of K562 can be specifically induced by antibodies to CD3 or δ-chains. Although this phenomenon may be caused by redirected lysis, it also raised the possibility that K562 may possess ligands capable of specifically interacting with the γ/δ receptor. We found that K562 specifically induced both CD3 and δ modulation as well as IL-2R expression and IL-2 production by γ/δ cells, supporting the idea that the TCR-γ/δ is specifically triggered by K562 cells. Moreover, although the γ/δ cell clones lysed other target cells (e.g., Molt 4, U937, Jurkat etc.), these latter targets did not induce δ modulation or IL-2R expression. In addition, F(ab)$_2$ anti-CD3 antibodies inhibited activated γ/δ T cells from killing K562 but did not inhibit the lysis of the other targets. Taken together, these results suggest that γ/δ cells lyse some targets by utilizing receptors (perhaps NK-like) distinct from the γ/δ receptor. We also found that triggering of the γ/δ receptor by K562 inhibited the capacity of resting γ/δ to lyse Molt 4 cells under conditions in which the K562 cells were not lysed. These findings suggest that the γ/δ receptor maybe directly involved in the lysis of certain targets (i.e., K562) and, importantly, may potentially regulate the function of NK-like receptors that are involved in the lysis of other targets.

Thymocyte development is characterized by the emergence of two separate cell lineages each expressing a distinct set of Ag-specific receptors (1, 2). The TCR expressed on the majority (>90%) of T cells is comprised of two disulfide-linked variable glycoproteins (termed α and β) noncovalently associated on the membrane with the CD3 complex of peptides (3–6). This TCR-α,β is predominantly concerned with recognition of peptide Ag bound to MHC molecules (7, 8). The TCR expressed on the other T cell lineage is analogous to the TCR-α,β, and is comprised of a CD3-associated heterodimer of variable γ and δ polypeptide chains (9–12). Yet, neither the precise ligands nor role of the TCR-γ,δ in the immune response is known.

Although a few reports have shown that γ,δ T cells may interact in an allospecific manner with MHC gene products (13–17), the majority of γ,δ cells are not MHC restricted. Search for other putative ligands have revealed that different γ,δ clones may interact with distinct cell surface molecules including CD1 (18), Qa (19), TL (20), and even surface Ig molecules (21). However, these specific reactivities are not general properties of the majority of γ,δ clones isolated, nor are they a consistent feature of unselected γ,δ T cell populations. Finally, a number of studies have suggested that certain γ,δ T cell clones may interact with products of mycobacteria and, specifically, with their heat shock proteins (22–26). Here again, it is currently unclear whether this specificity is generally applicable to the majority of γ,δ cells.

One reason for the difficulty in determining the ligands for the TCR-γ,δ is that the vast majority of γ,δ clones have the capacity to lyse a variety of immortalized cell lines in an MHC-nonrestricted manner analogous to previously described NK cells (9, 27–31). Therefore, inferences concerning the specificity of the TCR-γ,δ based on the cytotoxic potential of γ,δ cells could be misleading because these cells, like some α,β T cells, may coexpress a distinct NK-like receptor (14, 32, 33). In the present report we provide evidence that the killing of some but not all target cells by activated γ,δ clones is independent of the TCR-γ,δ despite the fact that specific activation of the γ,δ receptor regulates, by either amplification or down-regulation, the cells' killing potential. Furthermore, our results indicate that the cytotoxic activity of resting γ,δ cells differs from that of activated cells. Specifically, we demonstrate that the "classical" NK target cell, K562, is not efficiently lysed by resting γ,δ clones. However, K562 specifically induces resting γ,δ cells to express IL-2R, synthesize IL-2, and down-regulate the TCR, suggesting that K562 may express a ligand for the TCR-γ,δ.

MATERIALS AND METHODS

mAb. The mAb OKT3 (IgG2a), OKT4, OKT6, OKT8, OKT11 (IgG1), and OKM1 were produced from hybridomas obtained from the American Type Culture Collection (Rockville, MD) and were purified from ascites fluid on protein A columns (Bio-Rad, Rockville Center, NY). TCR-δ1 (IgG1) directed to the δ-chain of the TCR was the gift from M. Brenner (Boston, MA) (34). BMA-031 directed to TCR-αβ was purchased (Behringer, Mannheim, Germany). SPV-T3b (anti-CD3), III E5 (anti-CD1a), and M241 (anti-CD1c) were gifts from C. Terhorst (Boston, MA) (18). Leu-4 (anti-CD3) FITC and PE conjugated, WT31 (anti-TCR-αβ) FITC conjugated, and anti-IL-2R (CD25) FITC and PE conjugated were purchased (Becton Dickinson, Mountain View, CA).

Received for publication July 19, 1990.
Accepted for publication January 28, 1991.
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[1] This paper was supported in part by National Institutes of Health Grants AI 14969, AI 24748, and National Research Service Award AI 07132.
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Anti-luteinizing hormone (IgG2a) used as an isotype control was the gift of A. Kirchevsky (New York, NY). $F(ab)_2$ fragments of OKT3 were made via pepsin digestion and protein A column purification by standard techniques (35).

Lymphocyte preparation and isolation of T cells. Purified populations of $CD4^-$, $CD8^-$ T cells were isolated from consenting normal volunteers by Ficoll-Hypaque density gradient centrifugation. The methods for the isolation of lymphocyte subpopulations and distinct T cell subsets has been previously described (9, 36, 37). Briefly, T cells were isolated from PBL by sheep E-rosetting. Enriched populations of $CD4^-$, $CD8^-$ γδ cells were isolated by negative selection with the use of antibody (OKT4 and OKT8) and C depletion. In addition, antibody-coated cells were removed with magnetic goat anti-mouse Ig-coated beads according to the manufacturers instructions (Advanced Magnetics, Cambridge, MA). This procedure resulted in populations that were <5% CD4, CD8, or BMA31 positive and >60 to 90% TCR-δ1 positive when stained with anti-mouse Ig secondary antibody coupled to fluorescein (Jackson Immuno Research, West Grove PA). Cell membrane fluorescence was measured by using a FACScan cytofluorograph (Becton Dickinson).

Cell lines. K562 (erythroleukemia), Molt 4 (immature T cell leukemia), HSB2 (immature T cell leukemia), Jurkat (mature αβ T cell leukemia), and Peer (mature γδ T cell leukemia) were obtained from American Type Culture Collection and have been maintained in final medium. The B cell lines BA, PL, DM, and EK are Epstein-Barr-transformed B cell lines prepared in our laboratory as previously described (38) and maintained in final media. The CD1a- and CD1c-transfected murine T-T hybridomas, BY-A, and BY-C, as well as the parental T-T hybridoma, BY 155.16, were kind gifts of C. Terhorst (18).

Cloning and cultures. Final medium for all cultures consisted of Iscoves modified Dulbecco's medium supplemented with 1% penicillin-streptomycin (GIBCO, Grand Island, NY) and 10% FCS (Hyclone Laboratories, Logan, UT). To prepare γδ lines and clones, purified populations of enriched $CD4^-$, $CD8^-$ γδ cells were activated with either PHA (Sigma, St. Louis, MO) or OKT3 and irradiated feeder cells. After 2 wk of bulk culture, cells were cloned at limiting dilution in 96-well U-bottom (Costar, Cambridge, MA) plates and subsequently propagated with OKT3 and irradiated PBL and B cell lines (6000 rad) in final media containing 50 U IL-2 as previously reported (9, 39).

Cytotoxicity assays. Specific cytotoxicity was determined by ^{51}Cr release assay, as previously described (9). Briefly, 2 million viable targets cells were removed from the cultures and treated with ^{51}Cr-sodium chromate (Dupont, Wilmington, DE) washed and placed (5000 cells/well) in U-bottom 96-well plates (Linbro, Hamden, CT). After 4 h of incubation at 37°C in a humidified atmosphere containing 5% CO_2, 100 μl of the spun supernatants were removed and ^{51}Cr content was measured. Specific cytotoxicity was calculated from the formula: specific cytotoxicity = (experimental ^{51}Cr release − spontaneous ^{51}Cr release)/(total ^{51}Cr release − spontaneous ^{51}Cr release). Total ^{51}Cr release was assessed by Triton lysis of ^{51}Cr-labeled cells. In studies of antibody-induced activation or blocking of killing, a panel of antibodies was incubated (10 μg/ml) with T cell clones for 30 min at room temperature before addition of labeled targets. In studies of anti-CD1 antibodies (1/1000), however, labeled Molt 4 cells were preincubated with antibodies before the addition of effectors. In cold target blocking experiments, graded numbers of either unlabeled Molt 4, K562, U937, or a B cell line were added to radiolabeled target cells before the addition of the effector T cell clones.

Cell surface modulation assay. T cell clones were washed, and placed in 96-well U-bottom plates with irradiated (6 to 9000 rad) K562, Molt 4, or other cell lines at a 1:6 ratio (effector to stimulator cell) in media without IL-2. These cultures were incubated in a humidified atmosphere containing 5% CO_2 for 18 h. Cells were washed and analyzed for surface phenotype by either single or double color fluorescence as described (9).

IL-2 assay. The assay for IL-2 has been previously described (9). In brief, T cells were incubated for 18 h with stimulator cells as described above. A total of 100 μl of supernatant was placed in triplicate in 96-well flat-bottom plates containing 7.5×10^3 CTLL cells for 18 h at 37°C in a humidified 5% CO_2 atmosphere. ^3H-TdR (1 μCi) was then added for an additional 4 h and cultures were harvested by using a PHD cell harvester (Cambridge Technology, Cambridge, MA) and counted on a LKB scintilation counter (Pharmacia, Piscataway, NJ). A standard reference curve was obtained for each experiment by using rIL-2 (Biogen, Cambridge, MA).

RESULTS

Cell-surface phenotype and cytotoxic characteristics of isolated γ,δ clones. To study the ligand specificity of the TCR-γ,δ, we first isolated $CD4^-$, $CD8^-$ cells from normal peripheral blood and activated them with antibodies to CD3. The activated cells were propagated in IL-2 and after several weeks T cell clones expressing the γ,δ receptor were selected and analyzed with respect to cell-surface phenotype and cytotoxic function. In total, we utilized 7 normal blood donors to isolate 10 clones and 7 homogenous lines that expressed the CD2 and CD3 cell-surface molecules and reacted with TCR-δ1, an antibody to the δ-chain of the TCR-γ,δ but not with BMA 31, an antibody to a conformational determinant on the TCR-α,β (Table I). In addition, none of the clones expressed the CD4 molecule and only one of the clones expressed CD8. Thus, these clones displayed the known cell-surface phenotypic characteristics of previously described γ,δ cells (9, 10, 40). Similarly, two clones and seven homogenous α,β cell lines that reacted with BMA 31 and either OKT4 or OKT8 were established as controls from three normal donors.

All T cell lines isolated were tested for NK function against K562, Molt 4, and a B cell line several weeks after cellular activation with anti-CD3 antibodies. Table II, a representative study of cytotoxicity on a wide variety of transformed cell lines as targets, shows that many of the "rested" γ,δ clones killed the immature macrophage cell line, U937, and the immature T cell line, Molt 4, as well as other T cell lines of both mature (Jurkat, Peer) and immature phenotype (CEM, HSB2, Molt 4). Interestingly, the killing of K562, the "classical" NK target was quite variable. Moreover, we observed that the killing of K562 decreased with increasing length of time after activation by anti-CD3 antibodies, whereas the killing of U937, Molt 4, and other T cell lines was independent of the state of activation. In a representative experiment (Fig. 1), a resting clone was triggered with anti-CD3 antibodies and analyzed for killing specificity over time. Within 1 wk of cellular activation, this clone manifested a potent ability to kill K562 as well as Molt 4. After 4 wk, however, the killing of K562 was barely measurable, whereas the killing of Molt 4 was continuously expressed. A total of 14 of 16 γ,δ cell lines demonstrated this same phenomenon.

To determine if this property of cultured γ,δ cells was generalizable to freshly isolated γ,δ cells, homogeneous populations of $CD4^-CD8^-$ γ,δ cells were isolated from peripheral blood and utilized as effector cells in cytotoxicity assays before cell culture (Fig. 2). These fresh γ,δ cells manifested significant dose-dependent killing of Molt 4 whereas there was no significant killing of K562, even in the presence of IL-2. However, these freshly isolated γ,δ cells when cultured in IL-2 for several days could be induced to kill K562 by triggering the TCR because incubation of the γ,δ cells with OKT3 or TCR-δ1 on the day of the assay caused a significant increase in the level of killing of K562 (Fig. 3). Although this killing could be caused by redirected lysis, when taken together, our data suggested that the killing of K562 was linked to the state of activation of both freshly isolated γ,δ cells and IL-2-dependent γ,δ-expressing clones. Furthermore, the activation signal to kill K562 could be induced by antibodies to the CD3/TCR-γ,δ complex (6, 28, 35). In contrast, the killing of Molt 4 and other lines is constitutively expressed.

At least two receptors mediate the specificity of cy-

TABLE I
Phenotypic analysis of T cell lines

	mAb[a]						
	OKT3	OKT4	OKT8	BMA 31	TCR-δ1	OKT11	OKM1
γδ Lines[b]							
PLT8 δ[c]	98	<1	80	<1	99	100	28
PLDN 1.2[c]	98	<1	<1	<1	94	98	66
PLTN	96	1	3	<1	98	100	28
MF 5B7[c]	99	<1	<1	<1	99	100	8
MF 5C10[c]	99	<1	<1	<1	100	100	69
MF 5D10[c]	100	2	4	1	99	100	23
MF 5G5[c]	90	<1	<1	<1	82	98	61
MF 5G6[c]	97	<1	4	<1	93	98	73
MF 5G8[c]	97	<1	6	<1	100	100	22
WCDN	93	3	4	3	92	99	34
WC 1D2[c]	99	<1	8	<1	99	99	33
WC 5G6[c]	97	<1	3	<1	94	98	73
SGDN	99	<1	1	<1	95	100	37
TLDN	98	1	8	1	99	100	11
DMDN	99	<1	<1	<1	99	99	15
JBDN	93	<1	4	<1	99	100	31
αβ Lines[b]							
WCT4	98	95	3	93	3	95	19
WCT8	93	14	78	84	8	94	85
WC PBL	91	3	80	82	4	99	16
PLT4	100	99	<1	98	1	99	4
PLT8	90	<1	77	84	4	100	11
PL PBL	99	17	90	98	<1	99	81
PLT8 10F4[c]	95	<1	99	99	1	99	22
MF PBL	98	14	91	95	3	100	15
MFT4 5D3[c]	100	100	<1	100	<1	99	43

[a] Data shown as percentages of cells stained with the indicated antibodies.
[b] First two letters of name are initials of distinct donors.
[c] Clones isolated by limiting dilution.

TABLE II
Cytotoxic profile of resting γ/δ T cell lines[a]

Effector Cells	Target Cell Lines							
	Molt 4	Peer	HSB2	Jurkat	CEM	K562	U937	BCL
γδ Lines								
PLT8 δ	20	19	23	26	18	6	20	0
PLDN 1.2	7	6	N/A[b]	4	6	0	0	1
PLTN	23	15	1	11	19	2	3	0
MF 5C10	35	30	54	48	59	14	N/A	N/A
MF 5G5	33	26	53	49	55	9	5	5
MF 5G8	31	27	33	35	37	8	17	4
αβ Lines								
PL PBL	52	42	74	78	29	31	66	15
MF PBL	53[c]	N/A	N/A	17	20	52[c]	14	8

[a] Representative ^{51}Cr release cytotoxicity data from several experiments done with "resting" α/β and γ/δ T cell lines (i.e., assayed 4 wk or more after activation with anti-CD3 antibody). All cell lines are placed in media free of lymphokines 48 h before assay. Data is presented as % specific cytotoxicity. The E:T ratio is 5:1 unless otherwise indicated.
[b] N/A signifies data not available.
[c] E:T ratio is 20:1.

totoxicity effected by TCR-γ,δ-expressing clones. In order to investigate whether the activation dependent cytotoxicity of K562 is mediated by the γ,δ receptor we attempted to block the cytotoxicity of recently activated γ,δ cells by antibodies to the CD3/TCR complex. For these experiments F(ab)$_2$ fragments of antibodies to CD3-TCR were used because we recognized that the interpretation of studies utilizing whole antibodies to the CD3-TCR complex may be difficult given that several γ,δ clones as well as K562 are known to be FcR bearing which could potentially lead to either nonspecific activation of killing or redirected lysis. Thus, a representative γ,δ clone (MF 5D10) was triggered, cultured for 2 wk, and subsequently tested for cytotoxicity on both K562 and Molt 4 targets in the presence or absence of OKT3 or F(ab)$_2$ OKT3 or with control antibodies. The killing of K562 is blocked by F(ab)$_2$ OKT3 (Fig. 4*A*), however, the killing of Molt 4 is not changed (Fig. 4*B*). As an additional control F(ab)$_2$ OKT3 was also effective in blocking the killing of an alloreactive α,β clone (PLT8-10F4) (Fig. 4*C*). It is notable that immediately before the assay, flow microfluorometry analysis with FITC anti-Ig did not demonstrate residual anti-CD3 antibodies on our γ,δ cells, mitigating the possibility of redirected lysis. Furthermore, saturating amounts of TCR-δ1, whole and F(ab)$_2$ of OKT3 antibodies were utilized over a 4 log range (25 to .001 μg/ml) in cytotoxicity assays and were unable to block Molt 4 killing by γ,δ lines (data not shown). Taken together, these results support the idea that the recognition and killing of K562 is mediated by the TCR-γ,δ whereas an alternative NK-like receptor on γ,δ cells mediates the recognition of Molt 4.

TCR-γ,δ specifically interact with a ligand expressed on K562 cells. The experiments described above provided evidence that the TCR-γ,δ could specifically interact with a ligand expressed on K562 cells. Because triggering of

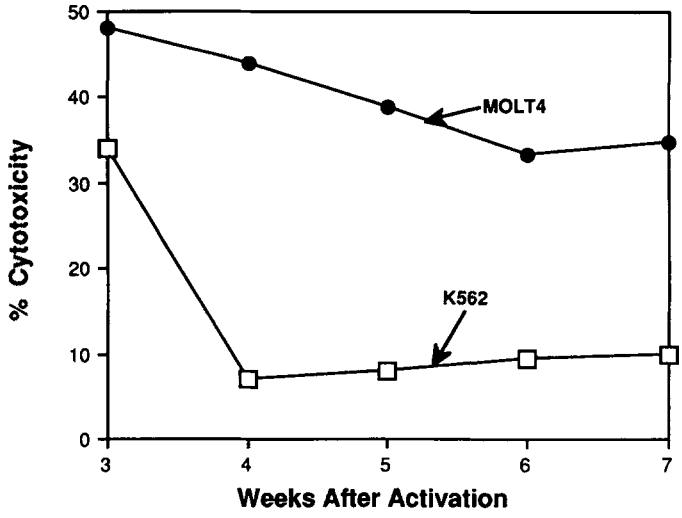

Figure 1. Kinetic analysis of cytotoxicity by γ,δ cells. A representative $CD4^-$, $CD8^-$ γ,δ clone, MF5C5, was assayed weekly for its ability to lyse K562 and Molt 4 in ^{51}Cr release assays. E:T ratio is 5:1.

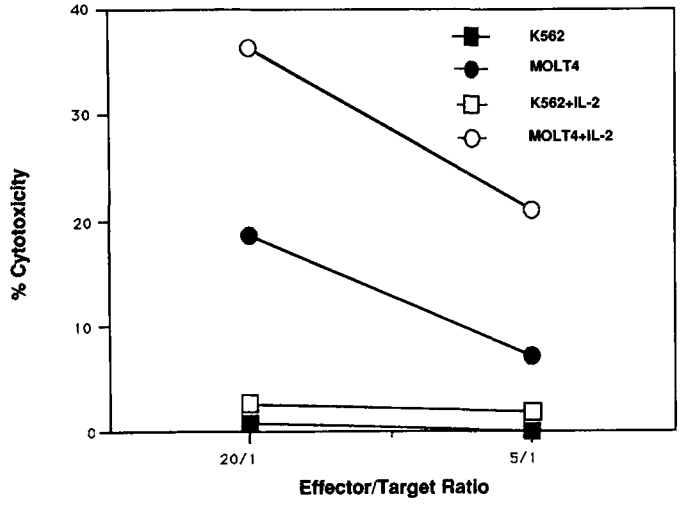

Figure 2. Freshly isolated γ,δ kill Molt 4 but not K562. An enriched $CD4^-$, $CD8^-$ population isolated from PBL containing 87% γ,δ cells was assayed for the ability to lyse K562 and Molt 4 in the presence or absence of IL-2 (50 U/ml) in ^{51}Cr release assays. The E:T ratio was either 20:1, or 5:1.

the TCR is known to induce IL-2R (CD25) expression and IL-2 synthesis as well as CD3-TCR modulation (9, 14, 41, 42, 43) we sought to determine if these phenomena could be induced by K562 cells. Therefore, resting γ,δ clones were cocultured with K562, Molt 4, or other cell lines and analyzed for CD3, δ-chain, and CD25 expression. Figure 5 illustrates our findings with representative γ,δ and control α,β clones. Notably, the populations of unstained cells seen in Figure 5 are not T cells but reflect residual stimulator cells used in overnight coculture with effector cells, as evidenced by control antibody staining (data not shown). K562 cells, although not efficiently lysed by unactivated γ,δ clones, caused modulation of CD3 (Fig. 5*A*) and δ-chains (Fig. 5*B*) and greatly increased IL-2R expression. In contrast, Molt 4, which is avidly killed by γ,δ cells regardless of their state of activation as well as control B cell lines, caused little or no CD3 and δ-chain down-regulation and a small, variable increase in IL-2R expression. To further assess the specificity of CD3 modulation we examined the magnitude of CD3 modulation relative to CD2. The modulation of CD3 was both significant and consistently greater than CD2 (Fig. 5*C*). To demonstrate the specificity of the effect of K562 on γ,δ cells we also studied seven α,β lines with NK-like activity that efficiently lyse K562, Molt 4 and B cell lines. Coculture of these α,β lines with K562 and other NK targets did not induce TCR modulation. A representative α,β NK-like clone is shown in Figure 5*A*. Moreover, an allospecific α,β clone without NK-like activity (e.g., PLT8-10F4, Fig. 5*B*) demonstrated both specific TCR down-regulation and IL-2R expression after culture with the appropriate allo-target but not with K562 or Molt 4. Furthermore, other cell lines that are lysed by γ,δ clones, including Jurkat, CEM, and U937, did not induce significant CD25 expression or TCR down-regulation (data not shown). Therefore, K562 is relatively unique in its capacity to induce specific TCR-γ,δ down-regulation and IL-2R expression in γ,δ clones. Figure 6 summarizes data from five γ,δ clones derived from three separate donors that were assayed for TCR/CD3 modulation and IL-2R expression. The data in Figures 5 and 6 clearly indicate that K562 induces specific CD3-TCR modulation and CD25 expression by γ,δ cells. Furthermore, this was a reproducible property of 14 of 16 γ,δ cell lines isolated (10 clones and 6 homogeneous lines isolated from 7 donors). In contrast, none of the α,β lines (7 homogeneous lines from 3 donors) that lysed K562 and Molt 4 modulated CD3 or induced CD25 expression upon coculture. These data indicate that K562 possesses a cell-surface determinant that interacts with and activates the TCR of the majority of γ,δ cell lines we isolated from normal PBL.

We next determined if the changes in cell-membrane expression of receptors induced by K562 cells are accompanied by other evidence of functional activation. Because IL-2 release is known to be accompanied by specific triggering of the γ,δ receptor with anti-CD3 or anti-δ antibodies (9, 44), we assayed supernatants derived from K562 stimulated γ,δ cells for IL-2 (Fig. 7). As shown, γ,δ but not α,β NK-like cells produced significant amounts of IL-2 in response to an 18-h coculture with K562. In contrast, γ,δ cells produced minimal to no IL-2 in response to Molt 4, Jurkat, or B cell lines. These data demonstrate that K562 functionally activates γ,δ cells and further support the idea that K562 but not the other cell lines specifically interacts with the TCR-γ,δ.

Although CD1 molecules have been reported to be a ligand for TCR-γ,δ (18), they are not present on K562. However, this does not exclude the possibility that CD1a and CD1c, present on Molt 4, could specifically interact with the TCR-γ,δ. Therefore, we utilized CD1a- and CD1c-transfected mouse L cell lines to perform TCR-modulation studies which showed that these cell lines neither induce TCR modulation nor IL-2 expression on γ,δ cells. Furthermore, none of the 11 γ,δ clones studied lysed the CD1-transfected mouse L cells despite the fact that the same CD1c-transfected L cells were shown previously to serve as targets for a γ,δ line (data not shown).

The interaction of K562 with the TCR-γ,δ may regulate NK function. It is paradoxical that K562 serves as an efficient target cell for NK cells in general and triggers the TCR-γ,δ and yet K562, unlike other NK targets (i.e., Molt 4 and U937), is not killed by the majority of resting γ,δ cells. Thus, we queried whether the interaction of K562 with the TCR-γ,δ may inhibit the NK functions of γ,δ cells.

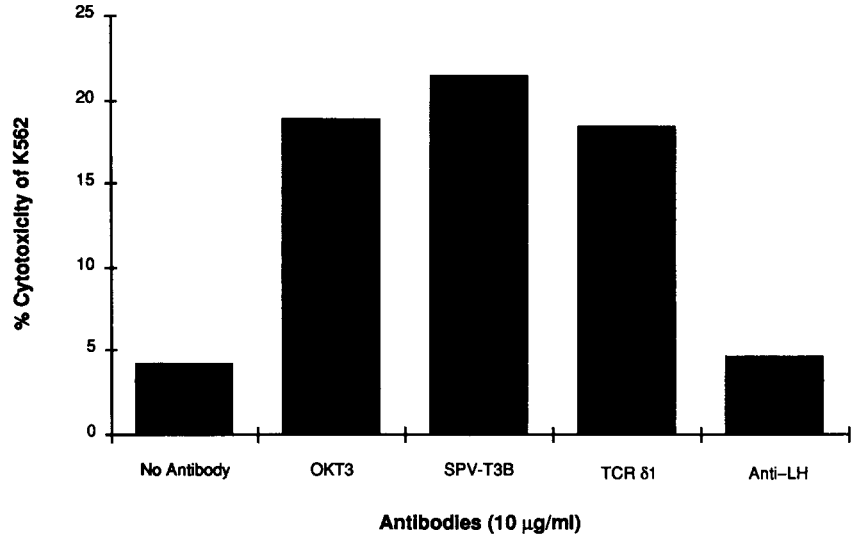

Figure 3. Antibodies to the CD3-TCR complex activate freshly isolated γ,δ to kill K562. A panel of antibodies (10 μg/ml) was incubated with a representative γ,δ line (WCDN) for 30 min at room temperature before addition of labeled K562 targets. OKT3 (IgG2a) and SPV-T3B (IgG2a) are directed to CD3, whereas TCR-δ1 (IgG1) is δ-chain specific. OKT11 (IgG1) directed to CD2 and anti-LH (IgG2a) directed to human luteinizing hormone were utilized as cell-surface binding and isotype controls. E:T ratio was 20:1.

Radiolabeled Molt 4 target cells were added to "rested" γ,δ effector cells, and graded numbers of either unlabeled Molt 4, K562, U937, or a B cell line. As expected, unlabeled Molt 4 and U937 efficiently blocked killing (data not shown). Because both of these cell lines are efficiently killed by γ,δ effectors it is likely that the same NK receptor mediates killing of both of these targets. Interestingly, K562, which is not killed by the γ,δ clone, also served as potent inhibitor of Molt 4 killing (Fig. 8*a*). Furthermore, to rule out that the potent inhibition by K562 was caused by some highly efficient steric effect, we simultaneously performed a competitive inhibition experiment with an αβ $CD8^+$ CTL clone known to kill BA B cell lines cells in an allospecific manner (Fig. 8*b*). Here, unlabeled K562, Molt 4, or B cell line cells were added to radiolabeled B cell targets. As expected, the unlabeled BA B cell line efficiently blocked killing. However, K562 and Molt 4 did not block the killing of the BA B cell target. Thus, under these conditions neither K562 nor Molt 4 nonspecifically block by steric mechanisms. Taken together, these observations suggest that the interaction between the ligand on K562 cells and the γ,δ receptor may inhibit NK function.

DISCUSSION

We have demonstrated that K562 cells specifically interact with the TCR-γ,δ expressed on multiple clones of γ,δ T cells as well as on highly purified γ,δ cells freshly isolated from human peripheral blood. The evidence that K562 cells trigger the γ,δ receptor is: 1) coculture of K562 with γ,δ cells reproducibly induces quantitatively significant down-regulation of both the CD3 peptides and the δ-chain of the TCR while causing only low level of down-regulation of other T cell surface molecules (e.g., CD2); 2) interaction of K562 with γ,δ T cells induces the expression of IL-2R and the release of functional IL-2 molecules, and 3) $F(ab)_2$ antibodies to CD3 specifically inhibit the killing of K562 cells by activated γ,δ effector cells. The triggering of the γ,δ receptor by K562 is most likely mediated by molecules on the cell surface because cell-free supernatants of K562 have no demonstrable effect on γ,δ T cells (data not shown).

It is of interest that although many cell lines serve as target cells for MHC nonrestricted killing by γ,δ cells, K562 induced the greatest degree of CD3 modulation and IL-2R expression. In addition, K562 was the only cell line that caused significant modulation of the δ-chain. Molt 4, U937, Jurkat, and the other NK targets caused little, if any, CD3 modulation and CD25 expression and no modulation of the δ-chain, suggesting that these target cells do not efficiently interact with the TCR-γ,δ. The simplest interpretation of these findings is that although the killing of K562 is mediated by the TCR-γ,δ, the killing of many other target cells (Molt 4, Jurkat, U937) is mediated predominantly by NK-like receptors distinct from the TCR-γ,δ. In support of this interpretation we found that $F(ab)_2$ anti-CD3 antibodies inhibit the killing of K562 but not Molt 4. Furthermore, the small degree of CD3 modulation upon coculture with Molt 4 may suggest that the receptor which recognizes Molt 4 may be distinct from the TCR and somehow associated with CD3 (e.g., the ζ-chain of CD3 is associated with FcR on classical NK cells) (45).

Previous studies of the cytotoxic function and specificity of γ,δ cells have also addressed the question of whether or not the TCR-γ,δ itself is used in the killing function (14, 27–33, 46). These studies, in general have approached this question by utilizing specific antibodies to the TCR-γ,δ complex in order to block cytotoxicity. Because in some of these studies whole and not $F(ab)_2$ antibodies were used, conflicting results have emerged in which some γ,δ clones were activated to kill by anti-TCR or anti-CD3 antibodies and other clones were markedly inhibited. Because of the known functionally important interactions of antibody molecules with FcR on cells, especially on cells like γ,δ cells capable of mediating antibody-dependent cellular cytotoxicity, these studies are difficult to interpret with respect to the precise role of the TCR-γ,δ in the killing phenomenon. Recently, for example, it has been shown that the FcR on γ,δ clones could be directly involved in cytotoxicity and that antibody-blocking data can vary between FcR positive and negative clones (31, 47). Our own experience has been concordant with these observations because we have shown that whole anti-TCR antibodies functionally activate γ,δ clones to kill nonspecifically and therefore any potential role in blocking cytotoxicity could not be easily interpreted.

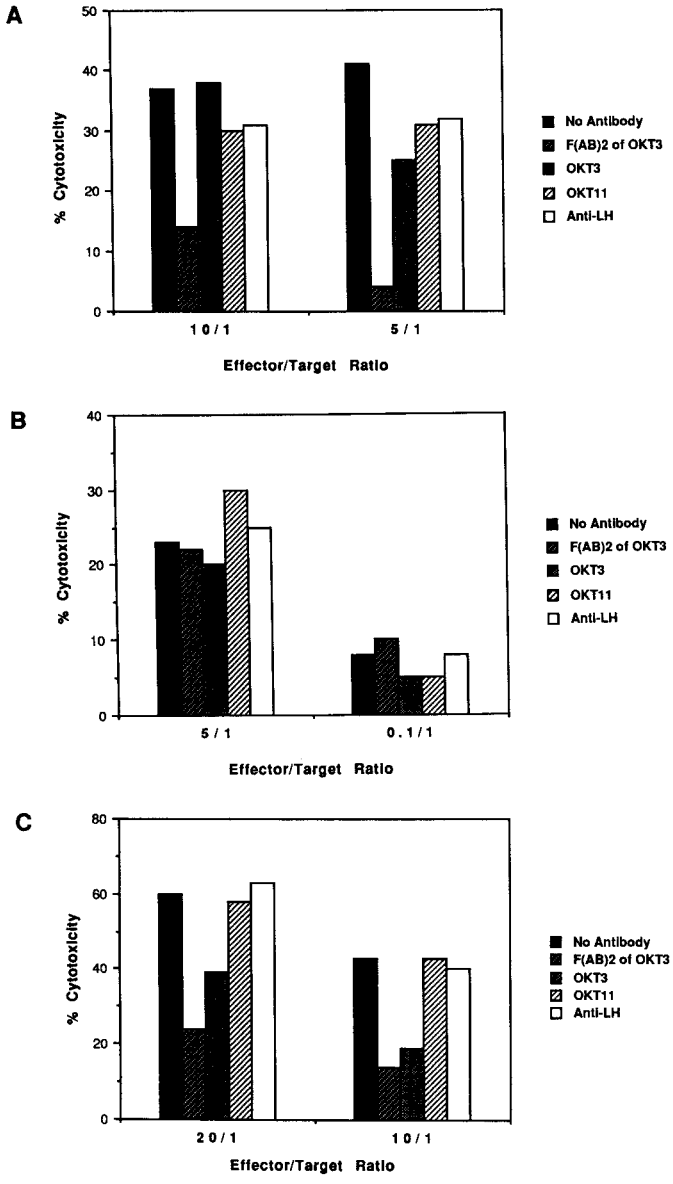

Figure 4. F(ab)2 fragments of OKT3 block the killing of K562 but not Molt 4 by γ,δ clones. A panel of antibodies (10 μg/ml) including whole and $(Fab)_2$ fragments of OKT3 (IgG2a), OKT11 (IgG1), and anti-LH (IgG2a) was incubated with effector cells for 30 min at room temperature before addition of labeled Molt 4, K562, or B cell targets. Graded numbers of the γ,δ clone (MF5D10) were incubated with K562 (Fig. 4*A*) or Molt 4 (Fig. 4*B*). Graded numbers of a $CD8^+$ α,β clone (PLT8-10F4) were incubated with its allospecific target (Fig. 4*C*). Cytotoxicity was assessed by ^{51}Cr release after 4 h.

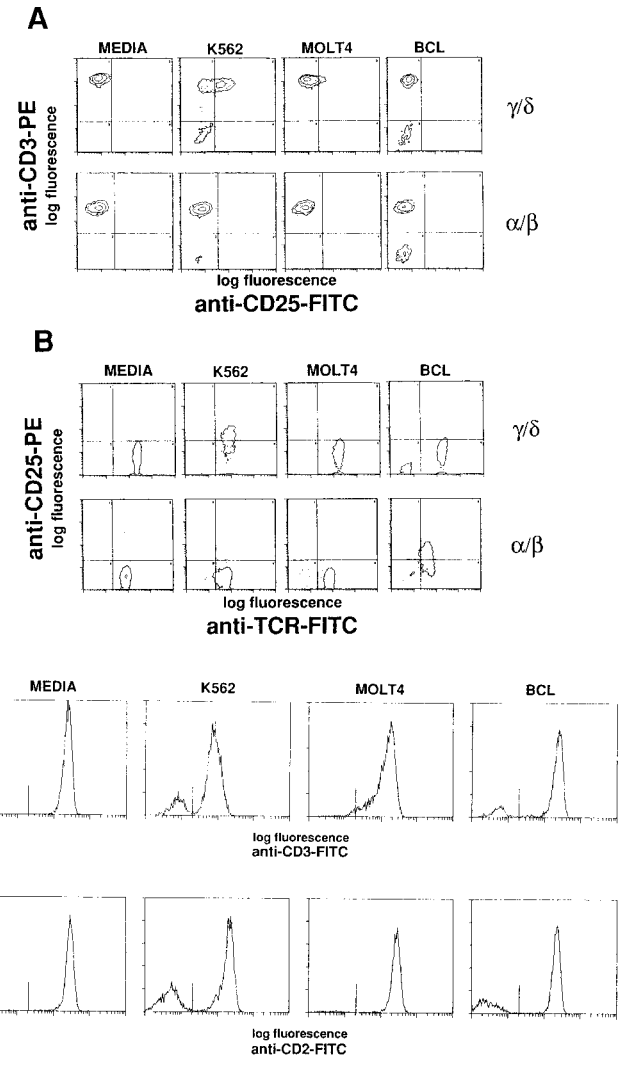

Figure 5. K562 specifically induces CD3 modulation and IL-2R expression by γ,δ cell lines. *A*, Two-color immunofluorescence study of representative γ,δ (PLT8 δ) and α,β NK-like (MFPBL) cell lines. The cells were incubated with either media, K562, Molt 4, or BCL for 20 h and then stained directly with phycoerythrin (*PE*)-conjugated anti-CD3 (*vertical*) and fluorescein (FITC)-conjugated anti-CD25 (*horizontal*). *B*, surface expression of TCR and IL-2R (CD25) by a representative γ,δ clone (MF5D10) and an allospecific α,β clone (PLT8-10F4) is shown after culture as in 5*A*. Both T cell clones were stained directly with PE-conjugated anti-CD25. The α,β clone was stained directly with FITC-conjugated anti-BMA31 (anti-TCR-2), whereas, the γ,δ clone was first stained with unconjugated TCR-δ1 (anti-TCR 1) and subsequently with FITC-conjugated anti-mouse Ig (*horizontal*). *C*, single color immunofluorescence study of a representative γ/δ clone (MF5D10). The cells were incubated with either media, K562, Molt 4, or BCL for 20 h and then stained with fluorescein (FITC)-conjugated OKT3 (*upper*) or OKT11 (*lower*).

On the other hand, studies using $F(ab)_2$ anti-TCR antibodies have suggested that the TCR-γ,δ is not involved in MHC nonrestricted killing because these reagents failed to block killing of some NK targets (Fig. 4, and (32, 47)). These studies utilized activated γ,δ clones and have not taken into consideration the possibility that different NK targets could be killed by distinct cell-surface receptors. Our data show that although the killing of K562 is blocked by $F(ab)_2$ anti-TCR antibodies the killing of other targets including Molt 4 and Jurkat is not. Furthermore, although the cytotoxicity directed against many tumor cell lines is constitutively expressed by γ,δ cells, the killing of K562 is observed largely only after cellular activation. Freshly isolated resting γ,δ cell populations and resting γ,δ clones are not efficient killers of K562 although they are quite efficient killers of Molt 4. Thus, the receptors utilized by γ,δ cells to kill K562 (i.e., the TCR-γ,δ) appears functionally separate from those used to kill Molt 4 or Jurkat. Taken together, our data provide evidence that TCR-γ,δ and distinct NK-like receptors are coexpressed on γ,δ cells and that the TCR-γ,δ is used in the lysis of some but not all NK targets. Consistent with our data are the findings on some γ,δ clones that specifically interact in an MHC-restricted fashion with certain targets and also display NK-like activity. In these cells, it was reported that antibodies to CD3 or the TCR-γ,δ inhibited the killing of allospecific targets but not NK targets (14). Therefore, our data and the work of others support the notion that γ,δ cells express distinct receptors that mediate the cytotoxicity of their targets.

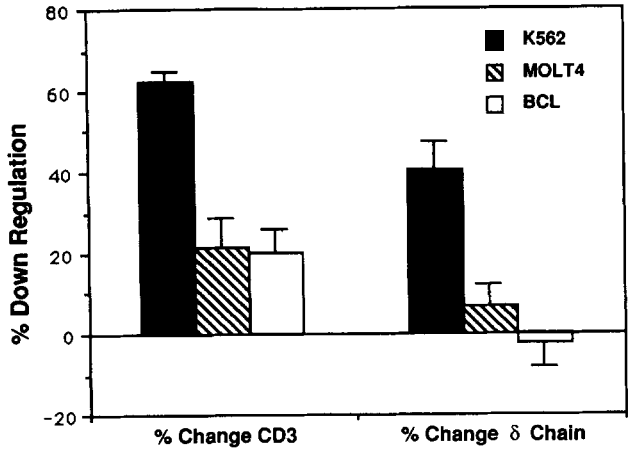

Figure 6. K562 specifically induces δ-chain and CD3 modulation by γ,δ clones. γ,δ Cells were cocultured with either K562, a B cell line, Molt 4, or media alone for 18 h and subsequently assayed for CD3 (Leu-4) or δ-chain (TCR-δ1) expression by immunofluorescence with the FACSscan. The % down-regulation (modulation) was calculated by the following equation: [(mean fluorescence observered without stimulator) − (mean fluorescence observered with stimulator)]/(mean fluorescence observered without stimulator) × 100. The % changes of CD3 and δ-chain expression of five different experiments were then averaged and expressed as % down-regulation ± SEM.

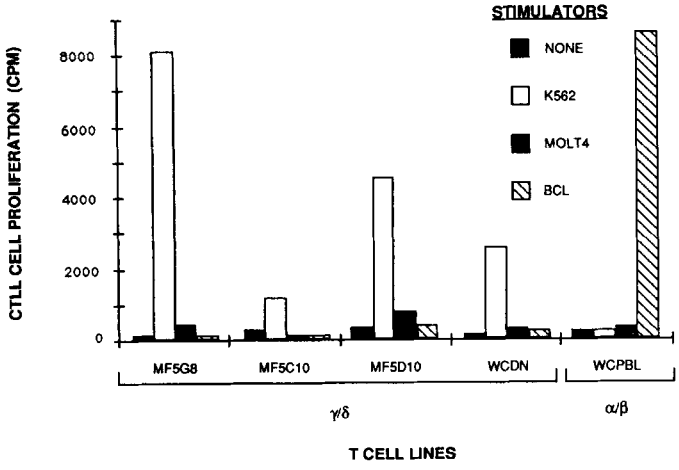

Figure 7. K562 specifically induces IL-2 production by γ,δ cell lines. γ,δ clones (MF5G8, MF5C10, and MF5D10), a γ,δ cell line (WCDN), and an alloreactive α,β cell line with NK-like activity (WCPBL) were incubated with K562, a B cell line, Molt 4 cells, or in media alone for 18 h. Supernatants were then assayed for IL-2 production on CTLL cells.

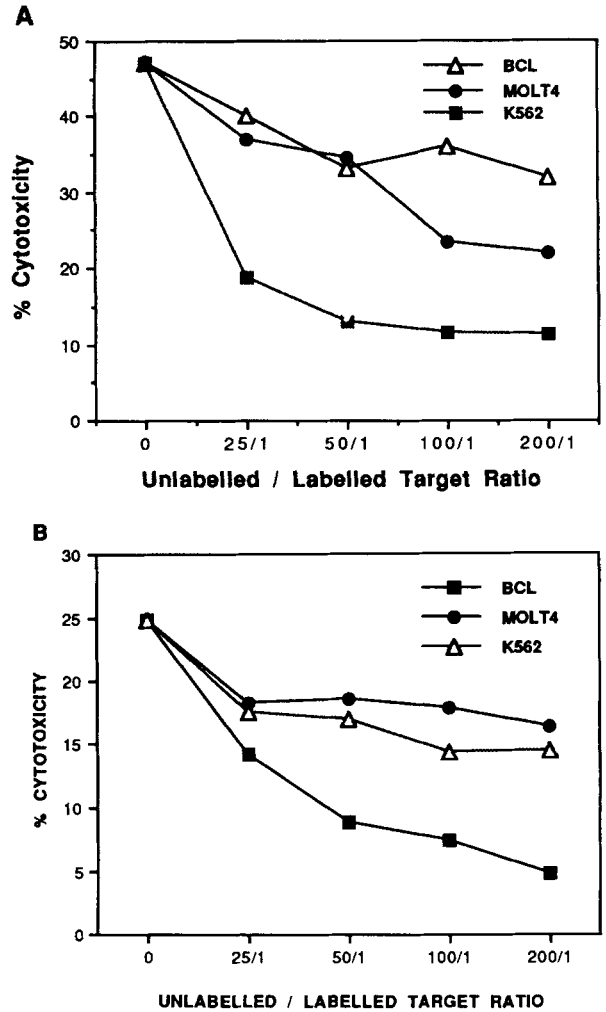

Figure 8. K562 inhibits the killing of Molt 4 by rested γ/δ cells. *A*, radiolabeled Molt 4 target cells were incubated with a γ/δ clone (MF5G8) in the presence of graded numbers of unlabeled Molt 4, K562, or a BCL line and were then assayed for % cytoxicity at a killer to target ratio of 5:1. *B*, radiolabeled BCL target cells were incubated with an allospecific α,β clone (PLT8-10F4) in the presence of graded numbers of unlabeled Molt 4, K562, or a B cell line (*BCL*) and then assayed for cytoxicity at a killer to target ratio of 5:1.

A paradoxical aspect of the specificity of γ,δ cells has been the consistently observed finding by us and others that although γ,δ cells display potent NK-like activity, resting γ,δ cells lyse K562 poorly, if at all. This paradox is amplified by the data reported here that K562 interacts with the TCR-γ,δ more effectively than other NK-like targets. The question arises as to why a cell capable of constitutively killing a variety of targets via putative NK-like receptors fails to lyse a cell that is known to express targets for these NK-like receptors as well as targets for the TCR-γ,δ. A clue to the answer to this question may be in our finding that K562 served as a potent inhibitor of Molt 4 killing even though K562 was not killed by these same γ,δ clones. In fact, quantitatively K562 cells inhibited the killing of Molt 4 better than Molt 4 or other NK-targets themselves. In addition, the inhibition of Molt 4 killing by K562 was not caused by nonspecific effects. One interpretation of these data is that the interaction of K562 with both the TCR-γ,δ and the NK-like receptors on γ,δ cells may trigger a negative signal and abrogate the killing program. In this way, it is conceivable that a general consequence of activation of the TCR-γ,δ may be to regulate the functional activity of coexpressed NK-like receptors. Activation of these NK receptors by certain autologous targets could well be counter-productive to other as yet unknown functions of γ,δ cells. Alternatively, the interaction of the TCR-γ,δ with K562 may impede access to a separate NK-like receptor or cause a cytotoxic "polarity", preventing the killer cell from mediating simultaneous destruction of a separate target while it is engaged with K562.

Although our studies have not specifically addressed the molecular structure on the surface of K562 that activates the TCR-γ,δ, it is of interest that K562 does not express any of the isoforms of CD1 molecules. CD1 molecules are MHC class I-like molecules expressed on the subpopulation of immature thymocytes, Langerhans' cells, and a subset of B cells and were reported to serve as important ligands for human γ,δ cells (18, 48). Because K562 does not express surface CD1 it is not surprising that antibodies to CD1 did not inhibit the killing of K562

(data not shown). However, because the majority of our γ,δ clones efficiently and constitutively killed the Molt 4 cell line that does express CD1a and CD1c molecules, we asked whether antibodies to these CD1 molecules inhibit killing of Molt 4. We were unable to show specific blocking of the killing of Molt 4 with these antibodies to CD1a or CD1c. Moreover, in other experiments CD1a- and CD1c-transfected murine L cells were not lysed by any of our γ,δ cell lines or clones and, as importantly, did not induce CD3 modulation or IL-2 expression (data not shown). Recently, Faure et al. (49) tested 43 cloned and 11 polyclonal γ,δ cell lines expressing various combinations of γ- and δ-chains for reactivity with CD1 transfectants. Only one clone was found to interact with target cells via a CD1c-dependent recognition pathway. These lymphocytes, like the clone described by Porcelli et al. (18), utilize a δ-chain that results from the frequent V1/J1 rearrangement whereas they use a distinct Vγ gene segment. Therefore, our data, taken together with the data of Faure, strongly suggest that CD1 is probably recognized by only a small fraction of the TCR-γ,δ.

In summary, it is clear that the key to the understanding of the role of γ,δ cells in the immune response is the precise determination of the natural ligands for the TCR-γ,δ. Our data suggest that K562 expresses a ligand recognized by the majority of TCR-γ,δ we isolated from peripheral blood, and that this ligand is distinct from ligands that trigger NK-like receptors also expressed on γ,δ cells. The precise biochemical nature of either of these ligands on K562 cells remains to be discerned. However, our data suggest that interaction of the TCR-γ,δ with its ligand on K562 may potentially regulate and abrogate killing functions induced by interaction of the NK-like receptor with its ligands.

Acknowledgments. We would like to thank Drs. Seth Lederman and Ned Braunstein for their scientific advice. We also thank Ruth Croson, Eva Glickman, and Christine McCarthy for their expert technical assistance.

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