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Specificity of HLA Class I Antigen Recognition by Human NK Clones: Evidence for Clonal Heterogeneity, Protection by Self and Non-self Alleles, and Influence of the Target Cell Type

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Summary

Prior studies using polyclonal populations of natural killer (NK) cells have revealed that expression of certain major histocompatibility complex (MHC) class I molecules on the membrane of normal and transformed hematopoietic target cells can prevent NK cell–mediated cytotoxicity. However, the extent of clonal heterogeneity within the NK cell population and the effect of self versus non-self MHC alleles has not been clearly established. In the present study, we have generated more than 200 independently derived human NK cell clones from four individuals of known human histocompatibility leukocyte antigens (HLA) type. NK clones were analyzed for cytolytic activity against MHC class I–deficient Epstein Barr virus (EBV) transformed B lymphoblastoid cell lines (B-LCL) stably transfected with several HLA-A, -B, or -C genes representing either self or non-self alleles. All NK clones killed the prototypic HLA-negative erythroleukemia K562 and most lysed the MHC class I–deficient C1R and 721.221 B-LCL. Analysis of the panel of HLA-A, -B, and -C transfectants supported the following general conclusions. (*a*) Whereas recent studies have suggested that HLA-C antigens may be preferentially recognized by NK cells, our findings indicate that 70% or more of all NK clones are able to recognize certain HLA-B alleles and many also recognize HLA-A alleles. Moreover, a single NK clone has the potential to recognize multiple alleles of HLA-A, HLA-B, and HLA-C antigens. Thus, HLA-C is not unique in conferring protection against NK lysis. (*b*) No simple patterns of HLA specificity emerged. Examination of a large number of NK clones from a single donor revealed overlapping, yet distinct, patterns of reactivity when a sufficiently broad panel of HLA transfectants was examined. (*c*) Both autologous and allogeneic HLA antigens were recognized by NK clones. There was neither evidence for deletion of NK clones reactive with self alleles nor any indication for an increased frequency of NK clones recognizing self alleles. (*d*) With only a few exceptions, protection conferred by transfection of HLA alleles into B-LCL was usually not absolute. Rather a continuum from essentially no protection for certain alleles (HLA-A*0201) to very striking protection for other alleles (HLA-B*5801), with a wide range of intermediate effects, was observed. (*e*) Whereas most NK clones retained a relatively stable HLA specificity, some NK clones demonstrated variable and heterogeneous activity over time. (*f*) NK cell recognition and specificity cannot be explained entirely by the presence or absence of HLA class I antigens on the target cell. For example, HLA-B*5801, an allele that strongly protected two different B-LCL targets, had no effect on NK cell susceptibility when expressed in two NK sensitive tumor targets, K562 and Jurkat. These results suggest the possibility that NK cells may possess cytotoxic mechanisms that are both MHC dependent and MHC independent, depending on the nature of the target cell.

Human NK cells are defined as membrane $CD3^-$, $CD16^+$, and/or $CD56^+$ lymphocytes (1). NK cells have the ability to lyse certain tumor cells and virus-infected cells and to secrete cytokines after stimulation (2). Traditionally, NK cell–mediated cytotoxicity has been defined as non-major MHC-restricted killing in that NK cells are capable of lysing both autologous and allogeneic tumor cell targets (3). However, an association exists between levels of NK sen-

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sitivity and the expression of MHC class I antigens on certain target cells. For many NK-sensitive targets, predominantly those of lymphoid origin, there is an inverse correlation between class I expression and susceptibility to NK cell–mediated cytolysis (4). Ljunggren and Kärre (5) have demonstrated that H-2 class I–deficient variants derived from murine lymphoma cell lines (EL-4 and RBL-5) were rejected by syngeneic animals, whereas, the wild-type MHC class I–bearing lymphomas were highly tumorigenic. This response was NK cell dependent and could be abrogated by the depletion of host NK cells (5, 6). Similarly, in vitro cytotoxicity assays showed that the MHC class I–deficient variants were more sensitive to NK cell lysis than the wild-type cells. In additional studies, up regulation of MHC class I on tumor cells by IFN-γ treatment was accompanied by a decreased susceptibility to NK cell–mediated cytotoxicity (7).

Formal proof that susceptibility to NK cell–mediated cytotoxicity is affected by expression of MHC class I on target cells has been provided using molecular genetic techniques. Transfection of MHC class I genes into class I–deficient cell lines rendered these targets relatively resistant to NK cell-mediated lysis. Storkus and co-workers (8–10) have demonstrated that transfection of certain MHC class I alleles into C1R, an HLA-deficient EBV-transformed B lymphoblastoid cell line (B-LCL),[1] rendered this cell line resistant to lysis by freshly isolated, polyclonal peripheral blood NK cell populations. Of particular note, only certain HLA alleles were protective, whereas others and all the murine MHC class I molecules tested (H2-K^b D^p or L^d) were ineffective (8, 9). Shimizu and DeMars (11) similarly demonstrated that transfection of HLA genes into 721.221, a class I–negative EBV transformed B-LCL, resulted in reduced susceptibility to NK cell–mediated lysis. Likewise, when murine YAC-1 and EL4 cells were transfected with β_2 microglobulin or H-2, the increased expression of H-2 was accompanied by a decrease in NK cell lysis (12–14). In these and other studies a correlation existed between the level of class I expression at the cell surface and the degree of protection from NK cell lysis. A threshold level of class I expression may therefore be required for protection to be observed (7, 9, 11). The property of MHC class I expression to render cells resistant to NK cell–mediated lysis has recently been extended to normal, nontransformed hematopoietic cells. Normal lymphoblasts from mice homozygous for disrupted β_2-microglobulin genes are susceptible to lysis by NK cells from wild-type or heterozygous littermates (15, 16). Moreover, bone marrow grafts from the β2-microglobulin–deficient mice are rejected by radioresistant NK cells in normal, syngeneic recipients (17).

Whereas expression of MHC class I molecules can certainly affect susceptibility to NK cell–mediated lysis, understanding of the specificity of the MHC recognition or of the mechanism of this process is limited. In this study, we have generated a large panel of human NK cell clones from donors of known MHC type and tested these effector cells for lysis of MHC class I–deficient EBV transformed B-LCL and other NK–sensitive cell lines transfected with either self- or nonself alleles of different MHC class I loci. This approach has provided new insights into the clonal heterogeneity of NK cells and has demonstrated that both MHC-dependent and MHC-independent modes of target cell recognition exist.

Materials and Methods

Preparation of NK Cells and NK Clones. Mononuclear cells from peripheral blood of healthy adults were isolated by standard Ficoll-Hypaque techniques. For cloning of NK cells, PBL were stained with PE-conjugated anti-CD56 mAb and FITC-conjugated anti-CD3 mAb. Membrane (m)$CD3^-$,$CD56^+$ NK cells were cloned at 1 cell per well using a single cell deposition system of the FACStar^{PLUS} flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). Clones were established using culture conditions described previously (18). All clones generated by this procedure were $mCD3^-$,$CD56^+$, and mediated NK cytolytic function. The HLA type of the NK clones are summarized in Table 1. NK cell clones established from fetal liver or thymus (19) were generously provided by Dr. María José Sánchez (DNAX).

Antibodies. Anti-CD3 (anti-Leu 4), CD16 (anti-Leu 11a), and CD56 (anti-Leu 19) were generously provided by Becton Dickinson Immunocytometry Systems. mAb MA2.1 reacts with HLA-A*0201, HLA-B*5701 and HLA-B*5801 (20, 21). 362.7 is a murine IgG1 mAb directed against a monomorphic determinant on human HLA-A,B,C molecules that was generated by immunizing BALB/c mice with human NK cells and fusing immune splenocytes with Sp2/0 myeloma cells.

Immunofluorescence and Flow Cytometry. Cells were stained with fluorochrome-conjugated mAb for detection of cell surface antigens by immunofluorescence, as described (22). Appropriate purified or fluorochrome-conjugated, isotype-matched control Ig were used in all experiments. Samples were analyzed using a FACScan® (Becton Dickinson).

Cell-mediated Cytotoxicity. Cytotoxic activity was measured in a 4-h ^{51}Cr-radioisotope release assay (23). Antibody-dependent cellular cytotoxicity (ADCC) was performed as described previously (24). For ADCC, targets were coated with saturating amounts of polyclonal rabbit antiserum against human hematopoietic cells (raised against K562 membranes), generously provided by Dr. John Ortaldo (National Cancer Institute, Frederick, MD).

Target Cell Panel. The NK-sensitive, HLA-negative K562 erythroleukemia cell line was purchased from American Type Culture Collection (Rockville, MD). The Jurkat T leukemia cell line (HLA-A9, -A25, -B7, -B41) was generously provided by Dr. Art Weiss (University of California, San Francisco, CA). C1R B-LCL ($HLA\text{-}A^-$,B^*3503^{low},Cw^*0401^+) (25, 26) was provided by Dr. Peter Cresswell (Yale University, New Haven, CT) and the 721.221 B-LCL ($HLA\text{-}A^-$,B^-,C^-) (27) was generously provided by Dr. Robert DeMars (University of Wisconsin, Madison, WI). Transfectants of 721.221 and C1R used in this study are summarized in Table 1 (10, 21, 25–33). C1R transfectants were generously provided by Drs. Peter Cresswell (Yale University), Jeffrey Dawson (Duke University, Durham, NC), Victor Engelhard (University of Virginia, Charlottesville, VA) and Paul Leibson (Mayo Clinic, Rochester, MN). 721.221.B*5101 and 721.221.Cw*0301 were generously provided by Dr. Robert DeMars (University of Wisconsin, Madison, WI) (33, 34).

PCR and Transfection. RNA was isolated as described by

[1] *Abbreviations used in this paper:* ADCC, antibody-dependent cellular cytotoxicity; B-LCL, B lymphoblastoid cell lines.

Chirgwin et al. (35), with minor modifications. cDNA was prepared in a 40-μl reaction volume containing 50 mM Tris-HCl, pH 8.3, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA, 0.1 μg/ml BSA, 1 mM dNTPs, 2 mM spermidine, 70 U RNAsin (Promega, Madison, WI), 0.4 mM Na pyrophosphate, 10 U AMV reverse transcriptase (Pharmacia, Piscataway, NJ), and 10 μg of total RNA. The reaction mixture was incubated at 42°C. After 45 min, RNA was hydrolyzed by adding 40 μl 0.3 N NaOH, 30 mM EDTA, and boiling for 5 min. cDNA was phenol/chloroform extracted and ethanol precipitated. PCR reactions were performed (43 cycles at 94°C, 1 min; 55°C 1 min; 72°C, 1 min) using a GeneAmp Kit (Perkin-Elmer Corp., Norwalk, CT). Primers for generation of HLA-B*5801 cDNA were: GGGCCTCGAGGGACTCAGAATCTCCCCAGACGCCGAG (sense) and CCGCAAGCTTCTGGGGAGGAAACACAGGTCAGCATGGGAAC (anti-sense). The PCR product was subcloned into the pBJ1-neo expression vector (36). B-LCL and Jurkat cells were transfected with HLA-B*5801-pBJ1-neo by electroporation, drug selected, and cells with high-density cell surface antigen expression were sorted by flow cytometry, as described previously (37).

HLA Typing. HLA class I typing was kindly performed by Dr. David Eckels and Ms. Susan Hackbarth (The Blood Center of Southeastern Wisconsin, Milwaukee, WI) using a standard microlymphocytotoxicity assay.

Results

Clonal Heterogeneity of NK Cell Recognition of MHC. Prior studies have reported that freshly isolated, polyclonal populations of NK cells lyse HLA class I–deficient EBV transformed B-LCL and that transfection with certain HLA class I genes can protect the target (8–11, 38). Such analysis of polyclonal populations however, would not reveal NK cell clonal heterogeneity or specificity. We have therefore, established an extensive panel of NK cell clones (Table 1) to investigate this question.

NK clones were established from single $mCD3^-,CD56^+$ lymphocytes, which were isolated by flow cytometry from the peripheral blood of adult donors. The target cell panel (Table 2) consisted of the parental C1R cell line ($HLA\text{-}A^-,B^*3503^{low}$, Cw^*0401^+) (9, 26), C1R transfected with HLA-A*0101, -A*0201, -A*0301, -A*6801, -A*6901, -B*0702, -B*2705, -B*3701, and -B*5801, the 721.221 cell line ($HLA\text{-}A^-,B^-,C^-$) (11, 39) and 721.221 transfected with HLA-B*5101, -B*5801, and -Cw*0301.

All of the transfectants expressed substantial levels of MHC class I (Fig. 1). Although some variation of expression between targets was observed, comparable levels of expression have been previously reported to confer resistance to NK cell lysis (8–10, 38). The levels of class I expression were routinely monitored by flow cytometry throughout the course of the study and only transfectants with stable surface antigen expression were analyzed.

To demonstrate that the HLA-transfected targets were susceptible to cell-mediated cytotoxicity, they were coated with a heterologous rabbit anti–human leukocyte antiserum and assayed for susceptibility to ADCC using freshly isolated NK cells and selected NK cell clones as effectors. All targets were equally susceptible to ADCC, demonstrating >60% specific lysis at an effector/target cell ratio of 6:1 in a 4-h ^{51}Cr radioisotope release assay (not shown).

Initial experiments were carried out with two NK cell clones, designated NK10 and NK11, which have been maintained in culture for extended periods (>2 yr). Both clones mediate high levels of lysis against K562, C1R and 721.221. Although these two clones were generated from the same donor and have the same NK cell phenotype ($mCD3^-$,-$CD16^+,CD56^+$) they display differential abilities to lyse the HLA-transfected targets (Fig. 2). Two targets, C1R.B*5801 and .221.Cw*0301, were protected from lysis by NK11 (Fig. 2 *B*). The lysis of other targets (C1R.A*0101, C1R.A*6901, C1R.B*2705, C1R.B*3701, and .221.B*5101) was only slightly decreased compared to that of the parental C1R and 721.221 cell lines. NK10 displayed a different specificity of HLA protection: C1R.A*0101, C1R.B*0702, C1R.B*2705, C1R.B*3701, C1R.B*5801, .221.B*5101 and .221.Cw*0301 targets were refractory to lysis (Fig. 2 *A*). These results indicate that similarly derived NK cell clones from the same individual mediate different interactions with MHC class I gene products. Moreover, the findings with clone NK10 clearly demonstrate that a single NK clone can potentially recognize HLA-A, HLA-B, and HLA-C antigens.

The killing by NK10 and NK11 of B-LCL and HLA transfectants was examined over a broad effector to target ratio (not shown). As high levels of killing were seen in the range of 6:1 and 3:1 (E/T ratios), these ratios were used for the remainder of the study. The overall pattern of HLA specificity for NK10 and NK11 was reproducible.

It should be appreciated that the relative levels of lysis of the HLA-transfected targets varied from high levels of lysis to complete resistance. To simplify the analysis, we consid-

Table 1. *HLA Class I Typing of NK Cell Clones*

Donor	Designation of clones	HLA class I serotype
287	NK287	A30, B14, B47, (W4/W6), Cw8
288	NK288	A25, B18, B51, (W4/W6), Cw--
315	NK315	A2, A3, B7, B62, (W6), Cw3, Cw7
186	NK186	A2, A3, B7, B58, Cw3, Cw7
1147	NK10, NK11	A2, A3, B7, (W6), Cw7
Three sources	Fetal clones	ND

Table 2. *Target Cell Panel*

Target designation	Parental cell		Transfected DNA (28)		
	Name	HLA expression	Type	HLA allele	Ref
K562	K562	A^-, B^-, C^-	None		
K562.B*5801	K562	A^-, B^-, C^-	cDNA	B*5801	21
C1R	C1R	A^-, $B^{*}3503^{low}$, $Cw^{*}0401^+$	None		25,26
C1R.A*0101	C1R	A^-, $B^{*}3503^{low}$, $Cw^{*}0401^+$	Genomic	A*0101	*
C1R.A*0201	C1R	A^-, $B^{*}3503^{low}$, $Cw^{*}0401^+$	Genomic	A*0201	29
C1R.A*0301	C1R	A^-, $B^{*}3503^{low}$, $Cw^{*}0401^+$	Genomic	A*0301	‡
C1R.A*6801	C1R	A^-, $B^{*}3503^{low}$, $Cw^{*}0401^+$	Genomic	A*6801	30
C1R.A*6901	C1R	A^-, $B^{*}3503^{low}$, $Cw^{*}0401^+$	Genomic	A*6901	30
C1R.B*0702	C1R	A^-, $B^{*}3503^{low}$, $Cw^{*}0401^+$	Genomic	B*0702	31
C1R.B*2705	C1R	A^-, $B^{*}3503^{low}$, $Cw^{*}0401^+$	Genomic	B*2705	10,32
C1R.B*3701	C1R	A^-, $B^{*}3503^{low}$, $Cw^{*}0401^+$	Genomic	B*3701	*
C1R.B*5801	C1R	A^-, $B^{*}3503^{low}$, $Cw^{*}0401^+$	Genomic	B*5801	21
721.221	721.221	A^-, B^-, C^-	None		27
.221.B*5101	721.221	A^-, B^-, C^-	Genomic	B*5101	33
.221.Cw*0301	721.221	A^-, B^-, C^-	Genomic	Cw*0301	34
721.221 .B*5801	721.221	A^-, B^-, C^-	cDNA	B*5801	21
JURKAT	JURKAT	A9, A25, B7, B41	None		
JURKAT .B*5801	JURKAT	A9, A25, B7, B41	cDNA	B*5801	21

* DNA clone generated in the laboratory of Peter Cresswell, Yale University.
‡ DNA clone generated in the laboratory of Elliot Cowan, National Institutes of Health.

ered the HLA-transfected targets to be "protected" from or refractory to NK cell–mediated cytolysis if lysis was 50% or less than that obtained with the untransfected parental cell. This criterion ensures that only substantial levels of protection are considered significant.

The observation that two NK clones generated from the same donor differentially lysed the panel of HLA-transfected target cells suggested that clonal heterogeneity exists within the NK cells of an individual. To further examine this possibility, a large number of $mCD3^-$,$CD16^{+/-}$,$CD56^+$ NK cell clones were generated from four donors of different HLA type (Table 1). All NK clones possessed cytolytic ability, as demonstrated by their ability to kill K562 cells. 71% (221 of 313) of the clones generated also displayed lytic capacity against the parental MHC class I–deficient C1R and 721.221 targets. NK clones able to lyse C1R or 721.221 at a level of 30% or greater were further tested for the ability to lyse the panel of HLA-transfected targets. A high percentage of the total clones tested were unresponsive against targets transfected with HLA-B alleles, in particular B*3701 (51%), B*5801 (71%) and B*5101 (49%) (Table 3). A lesser percentage (18–35%) of clones were unresponsive against HLA-Cw*0301, -A*0101, and -A*0301 transfected targets. However, only a small percentage (2–6%) of clones were prevented from lysing the A*0201, A*6801, and A*6901 transfectants. Therefore, in general, HLA-B alleles appear to be more protective than HLA-A alleles. Of the HLA alleles tested, B*5801, B*3701, and B*5101 protected target cells from NK lysis with the highest frequency.

These results were obtained using NK clones derived from adult peripheral blood. We have recently reported that functional NK cells arise early during human fetal ontogeny, being detectable from 6 wk gestation until birth (19). To determine whether HLA class I recognition is operative in fetal NK cell populations, NK clones derived from fetal liver or thymus were tested for the ability to lyse the HLA-transfected target cells. The results obtained with NK clones generated from three fetuses were pooled and are represented in Table 3. Like the adult NK clones, all fetal NK clones lysed K562 and most killed C1R and 721.221. Moreover, as with adult NK clones, HLA-B*3701, -B*5801, and -B*5101 alleles provided protection against a high percentage (42–59%) of

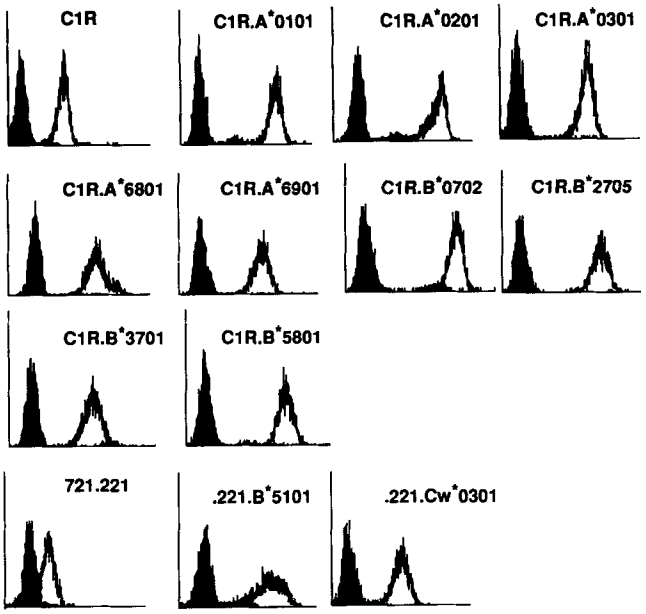

Figure 1. HLA expression on target cell panel. Cells were stained with FITC-conjugated anti-HLA class I-specific mAb 362.7 (*open histograms*) or FITC-conjugated isotype-specific control mAb (*shaded histograms*). X-axis, 4 decade log fluorescence; y-axis, relative number of cells. A slight shift was observed when 721.221 was stained with 362.7. This is most likely due to nonspecific binding as we were unable to immunoprecipitate MHC class I molecules from ^{125}I-labeled cells using either 362.7 or W6/32 mAb.

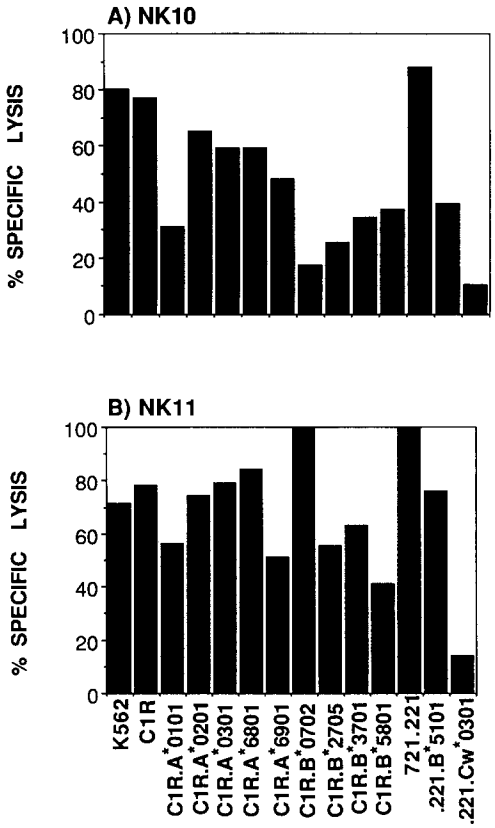

Figure 2. Lysis of HLA$^{+\backslash -}$ target cells by NK10 and NK11. Two NK clones were tested for the ability to lyse MHC class I-deficient cell lines (K562, C1R, 721.221) and HLA-transfected targets in a 4-h ^{51}Cr release assay. *A* and *B* show percent specific lysis for NK10 and NK11, respectively. The effector/target ratio (E:T) was 6:1.

the fetal NK clones with other alleles being less efficient. Thus, fetal NK cells behaved similarly to adult NK cells with regard to HLA class I recognition.

Do Self HLA Alleles Protect the Target Cell from NK Cell Lysis? Since the donors used to generate the NK clones in these studies were of known HLA type, it was possible to

Table 3. *Percentage of NK Clones Tested which Show Decreased Lytic Potential against Target Cells Transfected with Different MHC Class I Alleles*

Donor	C1R. A*0101	C1R. A*0201	C1R. A*0301	C1R. A*6801	C1R. A*6901	C1R. B*0702	C1R. B*2705	C1R. B*3701	C1R. B*5801	.221. B*5101	.221. Cw*0301
287	ND	0% (n = 32)*	6 (32)	0 (32)	ND	6 (32)	50 (32)	31 (32)	47 (32)	44 (32)	22 (32)
288	ND	3 (38)	47 (38)	26 (38)	ND	71 (38)	0 (38)	92 (38)	89 (38)	47 (38)	16 (38)
315	42 (62)	5 (62)	19 (62)	0 (62)	6 (62)	10 (62)	39 (62)	61 (62)	82 (62)	55 (62)	48 (62)
186	ND	ND	ND	ND	ND	4 (26)	25 (16)	4 (26)	73 (26)	37 (16)	23 (26)
Fetal‡	19 (27)	0 (59)	5 (59)	0 (59)	4 (27)	20 (59)	5 (59)	42 (59)	59 (59)	49 (59)	36 (59)
Total	35 (89)	2 (191)	18 (191)	5 (191)	6 (89)	22 (217)	23 (207)	51 (217)	71 (217)	49 (207)	33 (217)

* The percentage of NK clones which showed decreased lysis towards C1R or 721.221 HLA transfectants compared with the parental cells is indicated. The NK clone was considered to be "less lytic" against the HLA-transfected target if the percent cytotoxicity was 50% or less than that of the parental cell. In each case the NK specific lysis of the parental C1R or 721.221 targets was ≥30%. The absolute number of clones included in this analysis is shown in parenthesis. Alleles that represent self alleles for donors 288, 315, and 186 are underlined.
‡ NK clones derived from three fetuses (liver or thymic tissue).

address the issue of whether self HLA alleles expressed on the target cell would preferentially protect a target from lysis. When the HLA phenotype of the donors (Table 1) and the HLA specificity of the NK clones (Table 3) were compared, no obvious correlation was found. Most of the HLA alleles that provided the strongest protection against NK cell-mediated lysis (e.g., HLA-B*5101, -B*3701, and -B*5801) were allogeneic. Moreover, transfectants expressing certain self alleles were infrequently protected. These observations indicate that self (HLA-matched) alleles do not preferentially protect a target cell from lysis by NK clones. For example, the phenotype of donor 315 is HLA-A2, -A3, -B7, -B62, -Cw3, -Cw7. These antigens, with the exception of B62 and Cw7, were represented in the target cell panel. Of the self alleles tested, only Cw*0301 prevented NK cell lysis by a substantial number of the clones derived from donor 315, whereas several non-self alleles (e.g., B*5101, B*5801, and B*3701) were protective for most clones. A*0301 and B*0702, which are self alleles for the 315 NK cell clones, prevented lysis by only a minority of the NK clones. By contrast, these alleles were protective for many of the clones from the HLA disparate donor 288.

An EBV-transformed B-LCL was generated from donor 315 and NK clones from this donor were tested for cytotoxicity against the autologous cell line. Of 22 NK clones examined, none demonstrated substantial cytotoxicity against the autologous B-LCL. The autologous B-LCL presumably expresses all of the self HLA-A,B, and C antigens. Therefore, the presence of multiple self HLA antigens on normal autologous cells may provide complete resistance to NK clone lysis.

HLA-B*3701, -B*5801, and -B*5101 protected target cells from lysis by 50–70% of all clones tested, yet these alleles did not match the HLA type of donors 287, 288, or 315. Moreover, the levels of protection were the greatest with the transfectants expressing these alleles. In many cases, protection was almost complete. This protection is not due to nonspecific resistance to lysis as the HLA-B*3701, -B*5801, and -B*5101 transfectants were susceptible to ADCC mediated by these NK cell clones (data not shown).

Since HLA-B*5801 was observed to confer the most consistent and strongest protection when NK clones from allogeneic donors were tested, we examined whether B*5801 would also demonstrate such a profound effect using NK clones from a donor expressing this allele. NK clones were prepared from donor 186 and were tested for cytotoxicity against the target cell panel (Table 3). Note that the HLA type of this donor was identical to donor 315, with exception that donor 186 expressed HLA-B58 rather than HLA-B62 (Table 1). Similar to the results obtained with the donors 287, 288, and 315, HLA-B*5801 conferred protection to the B-LCL transfectants against 73% (19 of 26) of donor 186 NK clones. Therefore, irrespective of whether HLA-B*5801 is autologous or allogeneic, a high frequency of NK clones recognize this specificity.

Although there was no evidence for "fine specificity" of MHC recognition, NK clones from the four donors showed differential abilities to lyse the HLA-transfected target cells (Table 3). For example, 50% of the clones generated from donor 287 and 39% of those from donor 315 were unable to lyse the C1R.B*2705 target, whereas all of the clones from donor 288 were lytic against this target. 47 and 71% of the 288 clones were unable to kill the C1R.A*0301 and C1R.B*0702 targets, respectively. Conversely, most of the NK clones from donors 287 and 315 lysed these targets. In conclusion, NK clones from different individuals demonstrate different patterns of HLA specificity. However, there was no requirement for the HLA to be a self allele to mediate protection and frequently non-self alleles were more potent than self alleles in conferring target resistance. Additionally, there was no evidence for clonal deletion of NK clones reactive with self HLA alleles or any particular increase in frequency of NK clones reactive with self HLA alleles.

Are there Recognizable Subsets of NK Cells? Given the heterogeneity observed between NK clones derived from a single individual, we examined whether distinct subsets of NK clones could be identified on the basis of HLA preference. Of the 59 NK clones established from donor 315 that were able to lyse C1R or 721.221, a broad spectrum of clonal heterogeneity was evident in their reactions with the HLA transfectants (Table 4). It is evident that the NK clones established from a single individual and cultured under identical conditions were remarkably heterogeneous with respect to interaction with the HLA transfectants. Although the majority of the clones were unresponsive to targets expressing HLA-B*3701, -B*5801, and -B*5101, no simple patterns of HLA specificity emerge. Many clones demonstrated overlapping, yet distinct, patterns of reactivity when a sufficiently broad panel of HLA transfectants was examined. Moreover, note that in many cases a single NK clone had the ability to recognize multiple HLA-A,-B, and -C alleles.

To illustrate the individual behavior of the NK clones, cytotoxicity of representative clones from donor 315 against the HLA class I transfectant target cell panel is presented in Fig. 3. Clones NK 315.17 and NK 315.37 were unresponsive to only one HLA-transfected target each, C1R.B*5801 and .221.Cw*0301, respectively. By contrast, clone NK 315.33 was refractory to both C1R.B*3701 and C1R.B*5801 (Fig. 3, *A*, *B*, and *C*). Reduced levels of lysis by clone NK 315.41 were observed with all HLA-transfected targets (Fig. 3 *D*). The other clones shown in the Fig. 3 exemplify other distinct patterns of NK clonal heterogeneity.

A substantial proportion of human NK cells express CD8α (23, 40), a receptor for MHC class I molecules expressed on MHC class I–restricted T cells (41, 42). NK clones from donor 315 were examined for CD8α expression by flow cytometry and 15% of these NK clones expressed CD8α. However, both the CD8α^+ and the CD8α^- NK clones displayed similar HLA specificities (data not shown).

*Will HLA-B*5801 Protect other Target Cells from NK Cell Lysis?* HLA-B*5801 was noteworthy as this gene protected the C1R target cells from lysis by the majority of the NK clones and exhibited the strongest degree of protection. Therefore, we investigated whether this HLA allele would also exhibit protection when expressed in other cell types. To this

Table 4. *HLA Specificities of NK Clones from a Single Donor (315)*

	Targets protected from NK cell lysis										
No. of clones per group	C1R. A*0101	C1R. A*0201	C1R. A*0301	C1R. A*6801	C1R. A*6901	C1R. B*0702	C1R. B*2705	C1R. B*3701	C1R. B*5801	.221. B*5101	.221. Cw*0301
1								X*			
1									X		
3								X	X		
1									X		X
1										X	X
1	X										X
3								X	X		X
1								X	X	X	X
2	X							X	X		
3			X					X	X		
1	X						X	X			
1	X						X		X		
1									X	X	X
2							X		X	X	
1	X						X			X	X
1	X						X		X	X	
2						X		X	X		X
1	X				X				X	X	X
1	X				X			X			X
1	X							X	X	X	
2							X	X	X	X	
6							X	X	X	X	X
1	X		X		X			X	X		
2	X					X		X	X	X	
1	X				X			X	X		
1			X				X	X	X	X	
1			X				X	X	X	X	X
1	X	X	X					X	X		X
2	X		X					X	X	X	X
1						X	X	X	X	X	X
2	X				X		X	X	X	X	X
4	X	X	X		X			X	X	X	
2	X	X					X	X	X	X	X
1	X	X	X		X	X		X	X	X	X
1	X		X		X	X		X	X	X	X
1	X	X	X				X	X	X	X	X
1	X			X	X	X		X	X	X	X

* An "X" indicates that the HLA-transfected target was "protected" (see legend to Table 3) from lysis by the NK clones of each particular group. Groups were defined as clones that demonstrated a common pattern of HLA specificity. Note that many clones were unique.

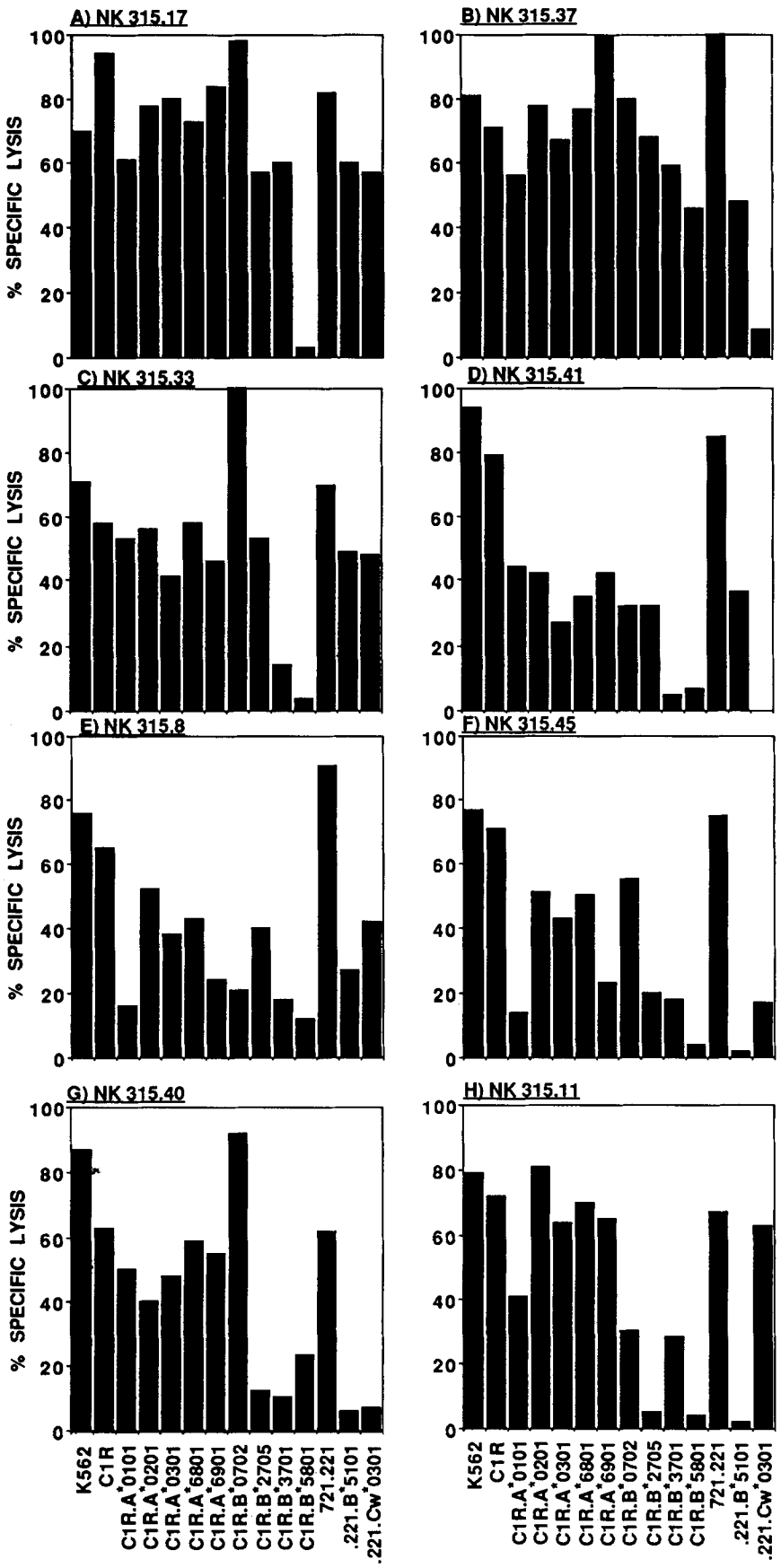

Figure 3. Patterns of HLA specificities of NK clones. The cytotoxicity of eight representative clones generated from donor 315. Assay conditions were as described in the legend to Fig. 2

end, HLA-B*5801 cDNA was prepared from the WT49 EBV B-LCL by PCR and was cloned into the pBJ1-neo expression vector. WT49 is the line from which the genomic B*5801 DNA was obtained and used to transfect the C1R cell line (21). The HLA-negative 721.221 B-LCL, the NK-sensitive Jurkat T leukemia cell line, and the HLA-negative erythroleukemia K562 were transfected with HLA-B*5801-pBJ1-neo. Cells expressing HLA-B*5801 were selected by flow cytometry using mAb MA2.1 that reacts with HLA-B*5801. Significant expression of HLA-B*5801 was achieved in the Jurkat and 721.221 cell lines; lower expression levels were obtained in the K562 cells (Fig. 4). Jurkat T cells express endogenous HLA-A9, -A25, -B7, -B41, but the mAb MA2.1 used to select for HLA-B*5801 transfectants does not react with these alleles.

Additional NK clones were generated from donor 315 and tested for cytolytic activity against the target cell panel as well as 721.221 transfected with B*5801 (not shown). In these experiments, all NK clones lysed K562 and 89% of the clones generated were lytic (>30% specific lysis) against C1R and 721.221. Consistent with the previous cloning from this donor (see Table 2), the HLA-B alleles, B*5101, B*3701, and B*5801, protected the targets from lysis by most of the clones. 52% of the clones failed to lyse the HLA-Cw*0301 transfected targets. Using this series of NK clones, none of the HLA-A alleles tested in this assay (A*0201, A*6801, A*6901) protected the transfectants from NK lysis.

When lysis of C1R.B*5801 and 721.221.B*5801 by the series of NK clones was compared, most clones (13 of 19, 68%) that failed to lyse C1R.B*5801 also did not kill the 721.221.B*5801 transfectant. However, certain clones demonstrated differential behavior against these transfectants. For example, clones NK 315.B6 and NK 315.B10 efficiently lysed 721.221.B*5801, but not C1R.B*5801 (Table 5). C1R expresses endogenous HLA-Cw*0401 and a low level of B*3503 (26), whereas 721.221 is class I HLA negative. It is therefore conceivable that the NK clones inhibited from lysing C1R.B*5801, but not 721.221.B*5801, require a cooperation of B*5801 and either Cw*0401 or B*3503 for target protection. Two clones (NK 315.B4 and NK 315.A9) efficiently lysed K562 and 721.221 and were less responsive to 721.221.-B*5801 (Table 5); however, neither clone lysed C1R. Thus, it is possible that these clones can be inhibited by either Cw*0401 or B*5801.

Selected NK 315 clones that were unable to lyse C1R.B*5801 or 721.221.B*5801 targets were retested in a subsequent experiment that also included K562.B*5801 and Jurkat.B*5801 transfectants as targets. Again, most of the clones were unresponsive to both C1R.B*5801 and 721.221.B*5801. Note that transfection of C1R with HLA-B*5801 conferred essentially complete resistance against certain NK clones (e.g., NK 315.A16) (Table 5). By contrast, transfection of K562 or Jurkat with HLA-B*5801 never protected these targets from NK cell-mediated lysis. HLA-B*5801 was also unable to protect Jurkat or K562 targets against lysis by donor 186 NK clones where HLA-B58 is a self antigen (not shown). Therefore, certain tumor targets which are very sensitive to NK cell lysis cannot be protected by the expression of an HLA allele which is strongly protective when expressed in other cell types. Note that Jurkat.B*5801 expresses levels of HLA-B*5801 comparable to other HLA transfectants (i.e., C1R.-B*3701 or .221.Cw*0301) which are refractory to NK cell

Table 5. *Lysis of Various Cell Types Transfected with HLA-B*5801*

Clone	K562	K562. B*5801	C1R	C1R. B*3701	C1R. B*5801	721.221	721.221. B*5801	JURKAT	JURKAT. B*5801
315.A5	71	64	49	**6**	**1**	68	**23**	68	63
315.A6	67	64	82	57	**1**	78	**28**	79	65
315.A14	72	69	53	**10**	**2**	55	**0**	77	69
315.A16	80	87	100	**30**	**0**	78	**6**	75	66
315.A17	71	70	75	**29**	**0**	81	**5**	78	59
315.A22	62	66	76	**14**	**0**	85	**8**	68	57
315.B13	75	75	65	**22**	**0**	74	**2**	73	68
315.B4	70	72	2	0	5	58	36	48	53
315.B1	68	76	35	**5**	**3**	100	**0**	63	70
315.B6	72	75	89	**10**	**25**	82	80	79	73
315.B8	66	75	76	**6**	**4**	75	**8**	66	61
315.B10	71	77	86	**17**	**0**	76	43	73	75
315.A9	100	82	9	0	2	74	**7**	80	76

Values for the percent specific lysis by NK 315 clones against parental cells and HLA-B*3701 and -B*5801 transfectants, are shown. Numbers in underlined, boldface denote "protection" from NK cell lysis as described in Table 3.

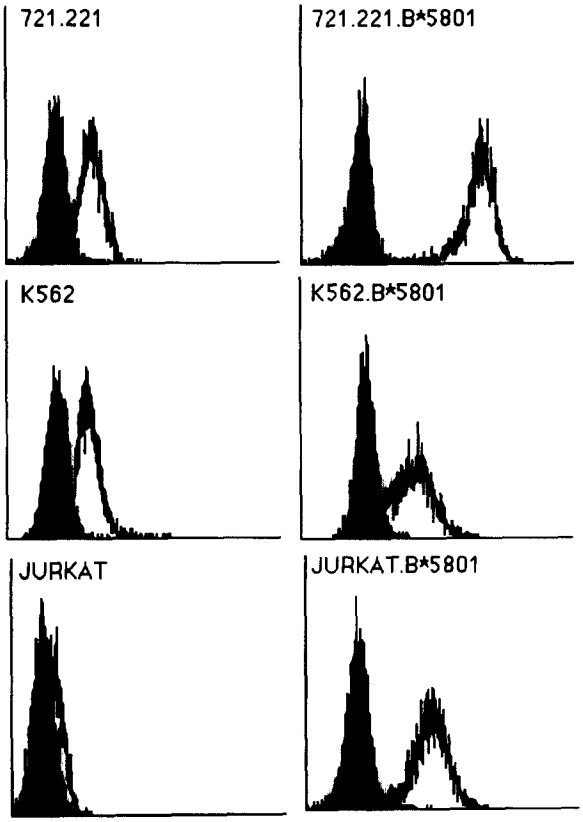

Figure 4. Expression of HLA-B*5801 on 721.221, K562, and Jurkat transfectants. 721.221 and K562 transfectants were stained with FITC-conjugated anti-HLA class I-specific mAb 362.7 (*open histograms*) or FITC-conjugated isotype-specific control mAb (*shaded histograms*). Jurkat cells were stained with mAb MA2.1 (*open histograms*) or isotype-matched control Ig (*shaded histograms*), followed by FITC conjugated goat anti-mouse Ig. X-axis, 4 decade log fluorescence; y-axis, relative number of cells. The slight staining of K562 with 362.7 is most likely nonspecific due to FcR binding as K562 cells are known to express high levels of FcR. Attempts have been made to obtain K562 with higher levels of HLA-B*5801 expression, but without success. Expression of HLA on the HLA-B*5801 K562 transfectant has been confirmed by immunoprecipitation of HLA from ^{125}I-surface-labeled cells using the MA2.1 mAb (not shown).

lysis. Although only low levels of HLA-B*5801 were expressed on K562 transfectants, there was no evidence of any HLA-conferred protection against this target in three separate assays. Prior studies using B-LCL as targets have indicated that resistance to NK lysis correlated with the surface density of HLA, but that even low levels of HLA expression conferred detectable protection (10).

Do NK Cells Maintain a Stable HLA Specificity Over Time? 48 NK clones were assayed in three experiments (over the course of a month) to determine if the HLA specificities of the clones would remain stable with time. NK clones were established from two donors and were cultured using identical conditions. In summary, 35 of 48 NK clones retained a qualitatively similar pattern of HLA specificity during the three assays. However, in most cases variation was observed with one to three of the nine targets tested. Four of 48 NK clones differed greatly on each assay date. Whereas nine of the clones lost lytic potential over time, they maintained the same pattern of HLA specificity. Interestingly, seven clones lost the ability to kill C1R, 721.221, and the HLA transfectants, yet remained lytic against K562, suggesting different mechanisms of recognition or lysis of these cell types. Data from eight NK clones representing the different types of behavior observed are shown in Fig. 5.

MHC-independent Recognition of Targets by NK Cells. Whereas MHC class I antigens can certainly influence recognition by most NK clones, some clones displayed a lytic capability which was apparently independent of this phenomenon. Clones NK 287.A15 and NK 287.A19 exhibit strong lysis of K562; however, none of the remaining targets, including the HLA-negative 721.221 cell lines, were lysed at significant levels (<20%) (Fig. 6, *A* and *B*). By contrast, NK 288.B26 and NK 287.A25 displayed ubiquitous cytotoxicity (Fig. 6, *C* and *D*). In each case, the percent specific lysis was >50% and none of the HLA-transfected targets were significantly refractory to NK cell-mediated cytolysis. Obviously since all HLA alleles were not tested, it is possible that these NK clones may be regulated by other alleles. However, together with the observation that transfection of Jurkat and K562 with HLA-B*5801 failed to affect target cell recognition and lysis, these findings also support the notion that NK clones may disregard either the lack of or presence of MHC class I on certain target cells.

Discussion

The mechanisms by which natural NK cells recognize susceptible targets remains elusive. Kärre et al. (6) have proposed that NK cells recognize cells lacking self MHC class I molecules. According to this "missing self" hypothesis, NK cells possess receptors for self MHC that when engaged transmit a "negative" signal that prevents initiation of cytolytic activity ("negative signal" model). An alternative possibility is that expression of MHC class I molecules on the target cell sterically prevents interaction with "target antigen" receptors on the NK cells ("target masking" model). Presently, most data support the former mechanism (43, 44). Karlhofer et al. (43) have identified a membrane glycoprotein, Ly49, expressed on a subset of murine NK cells in C57BL/6 mice that apparently is a receptor for H-2D^d and inhibits Ly49$^+$ NK cells from killing target cells expressing H-2D^d. Transfection of H-2D^d into tumor cell lines lacking this allele has formally demonstrated that Ly49$^+$ NK cells are inhibited from killing targets expressing polymorphic MHC class I molecules, indicating that interaction between Ly49 and H-2D^d conveys a dominant negative signal (43). A family of Ly49-related genes have been identified in mice (45), providing the possibility for at least a restricted repertoire of NK cell specificity. In humans, receptors for polymorphic MHC class I molecules have not as yet been identified, although Moretta et al. (46) have described mAbs against two NK cell-associated antigens that appear to correlate with the ability of NK cell clones to lyse allogeneic lymphoblasts.

Studies of signal transduction events in NK cells lends additional support to the negative signal model. Kaufman et

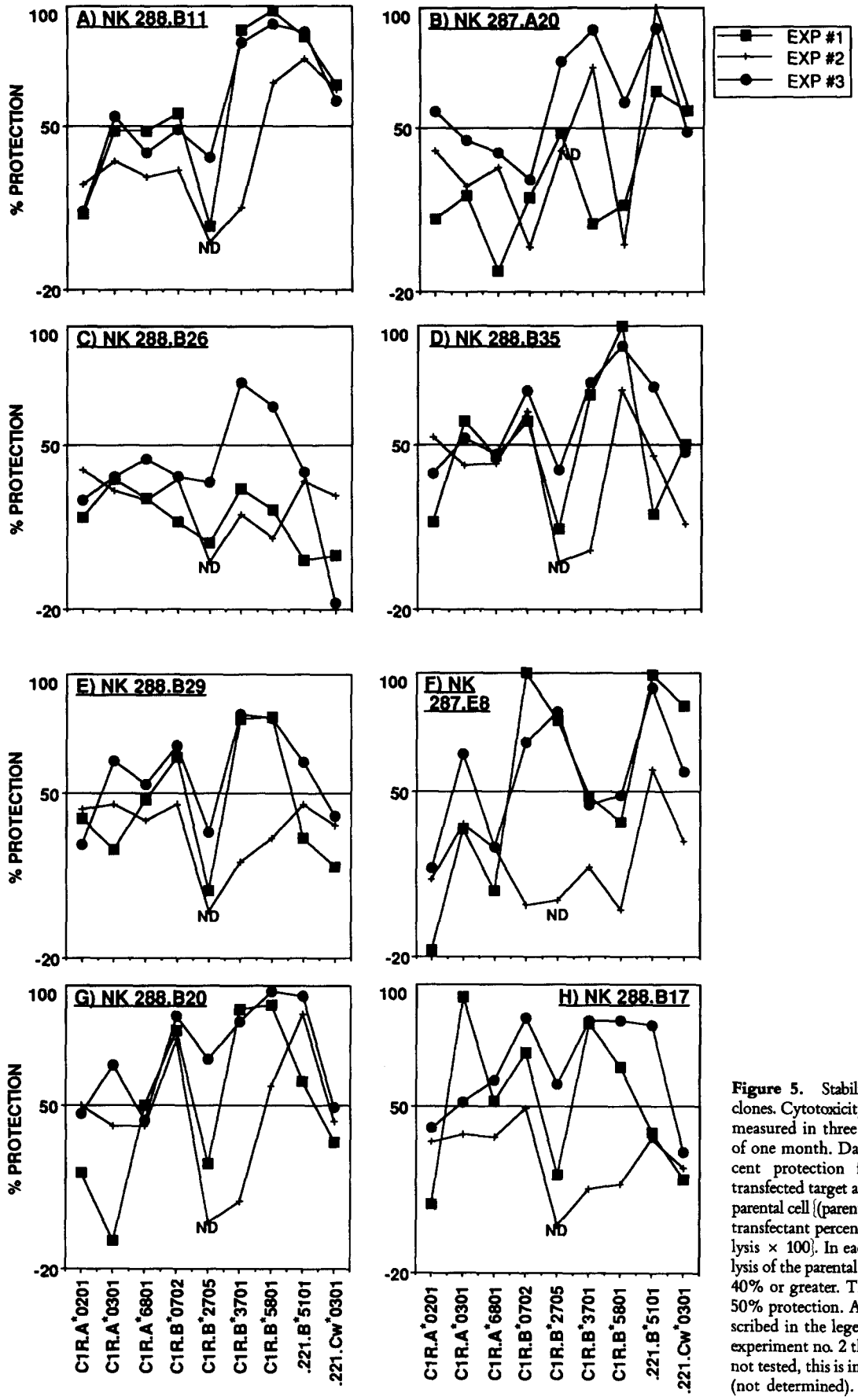

Figure 5. Stability of HLA specificity of NK clones. Cytotoxicity data from eight NK clones measured in three experiments over a period of one month. Data are expressed as the percent protection from lysis of the HLA-transfected target as compared with that of the parental cell [(parental cell percent lysis − HLA-transfectant percent lysis)/parental cell percent lysis × 100]. In each case, the percent specific lysis of the parental C1R or 721.221 targets was 40% or greater. The horizontal line indicates 50% protection. Assay conditions were as described in the legend to Fig. 2. Note that in experiment no. 2 the target C1R.B*2705 was not tested, this is indicated in the graph by ND (not determined).

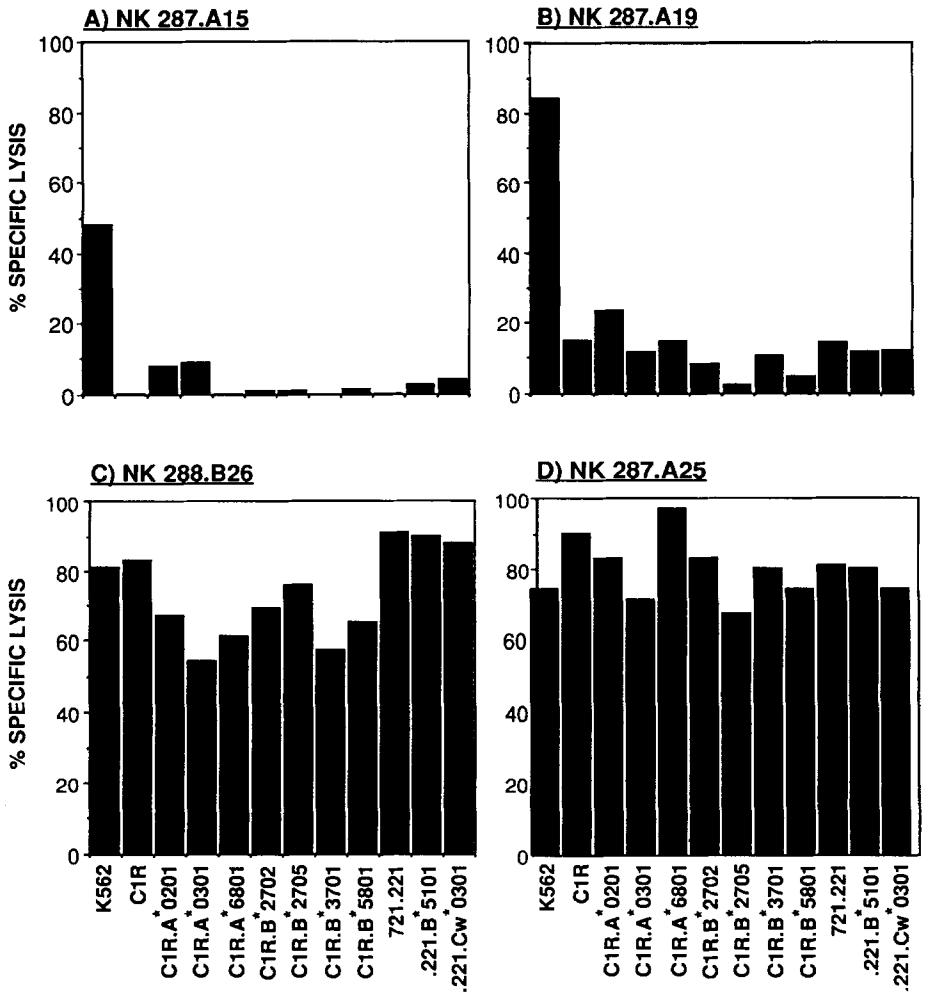

Figure 6. NK clones which only lysed K562 and NK clones which showed ubiquitous cytolytic activity. *A* and *B* show the cytotoxicity data for two NK clones which only have lytic potential against K562. *C* and *D* show two clones which have high lytic potential against all targets in the panel. Assay conditions were as described in Fig. 2 legend.

al. (44) found no differences in the early signal transduction events (i.e., phosphoinositide hydrolysis and Ca^{2+} flux) in NK cell binding of either C1R or HLA class I-transfected C1R. These early signaling events are known to occur when NK cells bind NK susceptible but not NK-resistant targets (47–50); therefore, Kaufman et al. (44) argue that class I expression on target cells does not mask a specific receptor interaction, but rather initiates inhibitory signals which do not alter early signaling events.

Analysis of the heterogeneity of NK cell recognition at the clonal level in mice has been hindered by the inability to generate NK cell clones. The cloning of human NK cells has provided an opportunity to explore the extent of heterogeneity in HLA recognition within the NK cell population. In the present study we established a large series of NK clones from four different individuals and tested these effector cells for interaction with HLA class I–deficient B-LCL transfected with several HLA alleles. Several conclusions were derived from these experiments. First, clonal heterogeneity was observed when NK clones from a single donor were assayed against this large target cell panel and differences existed between different individuals with respect to which HLA alleles provided protection. Whereas all NK clones lysed K562, demonstrating their cytolytic potential, there was considerable variability in lysis of the HLA class I–deficient B-LCL and HLA transfectants. However, no "fine" specificity of HLA recognition was evident. In general, the patterns of protection observed with the various HLA transfectants indicated that several alleles were capable of mediating inhibition of lysis by a single NK clone. Moreover, a single NK clone was able to recognize multiple alleles of HLA-A,-B, and -C. These results imply that either only a few receptors for HLA class I exist on NK cells that broadly cross react with multiple alleles or alternatively, that a single NK clone may express many receptors for MHC with distinct specificities. Resolution of this question awaits identification of these receptors. Our results are compatible with the possibility that specific structures may exist on NK cells which are involved in recognition of MHC class I gene products. If this indeed proves to be the case, our data suggest that these recognition structures are heterogeneously expressed and perhaps differentially regulated.

Another conclusion from these experiments is that the protection conferred by MHC class I on the target is usually

not absolute. With only a few exceptions, expression of the MHC alleles in the transfectants reduced levels of cytolysis only partially. While we arbitrarily designated >50% reduction of lysis as the criteria for HLA protection of a target, it is noteworthy that many alleles provided lesser degrees of inhibition. Overall, the levels of protection observed represent a continuum from essentially no protection for certain alleles (e.g., HLA-A*0201) to very striking protection for other alleles (e.g., HLA-B*5801), with a wide range of intermediate effects. These results are in contrast to the dominant negative signal observed when Ly49$^+$ NK cells interacted with H-2D^d bearing cells (43). Furthermore, using human NK clones as effectors the HLA transfectants demonstrating protection could be readily lysed by ADCC indicating that the effector cells were not permanently inactivated by interaction with the HLA-bearing targets. Again, this is in contrast to results with Ly49$^+$ murine NK cells which were also incapable of mediating ADCC against H-2D^d bearing targets (43).

Analysis of NK cells clones from donors of a defined HLA haplotype has indicated that expression of self MHC alleles is not necessary to protect a target cell from lysis. In fact, the general conclusion was that alleles with strong protective behavior (e.g., HLA-B*5801, -B*3701) provided resistance against NK clones from three independent donors of unrelated haplotype, none of which expressed HLA-B*5801 or -B*3701. Conversely, there was no evidence for permanent elimination or "clonal deletion" of NK clones that were potentially reactive with self MHC alleles (although we cannot exclude the possibility that the presence of HLA molecules might modulate expression of NK cell receptors for self HLA in vivo, similar to the situation with Ly 49 expression in murine allogeneic bone marrow chimeras) (51). For example, the majority of our NK clones failed to lyse HLA-B*5801 transfectants of C1R or 721.221, irrespective of whether this represented an autologous or allogeneic antigen. HLA-A*0201 rarely protected targets from lysis by NK clones obtained either from donors who expressed HLA-A*0201 (i.e., donors 315 or 186) or from donors who lacked this allele (i.e., donors 287 and 288). These findings are in contrast to the observation of Karlhofer et al. (43) who have reported the paradoxical observation that murine NK cells bearing Ly49 are not present in mice expressing the putative ligand for Ly49, i.e., H-2D^d. In man, self alleles were able to provide protection against certain NK clones, as illustrated by the fact that 48% of NK clones from donor 315 were unresponsive to 721.221 transfected with HLA-Cw*0301, a self allele. Likewise, ~73% of NK clones from donor 186 failed to lyse B-LCL transfectants expressing the self allele HLA-B*5801. Although self HLA alleles were protective, there was no apparent increase in the frequency of NK clones that recognized self HLA alleles compared to allogeneic HLA alleles, suggesting that "positive selection" for self alleles is unlikely.

Conclusions derived from our studies of the HLA specificity of NK clones contrast in several aspects with the reports of Ciccone, Moretta, and co-workers (46, 52–57) and Colonna and colleagues (58). These investigators have reported that NK clones can be readily placed into homogeneous groups that demonstrate exquisite target cell specificity, with susceptibility to lysis correlating with specific HLA-C alleles (54–58). By contrast, our findings indicate that NK clones can recognize HLA-A, HLA-B, and HLA-C antigens and that a single NK clone has the potential to recognize multiple alleles. However, there are several important differences in our experimental approach that might account for these discrepancies. It should be appreciated that Ciccone et al. (56) have indicated that the data presented to support the existence of NK clones with defined specificity represent only 36% of NK clones examined, leaving 64% of all NK clones unaccounted. By contrast, in the present study, we have presented the experimental findings obtained with all NK clones examined from several independent donors to give a more comprehensive representation of the total NK cell repertoire of HLA specificity.

While in our studies HLA-transfected B-LCL were analyzed, most of Ciccone, Moretta, and co-workers' experiments were performed using PHA lymphoblasts as targets (52–57). Presumably PHA lymphoblasts express both alleles of the HLA-A, B, and -C genes. Therefore, to identify an NK clone with specificity for a single HLA-C allele, all four HLA-A and B antigens present on the PHA blast must have no influence on that NK clone. Our results using HLA transfected B-LCL, as well as the prior studies of Storkus et al. (8–10), Shimizu and DeMars (11), and Kaufman et al. (44, 59) collectively support the conclusion that most NK cells can also recognize HLA-A or HLA-B antigens. Our present findings demonstrate that HLA-B*3701, -B*5101, and -B*5801 are recognized by >50% of all NK clones from several donors, irrespective of whether these alleles are autologous or allogeneic. Therefore, it is quite surprising that only an HLA-C effect was observed when PHA lymphoblasts were used as targets, since these targets also express a heterogeneous array of HLA-A and -B alleles. We have attempted to use PHA lymphoblasts as target cells for a direct comparison with the HLA transfected B-LCL. In agreement with Ciccone, Moretta, and co-workers we have observed that 48-h PHA lymphoblasts from certain donors are susceptible to lysis by certain NK clones, whereas lymphoblasts from other donors are resistant. However, we have noted that when susceptible PHA lymphoblasts are maintained in culture for longer time periods they very frequently convert from susceptible to totally resistant to lysis by the same NK clone (Phillips, J. H., unpublished observation). Since these resistant PHA lymphoblasts still express high levels of HLA antigens on the cell surface, unidentified variables other than HLA are undoubtedly responsible for this phenomenon. Moreover, we have also observed that certain NK clones can lyse autologous PHA lymphoblasts (Phillips, J. H., unpublished observation). As a consequence of these findings, together with the complication that PHA lymphoblasts potentially express six different HLA alleles, we have focused our studies on the analysis of stable HLA transfectants in B-LCL. The only transfectants used in Ciccone, Moretta, and co-workers' studies were HLA transfectants of a murine mastocytoma cell line P815 (56). One

group of NK clones with reactivity against NK "specificity 2" were unable to lyse murine P815 mastocytoma cell targets transfected with HLA-Cw*0301 (56). However, in these studies a more extensive analysis of self or non-self HLA alleles was not available for comparison, particularly with transfectants expressing allogeneic or autologous HLA-B alleles. In our experience, P815 cells are frequently resistant to lysis by NK clones without transfection with HLA (Phillips, J. H., unpublished observation). Nonetheless, the general conclusion that NK clones demonstrate the potential to recognize certain HLA molecules with resulting inhibition of target lysis are in accordance with findings of Ciccone, Moretta, and co-workers. In part, discrepancies in our results could be due to the complexity of HLA polymorphism in man. Significant individual variation with respect to the behavior of NK clones isolated from different donors might be expected and in fact has been noted in our study.

With respect to specificity of different HLA gene products, our results are more compatible with the conclusions of Shimizu and DeMars (11) and Storkus et al. (9) who analyzed freshly isolated, resting NK cells for the ability to lyse MHC class I–deficient B-LCL and HLA transfectants. Both groups concluded that HLA-A*0201 provided little protection and Shimizu and DeMars (11) noted that HLA-B alleles were generally more protective than HLA-A alleles in providing target protection. Our studies support both of these conclusions and are based on analysis of more than 200 individual NK clones derived from four donors of different haplotypes. However, several of our findings differ from those obtained in the earlier studies. First, we have noted that freshly isolated polyclonal NK cells from many individuals do not lyse the C1R cell line and in some cases do not lyse 721.221, whereas K562 was always efficiently lysed by these effector cells (our own unpublished observation). Therefore, individual variation apparently exists with respect to the ability of freshly isolated NK cells to lyse these MHC class I–deficient B-LCL targets. Additionally, Storkus et al. (8–10) reported that the HLA-A*0201 allele was not protective against NK cell lysis, whereas all other alleles tested (HLA-A*0301, -A*6801, -A*6901, -B*0702, -B*2705, -B*5801) were protective. In addition, the conversion of residue 74, His, in HLA-A*0201 to Asp, found in HLA-A*0301, -A*6801, -A*6901, -B*0702, and -B*2705, reversed the nonprotective phenotype of HLA-A*0201 (10). However, in our analysis of NK clones the HLA-A2/A28 family alleles represented in our target cell panel (HLA-A*0201, -A*6801, -A*6901) only infrequently protected against NK cell lysis. By contrast, members of the HLA-A1/A3/A11 family represented in our target cell panel (HLA-A*0101, -A*0301) were protective against ~20–30% of the NK clones analyzed. Thus, it appears that members of these HLA-A allele families are dissimilar in their ability to protect a target cell from NK cell lysis.

While expression of MHC class I molecules may certainly influence the ability of NK cells to lyse certain targets, other MHC-independent mechanisms of NK cell-mediated cytotoxicity apparently exist. We have demonstrated that HLA-B*5801 provides substantial protection for EBV-transformed B-LCL expressing this allele. However, transfection of the same allele into the NK-sensitive Jurkat and K562 cell lines provided absolutely no protection against lysis by the same NK cell clones. Leiden et al. (60) have also reported that transfection of HLA (A*0201 and B*0702) into NK-sensitive cell lines (K562 and MOLT-4) did not alter susceptibility to lysis by freshly isolated NK cells. Similarly, Ly49$^+$ murine NK cells efficiently lyse the NK-sensitive YAC-1 T leukemia cell line, despite the fact that these cells express the "protective" H-2D^d allele (43). Possibly the lack of protection of H-2D^d in YAC-1 and HLA-B*5801 in K562 and Jurkat is due to differential presentation of endogenous MHC-associated peptides in these cell lines. However, given the complexity of NK-target cell interactions an equally plausible explanation is that multiple NK recognition mechanisms exist, only some of which are strictly regulated by MHC class I expression.

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