

Expression of HLA-C Molecules Confers Target Cell Resistance to Some Non-major histocompatibility complex-restricted T Cells in a Manner Analogous to Allospecific Natural Killer Cells

By Christine S. Falk, Alexander Steinle, and Dolores J. Schendel

From the Institute for Immunology, University of Munich, D-80336 Munich, Germany

Summary

Specific HLA molecules have recently been shown to confer target cell resistance to lysis by some CD3⁻ natural killer (NK) cells. For certain NK clones, resistance is governed by two specificities (NK1 and NK2) that are associated with particular HLA-C alleles: in general, target cells expressing Cw1, Cw3, Cw7, or Cw8 are susceptible to NK1 but resistant to NK2 clones, whereas target cells expressing Cw2, Cw4, Cw5, or Cw6 are susceptible to NK2 and resistant to NK1 cells. These two clusters of HLA-C alleles are distinguished by a dimorphism in the $\alpha 1$ helical region, localized at amino acid positions 77 and 80. In this report, we show that highly enriched CD3⁺/CD56⁻ cytotoxic T cell sublines and CD3⁻/CD56⁺ NK sublines derived from the same donor have identical cytolytic specificities when tested against a panel of allogeneic LCL and various HLA-B and -C transfectant cell lines. The lysis pattern of the allogeneic cells appeared to be related to the NK2 specificity for both effector cells: LCL expressing HLA-Cw2, Cw4, Cw5, or Cw6 alleles were lysed, while LCL expressing HLA-Cw1, Cw3, or Cw7 molecules were resistant. Resistance to lysis could be conferred to susceptible target cells by transfection with a Cw*0702 gene, while expression of a Cw*0602 gene did not provide protection. Similar patterns of HLA-C-mediated resistance were also found with two polyclonal T cell lines generated from the peripheral blood lymphocytes of unrelated donors. Thus, major histocompatibility complex (MHC) molecules that induced resistance to particular NK cells also regulated target cell resistance to lysis by these non-MHC-restricted effector T cells. For both types of effector cells, direct binding to HLA-C molecules was necessary to achieve inhibition since preincubation with mAb specific for class I molecules destroyed the protection from lysis of HLA-Cw7 expressing target cells. mAbs specific for CD3 and CD8 molecules had no influence on lysis or inhibition of the NK-like T cells. Formation of MHC complexes with particular peptides did not appear to be essential to confer resistance, since a cell line with defective peptide transporter genes (TAP genes), when transfected with an appropriate HLA-C allele, was as resistant to lysis as HLA-C transfectant lines with normal TAP function. These results suggest that HLA-C molecules may deliver negative regulatory signals to some non-MHC-restricted T cells in a manner similar to that described previously for particular NK cells.

Cell surface phenotype, cytotoxic specificity, and mode of activation are three important parameters that distinguish the major classes of human cytotoxic effector cells. For classic CTL, expression of the coreceptor molecules CD4 or CD8 governs the interaction with MHC class II or class I molecules, respectively. Triggering of the TCR- $\alpha\beta$ -CD3 complex induces lysis that is specific for an MHC/peptide ligand. In contrast, CD16⁺/CD56⁺ NK cells do not express TCR/CD3, CD4, or CD8 molecules, with the exception that some NK cells, defined as CD8^{dim} NK, display low amounts of CD8 (1). NK cells do not show MHC-restricted target cell recognition, thus they recognize class I-negative target cells. Unlike CTL, no single re-

ceptor/ligand pair explaining NK target cell recognition has been identified. One positive signal for induction of NK cytotoxicity occurs through FcR γ III (CD16) binding of antibody-coated target cells (2). Interactions of CD2 with leukocytic function-associated-3 (CD58), and LFA-1 (CD11a) with intracellular adhesion molecule-1 (CD54), as well as CD27, have also been implicated in activation of NK lytic function (3, 4, reviewed in 5). NK cells are maintained in a functional state that enables them to directly mediate cytotoxic potential, thus formation of target cell conjugates via a number of different ligand interactions may be sufficient to induce target cell destruction. Although expression of lytic potential in NK cells does not

require presensitization, it can be enhanced by IL-2 and IL-12 (6); furthermore, stimulation with PHA or allogeneic cells can increase NK activity (7). These modes of activation also induce cytolytic activity in some CD3⁺ T lymphocytes; these NK-like T cells are not MHC-restricted and they can lyse MHC class I-negative target cells such as Daudi and K562 (2, 8); for some cells, an involvement of CD28 in activation is suggested (9).

While in many instances the molecules responsible for activation of NK cells remain undefined, increasing information is emerging about the role of MHC molecules as inhibitory elements in NK/target cell interactions (10–12). Attention to MHC involvement in NK regulation first came through the observation that increasing MHC expression on tumor cells induced NK resistance (13, 14), and transfection of some MHC genes into susceptible tumor cells conferred resistance to NK lysis (15). For human NK cells, an element of specificity in resistance was revealed with studies of defined HLA-A molecules (16). Involvement of HLA-C in providing resistance to particular NK cells was shown by conferring protection to susceptible target cells through transfection of the HLA-Cw3 allele (17). Further work pinpointed a dimorphism in HLA-C localized at positions 77 and 80 of the α 1-helical region that serves as a useful marker for HLA-C alleles capable of inducing resistance to selected NK cells (18–20). More recently, resistance to other NK clones was found to be associated with the Bw4 and Bw6 epitopes expressed by defined HLA-B alleles (7, 21, 22).

The finding that different HLA-A, -B, or -C alleles could provide resistance to particular NK populations suggests that the effector cells express variable receptor molecules involved in mediating inhibitory signals. Several molecules have been identified on murine and human NK cells that correlate with inhibition by MHC molecules, for example the murine Ly49 molecule (23), the p58 molecular family (24), the NKB1 receptor (22), and the CD94 molecule (25). Selective MHC inhibition has only been described for NK cells, but several NK-related molecules are also found on some T cells where they seem to be involved in regulating cytolytic activity (26, 27).

In this study, we compared the role of HLA molecules in conferring target cell resistance to lysis mediated by NK cells and non-MHC-restricted cytotoxic T cell populations. These studies show that MHC molecules that induced resistance to the NK cells also protected target cells from lysis by these particular non-MHC-restricted T cells. In addition, target cells deficient in peptide transporter genes were still protected if transfected with an appropriate HLA-C allele.

Materials and Methods

Panel Cells and Transfectants. PBLs of healthy Caucasian donors were HLA typed using local and International Histocompatibility Workshop sera by Dr. R. Wank (University of Munich, Munich, Germany). The HLA types of all effector and target cells are presented in Table 1. EBV-transformed B-lymphoblastoid cell

lines (LCL)¹ were established from PBL in RPMI 1640 medium with 10% FCS, 1% PHA (PHA-m; Difco Laboratories, Detroit, MI), and 50% supernatant medium of a 12-d culture of a marmoset cell line, producing the B95.8 strain of virus (American Type Culture Collection, Rockville, MD). Daudi and K562 are hematopoietic tumor lines that lack HLA class I expression; both are well known for their NK susceptibility. All LCL were maintained in RPMI medium with 5% FCS, 2 mM L-glutamine, 1 mM pyruvate, and antibiotics. The HLA mutant cell, C1R, was described previously to express only HLA-Cw4 molecules (Cw*0401) and very low levels of HLA-B35 molecules (B*3503) (28). The HLA-A, -B, and -C-deficient line L721.221 is hemizygous for chromosome 6, and it carries a deletion of the HLA-A and -B loci in the remaining MHC (29). The T2 line, kindly provided by Dr. R. Tampe (Max Planck Institute of Biochemistry, Martinsried, Germany), represents a hybrid obtained by fusion of L721.174 with the T-LCL CEM (30); T2 has lost both copies of chromosome 6 from CEM but retains the mutant HLA haplotype of L721.174, carrying the HLA alleles A*0201, B*5101, and Cw*0102 (31), and it has an extensive deletion in the class II region. T2 is therefore deficient in all copies of peptide transporter (TAP) genes and defective in peptide transport (32). It maintains residual expression of HLA-A2 (20–25% of wild-type level) through binding of a restricted set of signal peptides (33). Only low levels of HLA-B51 are found on T2 (34); due to the lack of specific mAb, no conclusions can be made about HLA-Cw1 surface expression.

L721.221, C1R, and T2 were transfected by electroporation with genomic DNA of HLA genes cloned into the eucaryotic expression vector pHEBo and selected by hygromycin B resistance (0.5 mg/ml) (Boehringer Mannheim GmbH, Mannheim, Germany). The HLA-B*2705 gene (35) was kindly provided by Dr. E. Weiss (Institute of Human Genetics, University of Munich, Munich, Germany), and the HLA-Cw*0702 gene (36) was provided by Dr. S. Weissman (Yale University, New Haven, CT). The HLA-B*3501 (37), B*3701 (38), and Cw*0602 (39) genes have been described previously by this laboratory. T2 cells transfected with HLA-B*3501 were kindly provided by Dr. M. Takiguchi (Institute of Medical Science Tokyo, Tokyo, Japan). Transfectant cell lines are designated as .221-Cw*0702 or T2-Cw*0702, for example.

Cytotoxic Effector Lines. Responding PBL were stimulated for 6 d with irradiated (20 Gy) allogeneic PBL (ratio 1:1) in 50 ml culture flasks in RPMI 1640 medium with 2 mM L-glutamine, 1 mM pyruvate, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 15% heat-inactivated pooled human serum (40). The effector lines were restimulated every 7–12 d and cultured in medium supplemented with 50–500 U/ml human rIL-2 (Proleukin; Cetus, Emeryville, CA) and 10% conditioned medium (41). A panel of sublines, including B.3D, B.3M, B.3N, B.3S, and B.3T, was established by limiting dilution (0.3 cells per well) from a 6-d B.3 culture in the presence of rIL-2 and irradiated feeder cells of donor 3.

Separation of CD3⁺ and CD56⁺ Subpopulations. To purify CD3⁺/CD56[–] T cells from CD3[–]/CD56⁺ NK cells, direct and indirect separations were performed using immunomagnetic beads (Dynal AS, Oslo, Norway). To deplete residual CD56⁺ cells from an NK-like T cell population (B.3S), cells were incu-

¹Abbreviations used in this paper: LCL, lymphoblastoid cell line; RCR, relative cytotoxic response; TAP, transporter associated with antigen processing (genes).

bated with the CD56-specific mAb NKH-1 (Coulter Corp., Hialeah, FL) diluted 1:5 in PBS/1% FCS for 90 min on ice. Thereafter, IgG1-coupled immunomagnetic beads (Dyna) were added for 30 min at 4°C; the cells binding beads were removed by magnetic adherence, leaving a negative fraction of CD3⁺/CD56⁻ cells (B.3S.1). Enrichment of CD56⁺/CD3⁻ NK cells was performed by direct depletion of the contaminating CD4⁺ cells from sublines B.3D and B.3T using CD4-coated immunomagnetic beads (Dyna) incubated for 45 min at 4°C, followed by removal through magnetic adherence resulting in lines B.3D.1 and B.3T.1. The CD56⁺ fraction of line B.3 was positively selected using the NKH-1 mAb and IgG1-coupled immunomagnetic beads (B.3P). All separated populations were tested for expression of surface markers and cytotoxic activity as described below.

Cell-mediated Lympholysis Assay. Cytotoxic activity was determined in a standard 4-h chromium release assay as described (42). Spontaneous release was determined by incubating target cells in medium alone. Total release was detected by directly counting an aliquot of labeled cells. The percent of lysis was calculated as follows: $\text{percent of lysis} = (\text{experimental cpm} - \text{spontaneous cpm}) / (\text{total cpm} - \text{spontaneous cpm}) \times 100$. All experiments used at least three step titrations of effector cells. Standard linear regression analysis was performed by plotting the percent of specific lysis against the log of the effector cell numbers. To combine data from separate experiments, a percent relative cytotoxic response (RCR) was calculated as follows: $\text{percent of RCR} = (\text{percent of lysis of experimental target} / \text{percent of lysis of reference target}) \times 100$ at a selected E/T ratio, as described earlier (42).

Cell-mediated Lympholysis Blocking Using mAbs. To evaluate the involvement of HLA class I, CD3, and CD8 molecules, respectively, in the interaction of effector cells with target cells, the

following mAb were added to the standard chromium release assay: the pan-HLA class I-specific mAb W6/32 (ATCC) and the HLA-B- and -C-specific 4E ascites (kindly provided by Dr. S. Y. Yang, Sloan Kettering Institute for Cancer Research, New York, NY) were used in dilutions ranging from 1:10 to 1:600 and were presented in the tables at 1:20. The MT-301 and MT-811 mAbs were kindly provided as purified Ig by Dr. E. P. Rieber (Institut für Immunologie der Technischen Universität, Dresden, Germany) and were used at concentrations of 20, 10, 1, and 0.1 µg/ml. Purified mouse myeloma protein MOPC21 was included at the same concentrations as a negative control. Effector cells were preincubated with the αCD3 and αCD8 mAbs, and the target cells were preincubated with the HLA class I-specific mAb for 30 min at room temperature. Thereafter, the effector or target cells were added, and chromium release was measured 4 h later.

Immunofluorescence Analysis. Effector cell lines were characterized for their lymphocyte composition with mAb specific for defined surface molecules. Directly labeled FITC- or PE-labeled mAb specific for CD3 (clone UCHT1), CD4 (clone 13B8.2), CD8 (clone B159) (all from Immunotech, Dianova, Hamburg, Germany), CD56 (clone NKH-1; Coulter), and CD16 (Leu11a; Becton Dickinson & Co., Mountain View, CA) were used. After 30 min incubation at 4°C and washing, staining was evaluated using a FACScan® analyzer (Becton Dickinson). Surface expression of class I molecules was determined using the class I-specific mAb W6/32 and the mouse myeloma protein UPC10 (Sigma Immunochemicals, St. Louis, MO) as a negative control. Cells were incubated with undiluted hybridoma supernatants or purified myeloma protein (20 µg/ml) for 90 min at 4°C and binding was detected with FITC-conjugated goat anti-mouse Ig (F261; Dakopatts, Copenhagen, Denmark) after 30 min incubation at 4°C.

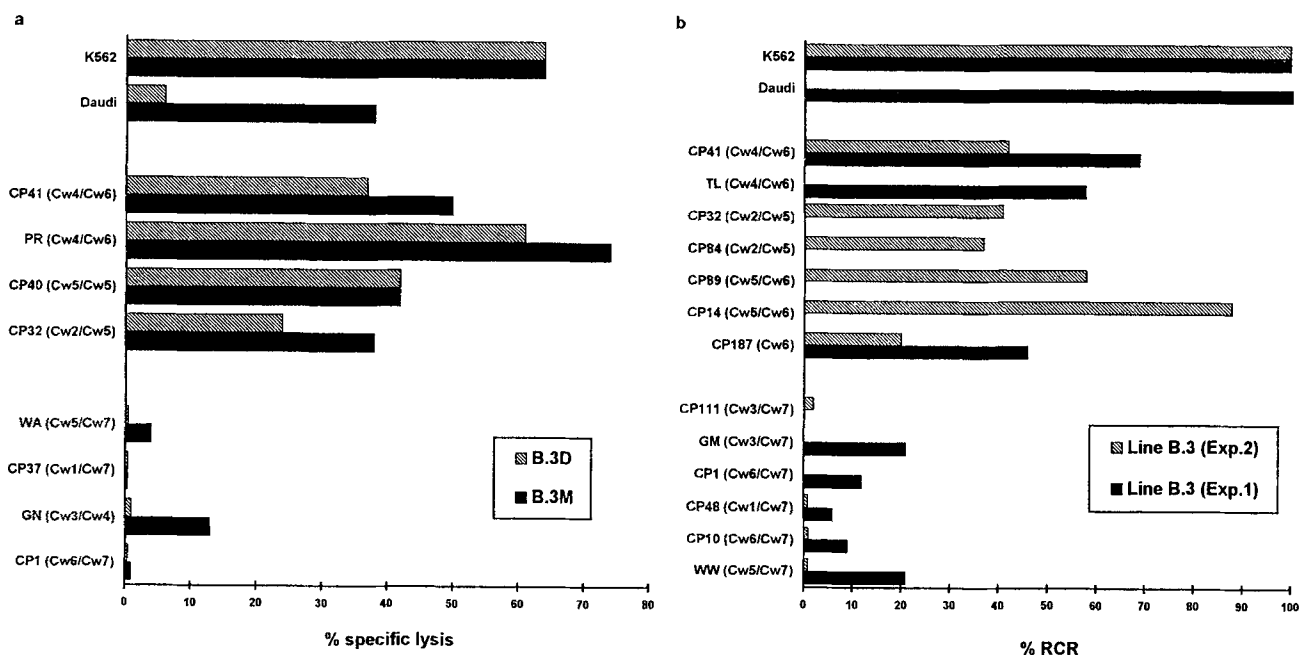


Figure 1. Cytotoxicity of the three effector populations of donor B against a panel of LCL targets. (a) Sublines B.3D and B.3M showed identical patterns of lysis with the exception of Daudi at an E/T ratio of 8:1. All LCL susceptible to lysis were members of the NK2 group (Cw2, Cw4, Cw5, or Cw6), while all resistant targets either belonged to the NK1 group (Cw1, Cw3, or Cw7) or were NK1/NK2 heterozygous. (b) For line B.3, two independent experiments demonstrating that these cells have the same specificity as B.3D and B.3M are presented. The data are given as the percentage of RCR standardized to the lysis of K562 (percent of specific lysis ranging from 59 to 65%) and using an E/T ratio of 20:1 in Exp. 1 and 30:1 in Exp. 2.

Results

HLA-C-associated Specificities of NK and Non-MHC-restricted CD3⁺ T Cell Populations. An allogeneic MLC, designated as B.3, was established using responding PBL of donor B and irradiated PBL of donor 3. From limiting dilution cultures of B.3, two sublines (B.3D and B.3M) were selected that showed strong lysis of class I negative target cells (Fig. 1 *a*), as did uncloned cells of line B.3 (Fig. 1 *b*). Line B.3 and subline B.3M lysed Daudi and K562 cells while Daudi cells were not recognized by subline B.3D in this experiment; however, lysis of >50% was observed using an even lower E/T ratio of B.3D cells in other experiments not shown. In addition, all three effector populations showed significant lysis of some allogeneic LCL, but recognition did not correlate with HLA molecules of donor 3 used for allogeneic stimulation (see Table 1).

FACS[®] analysis using mAbs directed against lymphocyte surface markers revealed that the two sublines were com-

posed of different lymphocyte subtypes. The dominant fraction of the B.3D subline was CD3⁺/CD56⁺ (93%) (Fig. 2 *a-c*) whereas the majority of B.3M cells was CD3⁺ (97%), with ~5% expressing CD56 (Fig. 2 *d-f*). The phenotype of line B.3 revealed a major CD3⁺ fraction (97%) of which 18% were also CD56⁺ (Fig. 2 *g-i*). No expression of CD16 was detected in these populations. The CD3⁺/CD56⁺ phenotype of subline B.3D prompted us to look for a correlation of its specificity with MHC molecules that govern NK resistance, including HLA-A (16), Bw4/Bw6 (7, 22), and subtypes of HLA-C, as defined by variations at positions 77 and 80 in the α 1-helical region (19, 20). No association was seen with HLA-A or -B, but classification of target cells according to their HLA-C alleles, based on the NK1 and NK2 specificities, correlated well with the pattern of lysis (Table 1). Thus, target cells expressing two alleles of the Cw2, Cw4, Cw5, and Cw6 group were strongly lysed, while target cells homozygous or heterozy-

Table 1. HLA Antigens of Cell Donors

Cell	HLA class I serotype						NK specificity*	
	HLA-A	HLA-B	HLA-Bw	HLA-C				
donor B (CP1) [‡]	A1	B8 B57	Bw4 Bw6	Cw6 Cw7			NK1	NK2
donor 3 (CP32) [‡]	A32	B44	Bw6	Cw2 Cw5			NK2	
donor A (GM) [‡]	A2	B8 B40	Bw6 Bw6	Cw7 Cw3			NK1	
donor 1 (CP41) [‡]	A2 A3	B35 B37	Bw6 Bw4	Cw4 Cw6			NK2	
father Le (CP47)	A2	B27 B13	Bw4 Bw4	Cw1 Cw6			NK1	NK2
mother Le (ML, CP48)	A24 A3	B35 B7	Bw6 Bw6	Cw4 Cw7			NK1	NK2
child 1 and 2	A2 A24	B13 B35	Bw4 Bw6	Cw6 Cw4			NK2	
child 3	A2 A24	B13 B7	Bw4 Bw6	Cw6 Cw7			NK1	NK2
child 4	A2 A3	B27 B7	Bw4 Bw6	Cw1 Cw7			NK1	
PR	A1 A3	B35 B70	Bw6 Bw6	Cw4 Cw4			NK2	
CP40	A2 A2	B44 B44	Bw4 Bw4	Cw5 Cw5			NK2	
WA	A1 A2	B7 B39	Bw6 Bw6	Cw7 Cw5			NK1	NK2
CP37	A3 A30	B51 B7	Bw4 Bw6	Cw1 Cw7			NK1	NK2
GN	A3 A31	B35 B55	Bw6 Bw6	Cw3 Cw4			NK1	NK2
TL	A2 A24	B13 B35	Bw4 Bw6	Cw4 Cw6			NK2	
CP84	A2 A24	B27 B44	Bw4 Bw4	Cw2 Cw5			NK2	
CP89	A1 A2	B17 B44	Bw4 Bw4	Cw5			NK2	
CP14	A2 A3	B13 B18	Bw4 Bw6	Cw5 Cw6			NK2	
CP187	A3 A31	B51 B35	Bw6 Bw6	Cw6			NK2	
CP111	A1 A11	B7 B35	Bw6 Bw6	Cw3 Cw7			NK1	
CP10	A2 A3	B13 B7	Bw4 Bw6	Cw6 Cw7			NK1	NK2
CP42	A1 A3	B35 B38	Bw6 Bw4	Cw4 Cw6			NK2	
AW	A2 A2	B15 B18	Bw6 Bw6	Cw3 Cw7			NK1	
C1R	— —	(B35) —	— —	Cw4 —			NK2	
L721.221	— —	— —	— —	— —			—	
T2	A2 —	— —	— —	— —			—	

* Cells are designed as NK1 or NK2, according to the classification of Colonna et al. (20).

[‡] Serological HLA typing was confirmed by HLA-C cDNA sequencing.

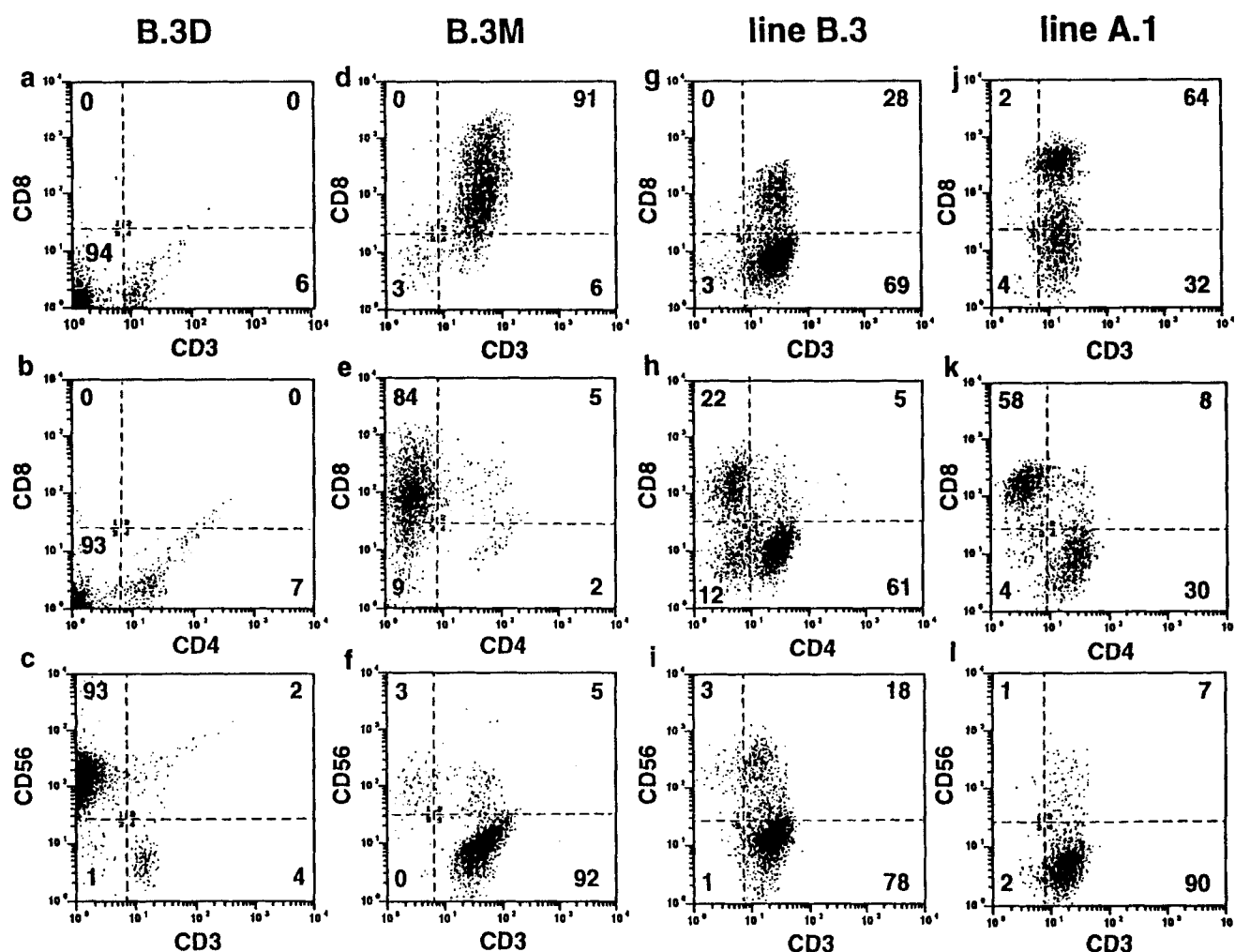


Figure 2. Phenotypic characterization of sublines B.3D and B.3M and lines B.3 and A.1. The double staining with the mAb combinations CD3(FITC)/CD8(PE), CD4(FITC)/CD8(PE), and CD3(FITC)/CD56(PE) allowed a clear distinction of the effector populations. B.3D cells expressing the CD56 surface molecule (95%) were not CD3, CD4, or CD8 positive, and clearly represented NK cells although the subline contained a small fraction (5%) of CD3⁺/CD4⁺ T cells (a–c); in contrast, B.3M expressed little CD56 and showed high levels of CD3 and CD8, thus representing a T cell population (e–f). Lines B.3 and A.1 contained mixtures of cells with a majority of CD3⁺/CD8⁺ or CD3⁺/CD4⁺ T cells and very few CD3[−]/CD56⁺ NK cells (g–i and j–l).

gous for Cw1, Cw3, or Cw7 alleles were poorly lysed (Fig. 1 a). Based on this pattern, the B.3D subline would be classified as having an NK2 specificity according to the criteria of Colonna et al. (19, 20). The B.3D subline was isolated from an NK1/NK2 heterozygous donor; assignment of HLA-Cw6 (NK2) and Cw7 (NK1) by serological typing was confirmed by cDNA sequencing of both HLA-C alleles (data not shown). Isolation of NK2-specific cells from this donor is in contrast to the findings of Colonna et al., who only isolated NK2-specific cells from NK1 homozygous donors (19, 20). To our surprise, the CD3⁺/CD8⁺ T cell subline B.3M showed a pattern of lysis identical to that of the NK subline (Fig. 1 a). In addition, cells of the CD3⁺ line B.3 cultured for various periods of time mediated lysis of allogeneic LCL that correlated with the NK2 group of HLA-C alleles: target cells lacking Cw1, Cw3, or Cw7 expression were more susceptible to lysis than target cells ex-

pressing one of these alleles (Fig. 1 b). This pattern of specificity emerged as the dominant characteristic of line B.3 after longer cultivation in vitro (Fig. 1 b, *Exp. 1* vs *Exp. 2*). Combined, these results suggest that the HLA-C specificities associated with inhibition of the NK cells might also explain the lysis pattern of these non-MHC-restricted T cells.

HLA-C Molecules Govern Target Cell Resistance to Lysis by both NK and Non-MHC-restricted T Cells. Two interpretations of the lysis patterns of these NK and T cells could be made: the effector cells may specifically recognize given HLA-C alleles; alternatively, target cells with particular HLA-C alleles may be resistant to lysis. The NK cells and the T cells recognized at least one target cell not known to express HLA-C molecules (Daudi or K562) and allogeneic cells heterozygous for NK1- and NK2-associated alleles were not lysed; these results argued in favor of HLA-C-

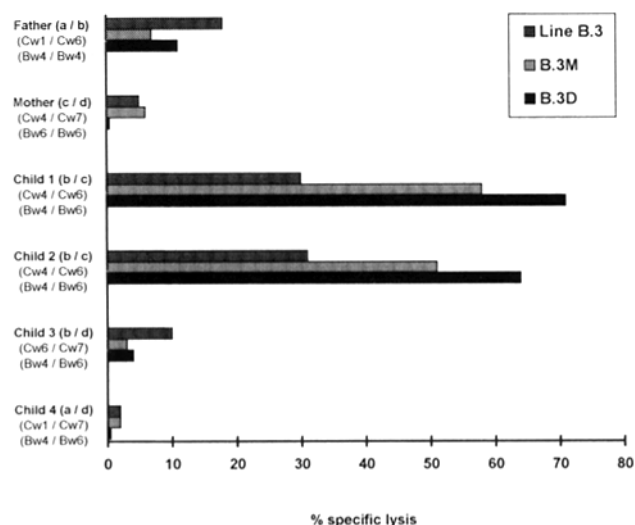


Figure 3. The cytolytic reactivity of the NK and T cell populations against LCL of family Le segregates with the HLA-C and not with HLA-Bw4/6 epitopes; a correlation with HLA-Bw4/Bw6 epitopes could be excluded because only cells expressing the HLA-bc haplotypes were well lysed. They shared HLA-Bw4/Bw6 with resistant cells expressing bd and ad haplotype combinations. Only the bc cells did not express an HLA-C molecule of the NK1 group. These results were obtained using an E/T ratio of 8:1 for B.3D and B.3M, and 10:1 for line B.3.

mediated resistance to lysis. This interpretation was directly supported by a segregation study in family Le (Fig. 3); the parents are NK1/NK2 heterozygous (Cw1/Cw6 and Cw4/Cw7) and four children have inherited different combinations of alleles (Table 1). All three effector cell lines preferentially lysed cells of the two children homozygous for NK2 alleles (Cw4/Cw6), while cells of the parents and the other two children were generally resistant, presumably because of Cw1 or Cw7 expression. HLA-Cw7 molecules seemed to be somewhat more effective in conferring protection from lysis by line B.3. An influence of the Bw4 and Bw6 epitopes in this family was excluded

since the parents are homozygous for Bw4 or Bw6, respectively. Segregation of individual HLA-B alleles expressing either the Bw4 or Bw6 epitope could not explain the lysis pattern. This study revealed that MHC-linked susceptibility to lysis by these non-MHC-restricted T cells was recessive, while resistance was dominant, a characteristic described previously for NK cells (11).

Transfection of Target Cells with HLA-Cw*0702 Provides Protection from Lysis by NK and Non-MHC-restricted T Cells. To prove a direct involvement of HLA-C in the inhibition of lysis, transfectant cell lines expressing different class I genes were tested as targets for the effector cells. A summary of several experiments is presented in Table 2. The MHC deletion mutant cell line L721.221 does not express HLA-A, -B, or -C molecules and thereby provides a defined system for the analysis of single HLA genes. Untransfected L721.221 cells were lysed efficiently by all three effector populations. The L721.221 cells were transfected with three different HLA-B genes: B*2705 and B*3701 both encode the Bw4 epitope while Bw6 is encoded by the B*3501 allele. None of these transfectant lines were resistant, confirming that their Bw4 and Bw6 epitopes did not influence these E/T combinations. In contrast, the transfectant line of L721.221 expressing HLA-Cw7 molecules showed strongly reduced levels of lysis as compared to the parental L721.221 line and the .221-Cw*0602 cells. These results provided proof that expression of Cw7 directly governed resistance to lysis by both the non-MHC-restricted effector T cell populations and the NK subline.

In family Le, resistance to lysis showed dominant segregation with HLA haplotypes carrying NK1-related alleles. To demonstrate that dominant resistance could be conferred by HLA-C alone, the LCL C1R was transfected with the HLA-Cw*0702 gene. Because of extensive deletions in the MHC, C1R only expresses HLA-Cw4 molecules at the cell surface in appreciable amounts. Since the Cw*0401 allele is classified in the NK2 group, transfection of C1R with HLA-Cw*0702 created a NK1/NK2 het-

Table 2. Inhibitory Effect of the L721.221-Cw*0702 Transfectant on the Cytotoxicity of the Three Effector Lines

Effector cells	E/T	Target cells									
		L721.221	.221-B*2705 (Bw4)	.221-B*3501 (Bw6)	.221-B*3701 (Bw4)	.221-Cw*0602 (NK2)	.221-Cw*0702 (NK1)				
		% RCR*	% RCR	% RCR	% RCR	% RCR	% RCR	%	%	%	%
Line B.3	5:1	100%	83%	17%	92%	8%	104%	0%	63%	37%	31%
B.3D	3:1	100%	124%	0%	116%	0%	128%	0%	120%	0%	37%
B.3M	3:1	100%	n.t. [§]	n.t.	143%	0%	104%	0%	n.t.	n.t.	44%

* The percent of specific lysis in different experiments ranged from 43 to 81%.

† The percent of inhibition was calculated as follows: % RCR (L721.221) - % RCR (transfectants); negative values are designed as 0%.

§ Not tested.

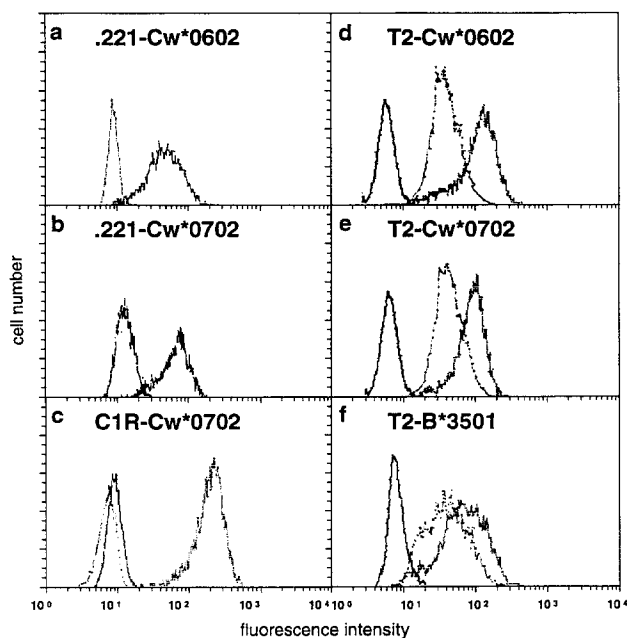


Figure 4. Surface expression of the transfected HLA class I genes in L721.221, C1R, and T2. The pan-HLA class I-specific mAb W6/32 clearly detected differences between the untransfected cell lines and the transfectants expressing Cw6 or Cw7 (a and b). Binding of W6/32 to untransfected L721.221 cells was identical to the binding of the negative control antibody (data not shown). Untransfected C1R cells showed a low level of W6/32 binding compared to the isotype control (c, left-hand curves); after transfection with Cw*0702, a 500-fold increase in W6/32 binding was observed (c, right-hand curve). B*3501, Cw*0602, and Cw*0702 transfectants of T2 showed a clear shift in W6/32 binding (d-f, right-hand curves) compared to W6/32 binding of untransfected T2 cells (d-f, middle curves) and the isotype control (d-f, left-hand curves).

erozygous cell line, although the levels of expression of the two alleles were very different (Fig. 4 c). Nevertheless, the Cw4 expression was sufficient to induce substantial resistance to lysis by an NK1-specific population that was comparable to the resistance of .221-Cw*0602 cells (data not shown). As shown in Fig. 5, untransfected C1R cells were strongly lysed by the NK subline B.3D and the CD3⁺ line B.3. Expression of the HLA-Cw*0702 gene in C1R led to complete resistance. Thus, HLA-Cw7 expression again conferred resistance to lysis by both the NK and T cells, which was dominant in an NK1/NK2 heterozygous cell, as surmised from the family study.

Highly Enriched CD3⁺ T Cell Populations Are Inhibited by HLA-Cw7 in a Manner Analogous to the Purified CD56⁺ NK Lines and the Polyclonal T Cell Lines. To demonstrate that enriched CD3⁺ T cells show the same pattern of cytotoxicity as CD56⁺ NK cells, four sublines were depleted of the contaminating subpopulations by immunomagnetic bead separation (Table 3). Depletion of CD3⁺ cells from two independently generated sublines, B.3T and B.3D, yielded highly enriched CD56⁺ NK lines with 90% and 98% CD3⁻/CD56⁺ cells, respectively (Fig. 6). The NK lines demonstrated efficient lysis of K562, C1R, and .221-

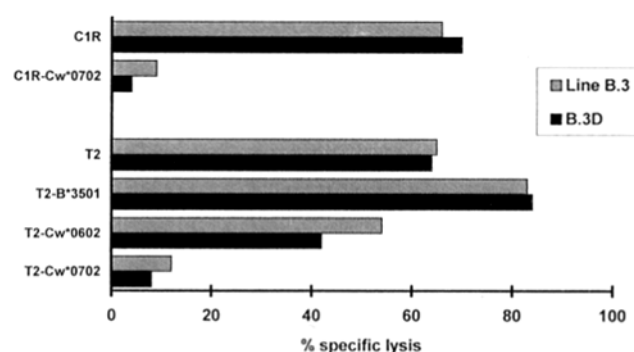


Figure 5. HLA-Cw7 confers dominant resistance and TAP function is not essential for inhibition. The NK line B.3D and the CD3⁺ line B.3 showed strong lytic activity for C1R cells expressing only Cw4 and the HLA-deficient variant cell T2. After transfection with HLA-Cw*0702, both target lines showed a dramatic resistance to lysis. T2 cells transfected with HLA-B*3501 or Cw*0602 remained susceptible to lysis. Data using an E/T ratio of 10:1 for both populations is presented.

Cw*0602 cells, but they were strongly inhibited by the HLA-Cw*0702 transfectants of C1R and L721.221. Linear regression analysis comparing the percent of lysis of positively recognized target cells with varying numbers of effector cells gave significant correlation coefficients, with *r* values that ranged from 0.9 to 1.00. In contrast, the inhibition of lysis by the HLA-Cw7 transfectants mostly gave such low values of lysis that linear regression analysis was not meaningful.

Subline B.3S was used to obtain highly enriched T cell populations through CD56 depletion; after two rounds of separation, CD56⁺ cells could no longer be detected in the B.3S.1 subline (Table 3 and Fig. 6). Nevertheless, both T cell sublines showed patterns of lysis exactly like those of the CD56⁺ NK lines: linear regression analysis gave significant *r* values for the quantitative lysis of K562, C1R, and .221-Cw*0602 cells, and strong inhibition was seen with the Cw*0702 transfectants of C1R and L721.221. The lytic activity of both T cell sublines was less than that of the NK cells; ~10-fold more cells were needed to achieve equivalent levels of cytotoxicity (compare B.3D.1 and B.3S, Table 3). In the absence of pure clones it is theoretically possible that a contaminating fraction of CD56⁺ NK cells like those of the B.3D.1 subline, yet accounting for 1% of the cells in subline B.3S, was responsible for the observed cytotoxicity. If this were the case, however, then the level of C1R lysis mediated by B.3D.1 cells at an E/T ratio of 0.1:1, for example, should equal that of the B.3S line at an E/T ratio of 10:1. Estimation of the expected lysis for B.3D.1 from the regression line would give a value of 0, whereas 27% lysis was seen with B.3S at a 10:1 ratio. In fact, the lysis of C1R by B.3D.1 at 1:1 is equivalent to that seen with B.3S at 10:1, yet only 1%, not 10%, of the cells had a CD3⁻/CD56⁺ phenotype. Therefore, it is highly unlikely that the activity in the T cell sublines was caused by residual CD3⁻/CD56⁺ NK cells.

Separation experiments performed to isolate CD3⁺/

Table 3. Cytotoxic Activity and Specificity of Separated CD3⁺ and CD56⁺ Effector Populations

Effector cells																
Phenotype							Target cells									
CD3 ⁺		CD3 [−]					K562	C1R	C1R-Cw*0702		.221-Cw*0602	.221-Cw*0702				
CD56 ⁺	CD56 [−]	CD56 ⁺	CD56 [−]				% Lysis	% Lysis	% Lysis	% Inhibition	% Lysis	% Lysis	% Inhibition			
2%	4%	90%	4%	B.3T	E/T	10:1	80	69	1	99	35	0	100			
						5:1	75	59	2	97	25	1	96			
						2.5:1	71	33	0	100	22	0	100			
						r	1.00	0.97			0.96					
1%	0%	98%	1%	B.3D.1	E/T	4:1	77	50	5	90	34	13	62			
						2:1	76	43	7	84	32	6	81			
						1:1	62	29	2	93	16	8	50			
						r	0.90	0.98			0.91					
1%	95%	1%	3%	B.3S	E/T	10:1	65	27	1	96	15	4	73			
						5:1	57	16	0	100	11	1	91			
						2.5:1	38	12	1	92	6	2	67			
						r	0.97	0.97			1.00					
0%	98%	0%	2%	B.3S.1	E/T	10:1	48	20	2	90	19	11	42			
						5:1	37	10	2	80	14	4	71			
						2.5:1	25	7	0	100	3	1	67			
						r	1.00	0.96			0.98					
13%	68%	8%	11%	B.3P	E/T	10:1	30	0	2		9	0	100			
						5:1	17	0	0		4	0	100			
						2.5:1	4	0	0		1	1	100			
						r	1.00				0.99					

CD56⁺ cells were unsuccessful since high enrichment for this phenotype was not achieved (Table 3). B.3P represents an example of one of three lines that were obtained after selection for CD56⁺ cells from line B.3 (see Fig. 2 g-i). Some reactivity directed against K562 was observed, but lysis of C1R and .221-Cw*0602 cells was very weak or absent. Since enrichment of CD3⁺/CD56⁺ cells required positive selection through the CD56-specific mAb, it may be that the separation procedure led to an alteration in their lytic capacity. Based on the results seen with the B.3S.1 subline, however, CD56 is not a characteristic marker for non-MHC-restricted T cells showing this pattern of specificity.

HLA Class I-specific Antibodies, but not CD3 or CD8 Antibodies, Can Restore Lysis of Resistant Target Cells. Blocking experiments were made using various mAb to evaluate the involvement of HLA-class I, CD3, and CD8 molecules in recognition and inhibition of lysis mediated by the NK-like T cells. For these experiments, target cells of two members of family Le used for the segregation study (Fig. 3) were selected since they expressed comparable levels of class I molecules, yet they behaved differently as target cells. The cell

TL (Cw4/Cw6) was lysed while the parental ML cells expressed HLA-Cw7 (Cw4/Cw7) and were resistant. For comparison, a CD56⁺ NK line was included. As a control for the proper functioning of the mAb, a CD3⁺/CD8⁺ HLA-B35-specific CTL line was tested against both target cells in the presence of various concentrations of mAb. The data in Table 4 are representative of three experiments and the values of lysis obtained in the presence of the highest concentration of each mAb used in each combination are given. Both target cells expressed HLA-B35 (Table 1) and were well lysed by line GM.1. The class I-specific reagents, W6/32 and 4E, inhibited lysis from 42 to 80%; the 4E mAb was more effective. 60–80% inhibition was observed with the CD3-specific mAb, whereas the CD8 mAb only caused 40% inhibition. In other experiments, half-maximal concentrations of the CD3 and CD8 mAb were used and shown to cause full impairment of lysis of an HLA-A2-specific T cell clone (data not shown). Thus, as expected, all mAb functioned to inhibit E/T interactions involving classical CTL.

Very different patterns of inhibition were seen with the NK-like T cells and the NK subline compared to this clas-

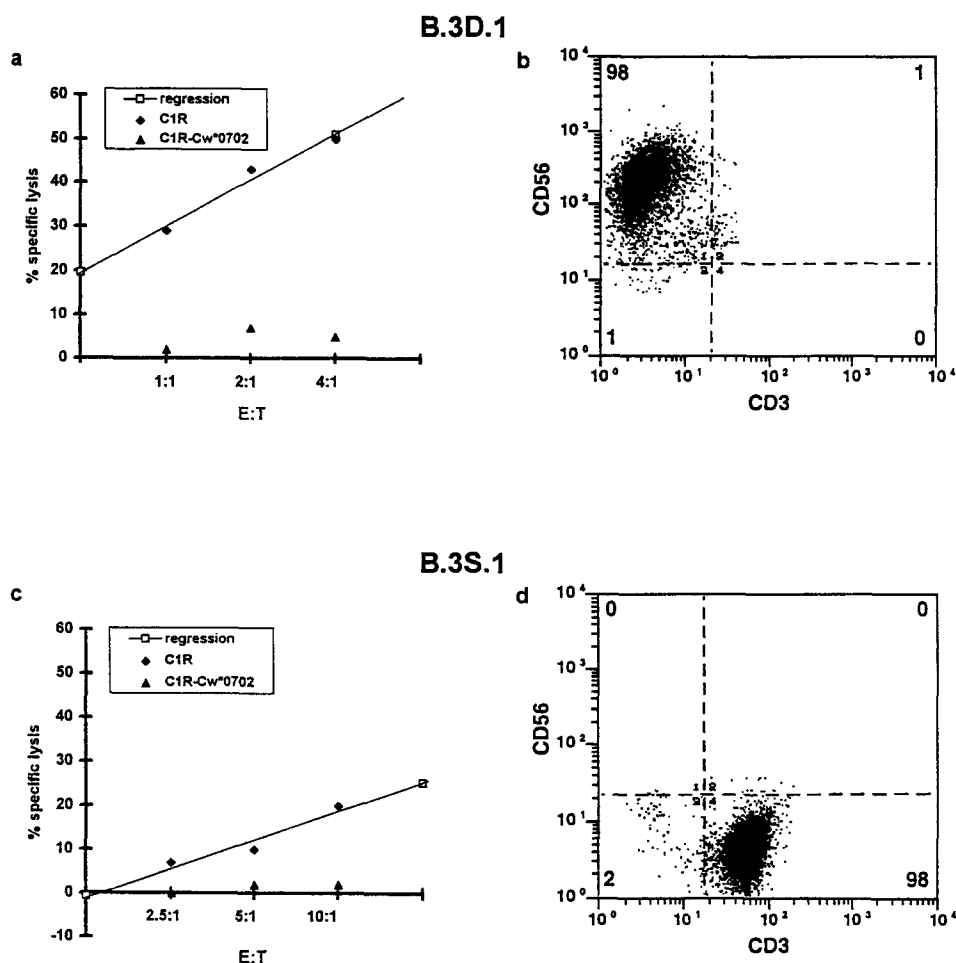


Figure 6. Linear regression analysis and phenotypic characterization of lines B.3D.1 and B.3S.1. Lysis of C1R gave a linear regression with r values of 0.98 for the NK line B.3D.1 (a) and 0.96 for the non-MHC-restricted T cell line B.3S.1 (c) and lysis of both was efficiently inhibited by the Cw*0702 transfectant. Line B.3D.1 represents a highly enriched CD3⁺/CD56⁺ population, (b) while line B.3S.1 is totally depleted of CD56⁺ cells thus only consisting of CD3⁺/CD56⁻ T cells (d).

sical CTL. No inhibition of the positive lysis of TL cells was seen with any mAb; thus, CD3 and CD8 molecules were not involved in positive recognition by the T cells. As expected, the ML cells caused strong inhibition of both effector cells. This inhibition was retained despite blocking of CD3 and CD8 molecules on the NK-like T cells. Once again, these molecules were not found to be involved in inhibition of lysis. In contrast, the inhibition of cytotoxicity by ML cells was lost when the target cells were preincubated with the class I-specific mAb. This could not be accounted for by antibody-dependent, cell-mediated cytotoxicity since neither population expressed CD16 (data not shown). This dramatic reversal of inhibition demonstrated that a direct interaction with Cw7 molecules was required to inactivate both the NK-like T cells and the NK subline.

Impaired Peptide Loading in T2 Does Not Influence the Ability of HLA-Cw*0702 to Confer Resistance to Lysis. The dimorphic variations at positions 77 and 80 that characterize the NK1/NK2 groups of HLA-C alleles lie in positions of the α 1-helical region, which could influence binding of endogenous peptides. Since T2 cells express no TAP1 and TAP2 genes, they present only a limited spectrum of endogenous peptides (33). T2 cells were found to be highly sensitive to lysis by both effector cell types (Fig. 5); thus the Cw*0102 allele present in this cell may be so poorly ex-

pressed that it cannot induce resistance. After transfection with HLA-B*3501, HLA-Cw*0602, or Cw*0702, T2 cells showed shifts in staining with the class I-specific mAb W6/32, revealing substantial surface expression of MHC molecules in all three transfectant lines (Fig. 4 d-f). T2-B*3501 and T2-Cw*0602 cells remained sensitive to NK and T cell cytotoxicity, but lysis of T2-Cw*0702 cells was abrogated. Thus, impaired peptide loading in T2 did not lead to failure of Cw7 molecules to provide protection to either effector cell type.

An NK2 Specificity Is Exhibited by a Second Non-MHC-restricted T Cell Population. A second allogeneic effector population (line A.1) was made using PBL of the NK1 homozygous donor A stimulated with PBL of donor 1. FACS[®] analysis demonstrated that line A.1 was 96% CD3⁺ but, like line B.3, it contained several T cell subtypes (Fig. 2 j-l). Line A.1 lysed K562 and Daudi; moreover, target cells expressing Cw2, Cw4, Cw5, or Cw6 were susceptible to lysis while cells expressing Cw7 were clearly resistant (Fig. 7 a). Segregation analysis in family Le gave the same results as seen with the effector populations of donor B (data not shown). The HLA-Cw*0702 transfectant of L721.221 was resistant to lysis, as were C1R-Cw*0702 cells (Fig. 7 b). Impaired peptide loading did not strongly influence resistance since T2-Cw*0702 cells conferred 65%

Table 4. *Effects of Class I-CD3- and CD8-specific mAb on Lysis and HLA-Cw7-mediated Inhibition*

Effector cells	E/T	mAb	Specificity	Target cells	
				TL Cw4/Cw6 % lysis*	ML (CP48) Cw4/Cw7 % lysis*
Line GM.1	10:1	None		49	53
B35-specific CTL		MOPC21	Control	49	52
		W6/32 [‡]	HLA-A, -B, -C	22	31
		4E	HLA-B, -C	8	17
		MT-811 [‡]	CD3	19	10
		MT-301 [‡]	CD8	28	31
B.3N	10:1	None		61	3
NK subline		MOPC21	Control	70	0
		W6/32 [‡]	HLA-A, -B, -C	85	63
		4E	HLA-B, -C	72	66
		MT-811 [‡]	CD3	59	3
		MT-301 [‡]	CD8	57	0
Line B.3	5:1	None		42	7
NK-like T cell line		MOPC21	Control	40	8
		W6/32 [‡]	HLA-A, -B, -C	57	34
		4E	HLA-A, -C	51	34
		MT-811 [‡]	CD3	30	3
		MT-301 [‡]	CD8	41	3

*Percent of lysis is given for the highest concentration of antibody tested as designated in Materials and Methods.

[‡]These mAbs were shown to inhibit target cell lysis of an HLA-A2-specific clone by 80–89% at 50% of the concentration used here with the NK line B.3N and the NK-like T cell line B.3.

inhibition of lysis while T2-Cw*0602 cells caused only 19% inhibition. Thus, this second allogeneic combination produced another CD3⁺ line showing a similar specificity to the B.3 T cells: lysis was not related to the HLA molecules of the allogeneic stimulating cells (Table 1); rather, it was dominated by HLA-Cw7-mediated resistance.

Discussion

Extensive studies of NK clones revealed complex patterns of specificity with respect to involvement of HLA-A, -B, or -C molecules in delivering inhibitory signals (43), yet numerous NK clones seem not to be susceptible to MHC regulation (5). The various effector populations described in this study were highly divergent with respect to lymphocyte composition, ranging from a highly enriched (>95%) CD3⁺/CD56⁺ subline (B.3D) to the mixed CD4⁺, CD8⁺, and CD56⁺ phenotypes of the polyclonal CD3⁺ lines B.3 and A.1 and the clear CD3⁺/CD8⁺ phenotype of the T cell subline B.3M. Additional depletion of CD56⁺ cells yielded the CD3⁺/CD56[−] subline B.3S.1 (99% CD3⁺/CD56[−]), which showed the same specificity as the CD3-depleted CD56⁺ (99%) subline B.3D.1. It is therefore astonishing that such homogenous patterns of

cytolytic specificity were found in these populations that differed 100-fold in their content of CD3⁺ non-MHC-restricted T cells and CD56⁺ NK cells.

The clear results obtained using single HLA gene transfectants demonstrated that HLA-Cw7 molecules not only conferred resistance to the B.3D NK cells in a manner like that shown for other NK cells with various HLA-C transfectants (17, 19), but they also protected target cells from lysis by non-MHC-restricted CD3⁺ populations. Since one target cell with HLA-Cw1 (father Le) and one with Cw3 (GN) were not lysed, the NK1 group of HLA-C alleles described by Colonna et al. (20) may determine resistance; nevertheless, studies of larger panels of allogeneic LCL and HLA-C transfectant lines will be important to establish the fine specificity of protection. We could block the inhibition of lysis by HLA-Cw7 of an NK line and a non-MHC-restricted T cell line with two HLA class I-specific mAb. Direct binding of the effector cell to the HLA-C molecules expressed by the target cell is therefore necessary to inhibit their lysis. In contrast, lysis of targets lacking a protective HLA-C allele was not influenced by class I-specific mAbs for either effector type. The CD3 and CD8 molecules that are involved in classical CTL lysis appear to be irrelevant for lysis as well as for inhibition since neither

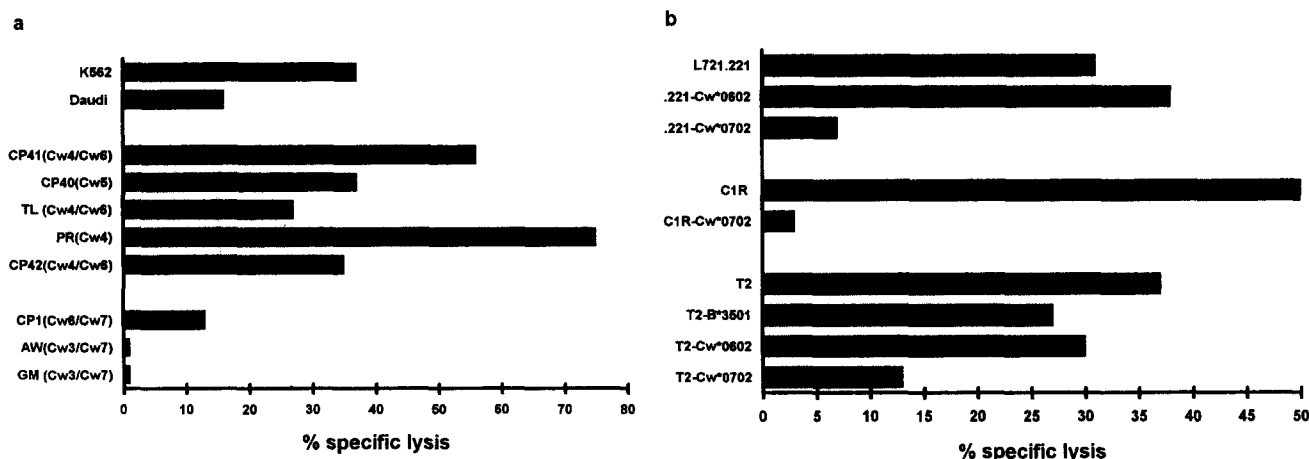


Figure 7. Detection of HLA-Cw7-mediated resistance to line A.1. (a) Analogous to the results of B.3D, B.3M, and line B.3, HLA-Cw7 was defined as an inhibitory molecule. (b) This resistance was confirmed in the three transfectant lines expressing Cw7, .221-Cw*0702, C1R-Cw*0702, and T2-Cw*0702. The E/T ratio was 10:1.

the non-MHC-restricted T cells nor the NK cells were influenced by blocking antibodies. Thus, for the NK-like T cells, the set of molecules interacting with HLA-Cw7 on the target cells is different from those governing classical CTL recognition.

Qualitative changes in the conformation of the class I heavy chain-peptide- β 2m complex are reported to influence NK resistance, and an increased sensitivity has been observed for some NK cells because of peptide loading to MHC molecules (5, 44, 45) or by addition of β 2-microglobulin (46). It is still unclear whether or not specific peptides may be required to induce resistance, but TAP genes could be involved in NK resistance (34). Limited peptide specificity in inhibition mediated by HLA-B27 to some NK cells was recently demonstrated (47), leading to different models for peptide involvement in selective NK recognition (48). Information with respect to HLA-C-mediated resistance is not yet available. In our studies, the comparison of the TAP-deficient cell line T2 and the related L721.221 cell line, after transfection with Cw*0702, revealed no differences in resistance to NK and T cell lysis. It is not known whether the HLA-C molecules occurring on T2 are devoid of peptides or are occupied with a special subset of endogenous peptides, such as signal peptides like those associated with HLA-A2 (33). The binding of the mAb W6/32 to the T2 transfectant cells indicates that some HLA-C molecules are associated with β 2-microglobulin since the epitope recognized by W6/32 is only displayed when class I heavy chains are associated with β 2-microglobulin (49). At least for HLA-A and -B molecules this association also requires peptide to maintain stable surface expression at 37°C (50). If the same rules govern HLA-C expression, then peptides are likely to be associated with these HLA-C molecules on T2; nevertheless, substantial alterations in their composition, as expected from the TAP deficiency, did not influence protection by Cw7. Perhaps many peptides allow folding and display of Cw7 mol-

ecules in a form that confers resistance. On the other hand, it is also possible that Cw7 heavy chains alone provide protection; if so, then such heavy chains must also be present on other transfectant cell lines as well as on normal cells that are resistant to lysis.

Increasing evidence suggests that many viral pathogens and tumor cells use a variety of mechanisms to downregulate MHC expression, thereby escaping detection by MHC-restricted CTL (51). An important function of NK cells and NK-like T cells could be to serve as sensitive biological densitometers detecting aberrations in MHC expression. Since HLA-C molecules are expressed at substantially lower levels than HLA-A and -B (52), slight differences in expression may alter the inhibitory signal in the effector cells that provides protection from lysis for normal cells. Even minor alterations in HLA-C expression in vivo might trigger the attack of cells harboring such pathogens or carrying mutations affecting MHC expression by effector cells, such as those described here.

Others have shown that the HLA-C specificity of NK clones correlates with expression of various molecules of the p58 family (53, 54). Direct binding of a defined HLA class I molecule to a given p58 receptor could protect target cells and reversion to lysis was detected in the presence of anti-p58 antibody (55). Similarly the NKB1 and CD94 (Kp43) molecules, expressed by subsets of NK cells, are receptor candidates involved in NK inactivation by particular HLA-B molecules; such NK cells are not inhibited by HLA-C. Some NK clones that express both CD94 and p58 molecules have been described; they display dual specificity for HLA-B and -C inhibitory signals (25). Recently, other antibodies directed against p58 molecules were found to bind to both NK cells and a limited subset of CD8⁺ T lymphocytes that show non-MHC-restricted lytic activity (26). The common expression of some surface receptors (26, 27) and overlapping cytotoxic specificities support the contention that some non-MHC-restricted T cells may be

related to NK cells in their mode of activation and/or regulation. NK and T cells may also be derived from a common progenitor cell (56, 57). Our observation that HLA-Cw7-mediated resistance to cytolysis is not only associated with a CD3⁻ NK cell subline but also with a CD3⁺ T cell subline and polyclonal, non-MHC-restricted T cell populations provides evidence for a regulatory relationship between these different types of effector cells. It will be of interest to investigate whether the T cells described here express any molecules of the p58 family that are associated with HLA-C regulation of NK cells.

The frequencies and specificities of the NK and NK-like T cells present in an individual could reflect his pathogen history: intracellular pathogens that downregulate or qualitatively alter MHC expression in a locus- or allele-specific manner could lead to preferential selection of one group of

effector cells, while generalized downregulation of MHC molecules might select cells with several different specificities. This could contribute to the wide variations in the numbers of cells binding the anti-p58, NKB1, or CD94 antibodies found in different individuals (22, 24, 25). If similar molecules are involved in T cell interactions, this might help to explain why distinct specificities could be identified in the two polyclonal T cell lines described here. Alternatively, it has been shown that individual NK clones can express simultaneously different receptor molecules associated with HLA-B- or -C-mediated inhibition (25). This may not be the case for T cells, thereby limiting their specificities and enabling these to be defined in uncloned T cell lines, while this is not possible with mixed NK populations (5).

We thank Barbara Maget for excellent technical help, Dr. R. Wank for HLA typing, and Dr. H. Engelmann and Dr. H. Fricke for support in graphic production.

This work was supported by the Thyssen Foundation (1992/2/54) and the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 217, B2).

Address correspondence to Dolores J. Schendel, Institut für Immunologie, Ludwig-Maximilians-Universität München, Goethestr. 31, 80336 München, Germany.

Received for publication 28 October 1994 and in revised form 17 March 1995.

References

- Gouttefangeas, C., I. Mansur, M. Schmid, H. Dastot, C. Gelin, G. Mahouy, L. Boumsell, and A. Bensussan. 1992. The CD39 molecule defines distinct cytotoxic subsets within allo-activated human CD8-positive cells. *Eur. J. Immunol.* 22: 2681-2685.
- O'Shea, J., and J.R. Ortaldo. 1992. The biology of natural killer cells: insights into the molecular basis of function. In *The Natural Killer Cell*. C.E. Lewis and J.O. McGee, editors. Oxford University Press, New York. pp. 2-40.
- Sugita, K., M.J. Robertson, Y. Torimoto, J. Ritz, S.F. Schlossman, and C. Morimoto. 1992. Participation of the CD27 antigen in the regulation of IL-2-activated human natural killer cells. *J. Immunol.* 149:1199-1203.
- Zarcone, D., O. Viale, G. Cerruti, C. Tenca, W. Malorni, G. Arancia, F. Iosi, R. Galandrini, A. Velardi, A. Moretta, and C.E. Grossi. 1992. Antibodies to adhesion molecules inhibit the lytic function of MHC-unrestricted cytotoxic cells by preventing their activation. *Cell. Immunol.* 143:389-404.
- Trinchieri, G. 1994. Recognition of major histocompatibility complex class I antigens by natural killer cells. *J. Exp. Med.* 180:417-421.
- Perussia, B., S.H. Chan, A. D'Andrea, K. Tsuji, D. Santoli, M. Pospisil, D. Young, S.F. Wolf, and G. Trinchieri. 1992. Natural killer (NK) cell stimulatory factor or IL-12 has differential effects on the proliferation of TCR- α β ⁺, TCR- γ δ ⁺ T lymphocytes, and NK cells. *J. Immunol.* 149: 3495-3502.
- 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.* 180:1235-1242.
- Reinhardt, C., C. Falk, A. Steinle, and D.J. Schendel. 1993. MHC class I allorecognition: the likes and dislikes of CTL and NK cells. *Behring Inst. Mitt.* 94:61-71.
- Azuma, M., M. Cayabyab, D. Buck, J.H. Phillips, and L.L. Lanier. 1992. Involvement of CD28 in MHC-unrestricted cytotoxicity mediated by a human natural killer leukemia cell line. *J. Immunol.* 149:1115-1123.
- Kärre, K., H.G. Ljunggren, G. Piontek, and R. Kiessling. 1986. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature (Lond.)* 319:675-678.
- Ciccone, E., D. Pende, O. Viale, C. Di Donato, G. Tripodi, A.M. Orengo, J. Guardiola, A. Moretta, and L. Moretta. 1992. Evidence of a natural killer (NK) cell repertoire for (allo) antigen recognition: definition of five distinct NK-determined allospecificities in humans. *J. Exp. Med.* 175:709-718.
- Moretta, L., E. Ciccone, A. Moretta, P. Höglund, C. Öhlen, and K. Kärre. 1992. Allorecognition by NK cells: nonself or no self? *Immunol. Today* 13:300-306.
- Piontek, G.E., K. Taniguchi, H.G. Ljunggren, A. Gronberg, R. Kiessling, G. Klein, and K. Kärre. 1985. YAC-1 MHC class I variants reveal an association between decreased NK

- sensitivity and increased H-2 expression after interferon treatment or in vivo passage. *J. Immunol.* 135:4281–4288.
14. Rager-Zisman, B., M. Aboud, J. Gopas, I. Har Vardi, G.J. Hammerling, and S. Segal. 1991. Resistance to NK and metastatic potential of fibrosarcoma cells is associated with products encoded by the H-2D region. *Sem. Cancer Biol.* 2:329–336.
 15. Storkus, W.J., J. Alexander, J.A. Payne, J.R. Dawson, and P. Cresswell. 1989. Reversal of natural killing susceptibility in target cells expressing transfected class I HLA genes. *Proc. Natl. Acad. Sci. USA.* 86:2361–2364.
 16. Storkus, W.J., R.D. Salter, J. Alexander, F.E. Ward, R.E. Ruiz, P. Cresswell, and J.R. Dawson. 1991. Class I-induced resistance to natural killing: identification of nonpermissive residues in HLA-A2. *Proc. Natl. Acad. Sci. USA.* 88:5989–5992.
 17. Ciccone, E., D. Pende, O. Viale, A. Than, C. Di Donato, A.M. Orenco, R. Biassoni, S. Verdiani, A. Amoroso, A. Moretta, and L. Moretta. 1992. Involvement of HLA class I alleles in natural killer (NK) cell-specific functions: expression of HLA-Cw3 confers selective protection from lysis by alloreactive NK clones displaying a defined specificity (specificity 2). *J. Exp. Med.* 176:963–971.
 18. 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 cell clones is associated with HLA-C or a closely linked gene. *Proc. Natl. Acad. Sci. USA.* 89:7983–7985.
 19. 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. Sci. USA.* 90:12000–12004.
 20. 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.
 21. Aramburu, J., M.A. Balboa, A. Rodriguez, I. Melero, M. Alonso, J.L. Alonso, and M. Lopez-Botet. 1993. Stimulation of IL-2-activated natural killer cells through the Kp43 surface antigen up-regulates TNF- α production involving the LFA-1 integrin. *J. Immunol.* 151:3420–3429.
 22. Litwin, V., J. Gumperz, P. Parham, J.H. Phillips, and L.L. Lanier. 1994. NKB1: A natural killer cell receptor involved in the recognition of polymorphic HLA-B molecules. *J. Exp. Med.* 180:537–543.
 23. Karlhofer, F.M., R.K. Ribaud, and W.M. Yokoyama. 1992. MHC class I alloantigen specificity of Ly-49⁺ IL-2-activated natural killer cells. *Nature (Lond.).* 358:66–70.
 24. Moretta, L., E. Ciccone, M.C. Mingari, R. Biassoni, and A. Moretta. 1994. Human natural killer cells: origin, clonality, specificity, and receptors. *Adv. Immunol.* 55:341–380.
 25. Moretta, A., M. Vitale, S. Sivori, C. Bottino, L. Morelli, R. Augugliaro, D. Barbaresi, D. Pende, E. Ciccone, M. Lopez-Botet, and L. Moretta. 1994. Human natural killer cell receptors for HLA-class I molecules. Evidence that the Kp 43 (CD94) molecule functions as receptor for HLA-B alleles. *J. Exp. Med.* 180:545–555.
 26. Ferrini, S., A. Cambiaggi, R. Meazza, S. Sforzini, S. Marciano, M.C. Mingari, and L. Moretta. 1994. T cell clones expressing the natural killer cell-related p58 receptor molecule display heterogeneity in phenotypic properties and p58 function. *Eur. J. Immunol.* 24:2294–2298.
 27. Melero, I., A. Salmeron, M.A. Balboa, J. Aramburu, and M. Lopez-Botet. 1994. Tyrosine kinase-dependent activation of human NK cell functions upon stimulation through a 58-kDa surface antigen selectively expressed on discrete subsets of NK cells and T lymphocytes. *J. Immunol.* 152:1662–1673.
 28. Zemmour, J., A.-M. Little, D.J. Schendel, and P. Parham. 1992. The HLA-A,B “negative” mutant cell line C1R expresses a novel HLA-B35 allele, which also has a point mutation in the translation initiation codon. *J. Immunol.* 148:1941–1948.
 29. 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.
 30. Salter, R.D., and P. Cresswell. 1986. Impaired assembly and transport of HLA-A and -B antigens in a mutant TxB cell hybrid. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:943–949.
 31. Steinle, A., and D.J. Schendel. 1994. HLA class I alleles of LCL 721 and 174xCEM.T2 (T2). *Tissue Antigens.* 44:268–270.
 32. Hosken, N.A., and M.J. Bevan. 1990. Defective presentation of endogenous antigen by a cell line expressing class I molecules. *Science (Wash. DC).* 248:367–370.
 33. 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.
 34. Salcedo, M., F. Momburg, G.J. Hammerling, and H.G. Ljunggren. 1994. Resistance to natural killer cell lysis conferred by TAP1/2 genes in human antigen-processing mutant cells. *J. Immunol.* 152:1702–1708.
 35. Weiss, E.H., W. Kuon, C. Dörner, M. Lang, and G. Riethmüller. 1985. Organization, sequence and expression of the HLA-B27 gene: a molecular approach to analyze HLA and disease associations. *Immunobiology.* 170:367–380.
 36. Duceman, B.W., D. Ness, R. Rende, M.J. Chorney, R. Srivastava, D.S. Greenspan, J. Pan, S.M. Weissman, and F.C. Grumet. 1986. HLA-JY328: mapping studies and expression of a polymorphic HLA class I gene. *Immunogenetics.* 23:90–99.
 37. Steinle, A., C. Reinhardt, E. Nöbner, B. Uchanska-Ziegler, A. Ziegler, and D.J. Schendel. 1993. Microheterogeneity in HLA-B35 alleles influences peptide-dependent allorecognition by cytotoxic T cells but not binding of a peptide-restricted monoclonal antibody. *Hum. Immunol.* 38:261–269.
 38. Nelson, P.J., E. Nöbner, and D.J. Schendel. 1991. Genomic cloning and expression of HLA-B37 in the mouse mastocytoma cell line P815-HTR. *Tissue Antigens.* 38:228–230.
 39. Steinle, A., E. Nöbner, and D.J. Schendel. 1992. Isolation and characterization of a genomic HLA-Cw6 clone. *Tissue Antigens.* 39:134–137.
 40. Schendel, D.J., C. Reinhardt, P.J. Nelson, B. Maget, L. Pullen, G.W. Bornkamm, and A. Steinle. 1992. Cytotoxic T lymphocytes show HLA-C-restricted recognition of EBV-bearing cells and allorecognition of HLA class I molecules presenting self-peptides. *J. Immunol.* 149:2406–2414.
 41. Schendel, D.J., and R. Wank. 1981. Production of human T cell growth factor. *Hum. Immunol.* 2:325–332.
 42. Schendel, D.J., R. Wank, and B. Dupont. 1979. Standardization of the human in vitro cell-mediated lympholysis technique. *Tissue Antigens.* 13:112–120.
 43. Litwin, V., J. Gumperz, P. Parham, J.H. Phillips, and L.L. Lanier. 1993. 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. *J. Exp. Med.* 178:1321–1336.

44. Chadwick, B.S., S.R. Sambhara, Y. Sasakura, and R.G. Miller. 1992. Effect of class I MHC binding peptides on recognition by natural killer cells. *J. Immunol.* 149:3150–3156.
45. Storkus, W.J., R.D. Salter, P. Cresswell, and J.R. Dawson. 1992. Peptide-induced modulation of target cell sensitivity to natural killing. *J. Immunol.* 149:1185–1190.
46. Carbone, E., G. Stuber, S. Andree, L. Franksson, E. Klein, A. Beretta, A.G. Siccardi, and K. Karre. 1993. Reduced expression of major histocompatibility complex class I free heavy chains and enhanced sensitivity to natural killer cells after incubation of human lymphoid lines with beta 2-microglobulin. *Eur. J. Immunol.* 23:1752–1756.
47. Malnati, M.S., M. Peruzzi, K.C. Parker, W.E. Biddison, E. Ciccone, A. Moretta, and E. Long. 1995. Peptide specificity in the recognition of MHC class I by natural killer cell clones. *Science (Wash. DC)*. 267:1016–1018.
48. Kärre, K. Express yourself or die: Peptides, MHC molecules, and NK cells. *Science (Wash. DC)*. 267:978–979.
49. Parham, P., C.J. Barnstable, and W.F. Bodmer. 1975. Properties of an anti-HLA-A, -B, -C monoclonal antibody. Use of a monoclonal antibody (W6/32) in structural studies of HLA-A, -B, -C antigens. *J. Immunol.* 123:342–349.
50. Elliott, T., V. Cerundolo, J. Elvin, and A. Townsend. 1991. Peptide-induced conformational change of the class I heavy chain. *Nature (Lond.)*. 351:402–406.
51. Maudsley, D.J., and J.D. Pound. 1991. Modulation of MHC antigen expression by viruses and oncogenes. *Immunol. Today* 12:429–431.
52. Snary, D., C.J. Barnstable, W.F. Bodmer, and M.J. Crumpton. 1977. Molecular structure of human histocompatibility antigens: the HLA-C series. *Eur. J. Immunol.* 7:580–585.
53. Moretta, A., C. Bottino, D. Pende, G. Tripodi, G. Tambussi, O. Viale, A. Orengo, M. Barbaresi, A. Merli, E. Ciccone, and L. Moretta. 1990. Identification of four subsets of human CD3-CD16⁺ natural killer (NK) cells by the expression of clonally distributed functional surface molecules: correlation between subset assignment of NK clones and ability to mediate specific alloantigen recognition. *J. Exp. Med.* 172:1589–1598.
54. Moretta, A., G. Tambussi, C. Bottino, G. Tripodi, A. Merli, E. Ciccone, G. Pantaleo, and L. Moretta. 1990. A novel surface antigen expressed by a subset of human CD3-CD16⁺ natural killer cells. Role in cell activation and regulation of cytolytic function. *J. Exp. Med.* 171:695–714.
55. 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.
56. Rodewald, H.R., P. Moingeon, J.L. Lucich, C. Dosiou, P. Lopez, and E.L. Reinherz. 1992. A population of early fetal thymocytes expressing FcγRII/III contains precursors of T lymphocytes and natural killer cells. *Cell*. 69:139–150.
57. Sanchez, M.J., M.O. Muench, M.G. Roncarolo, L.L. Lanier, and J.H. Phillips. 1994. Identification of a common T/natural killer cell progenitor in human fetal thymus. *J. Exp. Med.* 180:569–576.