NK3-specific Natural Killer Cells Are Selectively Inhibited by Bw4-positive HLA Alleles with Isoleucine 80

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Summary

Natural killer (NK) cell clones have been previously described which are inhibited by HLA-C alleles with Asn77-Lys80 (NK1-specific cells) or by HLA-C alleles with Ser77-Asn80 (NK2-specific cells). In the present work, the generation of NK cells with HLA-B-related specificities was attempted by stimulation of a Bw4 homozygous responder by a Bw6 homozygous donor. Two NK clones were found, which were inhibited by HLA-Bw4 (but not by HLA-Bw6) allotypes and by some HLA-A allotypes that share the Bw4 public epitope. Inhibition of NK cell–mediated lysis strongly correlated with the presence of an Ile residue at position 80 of the protective allele. These NK cell clones define a new specificity termed NK3.

NK cells display cytolytic activity against tumor and virally infected cells that is not restricted by the MHC (1, 2). However, target susceptibility of NK cell-mediated lysis is influenced by the expression of MHC class I proteins on target cells (3). NK-resistant cells become susceptible to NK cytotoxicity as a result of deletion of class I genes by mutagenesis; transfection of class I genes into these mutated cell lines restores the original resistance to lysis (4). This protective effect of class I antigens on NK cell–mediated lysis is influenced by their polymorphism. While HLA-A2 does not protect from NK lysis in one system, a single amino acid change at position 74 confers a protective effect on HLA-A2 (5). Moreover, five different subsets of NK cells with apparent allospecificities have been defined based on differential patterns of lysis on PHA-activated T cell blasts (6). For two of these subsets a dimorphism of HLA-C at positions 77-80 has been shown to control susceptibility/resistance of targets to lysis (7–10).

Recently, a variable degree of protection from lysis by NK cell clones has been described for several HLA-A and -B alleles, although the polymorphic amino acid residues responsible for these differences have not been identified (11). In the present work, the role of residues 77-83 of HLA-B was investigated with respect to target susceptibility/resistance to lysis by NK cells. These positions are characterized by a dimorphism as found in HLA-C, which is associated with the Bw4/Bw6 epitopes identified by human alloantisera (12–15). Bw4 and Bw6 are supertypic (public) specificities that are shared by several HLA-B and a few HLA-A and -C alleles (rather than characterizing individual alleles) and are the earliest HLA public specificities described. No comparable HLA alloantisera that recognize the HLA-C dimorphism have been identified. A number of NK clones were derived from a Bw4-positive donor and tested for their cytotoxicity on HLA transfectants of MHC class I–deficient cell lines and on PHA-activated T cell blasts. Cytotoxicity patterns of two clones revealed an inhibitory role of the Bw4 epitope on NK lysis that particularly correlated with the presence of an Ile residue at position 80. These clones define a new HLA-B–related NK specificity, termed NK3.

Materials and Methods

NK Cell Clones. $CD3^-$, $CD4^-$, $CD8^-$ NK cells were prepared from an HLA-typed blood donor (HLA-A2, A3, B5, B58, Bw4, Cw7, DR5, DR8, DR52, DQ7) as previously described (9). They were cloned by limiting dilution at a concentration of one to five cells/well in 96-well U-bottom plates in the presence of PHA (0.1% vol/vol; Difco Laboratories, Detroit, MI), rIL-2 (1,000 U/ml, Hoffman-La Roche, Nutley, NJ), 10% leukocyte-conditioned medium (LCM)[1] (16) and feeder cells. These consisted of 10^4 irradiated (5,000 rad) cells/well of the B-lymphoblastoid cell line (B-LCL) RPMI 8866, which promotes NK cell proliferation (17), and 3×10^4 irradiated (3,000 rad) PBMC/well from an HLA-typed donor (HLA-A2, A26, B35, B61, Bw6, Cw2, Cw4, DR4, DR5). After 10 d, proliferating cultures were transferred to 96-well

[1] *Abbreviations used in this paper:* B-LCL, B-lymphoblastoid cell line; LCM, leukocyte-conditioned medium.

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flat-bottomed plates and further expanded in complete medium. NK cell clones were restimulated with feeder cells every 3–4 wk. 1 wk after the last stimulation, the clones were screened in functional assays. Cytofluorometric analysis and cytotoxic assays were as previously described (9). The target cell panel included the proerythroblastic cell line K562, HLA class I–deficient B-LCLs C1R and 721.221 (4, 18), HLA-B transfectants of C1R (4, 11) (generously provided by Dr. Lewis L. Lanier, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA), HLA-C transfectants of 721.221 (10, 19, 20), and PHA-activated T cell blasts from HLA-typed random donors. These were prepared by culturing PBMC in the presence of PHA (0.1% vol/vol) and recombinant IL-2 (100 U/ml) for 4 d. HLA class I typing was performed using a standard microlymphocytotoxicity assay.

PCR-Oligonucleotide Typing. A 188-bp fragment of the second exon of HLA-B was selectively amplified from genomic DNAs by the PCR. The primers used for amplification were GGACG(A/G)-CAC(G/C)C(A/T)GTTCGTGA (5', nucleotides 83-102 and CGG-CCTCGCTCTGGTTGTAG (3', nucleotides 251–270) (21). Degenerate positions are shown in parenthesis. PCR was performed for 33 cycles, each of 30-s steps at 96°, 55°, and 72°C. Amplified fragments were denatured in 0.4 M NaOH/25 mM EDTA and applied to a nylon membrane (Zeta-probe; Bio Rad Laboratories, Cambridge, MA) with a dot blot apparatus (Bio Dot; Bio Rad Laboratories). Membranes were hybridized with ^{32}P-labeled oligonucleotides specific for Ile 80 (CCTGCGGATCGCGCTCCG-CTAC, nucleotides 230-251), Thr 80 (CCTGCGGACCCTGCT-CCGCTAC, nucleotides 230-251 of HLA-B27, -B37, -B47 and AACCTGCGCACCGCGCTCC, nucleotides 228-246 of HLA-B13 and -B44), and Asn80 (CCTGCGGAACCTGCGCGGC, nucleotides 230–248) (21). Hybridization was carried out as described (7). Samples that were negative for Ile 80 in HLA-B were subsequently typed for the presence of Ile 80 in HLA-A. A 81-bp fragment of the second exon of HLA-A was amplified from genomic DNAs by PCR using the 5' primer CA(C/G)GGAA(T/A)(A/G)TGA-AGGCCCA(G/C)T (nucleotides 190-210), which selectively amplified HLA-A. The 3' primer was as above.

Stastical Methods. The correlation between resistance to lysis by NK3-specific cells and the presence of a Bw4-positive HLA-B or -A allele or the presence of Ile 80 on the target cell was tested for its significance by the Wilcoxon Rank Sum test (22). The association between Ile, Thr, Asn at position 80 and resistance, intermediate lysis and susceptibility to lysis, respectively, was tested by the Kruskal-Wallis test (22).

Results

NK Cell Clones That Are Inhibited by HLA-B Alleles of the Bw4 Group. Alloreactive NK cells can be generated by stimulation across an HLA-C dimorphism involving residues 77 and 80, i.e., by stimulating Asn77-Lys80 homozygous NK cells with Ser77-Asn80 homozygous cells (NK1-specific cells) and vice versa (NK2-specific cells) (9). HLA-B is characterized by a similar polymorphism at residues 77-83 that encodes the Bw4/Bw6 epitopes recognized by human alloantisera and monoclonal antibodies. However, this polymorphism is more complex at the DNA and protein sequence level than that found in HLA-C. For example, Ile, Thr, and Asn are all found at position 80, and at least five different Bw4 sequences occur in this region (15).

Nevertheless, an attempt to establish alloreactive NK cells by stimulation across the Bw4/Bw6 epitopes was made, as had been done for HLA-C. CD3-, CD4-, CD8- lymphocytes were purified from a HLA-Bw4 homozygous donor (HLA-B5, B58) and cloned by limiting dilution in the presence of a feeder cell mixture, which included irradiated PBMC

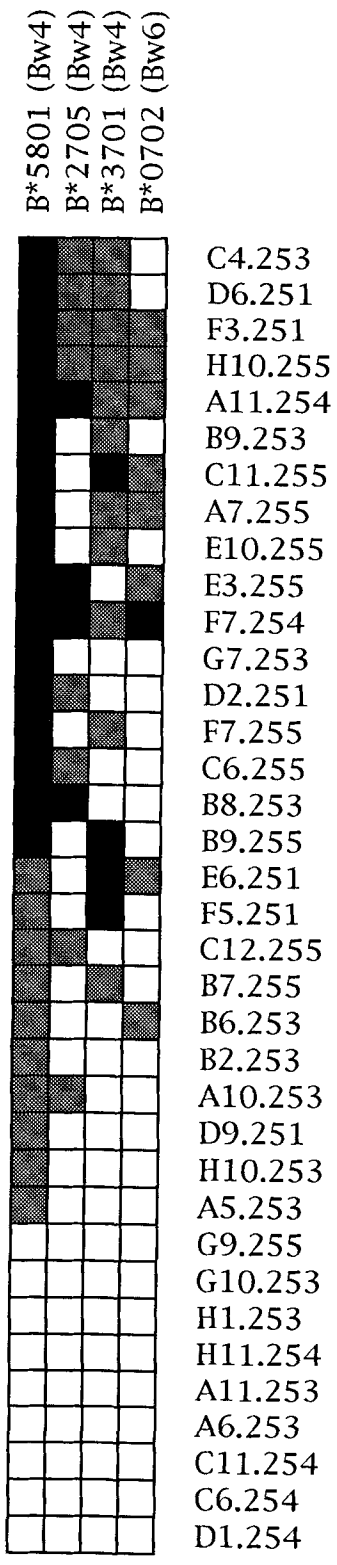
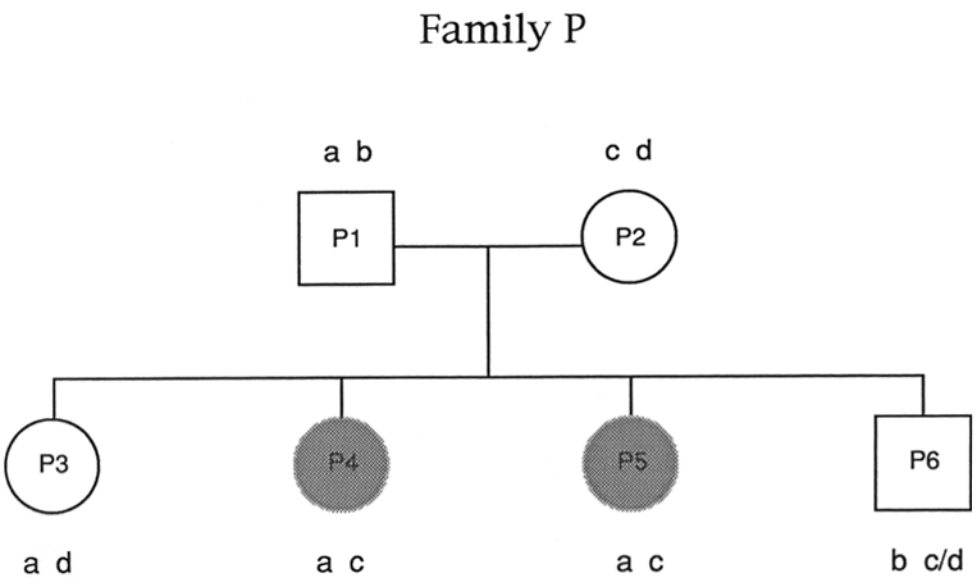


Figure 1. Sensitivity of 36 NK cell clones to inhibition by HLA-B alleles transfected into C1R cells. The cytotoxic activity of NK cell clones against the B lymphoblastoid cell line C1R and the HLA-B transfectants of C1R was determined by a 4-h ^{51}Cr release assay. Effector/target ratio was 10:1. *Solid squares* indicate a strong inhibition of NK lysis of C1R by a transfected HLA-B allele (the lysis of the transfectant was 50% or less than the lysis of C1R); *shaded squares* indicate weak inhibition (lysis between 50–80% of C1R lysis); *open squares* represent no inhibition (lysis <80%).



HLA haplotypes

a	A2; Cw4; B35; Bw6
b	A2; Cw -; B51; Bw4
c	A26; Cw2; B61; Bw6
d	A11; Cw -; B51; Bw4
c/d	A26; Cw -; B51; Bw4

Figure 2. Segregation of the NK3 specificity in the MHC recombinant family P. Cytotoxic activity of C4.253 and D6.251 cell clones against PHA blasts derived from family members was determined by a 4-h ^{51}Cr release assay. Effector/target ratio was 10:1. Both clones gave identical patterns. *Squares*, males; *circles*, females; *solid symbols*, individuals susceptible to lysis; *open symbols*, individuals resistant to lysis. Small letters indicate HLA haplotypes. These were determined by microlymphocytotoxicity assay and are shown below.

from a Bw6 homozygous donor (HLA-B35, B61) and the lymphoblastoid cell line RPMI 8866, which selectively stimulates NK cell proliferation (17). 36 CD3-CD56+ cell clones were obtained and screened for their cytotoxicity in a 4 h ^{51}Cr-release assay against a target panel including the NK-sensitive K562 cell line, the class I–deficient mutant C1R (which expresses the residual HLA-Cw4 gene) and 4 HLA-B transfectants of C1R (HLA-B*5801, -B*2705, -B*3701, and -B*0702). All the NK clones lysed K562 and C1R cells (data not shown). Transfection of HLA-B*5801 (Bw4) strongly inhibited C1R lysis by 17 clones and weakly inhibited C1R lysis by 10 additional clones (Fig. 1). Among these 27 clones, only 4 were strongly inhibited by HLA-B*2705 (Bw4), 4 by -B*3701 (Bw4), and 1 by HLA-B*0702 (Bw6). The cytotoxicity of the nine remaining clones was not significantly influenced by any of the transfected HLA-B alleles (Fig. 1). Thus, the tested NK cell clones showed a strong sensitivity to inhibition by HLA-B*5801, low sensitivity to HLA-B*2705 and HLA-B*3701, and almost no sensitivity to HLA-B*0702. These results are in agreement with the previously observed strong inhibitory role of HLA-B*5801, whereas a strong inhibitory role of HLA-B*3701 was not observed in this study (11). The high frequency of clones inhibited by HLA-B*5801 as compared to HLA-B*2705 or HLA-B*3701, all of which are Bw4 positive, may be related to differences in the precise sequences at residues 77–83 in these different alleles (15).

A New Specificity (NK3) Is Defined by Cytotoxicity of NK Cell Clones on PHA Blasts Which Is Recessively Inherited and Controlled by HLA-B. All the NK cell clones derived from the Bw4-positive donor were subsequently analyzed for their cytotoxicity against a target panel of PHA-activated T cell blasts obtained from members of HLA-typed families. Of the 36 cell clones tested, all of which were cytolytic on C1R cells, only 2 clones, C4.253 and D6.251, were cytotoxic against these PHA blasts. In family P, targets from both parents (P1 and P2) were resistant to lysis by both of these clones, whereas targets from the children included both susceptible (P4 and P5) and resistant individuals (P3 and P6), indicating that susceptibility to NK cell–mediated lysis is recessive, whereas resistance to lysis is dominant, as shown for all NK allospecificities previously examined (6) (Fig. 2). In addition, recessive susceptibility and dominant resistance were associated with

Table 1. *The Specificity of the NK Cell Clone D6.251 Does Not Correlate with the HLA-C Dimorphism That Defines NK1 and NK2 Specificities*

	HLA-A	HLA-B	Bw*	HLA-C	HLA-C 77-80	Percent lysis
BL	2, 30	35, 50	6	3, –	S77-N80	54
AG	2, 2	15, 18	6	3, 7	S77-N80	34
ME	1, 2	8, 62	6	3, 7	S77-N80	29
SG	2, 28	44, 61	4, 6	3, 7	S77-N80	0
MM	24, –	7, 62	4, 6	3, 7	S77-N80	3
BP	25, 25	51, 51	4	1, 1	S77-N80	0
PE	2, 26	35, 61	6	2, 4	N77-K80	46
VL	2, 30	13, 41	4, 6	4, 6	N77-K80	23
RG	2, 23	44, 18	4, 6	4, 5	N77-K80	0
AE	2, 33	44, 17	4	3, 4	S/N77-N/K80	0
AF	2, 33	5, 58	4	1, 6	S/N77-N/K80	0
FL	1, 3	8, 35	6	4, 7	S/N77-N/K80	24
C1R	–	–	–	4	N77-K80	43
C1R tr	–	*5801	4	4	N77-K80	4
C1R tr	–	*2705	4	4	N77-K80	20
C1R tr	–	*3701	4	4	N77-K80	25
C1R tr	–	*0702	6	4	N77-K80	35
721.221	–	–	–	–	–	63
721.221 tr	–	–	–	*0101	S77-N80	52
721.221 tr	–	–	–	*0501	N77-K80	61
721.221 tr	–	–	–	*0601	N77-K80	61

Cytotoxicity of the D6.251 cell clone was determined by a 4-h ^{51}Cr-release assay. The target panel included PHA blasts from 12 random donors, C1R, HLA-B transfectants (*tr*) of C1R, 721.221, and HLA-C transfectants (*tr*) of 721.221. Effector to target ratio was 3:1. Typing of Ser (*S*), Asn (*N*), and Lys (*K*) at positions 77 and 80 was described (7). A similar cytotoxicity pattern was observed with clone C4.253.
* See note to Table 2.

specific MHC haplotypes (a/c and b/d, respectively), suggesting that the locus controlling susceptibility/resistance to lysis maps within the MHC. This study did not allow us to position the locus controlling susceptibility/resistance to lysis with respect to the recombination breakpoint between HLA-A and -C of haplotype c/d identified in individual P6 by HLA typing, because the paternal haplotype b is responsible for the resistance of this individual, independently of the maternal haplotype c/d.

Cytotoxicity patterns of the D6.251 and C4.253 clones were also tested on PHA blasts from HLA-typed random donors. These patterns did not correspond to those previously characterized for NK1-specific cells (only HLA-C Ser77-Asn80 homozygous cells lysed) or for NK2-specific cells (only HLA-C Asn77-Lys80 homozygous cells lysed) (Table 1) (7, 9). Therefore, the C4.253 and D6.251 clones define a new specificity that is termed here as NK3. The role of HLA-B and HLA-C in this specificity was directly investigated by testing the cytotoxicity of NK3-specific cell clones against C1R (which expresses only one class I MHC protein, HLA-Cw4), HLA-B transfectants of C1R, the class I–negative cell line 721.221, and the HLA-Cw*0101 (NK1 target), -Cw*0501, and -Cw*0601 (NK2 targets) transfectants of 721.221 (Table 1). Lysis by both clones were strongly inhibited by B*5801 and partially by B*2705 and by B*3701, whereas cytolytic activity was only slightly affected by B*0702. In addition, there was no inhibition by HLA-C molecules as 721.221 and the HLA-C transfectants of 721.221 were lysed to the same extent. These results suggest that the NK3 specificity on PHA blasts is controlled by HLA-B but not by HLA-C.

Susceptibility/Resistance of T Cell Blasts to Cytotoxicity by NK-3–specific NK Cell Clones Correlates with the Polymorphisms at Residue 80 of HLA-A and -B. Cytotoxicity of NK cell clones C4.253 and D6.251 was tested against PHA-activated T cell blasts obtained from a large panel of HLA-typed random donors to identify the epitope controlling the NK3 specificity (Table 2). A good lysis (>40%) was observed in 10 Bw6 homozygous targets (PHA blasts 1 to 10); none of the Bw6

Table 2. *Inhibition of Cytolytic Activity of the NK3-specific Cell Clone C4.253 by PHA Blasts Carrying the Bw4 Motif*

PHA blasts	HLA-A	HLA-B	Bw*	HLA-C	Residue 80		Percent lysis
					HLA-B	HLA-A	
1. GE	1, 3	8, 41	6	7, -	NN	–	54
2. FG	11, 30	8, 55	6	3, 7	NN	–	52
3. AA	2, 31	35, 50	6	2, 6	NN	–	59
4. BR	2, 33	55, 65	6	3, -	NN	–	63
5. AG	2, 2	15, 18	6	3, 7	NN	–	43
6. BL	2, 30	35, 50	6	3, -	NN	–	60
7. ME	1, 2	8, 62	6	3, 7	NN	–	58
8. FL	1, 3	8, 35	6	4, 7	NN	–	49
9. CT	2, 3	35, 61	6	2, 4	NN	–	51
10. RGE	2, 3	35, –	6	6, 4	NN	–	54
11. SA	1, 30	13, 37	4	6, -	TT	–	28
12. RO	2, 28	18, 47	4, 6	6, 7	TN	–	22
13. OG	2, 28	44, 62	4, 6	3, 7	TN	–	25
14. DG	2, 2	18, 27	4, 6	1, 7	TN	–	29
15. VC	3, 30	13, 62	4, 6	3, 6	TN	–	23
16. FM	1, 29	27, 45	4, 6	2, 6	TN	–	17
17. AE	2, 33	44, 17	4	3, 4	IT	–	1
18. SP	2, 11	51, 57	4	1, 6	II	–	0
19. DE	2, 32	5, 18	4, 6	ND	IN	I	1
20. BS	25, 31	51, 51	4	1, -	II	I	0
21. RM	2, 24	55, 49	4, 6	3, 7	IN	I	3
22. BG	2, 24	5, 40	4, 6	2, 5	IN	I	0
23. CA	2, 24	35, 27	4, 6	1, -	TN	I	13
24. MM	24, –	7, 62	4, 6	3, 7	NN	I	0
25. RGI	24, –	35, 62	4, 6	3, 4	NN	I	8
26. GA	25, 32	35, 61	4, 6	2, 4	NN	I	1
27. GG	3, 24	35, –	4, 6	4, -	NN	I	8
28. NM	29, 32	44, 35	4, 6	4, -	TN	I	0
29. RG	2, 23	44, 18	4, 6	4, 5	TN	I	0
30. PR	28, 26	12, 27	4	2, 7	TT	–	9
31. DGA	29, 31	35, 44	4, 6	4, -	TN	–	2
32. BG	2, 29	44, 50	4, 6	6, -	TN	–	4
33. RU	28, 29	47, 44	4	6, -	TT	–	1
34. LL	11, 29	35, 44	4, 6	4, 5	TN	–	1

Cytotoxic activity of the C4.253 cell clone against PHA blasts derived from 34 random donors was determined by a 4-h ^{51}Cr-release assay. Effector/target ratio was 10:1. A similar cytotoxicity pattern was observed with clone D6.251. HLA class I typing was performed by standard microlymphocytotoxicity assay. (–) The sample was either homozygous or carried a blank allele. Ile (*I*), Thr (*T*), and Asn (*N*) at position 80 in HLA-B or Ile (*I*) 80 at HLA-A were determined by oligotyping (see Materials and Methods). The difference in level of lysis between Bw6 homozygous cells (PHA blasts 1 to 10) and Bw4 homozygous or heterozygous cells (PHA blasts 11-34) was highly significant ($p < 0.0001$ by the Wilcoxon Rank Sum test); similarly, the difference between I 80 positive (PHA blasts 17 to 29) and I 80 negative (PHA blasts 1-16 and 30-34) was highly significant ($p < 0.0001$). Comparison of level of lysis in three groups, I 80 positive (PHA blasts 17-29), T 80 positive (PHA blasts 11-16 and 30-34), and N80 homozygous (PHA blasts 1-10), revealed a p value <0.0005 by the Kruskal-Wallis test.

* The Bw4 motifs present at residues 77-83 were: NLRIALR in A9 (23, 24), B5 (51, 52), B17 (57, 58), B49; SLRIALR in A25, A32; NLRTALR in B13, B12 (44); and DLRTLLR in B27 (B*2705 subtype), B37, B47. Other B27 subtypes can present NLRIALR, NLRTALR, and a fifth motif SLRTLLR. An additional Bw4 motif NLRTAAR may be found in a variant of HLA-B44 (B*4401) (23, 24). The Bw6 motif was SLRNLRG in B7, B8, B18, B41, B12 (45), B50, B55, B40 (60, 61), B15 (62), B65. HLA-A and -B alleles shown in parentheses represent a "split" serological specificity; for instance, B12 has been split into B44 (Bw4) and B45 (Bw6).

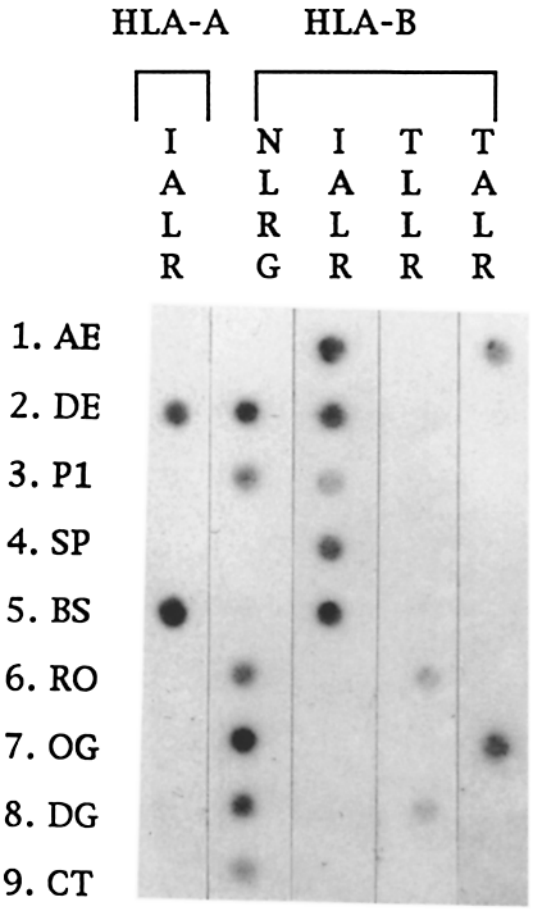


Figure 3. Oligotyping of HLA-B and HLA-A polymorphism at position 80. Representative samples are shown.

homozygous targets was resistant. A low degree of lysis (PHA blasts 11 to 16) or no lysis at all (PHA blasts 17 to 34) was observed in 24 targets; these carried a Bw4 sequence motif either on a HLA-B allele or on a HLA-A allele or on both (Table 2). Amino acid sequence comparison of alleles conferring high resistance revealed that 13/18 cells (PHA-blasts 17 to 29) shared Ile at position 80. The five remaining cells that were highly resistant did not express HLA-A or HLA-B alleles with Ile 80 (PHA blasts 30 to 34), but all typed serologically as HLA-B44 positive. The HLA-B44 specificity is heterogeneous at residues 80-83, and some subtypes present a unique sequence (TAAR; Table 2) (23, 24). Most alleles of the Bw4 group with Thr at position 80 (including HLA-B13, -B27, -B37, -B47) gave an intermediate degree of lysis (PHA blasts 11 to 16). This correlation between susceptibility/resistance to lysis by NK3-specific cell clones and the amino acid residues at position 80 was confirmed by genomic DNA typing with sequence-specific oligonucleotides (Fig. 3). Samples resistant to NK lysis were heterozygous or homozygous for Ile 80 (or carried HLA-B44). All highly susceptible samples were homozygous for Asn 80, while samples with intermediate degree of lysis were Thr 80/Asn 80 heterozygous or Thr 80 homozygous. All observed correlations were highly significant statistically (Table 2, see legend).

Discussion

An inhibitory role of the Bw4 motif on NK cell–mediated lysis was initially suggested by the observation that 17 out of the 36 NK cell clones obtained from a Bw4 homozygous individual in the presence of Bw6 homozygous feeder cells were selectively inhibited by the Bw4-positive allele B*5801. More conclusive evidence was provided by detailed analysis of two of these NK clones, which define the new specificity NK3. Analysis of the cytotoxicity of these NK3-specific cells against class I transfectants and PHA blasts demonstrated that HLA-B and a few HLA-A alleles with the Bw4 motif at positions 77-83 conferred a dominant protection to NK3-specific lysis. However, the inhibitory role of these HLA-A alleles requires confirmation by transfection. Heterogeneity in the Bw4 group of alleles was also observed, which correlated with the amino acid residue at position 80. Ile 80 was associated with NK inhibition, Thr 80 with an intermediate degree of lysis, and Asn 80 with complete susceptibility to lysis. This observation is consistent with the previously observed inhibitory role of positions 77-80 of HLA-C, may focus attention on residue 80 and indicates that this (these) residue(s) in the α1 domain of HLA-A, -B, and -C may have in a predominant role in protecting target cells from NK lysis. Residue 80 of the HLA heavy chain is located on the α1 helix just beyond the COOH end of the peptide binding cleft and is pointing upward, accessible to recognition (25).

Out of 17 clones which lysed C1R but not C1R/HLA-B*5801 transfectant, only two gave positive results in cytotoxicity on the PHA blasts. This observation may explain the discrepancy between previous work identifying MHC-specific NK cells (6) and a more recent study describing the lack of specific MHC recognition patterns in 120 NK clones tested against HLA transfectants (11). NK cells with defined MHC recognition pattern can be found, although these are present in a very low percentage of the total NK population (5% in the present study) and can only be identified by analyzing NK cytotoxicity on PHA blasts. In some cases, the percentage of these cells in the NK population may be increased by allogeneic stimulation, as previously shown for NK1- and NK2-specific cells, although it is not known why proliferation occurred in this situation (9). There are several possible explanations for the different sensitivity of NK cells to inhibition by HLA alleles transfected in B cell lines or expressed on PHA blasts. First, HLA molecules may display a different expression level or conformation on the two cell types. Second, molecules other than MHC proteins may influence NK cytotoxicity in a tissue specific fashion. Transfection of the same class I allele in several NK susceptible cell lines of different lineages has been shown to induce resistance only in some (11).

By far the most interesting hypothesis is that multiple receptors with distinct MHC specificities may be displayed

by NK cells. The NK1-, NK2-, and NK3-specific cell clones may preferentially express only one receptor that has high affinity for specific class I HLA epitopes. Thus, these clones display a cytotoxic pattern on PHA blasts which appears to be regulated by only one HLA molecule or epitope independently of other class I alleles or epitopes on the cell target. However, other NK cells, perhaps the majority, may express several receptors for MHC specificities. Thus, these cells are more likely to kill transfectants expressing only one allele, than PHA blasts, which may express up to six different HLA alleles. Recently, a multigene family has been described, which is primarily expressed in human NK cells and encodes several type II integral membrane proteins with an extracellular lectin domain (26). These proteins are distantly related to the rat NKR-P1 molecule (27) and to the Ly-49 protein, which functions as receptor for class I molecules on murine NK cells (28). Thus, these or related molecules (29) may be candidates for the putative multiple inhibitory receptors for class I molecules on human NK cells.

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