Protection from Lysis by Natural Killer Cells of Group 1 and 2 Specificity Is Mediated by Residue 80 in Human Histocompatibility Leukocyte Antigen C Alleles and Also Occurs with Empty Major Histocompatibility Complex Molecules

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Summary

Recognition of major histocompatibility complex class I molecules by natural killer (NK) cells leads to inhibition of target cell lysis. Based on the capacity of different human histocompatibility leukocyte antigen (HLA)-C and HLA-B molecules to inhibit target cell lysis by NK lines and clones, three NK allospecificities have been defined: NK1 and NK2 cells are inhibited by different HLA-C allotypes and NK3 cells by some HLA-B allotypes. The NK1 and NK2 inhibitory ligands on target cells correspond to a dimorphism of HLA-C at residues 77 and 80 in the α1 helix: Asn77-Lys80 in NK1 and Ser77-Asn80 in NK2 inhibitory ligands. It has been reported that protection from NK1 killers depended on the presence of the Lys residue at position 80, an upward pointing residue near the end of the α1 helix (and not on Asn77), whereas inhibition of NK2 effector cells required Ser77, a residue deep in the F pocket and interacting with the peptide (and not Asn80). As part of ongoing experiments to investigate the structural requirements for NK cell inhibition by HLA-C locus alleles, we also examined the effects of mutations at residues 77 and 80 on the ability of HLA-C alleles to confer protection from NK lysis. We present data confirming that the NK1 specificity depended on Lys80 (and not on Asn77); however recognition of NK2 ligands by NK cells was also controlled by the amino acid at position 80 (Asn), and mutation of Ser77 had no effect. Furthermore, bound peptide was shown to be unnecessary for the inhibition of NK cell–mediated lysis since HLA-C molecules assembled in the absence of peptide in RMA-S cells at 26°C were fully competent to inhibit NK cells specifically. The implications of these data for peptide-independent recognition of HLA-C by NK receptors are discussed.

One recognized function of NK cells is in mediating the destruction of cells that have lost expression of HLA class I molecules (1), for example, virus-infected cells that have downregulated surface class I expression by one of several possible mechanisms. Recognition of HLA determinants by human NK cells is mediated by a family of inhibitory receptors (NKIR)[1] that are members of the Ig superfamily (2–4). Recognition of MHC class I molecules by these receptors leads to the generation of a negative signal in NK cells and thus inhibition of the lytic process (5, 6). To date, four NK cell specificities have been identified

(7–11; Luque, I., and J. Pena, manuscript submitted for publication and personal communication). NK1 (group 1)-specific cells are unable to kill target cells expressing any HLA-C allele with Asn at position 77 (N77) and Lys at position 80 (K80) (Cw2, w4, w5, w6), whereas NK2 (group 2) cells are inhibited by HLA-C alleles expressing Ser77 and Asn80 (Cw1, w3, w7, w8). NK3-specific cells recognize HLA-B alleles expressing the serologically defined Bw4 determinant and are inhibited by allotypes containing Ile80 (11). NK4-specific cells also recognize Bw4-expressing cells, but require Thr80 (Luque, I., and J. Pena, personal communication).

The precise structural features required for HLA-C molecules to be able to inhibit NK cells are not clear. Does the nature of the bound peptide affect the NKIR–HLA inter-

action? Correa and Raulet (12), in a murine system, suggested that the role of peptide was merely to promote the assembly and cell surface expression of MHC class I molecules and that there was no peptide specificity in NK recognition of MHC class I (13). In contrast, Malnati et al (14) identified some peptides that could promote assembly of HLA-B27 and confer protection from B27-specific NK cells, whereas other B27-binding peptides could not protect. Further, since in HLA-C alleles residues 77 and 80 segregate together, which of these dimorphic residues is actually important for NK recognition? Biassoni et al. (15) used site-directed mutagenesis to define which of these positions is important for inhibition by NK1 and NK2 cells. These experiments suggested that Lys80 in Cw4 was important for NK1 inhibition whereas Ser77 in Cw3 defined the NK2 specificity; mutations at residues 77 (NK1) and 80 (NK2) had no effect. Given that the majority of NK specificities correlate with the amino acid found at position 80, the result obtained was unexpected.

To identify the precise structural characteristics required for inhibition of specific NK cells by HLA-C, we have mutated residues 77 and 80 in HLA-Cw4 and Cw6 (NK1 protection) and HLA-Cw3 and Cw7 (NK2 protection). The ability of these mutants to inhibit lysis by NK1- and NK2-specific lines and clones was then tested. In the present experiments, position 80 was defined as a key residue for control of recognition by both NK1 and NK2 effector cells. To assay whether specific peptide has any role in protecting cells from NK killing, we have tested whether HLA-Cw6 (NK1) or HLA-Cw7 (NK2), when transfected into RMA-S cells, were able to inhibit NK killing when stabilized by culture at reduced temperature in the absence of exogenously added peptide. The implications of these data for recognition of HLA class I molecules by specific NK cells are discussed.

Materials and Methods

The MHC class I–negative human B cell line 721.221 (16) was obtained from the American Type Culture Collection, Rockville, MD. RMA-S cells, which are TAP-deficient and therefore unable to load MHC class I molecules with peptide (13), were a kind gift of Kirsten Falk (Harvard University, Cambridge, MA). mAb HP3E4 (17) was a kind gift of M. Lopez-Botet (Hospital de la Princesa, Madrid, Spain) and mAb GL183 (18) was purchased from Immunotech (Marseille, France).

NK cells were obtained from healthy adult donors MV (A2, A11; B18, B44; Cw5), NQ (A1, A29; B35, B51, Cw4), HTR (A1, A2; B7, B8; Cw7), DP (A1, A3, B7, B8, Cw7), and AH (A1, A3, B8, B62, Cw9). PBMC were purified from heparinized venous blood by centrifugation on Ficoll-Hypaque (Pharmacia, Piscataway, NJ). NK lines were generated by culture of PBL with irradiated (3,000 rad) 721.221 cells (3:1 PBMC:721.221, 7.5 × 10^5 PBMC/ml in RPMI 10% AB$^-$ human serum). After 5–6 d of culture, pure preparations of CD3$^-$, CD56$^+$, CD16$^+$ cells were obtained by depletion of contaminating T cells with mAbs against CD3 (T3D) and CD4 (OKT4) and rabbit complement (Cedarlane Laboratories, Hornby, ON, Canada). NK lines were cultured in RPMI, 10% human serum supplemented with 1 mM
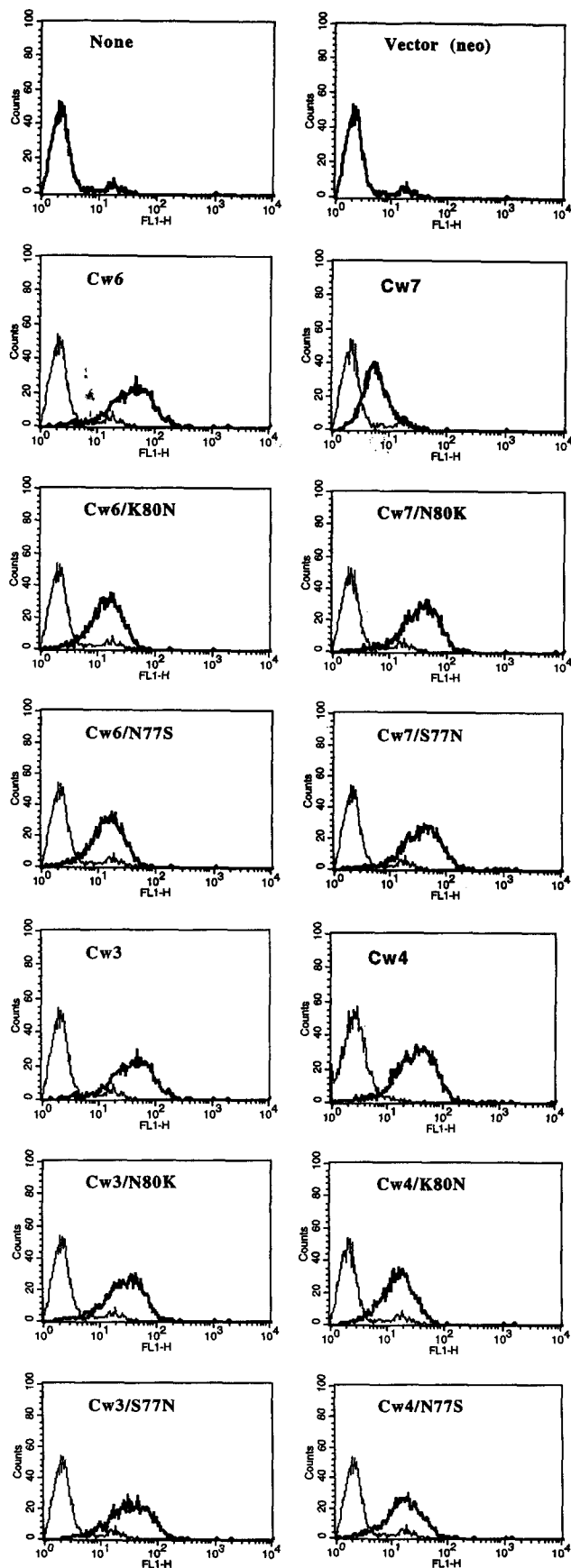
glutamine, 1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 × 10^{-5} M β-ME (all from GIBCO BRL, Gaithersburg, MD), and 50 U/ml rhuIL-2 (Boehringer Mannheim, Indianapolis, IN). NK clones were obtained by seeding NK lines at one or five cells/well in 96-well U-bottomed plates in complete medium supplemented with 10% FCS, 10% leukocyte-conditioned medium (19), and 1 μg/ml PHA (Wellcome, Greenville, NC). Irradiated feeder cells (2.5 × 10^4 allogeneic PBMC from two donors and 5 × 10^3 RPMI 8866 B cell line in each well) were added. Proliferating clones, as defined by growth at cell densities where growth of cells occurred in less than one third of the wells plated, were expanded in complete medium in 96-well plates.

Mutagenesis. cDNAs encoding HLA-Cw*0303, HLA-Cw* 0401, HLA-Cw*0602, and HLA-Cw*0702 were generated, via reverse transcriptase (RT)-PCR, using RNA isolated from the B cell lines Boleth (Cw3), C1R (Cw4), LBF (Cw6), and JY (Cw7), cloned into M13mp18, and sequenced fully. Mutagenesis was carried out using the Sculptor in vitro mutagenesis kit (Amersham Corp., Arlington Heights, IL) according to the manufacturer's instructions. The sequences of the mutagenic oligonucleotides used were K80N (to replace Lys with Asp at position 80) 5'-GCC-GCGCAGGTTCCGCAGGTT-3', N77S 5'-TTTCCGCAG-GCTCACTCGGTC-3', N80K 5'-GCCGCGCAGTTTCCG-CAGGCT-3', and S77N 5'-GTTCCGCAGGTTCACTCG GGTC-3'. Primers K80N and N77S were used to mutagenize Cw4 and Cw6, whereas primers N80K and S77N were used for mutagenesis of Cw3 and Cw7. The wild-type Cw6 and Cw7 genes and the mutants were subcloned from M13 into pCDNA3 that encode *neo* (Invitrogen, San Diego, CA) for transfection. The sequences of all mutants were verified by sequencing three times: during mutagenesis in M13, after introduction into the transfection vector, and after transfection into 721.221 cells.

Transfection. Plasmids were linearized with BglII, and 100 μg of each plasmid was used to electroporate the HLA-A, -B, -C negative cell line 721.221 (16) with a gene pulser (Bio-Rad Laboratories, Richmond, CA) set at 230 V and 250 μFd. After electroporation, cells were seeded in 24-well plates (Costar Corp., Cambridge, MA) in RPMI medium containing 10% FCS, glutamine, nonessential amino acids, pyruvate, and mercaptoethanol and transfectants selected using 1.6 mg/ml Geneticin (GIBCO BRL). Surface expression of HLA-C molecules was analyzed by flow cytometry using the pan-class I mAb W6/32. Clones exhibiting the highest levels of HLA-C expression were used as targets in cytolytic assays.

Using the above protocol, RMA-S cells were cotransfected with 100 μg of a plasmid encoding the human β$_2$-microglobulin (β$_2$m) gene and 10 μg of either the Cw6 or Cw7 plasmids. Surface expression of HLA-C molecules was induced by culture at 26°C in a humidified atmosphere with 5% CO$_2$ and was monitored by flow cytometry using the mAbs BB7.7 (heavy chain specific) or BBM1 (β$_2$m specific).

Cytolytic Assays. The cytolytic activity of NK lines and clones against the various HLA-C transfectants and mutants was assessed in 5-h ^{35}S-release assays (4 h for the RMA-S targets) in which effector cells were admixed with 5 × 10^3 [^{35}S]methionine–labeled targets at different E/T ratios in U-bottomed microtiter plates. Assays were terminated by centrifugation at 1,000 rpm for 10 min at 4°C and 100 μl of the supernatant was collected for liquid scintillation counting. Percent specific lysis was calculated as follows: % lysis = [(cpm experimental well − cpm spontaneous release)/ (cpm maximal release − cpm spontaneous release)] × 100. Spontaneous release was determined by incubation of the labeled target cells with medium. Maximal release was determined by solubiliz-

None

Vector (neo)

Cw6

Cw7

Cw6/K80N

Cw7/N80K

Cw6/N77S

Cw7/S77N

Cw3

Cw4

Cw3/N80K

Cw4/K80N

Cw3/S77N

Cw4/N77S

ing target cell in 0.1 M NaOH. In all presented experiments, the spontaneous release was <25% of maximal release. Each experiment was repeated three to six times. Error was <5% of the mean of the triplicates. In experiments where NKIR-specific mAbs were used to block MHC–NKIR interaction, mAb was included in the medium to a final concentration of 2.5 μg/ml.

Results

Generation and Expression of HLA-Cw4/Cw6 and -Cw3/ Cw7 Molecules Mutated at Positions 77 and 80. To investigate the role of residues 77 and 80 in protection from NK1 and NK2 effector cells, site-directed mutagenesis was carried out in which the amino acids at either position 77 or 80 in the NK1-protecting allele (Cw4 or Cw6) was replaced with the residue found at the homologous position in the NK2 protecting allele (Cw3 or Cw7) and vice versa. For example, mutants Cw6/K80N and Cw6/N77S have the Cw6 backbone, but express Asn at position 80 and Ser at position 77, respectively, whereas mutants Cw7/N80K and Cw7/S77N have the Cw7 backbone, but express Lys at position 80 and Asn at position 77, respectively. These mutant constructs and the wild-type HLA-C genes were transfected into 721.221 cells. The levels of HLA-C expression of the various transfectants were monitored by staining using the mAb W6/32 (Fig. 1). 721.221 cells, as well as cells transfected with the empty pCDNA3 vector, did not express detectable levels of MHC class I molecules. The existence of specific HLA-C transcripts in all of the 721.221 transfected cells, including specific examination of the mutations introduced, was confirmed at least twice in each line using RT-PCR (data not shown).

Recognition of HLA-C by NK Lines and Clones Generated from NK1 and NK2 Donors Is Controlled by Residue 80. To test the role of residues 77 and 80 in NK recognition of HLA-C, NK lines and clones were generated from donors MV and NQ (N77, K80 homozygous; NK1-specific cells) and from donors HTR, DP and AH (S77, N80 homozygous; NK2-specific cells) as described in Materials and Methods. All NK lines and clones (except two clones noted below) were CD3 negative, 100% CD56/CD16 positive, and expressed NKIR molecules recognized by the mAbs HP3E4 and GL183 (data not shown). NK inhibition assays were performed with the MV NK1 (Fig. 2 *A*) or the DP NK2 line (Fig. 2 *B*). 721.221 cells were killed by both lines to the same extent. 721.221 cells transfected with Cw4 or Cw6 were protected from lysis by NK1-, but not by NK2-specific lines. HLA-Cw3 and Cw7 transfectants were killed by NK1 lines, but protected from killing by NK2 lines. Next, position 80 in the α1 domain was shown to be the important position controlling recognition by

Figure 1. HLA-C expression on the various transfectants. 721. 221 cells were transfected with various HLA-C genes as described in Materials and Methods. Wild-type cells (721.221) and the various transfectants were stained with W6/32 antibody and than stained with anti–mouse IgG antibody bound to FITC (*bold lines*). Controls were the same cells stained only with anti–mouse IgG bound to FITC (*light lines*). One out of four representative experiments is shown.
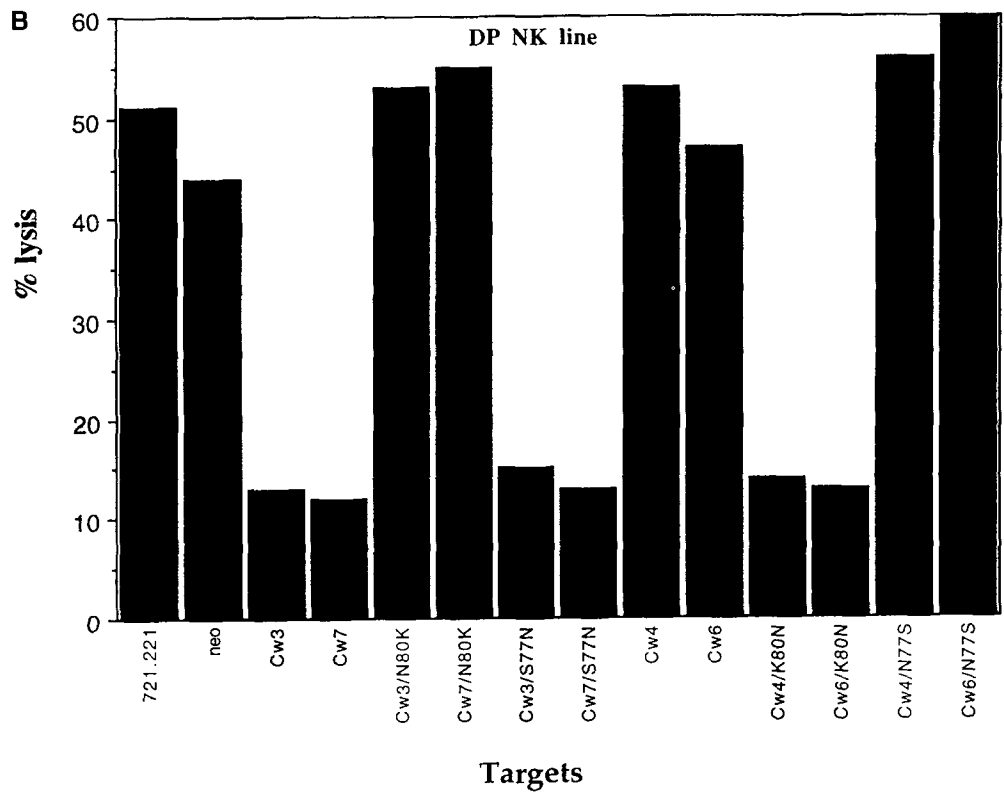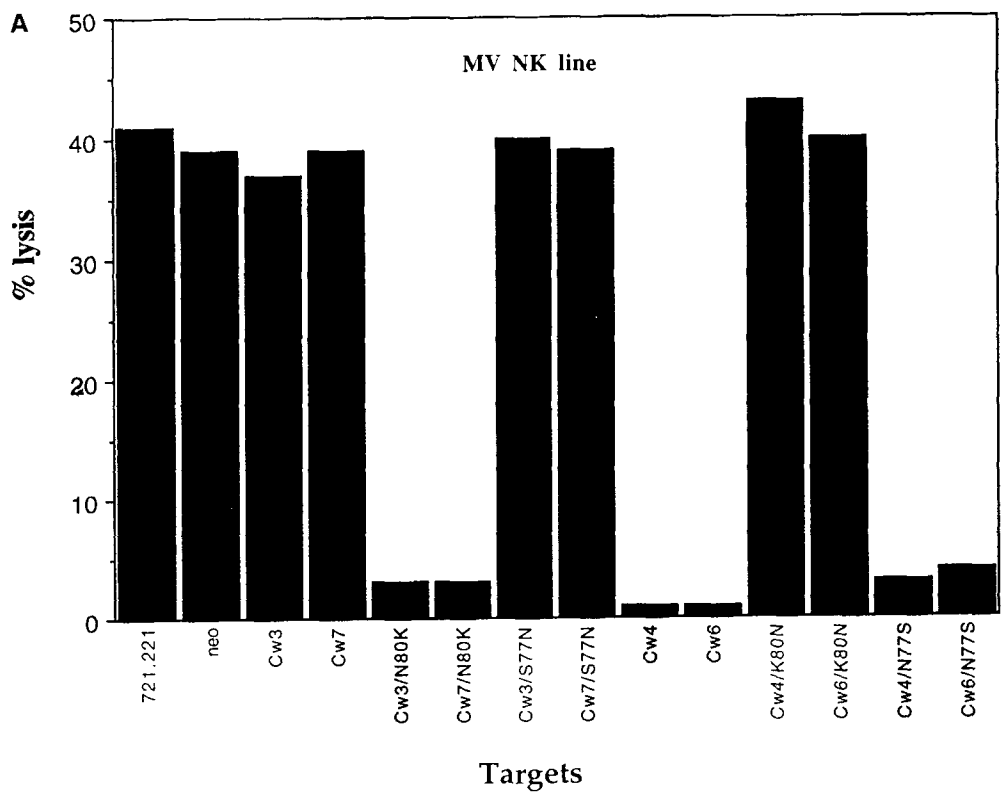
A MV NK line

B DP NK line

Figure 2. Residue 80 of HLA-C allotypes determines inhibition in NK lines. (A) Group 1 specificity. (B) Group 2 specificity. NK lines prepared from donor MV, NK1 (A) or from donor DP, NK2 (B) were reacted with various [^{35}S]-methionine-labeled target cells for 5 h at various E/T ratios. Only the E/T ratio of 10:1 is shown. One out of six representative experiments is shown.

both NK1 and NK2 lines. Target cells expressing K80 (Cw4 wild-type, Cw6 wild-type, Cw4/N77S, Cw6/N77S, Cw3/N80K, and Cw7/N80K) were protected from lysis by NK1 lines and killed by NK2 lines (Fig. 2 A), whereas target cells expressing N80 (Cw3 wild-type, Cw7 wild-type, Cw4/K80N, Cw6/K80N, Cw3/S77N, and Cw7/S77N) were protected from lysis by NK2-specific lines (Fig. 2 B). In both sets of experiments, lysis was reduced by >70% in

Table 1. *Residue 80 of HLA-C Allotypes is the Dominant Residue in Inhibition of NK1- and NK2-specific Clones*

NK clones	Controls		Transfectants											
	neo	721.21	Cw3	Cw3 N80K	Cw3 S77N	Cw4	Cw4 K80N	Cw4 N77S	Cw6	Cw6 K80N	Cw6 N77S	Cw7	Cw7 N80K	Cw7 S77N
A	NK1													
MV1	27	28	29	9	27	0	30	0	0	29	0	28	0	29
MV2	30	32	33	6	30	0	34	0	0	39	0	33	0	30
MV3	50	54	56	6	45	0	49	0	0	44	0	43	0	40
MV4	60	61	60	4	66	0	62	0	0	63	0	65	0	59
MV5	31	39	33	0	34	0	29	0	0	32	0	31	0	33
MV6	34	36	37	0	33	0	31	0	0	34	0	33	0	30
Aver.	**39**	**42**	**41**	**4**	**39**	**0**	**39**	**0**	**0**	**40**	**0**	**39**	**0**	**37**
B	NK2													
AH1	37	37	8	50	15	42	17	51	47	17	47	16	50	12
AH2	23	24	0	20	0	19	0	19	24	0	24	0	25	0
HTR31	31	32	0	27	0	30	0	33	32	0	23	6	20	0
DP1	47	46	17	49	15	48	16	49	54	19	57	20	55	20
DP2	73	74	19	79	33	78	30	81	86	32	90	22	79	14
DP3	85	90	18	49	6	50	8	51	43	5	42	4	51	3
DP4	43	44	0	40	3	41	0	43	39	3	46	2	43	1
Aver.	**48**	**50**	**9**	**45**	**10**	**44**	**10**	**47**	**46**	**11**	**47**	**10**	**46**	**7**

NK clones were prepared from NK1 donor (MV 1-6) (*A*) and from NK2 donors (AH-1-2, HTR-31, DP-1-4 (*B*) as described in Materials and Methods. Clones were reacted with the indicated [^{35}S]-methionine–labeled target cells for 5 h at various E/T ratios. Only data obtained at an E/T ratio 1:1 are shown. One representative experiment out of three is shown. In each group, Aver. refers to the average killing or inhibition (in **bold**) of the panel of NK clones on the indicated target cell.

relation to the killing of 721.221 cells. Experiments were performed six times with three independently derived NK2 lines from donors HTR, DP, and AH and with two NK lines derived from donors MV and NQ.

To confirm the above data at the clonal level, NK clones were generated from donors NQ, MV, AH, DP, and HTR. Table 1 summarizes experiments performed with 5 NK1 clones and 7 NK2 clones. MV NK1 clones were derived from NK1-specific lines and stained with mAb HP-3E4 (18), but not GL183 (NK2 specific) (19), or DX9 (NK3 specific) (20). These clones showed the same pattern of lysis as the NK1-specific lines; all transfectants expressing K80 were protected from lysis (Table 1 *A*). The reciprocal picture was seen in killing experiments using the NK2 clones. As in the case of the NK2-specific lines, target cells expressing N80 (Cw7 wild-type, Cw6/K80N, and Cw7/S77N) were protected from lysis (Table 1 *B*).

To provide direct proof that p58 (NKIR) molecules were involved in recognition of the mutated HLA-C alleles, the NKIR-specific mAbs HP3E4 and GL183 were tested for their ability to specifically reverse HLA-C–mediated inhibition of NK killing (Fig. 3). Inclusion of mAb GL183 (which stains NK cell lines and clones inhibited by NK2 alleles), but not mAb HP3E4 (which stains NK cell lines and clones inhibited by NK1 alleles) in the assay abrogated the

ability of HLA-C alleles expressing Asn80 to inhibit killing by NK2 lines and clones. Conversely, addition of mAb HP3E4, but not mAb GL183, to the assay blocked the ability of HLA-C alleles expressing Lys80 to inhibit lysis mediated by NK1-specific effector cells.

2 out of 16 NK 2-specific clones tested were inhibited by either Cw6/N77S- or Cw6/K80N-expressing cells and 1 out of 8 NK2 clones was inhibited by either Cw4/N77S or Cw4/K80N. These clones did not express any of the NKIR defined by the available mAb to these receptors, and since NK clones may express multiple receptors (21), other as yet unidentified receptors may account for these observations. Given the discrepancy with the result of Biassoni et al. (15), we considered that the use of NK cell lines as well as clones was important to establish that these properties were representative of the majority of the NK cell population.

The present data on the relative importance of residues 77 and 80 in controlling recognition by NK2-specific effector cells are in conflict with the recent data of Biassoni et al. (15). We have no explanation for this discrepancy other than to suggest that it may be a result of the limited number of NK clones tested in those experiments.

The Role of Peptide in Recognition of HLA-C by NK Lines and Clones. Experiments in human and murine model systems of NK recognition of MHC class I molecules have
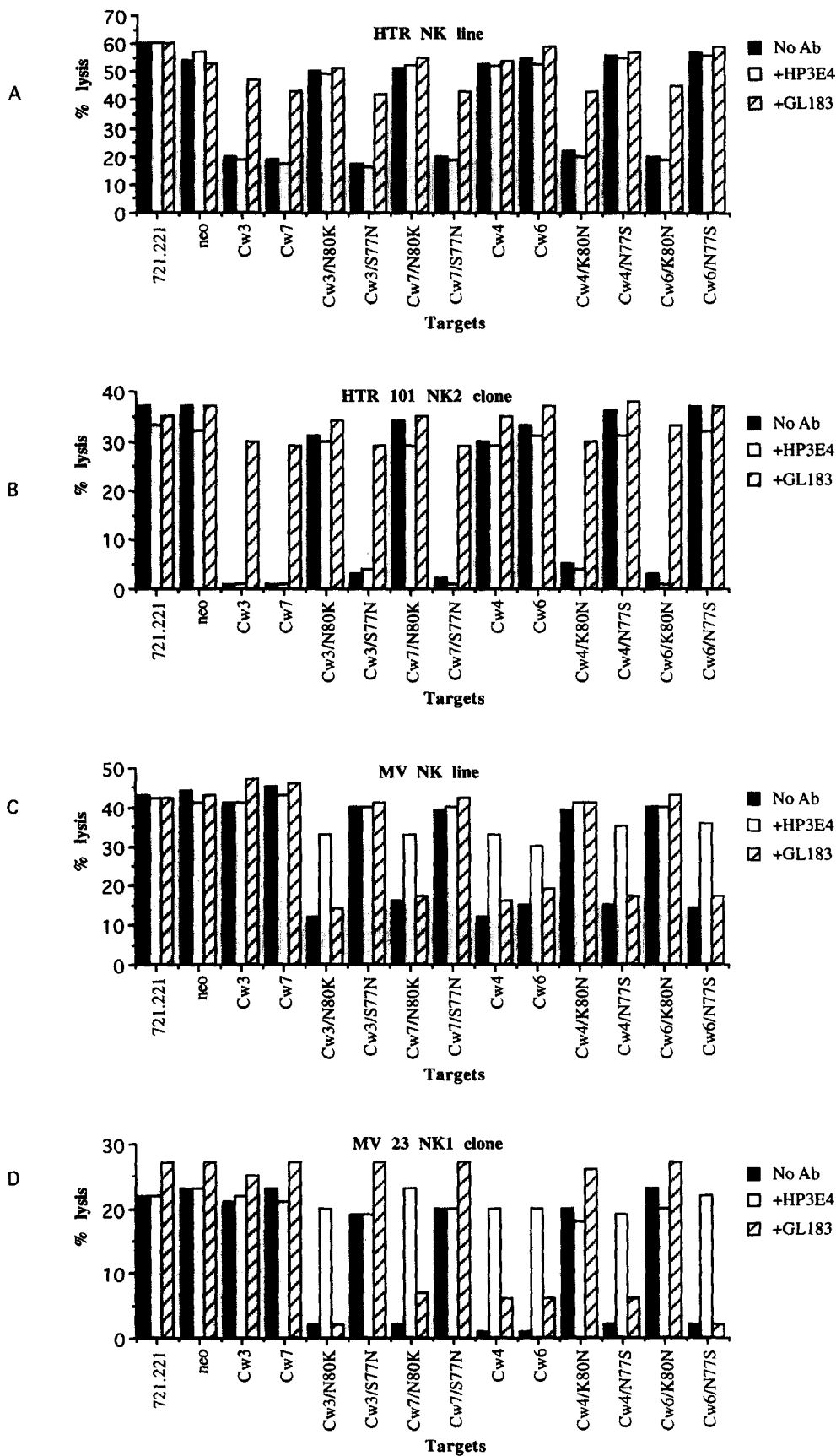
Figure 3. Reversal of inhibition of NK killing by p58-specific antibodies. mAb GL183, but not HP3E4, reverses HLA-C Asn80-mediated inhibition of (*A*) an NK2-specific line and (*B*) an NK2-specific clone. mAb HP3E4, but not GL183, reverses HLA-C Lys80-mediated inhibition of (*C*) an NK1-specific line and (*D*) an NK1-specific clone. NK lines and clones prepared from donor MV (NK1) or from donor HTR (NK2) were reacted at E/T ratios of 10:1 for the lines, or 1:1 for the clones with various [^{35}S]methionine-labeled target cells for 5 h in the presence or absence of the indicated antibodies (2.5 μg/ml).
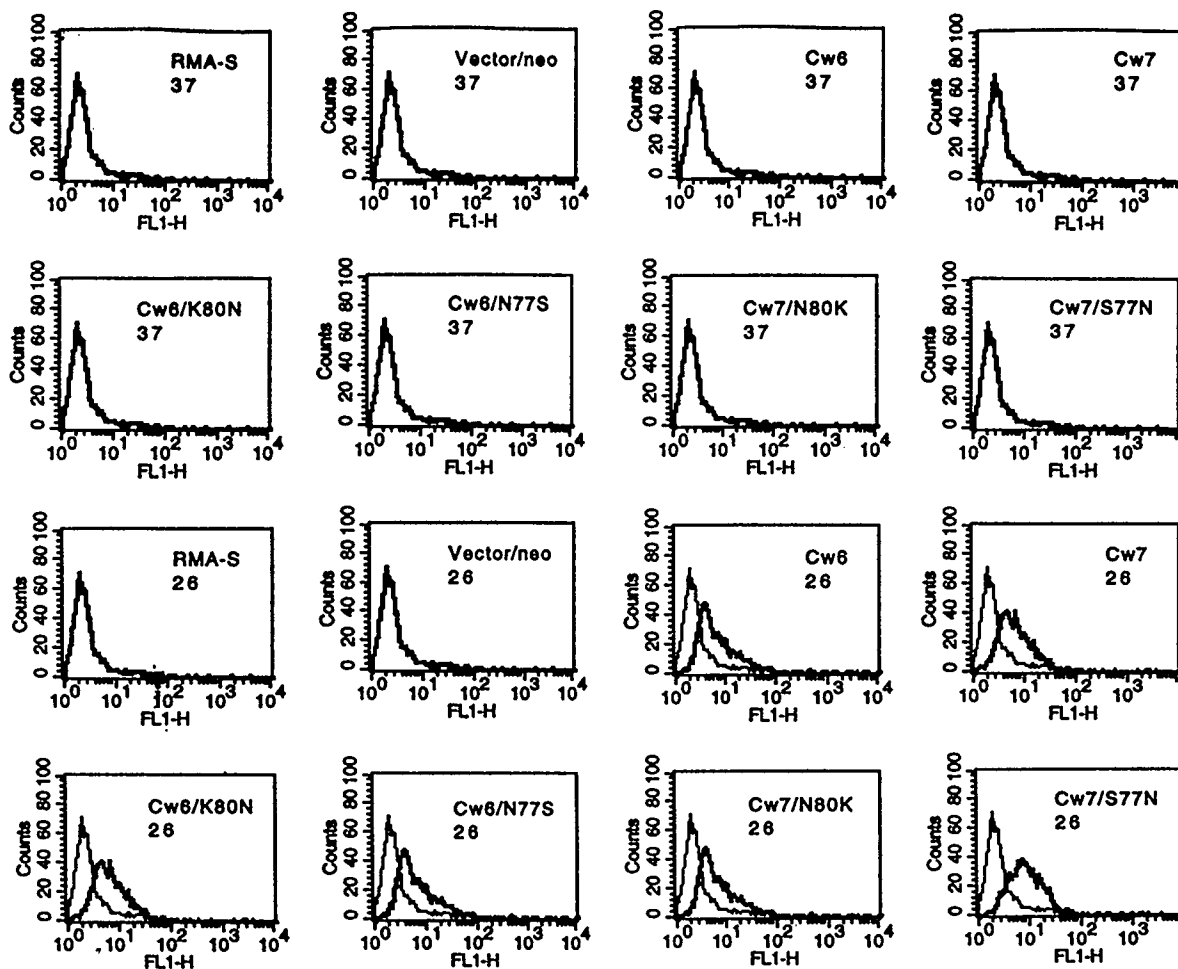
Figure 4. Cell surface expression of HLA-C molecules on RMA-S cells after overnight culture at 26°C and 37°C. RMA-S cells either untransfected or transfected with the indicated HLA-C genes, and human β_2m were cultured overnight in a humidified atmosphere with 5% CO_2 at either 37 or 26°C. Cells were stained with mAb BB7.7 and analysed by FACS®.

produced discordant results with regard to the role of peptide in allorecognition by NK cells. Correa and Raulet (12) reported that any peptide that induced stabilization, and hence cell surface expression, of the MHC molecule was able to induce protection from NK cell–mediated lysis. In contrast, Malnati et al. (14) have reported that NK clones inhibited by HLA-B2705 can exhibit a degree of peptide specificity in recognition of HLA. In the present study, this issue was addressed by transfecting HLA-C alleles into RMA-S cells, which are deficient in TAP function and thus unable to load peptides onto MHC molecules. Culture of RMA-S at 26°C results in surface expression of empty MHC molecules that are apparently devoid of peptide (13). Various RMA-S transfectants were incubated at either 37 or 26°C and then stained with mAb BB7.7. RMA-S transfectants cultured overnight at 26°C expressed HLA-C (Fig. 4). Cell surface expression of HLA-C was maintained for at least 2 h after shift to culture at 37°C (data not shown). RMA-S cells transfected with either the wild-type HLA-C genes or the various mutant molecules were protected from NK lysis when cultured at 26°C in the ab-

sence of exogenously added peptide (Fig. 5). RMA-S cells and the various transfectants were not protected from NK lysis if cultured at 37°C. This protection was specific since only RMA-S cells transfected with an HLA-C gene expressing Lys80 (Cw6, Cw6/N77S, Cw7/N80K) were protected from lysis by a NK1 line (Fig. 5 *A*). In the reciprocal experiment, only HLA-C molecules with Asn80 (Cw7, Cw7/S77N, Cw6/K80N) are able to protect from a NK2 line (Fig. 5 *B*). Identical results were obtained in killing/inhibition assays using these transfectants and the panel of NK1 and NK2 clones (data not shown).

Discussion

The correlation between the residue found at position 80 and susceptibility to NK cell lysis was first observed in the relationship between the dimorphism at residues 77 and 80 in HLA-C alleles and susceptibility to lysis (7). Subsequently, a similar correlation between susceptibility to lysis by NK3 cells and the presence of I80, T80, or N80 in HLA-B alleles was also observed (11). Residue 80 is lo-
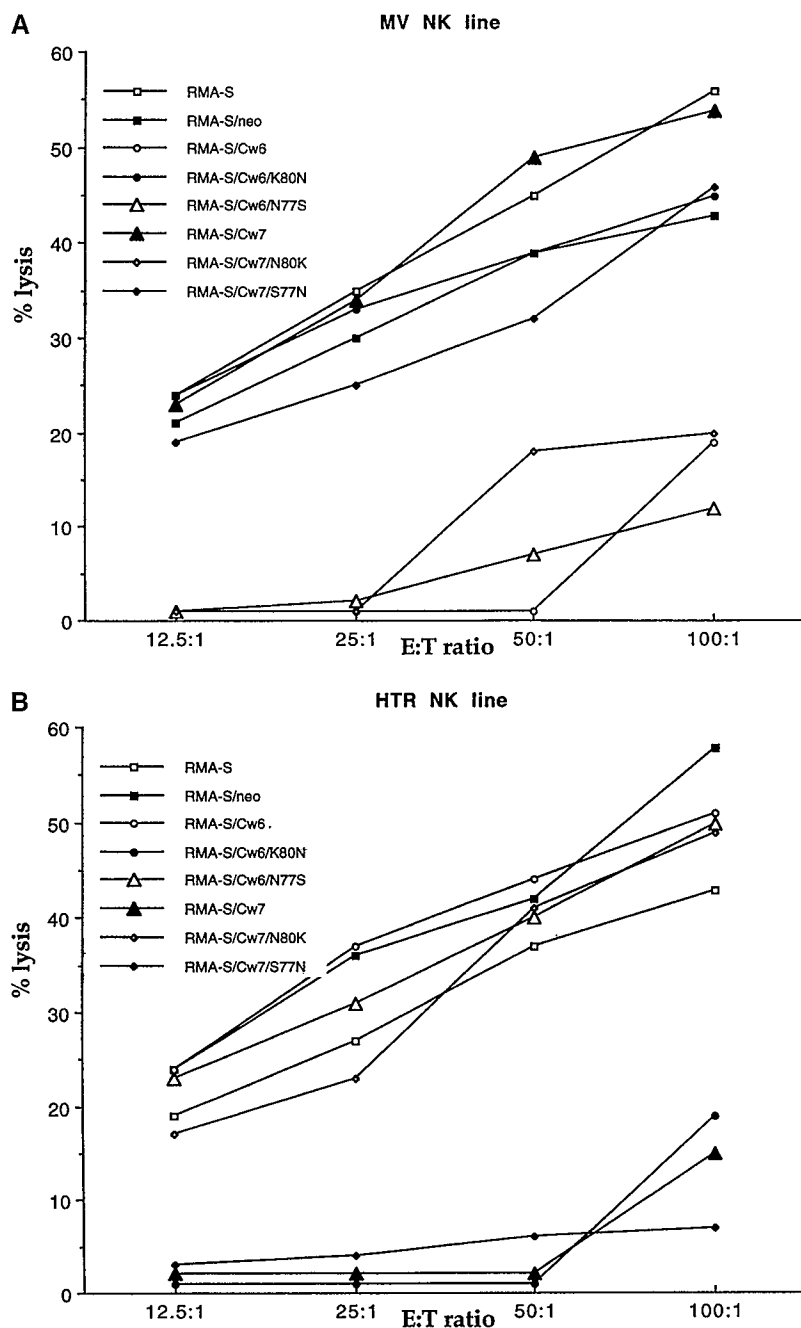
A

MV NK line

Legend:
- RMA-S
- RMA-S/neo
- RMA-S/Cw6
- RMA-S/Cw6/K80N
- RMA-S/Cw6/N77S
- RMA-S/Cw7
- RMA-S/Cw7/N80K
- RMA-S/Cw7/S77N

% lysis

E:T ratio: 12.5:1, 25:1, 50:1, 100:1

B

HTR NK line

Legend:
- RMA-S
- RMA-S/neo
- RMA-S/Cw6.
- RMA-S/Cw6/K80N
- RMA-S/Cw6/N77S
- RMA-S/Cw7
- RMA-S/Cw7/N80K
- RMA-S/Cw7/S77N

% lysis

E:T ratio: 12.5:1, 25:1, 50:1, 100:1

Figure 5. Killing of RMA-S and transfectants by (*A*) a NK1-specific line and (*B*) a NK2-specific line. RMA-S and the indicated transfectants were cultured overnight at either 26 or 37°C and then used as targets in cytotoxicity assays. None of the various HLA-transfected RMA-S cell lines incubated at 37°C were protected from NK killing (data not shown). (*A*) Pattern of killing and inhibition obtained by assaying the indicated targets (cultured at 26°C) for susceptibility to a NK1-specific line (*MV*). (*B*) Results obtained when the effector cells were a NK2-specific line (HTR). Percent specific lysis is shown at various E/T ratios.

cated at the far side of the lip of the P9 pocket (F pocket) of class I MHC molecules, and its side chain points upwards (22); thus, its role in recognition by NK receptors can be readily understood. On the other hand, residue 77 is located deep in the P9 pocket and its side chain is not accessible to recognition. The data of Biassoni et al. (15; suggesting that a change in residue 80 did not affect NK2 allorecognition, whereas a change at residue 77 abrogated recognition), which we have been unable to reproduce were, therefore, extremely surprising. Residue 77 does, however, form an important residue in interaction with the P9 residue of the peptide. Its nature (S or N in all HLA-C alleles) could indirectly affect NK recognition by determining the peptide

that can be bound, and possibly the conformation of the molecule. The present data, however, show that cell surface expression of HLA-C molecules, even in the absence of peptide, is both necessary and sufficient to inhibit HLA-C-specific NK lines and clones, and that residue 80 plays a critical role in determining specificity of both NK1- and NK2-specific cells. This result strongly suggests that the site of the interaction between HLA molecules and the NKIR localizes to the top of the α1 helix.

The actual epitope recognized by NKIR on NK cells is, however, likely to be larger than a single amino acid. It has been suggested that NK cells interact with the "side" of the MHC molecule and recognize polymorphic residues in the

α1 helix (23). We propose that the recognition site might be similar in position to the site occupied by Staphylococcal enterotoxin B and toxic shock syndrome toxin 1 (TSST-1) on top of and behind the α1 helix on class II MHC molecules, but further towards the COOH terminus of the α1 helix than either of these superantigens. α1-V65 in HLA-DR with which TSST-1 interacts is equivalent in position to α1-73T/A in HLA-Cw3/7, respectively (24). Residue 80 in HLA-C is thus two turns of the helix further toward the COOH terminus than α1-V65 in class II MHC molecules. The precise epitope recognized by NK cells in this region requires much further study, and ultimately will be defined by detailed structural analysis.

Moreover, from the present results, peptide(s) are unlikely to be involved directly in recognition of the HLA-C molecule since the empty molecules expressed in RMA-S cells at 26°C are inhibitory in a manner related only to expression of the appropriate amino acid at position 80. RMA-S cells do not express TAP, the peptide transporter through which most peptides enter the endoplasmic reticulum (ER) for binding to class I MHC proteins. The entry of hydrophobic peptides formed through cleavage of signal sequences within the ER is TAP independent, and in at least one case, HLA-A2 expressed in TAP-deficient human cells (but not HLA-B5 [25]), some HLA-A2 folds appropriately with such hydrophobic peptides. However, in that unusual case, low level HLA-A2 expression is seen at the cell surface at 37°C, as well as at 26°C (26, 27). In the present case, the FACS® profile for HLA-C expression in RMA-S at 37°C cells can be superimposed on that of control cells, i.e., no HLA-C appears to be expressed. It is, therefore, unlikely that these class I MHC proteins bind TAP-independent peptides.

References

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