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

By Ofer Mandelboim, Hugh T. Reyburn, MarValés-Gómez, Laszlo Pazmany, Marco Colonna, Giovanna Borsellino, and Jack L. Strominger

From the Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 01238

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 effectors. 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 (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 blood by centrifugation on Ficoll-Hypaque (Pharmacia, Piscataway, NJ). 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 × 10⁴ allogeneic PBMC from two donors and 5 × 10⁴ 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 Boletis (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'-GCCGCGACTGGTGCCAGGT-3', N77S 5'-TTTCCCGAG- GTTACTCGTGTC-3', N80K 5'-GCCGCGACTGGTGCCAGGGT-3', and S77N 5'-GTTCCCGAGGTTCGACCTCGGGCT-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 pulse (Bio-Rad Laboratories, Richmond, CA) set at 230 V and 250 μF. 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 transfected cells selected using 1.6 mg/ml Genetec (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 β₂-microglobulin (β₂m) 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% CO₂ and was monitored by flow cytometry using the mAbs BB7.7 (heavy chain specific) or BBM1 (β₂m specific).

**Cytolytic Assays.** The cytolytic activity of NK lines and clones against the various HLA-C transfectants and mutants was assessed in 5-h ³¹P-release assays (4 h for the RMA-S targets) in which effector cells were admixed with 5 × 10⁴ [³¹P]methionine-labeled targets at different E/T ratios in U-bottomed microtiters 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-
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 then 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.
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

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 [35S]-methioninelabeled 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.
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 | Cw3 | Cw4 | Cw4 | Cw4 | Cw6 | Cw6 | Cw6 | Cw7 | Cw7 | Cw7 |
| 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 [35S]-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.

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 [35S]methionine-labeled target cells for 5 h in the presence or absence of the indicated antibodies (2.5 μg/ml).
produced discordant results with regard to the role of peptide in allore cognition 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 absence 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-
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.

ated 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
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 α1-737/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.

We thank José Aramburu and Juan J. Pérez-Villar for much helpful advice with regard to the generation and culture of NK lines and clones and Miguel Lopez-Botet for his generous gift of the HP3E4 mAb.

This work was supported by National Institutes of Health grant CA-47554. O. Mandelboim is an European Molecular Biology Organization fellow and a Fulbright scholar, H.T. Reyburn holds a Wellcome Trust International Prize Travelling Research Fellowship, L. Pazmany is a recipient of an Arthritis Foundation postdoctoral fellowship, and G. Borsellino is supported by I Clinica Neurologica, Universite di Roma “La Sapienza.”

Address correspondence to Dr. Jack L. Strominger, Department of Molecular and Cellular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 01238.

Received for publication 22 January 1996 and in revised form 6 June 1996.

References

1. Ljunggren, H.G., and K. Karre. 1990. In search of the “missing self”: MHC molecules and NK cell recognition. Immunol. Today. 11:237–242.

2. Colonna, M., and J. Samaridis. 1995. Cloning of immuno-globulin-superfamily members associated with HLA-C allele and HLA-B recognition by human natural killer cells. Science (Wash. DC). 268:405–408.

3. Wagtmann, N., R. Biassoni, C. Cantoni, S. Verdianni, M.S. Maltini, M. Vitale, C. Bottino, L. Moretta, A. Moretta, and E.O. Long. 1995. Molecular clones of the p88 natural killer cell receptor reveal Ig related molecules with diversity in both the extra- and intracellular domains. Immunity. 2:439–458.

4. D’Andrea, A., C. Chang, K. Franz-Bacon, T. McClanahan, J.H. Philips, and L.L. Lanier. 1995. Molecular cloning of NKB1. A natural killer cell receptor for HLA-B allotypes. J. Immunol. 155:2306–2313.

5. Moretta, L., E. Ciccone, M.C. Mingari, R. Biassoni, and A. Moretta. 1994. Human NK cells: origin, clonality, specificity and receptors. Adv. Immunol. 55:341–390.

6. Trinchieri, G. 1994. Recognition of major histocompatibility complex class I antigens by natural killer cells. J. Exp. Med. 180:417–420.

7. Colonna, M., T. Spies, J.L. Strominger, E. Ciccone, A. Moretta, L. Moretta, D. Pende, and O. Viale. 1992. Alloantigen recognition by two human natural killer cells clones is associated with HLA-C or a closely linked gene. Proc. Natl. Acad. Sci. USA. 89:7983–7985.

8. Cristiano, F.T., C.S. Witt, E. Ciccone, D. Townsend, D. Pende, O. Viale, L.J. Abraham, P.L. Dawkins, and L. Moretta. 1993. Human natural killer (NK) alloreactivity and its association with the major histocompatibility complex: ancestral haplotypes encode particular NK-defined haplotypes. J. Exp. Med. 178:1033–1039.

9. Colonna, M., E.G. Brooks, M. Falco, G.B. Ferrara, and J.L. Strominger. 1993. Generation of allospecific natural killer cells by stimulation across a polymorphism of HLA-C. Science (Wash. DC). 260:1121–1124.

10. Colonna, M., G. Borsellino, M. Falco, G.B. Ferrara, and J.L. Strominger. 1993. HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells. Proc. Natl. Acad. USA. 90:12000–12004.

11. Cella, M., A. Longo, G.B. Ferrara, J.L. Strominger, and M. Colonna. 1994. NK3-specific natural killer cells are selectively inhibited by Bw4-positive HLA alleles with isoleucine 80. J. Exp. Med. 180:1235–1241.

12. Correa, J., and D.H. Raulet. 1995. Binding of diverse peptides to MHC class I molecules inhibits target cell lysis by activated natural killer cells. Immunol. 2:61–71.

13. Ljunggren, H.G., N.J. Stan, C. Ohlen, J.J. Neefjes, J. Bastin, T.N.M. Schumacher, A. Townsend, K. Karre, and H.L.
Ploegh. 1990. Empty MHC molecules come out in the cold. Nature (Lond.). 346:476–480.

14. Malnati, M.S., M. Peruzzi, K.C. Parker, W.E. Biddison, E. Ciccone, A. Moretta, and E.O. Long. 1995. Peptide specificity in the recognition of MHC class I by natural killer cell clones. Science (Wash. DC). 267:1016–1018.

15. Biassoni, R., M. Falco, A. Canbiaggi, P. Costa, S. Verdiani, D. Pende, R. Conte, C. Di Donato, P. Parham, and L. Moretta. 1995. Amino acid substitutions can influence the natural killer (NK)-mediated recognition of HLA-C molecules. Role of serine-77 and lysine-80 in the target cells protection from lysis mediated by "group 1" or "group 2" NK clones. J. Exp. Med. 182:605–609.

16. Shimizu, Y., and R. DeMars. 1989. Production of human cells expressing individual transferred HLA-A, -B, -C genes using an HLA-A,-B,-C null human cell line. J. Immunol. 142: 3320–3328.

17. Melero, I., A. Salmeron, M.A. Balboa, J. Aramburu, and M. Lopez-Botet. 1994. Tyrosine kinase–dependent activation of human NK cells function upon stimulation through a 58-kDa surface antigen selectively expressed on discrete subset of NK cells and T lymphocytes. J. Immunol. 152:1662–1673.

18. Moretta, A., M. Vitale, C. Bottino, A.M. Orengo, L. Morelli, R. Augugliaro, M. Barbaresi, E. Ciccone, and L. Moretta. 1993. P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-P58 antibodies reconstitute lysis of MHC class-I protected cells in NK clones displaying different specificities. J. Exp. Med. 178:597–604.

19. Robertson, J.R., T.J. Manley, C. Donahue, H. Levine, and J. Ritz. 1993. Costimulatory signals are required for optimal proliferation of human natural killer cells. J. Immunol. 150: 1705–1714.

20. Gumperz, J.E., V. Litwin, J.H. Phillips, L.L. Lanier, and P. Parham. 1995. The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor. J. Exp. Med. 181:1133–1144.

21. Moretta, A., S. Sivori, M. Vitale, D. Pende, L. Morelli, R. Augugliaro, C. Bottino, and L. Moretta. 1995. Existence of both inhibitory (p58) and activatory (p50) receptors for HLA-C molecules in human natural killer cells. J. Exp. Med. 182:875–884.

22. Saper, M.A., P.J. Bjorkman, and D.C. Wiley. 1991. Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. J. Mol. Biol. 219:277–319.

23. Gumperz, J.E., and P. Parham. 1995. The enigma of the natural killer cells. Nature (Lond.). 378:245–248.

24. Kim, J., R.G. Urban, J.L. Strominger, and D.C. Wiley. 1994. Toxic shock syndrome toxin-1 complexed with a class II major histocompatibility molecule HLA-DR1. Science (Wash. DC). 266:1870–1874.

25. Baas, E.J., H.-M. van Santen, M.J. Kleijmeer, H.J. Geuze, P.J. Peters, and H.L. Ploegh. 1992 Peptide-induced stabilization and intracellular localization of empty HLA class I complexes. J. Exp. Med. 176:147–156.

26. Henderson, R.A., H. Michel, K. Sakaguchi, J. Shabanowitz, E. Appella, D.F. Hunt, and V.H. Engelhard. HLA-A2.1-associated peptides from a mutant cell line: a second pathway of antigen presentation. Science (Wash. DC). 255:1264–1266.

27. Wei, M.L., and P. Cresswell. 1992. HLA-A2 molecules in an antigen-processing mutant cell contain signal sequence-derived peptides. Nature (Lond.). 356:443–446.