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Alloreactivity and Association of Human Natural Killer Cells with the Major Histocompatibility Complex

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All NK cells potentially lytic for autologous cells but not expressing self-major histocompatibility complex (MHC)-reactive receptors could be eliminated by a negative selection mechanism during ontogeny. This idea is based on the existence of a NK cell subset expressing a specific inhibitory receptor for allogeneic MHC alleles. As ancestral haplotypes of the MHC appear to define identical MHC haplotypes in unrelated individuals, unrelated individuals having the same ancestral haplotype should also have the same NK-defined allospecificities that have been shown to map to the human MHC. To test this prediction, multiple cell lines from unrelated individuals having the same ancestral haplotypes were tested for the NK-defined allospecificities. It was found that cells having the same ancestral haplotypes do have the same NK-defined specificities. Furthermore, the NK-defined phenotype of cells that possess two different ancestral haplotypes can be predicted from the NK-defined phenotypes of unrelated cells that are homozygous for the ancestral haplotypes concerned. Although the group 1 and 2 NK-defined allospecificities can be explained to some extent by HLA-C alleles, evidence is presented that additional genes may modify the phenotype conferred by HLA-C.

With the expanding use of bone marrow transplantation, an increasing number of patients lack HLA genotypically identical sibling donors. Unrelated donors identified from large panels are being used. Current strategies for donor-recipient matching involve detailed matching for alleles at HLA class I and II loci, but this approach is evidently inadequate. Graft rejection can occur despite apparently good matching, and the outcome can be successful despite mismatches at these loci (3). Current methods may not allow adequate matching of the class I and II alleles, as has been demonstrated in a case report of T-cell rejection involving mismatching at HLA-B (24). In addition, however, other polymorphic non-HLA genes within the major histocompatibility complex (MHC) may be involved, and matching for HLA alone does not ensure matching for these genes. There is direct evidence in the mouse for the presence of at least one set of such genes (45).

The hemopoietic histocompatibility system in mice has been shown to determine F1 hybrid resistance to a bone marrow graft from either parent with graft rejection mediated by radioresistant NK cells (4). Unlike classical MHC antigens, hemopoietic histocompatibility antigens are inherited in a recessive fashion (4). It has been suggested that the major hematopoietic histocompatibility locus (Hh-1) maps within the H-2 complex between H2-S and H2-D and can be dissociated from class I genes (5). However, class I MHC antigens may play a role in the function or expression of Hh-1 antigens (41). One model suggests that two genetic loci, Hh-1r and Hh-1s, control the expression of Hh-1 antigens. The Hh-1s genes encode the structural antigen, whereas the Hh-1r gene downregulates expression of the Hh-1s genes. Complex Hh-1 haplotypes have been suggested previously (50). Compatibility between the donor and recipient at Hh-1 is required to prevent NK-mediated graft rejection.

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There is evidence that the equivalent of the Hh-1 system exists in humans. A series of studies has demonstrated that NK cells can mediate specific allogeneic target cell lysis (11); NK clones derived from single donors can recognize different allospecificities (12), and five different allospecificities have been defined (14). Susceptibility to lysis by NK clones recognizing specificities 1, 2, and 3 and probably specificities 4 and 5 has been shown to be inherited in an autosomal recessive manner, whereas resistance to lysis is a dominant genetic trait (15). Segregation studies and mapping with families with recombinant haplotypes have shown that the genes controlling susceptibility or resistance to lysis are localized within the MHC between complement factor Bf and the HLA-A locus (13). The nature of the target molecules is uncertain. However, in another series of studies, Ciccone et al. have provided evidence that HLA-Cw3 can provide specific protection of target cells against lysis mediated by group 2-reactive NK clones (14) and evidence that group 1 and 2 specificities are reciprocally associated with homozygosity for a diallelic polymorphism at amino acid positions 77 and 80 on HLA-C (15). Killer inhibitory receptors (KIRs) are transmembrane glycoproteins, expressed on NK cells and a small subset of T cells, that inhibit cell-mediated cytotoxicity upon binding to polymorphic MHC class I determinant on target cells. A member of the KIR cDNA family was recently discovered (24). The aqueous humor inhibits NK cell-mediated cytotoxicity in vitro but does not affect cytotoxic T lymphocyte-mediated lysis (1). The existence of human inhibitory NK cell receptors for polymorphic MHC class I molecules was predicted based on the observation that NK cells killed HLA class I-deficient B lymphoblastoid cell lines but did not lyse these target cells when transfected with certain HLA class I genes (43, 44). Membrane glycoproteins on NK cells involved in the recognition of HLA-A (24, 40), HLA-B (30), and HLA-C (36) were subsequently identified by using monoclonal antibodies that disrupted interactions between the inhibitory receptors on the NK cells and their class I ligands on targets. Cloning of the cDNAs encoding these receptors (18, 19, 48) revealed the existence of a family of

genes, designated KIR (31), on human chromosome 19 q13.4 (2). Unlike the Ly49 or CD94/NKG2A receptors, KIRs are type I glycoproteins related to the immunoglobulin superfamily (18, 20, 48). Like Ly49, KIRs recognize a region in the α1 domain of the HLA class I heavy chain (6, 33). The three-immunoglobulin domain KIR designated NKB1 recognizes the HLA-Bw4 motif, which is conferred by amino acids 77 to 83 in the α1 domain of certain HLA-B heavy chains (27). Other three-immunoglobulin domain KIRs, recognized by the 5.133 and Q66 monoclonal antibodies (MAbs), recognize HLA-A3, although the structural properties of this specificity have not been well characterized (24, 40). The two-immunoglobulin domain KIRs recognize a polymorphism at positions 77 and 80 of the HLA-C heavy chain (17). KIRs reactive with the EB6 (36) or HP-3E4 (28) MAbs recognize HLA-Cw4 and related alleles, whereas KIRs detected with the GL183 MAb (36) bind HLA-Cw3 and related alleles.

Given the existence of the human equivalent of Hh-1, NK allorecognition is likely to be involved in human bone marrow graft rejection. Therefore, a simple means of matching for these determinants and of retrospectively analyzing cases for such matching is required. It is increasingly evident that these ancestral haplotypes (AH) have been maintained as a whole from remote ancestors and that each haplotype defines a continuous specific sequence of DNA (52). It follows, therefore, that AH provide markers for alleles at unknown genes as well as at known genes in the MHC. We therefore predicted that each AH would be associated with particular sets of NK-defined determinants (NK haplotypes). Consequently, the identification of AH should provide an effective means of matching for the NK-defined specificities before bone marrow transplantation. It has been shown that HLA-E, a nonclassical molecule, is involved in regulating NK cell-mediated cytotoxicity both positively and negatively (9). Therefore, in this study we determined the NK-defined specificities present on target cells carrying various AH and related the findings to the unknown alleles present on these haplotypes.

MATERIALS AND METHODS

Target cells for NK allorecognition. A panel of 34 Epstein-Barr virus-transformed lymphoblastoid cell lines (LCLs) served as targets. These cells were selected from an extensive local panel of LCLs based on their homozygosity or heterozygosity for the AH listed in Table 1. Several of these cells were included in the 10th International Histocompatibility Workshop cell panel held in Princeton, N.J., and New York, N.Y., 1987. Each cell was characterized by using all the MHC markers listed in Table 2 to confirm the presence of the particular AH. HLA-A, -B, and -C and DR, DQ serological typing was performed by a complement-mediated microlymphocytotoxicity assay with a panel of antisera extensively characterized against standard cells included in previous International Histocompatibility Workshops. DNA-based HLA class II typing was performed according to the methods detailed in the 11th International Histocompatibility Workshops held in Yokohama, Japan, in 1991 by using a series of sequence-specific oligonucleotide probes labeled with derivatized horseradish peroxidase suitable for detection by enhanced chemiluminescence (49). Complement components C4 and Bf allotyping was performed by immunofixation with appropriate antisera after electrophoresis described previously (51). Methods for the typing of the alleles at tumor necrosis factor (TNF) (21), BAT3 (22), and XYV (49) have been described previously.

Isolation of NK clones and evaluation of NK cytotoxicity. NK alloreactivity against the LCL target cells was evaluated by using previously described methods (14). In brief, peripheral blood lymphocytes from normal donors were isolated on Ficoll-Hypaque gradients, and NK cells were enriched after the depletion of T cells by using a mixture of MAbs against CD3, CD4, and CD8 (11, 12). The viable cells were then separated on a Ficoll-Hypaque gradient. These viable NK-enriched cells were then cloned under limiting dilution conditions in the presence of irradiated feeder cells, 0.1% phytohemagglutinin, and recombinant interleukin-2. The NK-defined specificities present on the LCL target cells were determined in a 4-h ^{51}Cr release assay by using cloned NK effector cells reacting specifically with group 1 (ES2 or ES10), group 2 (AM25, Mauro P), group 3 (A51-8), and group 5 (OA64) specifities. Target cells were used at 5×10^3/well, for a final effector/target cell ratio of 10:1. The percent specific lysis was determined as described previously (11, 12).

TABLE 1. Haplotype specificity

AH	No. of haplotypes tested	No. of haplotypes positive for indicated group	
		1	2
7.1	9[a]	9	0
8.1	9	9	0
18.1	2	2	0
44.2	4	4	0
46.1	6	6	0
52.1	4	4	0
54.1	3	3	0
60.1	2	2	0
60.3	4	4	0
62.1	6	6	0
13.1	3	0	3
18.2	4	0	4
44.1	4	0	4
57.1	4	0	4
35.1	2	ND[c]	2[b]
35.4	2	ND	2[b]

[a] Cells homozygous for a single AH were considered two examples of the same AH.
[b] Based on group 1 reactivity only.
[c] ND, not determined.

The use of this assay cytotoxicity is usually clearly bimodal. Indeed, it was previously demonstrated that GL183 and EB6 MAbs recognize two triggering molecules with common biochemical and functional properties on the surface of human NK cells. Target cells considered negative for a specificity give <10% lysis, whereas targets considered positive give >20% lysis with the specific NK clone (14).

HLA-C alleles and sequencing. All target cells were HLA typed for the presence of Cw antigenes 1 to 7. The Cw allele associated with each AH has been previously established based on typing of many examples of each AH. The presence of the amino acids at residues 77 and 80 on the α chain of the Cw molecule present on each AH was established by review of published nucleotide sequences (23), and that for Cw4 was established by review of a sequence submitted to GenBank (sequence no. M84386). This sequence was derived from an HLA-B35-positive cell and has therefore been provisionally assigned to the 35.1 and 35.4 AH.

The HLA-C allele on the 44.2 AH was sequenced by the following method. HLA-C was specifically amplified by PCR with primers and conditions described previously (16). The resulting PCR product was diluted 1:25 in distilled water and reamplified with nested degenerate primer CACAGAAGTACAA(C/G)CG CCAGG (5′, nucleotides 189 to 209, exon 2) and the same 3′ primers used in the original PCR. The nested PCR was performed by a standard PCR, with 25 μl of diluted product in 50 μl of final reaction mix, with 1-min steps at 94, 60, and 72°C for 30 cycles with 2-s increments every cycle. The resulting PCR product was purified by column centrifugation (Filtron 30 microconcentrator; Polylabo, Strasbourg, France) and sequenced by using fluorescence-labeled dideoxy termination reaction mixtures on an automated DNA sequencer (model ALF express; Pharmacia Biotech S.A., St- Quentin en Yvelines, France) with the primers used to produce the nested PCR product.

RESULTS

NK group 1 and 2 specificities are associated with specific AH. The cytotoxicity of the NK clones defining group 1 and 2 specificities against the 34 LCL target cells are shown in Table 3. Several points are evident. The specific lysis is bimodal, with most target cells being clearly positive (>20% lysis) or negative (<10%). All cells homozygous for an AH express either group 1 or 2 specificity, and these specificities behave as alleles at a single locus. The results for cells heterozygous for two AH are predictable from the results obtained with the cells that are homozygous for these AH and a recessive model of susceptibility to lysis. For example, both the 8.1 and 7.1 AH carry the group 1 specificity, and cells R7/17219 and Q9/20920, which are heterozygous for these two AH, are group 1 positive. On the other hand, cell Q6/8187 is heterozygous for AH 8.1 and

TABLE 2. MHC alleles and restriction fragment length polymorphism markers

AH	MHC allele or RFLP marker[a]																				
	A	Cw	B	Ya	Yba	X	V	TNF	B144	BAT3	C2	Bf	C4A	C4B	DR	DRB1	DRB3	DRB4	DRB5	DQA1	Dqw	DQB1
7.1	3	7	7	A	S	L	A	L	S	L	C	S	3	1	15	1501			0101	0102	6	0602
8.1	1	7	8	B	S	L	B	S	S	S	C	S	Q0	1	3	0301	0101			0501	2	0201
13.1	30	6	13	D	L	L	C	L		S	C	S	3	1	7	07		0101		0201	2	0201
18.1	25		18	A	S	S	B	L	S	L	Q0	S	4	2	15	1501			0101	0102	6	0602b
18.2	30	5	18	C	S	M	A	L	S	S	C	F1	3	Q0	3	0301	0202			0501	2	0201
18.3			18					L				S	3	1	11	(1102)	(0202)			(0501)	7	(0301)
35.1		4	35	C	S	L	A	S	S	L		S	3	1	11	1104	0202			0501	7	0301
35.4		4	35	G		L	B	S		L		S	3	1	11	1103	0202			0501	7	0301
44.1	2	5	44	A	S	L	C	S	S	L	C	S	3	Q0	4	0401		0101		(0301)	7	(0301)
44.2	29		44	D	L	L	C	L		L	C	F	3	1	7	07		0101		0201	2	0201
46.1	2	1	46	A	S	L	C	S		L	C	S	4	2	9	0601		(0101)		(0301)	9	(0303)
52.1	24		52	A		L	A	L			C	S	3+2	Q0	15	1502		(0102)		(0103)	6	(0601)
54.1		1	54	E	L	M	C	L	S	L	C	S	3	5	4	0405		(0101)		(0301)	4	(0401)
57.1	1	6	57	E	L	S	C	L	S	L	C	S	6	1	7	07		0101		0201	9	0303
58.1	33		58		S	S		S		S		S	3	Q0	3	0303		02		0501	2	(0201)
60.1		3	60						S	L	C	S	3	1	4	(0404)		(0101)		(0301)	3	(0302)
60.3	2	3	60	A		L	B	S		S	C	S	Q0	2	13	1302		(0301)		(0102)	6	(0604)
62.1	2	3	62	A		L	B	L	S	L	C	S	3	3	4	0401		0101		0301	8	0302

[a] TNF, B144, and BAT3 were determined by restriction fragment length polymorphism (RFLP) typing. DRB1, DRB3, DRB4, DRB5, DQA1, and DQB1 were determined by DNA typing. Probes were as follows: for Ya, Y/*Taq*I + *Rsa*I; for Yb, Y/*Bst* EII; for X, X/*Taq*I; for V, V/*Taq*I. S, short fragment; M, medium fragment; L, long fragment. Parentheses indicate provisional assignments.

57.1, which possess group 1 and 2 specificity, respectively, and expresses neither group 1 nor group 2 specificity. Cell R6/12336, which is homozygous for the 44.2 AH, gave indeterminate cytotoxicity of 14% with the group 1 cone. This cell has been considered group 1 positive. This classification is supported by the results of two other cells (R8/5618 and Q5/7952) that are heterozygous for 44.2 and another AH which is clearly group 1 positive based on the results for homozygous cells. Both these cells are group 1 positive (37 and 27% cytotoxicity, respectively), indicating that the 44.2 AH must also be group 1 positive under a recessive model of susceptibility to lysis. However, the cytotoxicities of both these cells and that of the homozygous cell are lower than those of most of the other group 1-positive cells. These data suggest that the Cw allele present on the 44.2 AH is different from the other group 1 alleles.

The results for the 68 haplotypes present on the 34 cells, summarized in Table 3, indicated that all AH tested carry either group 1 or group 2 specificity. All examples of the same AH from unrelated individuals possess the same NK-defined specificity, i.e., these specificities are AH haplotypic. Therefore, AH identifies specificity, and the NK-defined phenotype of a cell can be predicted by AH.

NK-defined haplotypes occur and are associated with specific AH. Having shown that the group 1 and 2 determinants are AH haplotypic, we examined whether more-complex NK-defined haplotypes are also associated with specific AH. A subset of 12 of the 34 target cells was therefore tested against NK clones defining group 3 and 5 specificities. This subset included cells homozygous for 7.1, 8.1, 18.2, 60.1, 57.1, and 44.1 AH and three heterozygous cells. All 12 cells were positive for group 3 specificity, so the nature of the group 3 inheritance could not be determined. The 7.1, 8.1, and 44.1 homozygous cells were all positive, and the 18.2 and 57.1 homozygous cells were negative for group 5 specificity. However, the results for the three heterozygous cells cannot be explained by the same genetic model as group 1 and 2 specificities.

From the results obtained above, several points are evident as follows. (i) Target cells homozygous for an AH can encode several specificities. (ii) Sets of NK-defined specificities (i.e, NK-defined haplotypes) occur. (iii) Unrelated individuals homozygous for the same AH express the same NK-defined haplotype, i.e., NK-defined haplotypes are AH haplotypic; and (iv) the same NK-defined haplotype can be common to several different AH.

Residues 77 to 80 on the HLA-C molecule do account for NK-defined specificities. Having demonstrated that NK specificities associate with specific AH, we determined whether these associations could be accounted for by the two alternate epitopes at amino acid residues 77 and 80 on HLA-C associated with group 1 and 2 specificities (15, 16). Therefore, the HLA-C allele and the epitope present on each AH was examined. These results are summarized in Table 4. In all cases for which data are available, the results fit the hypothesis that the alternative epitopes defined at residues 77 to 80 are associated with the NK-defined group 1 and 2 specificities. However, the NK specificity associated with the 44.2 AH is unusual in expressing group 1 target relatively weakly. In view of the different phenotype of the 44.2 AH, the HLA-C allele of this cell was sequenced from nucleotides 190 to 269 of exon 2, with the expectation that this allele would possess a different epitope from those shown to correspond to groups 1 and 2. Surprisingly, this cell carries the S--N epitope expected for group 1-positive cells. However, its sequence apparently describes a new HLA-Cw allele. This new allele is identical to Cw1 and HLA-Cw*1401 in this region, except that it has an A at nucleotide 267.

It is also evident that the group 3 and 5 specificities are not associated with any particular C allele or with either of the two alternative Cw epitopes at residues 77 and 80.

DISCUSSION

In this study we utilized cells that are homozygous or heterozygous for specific AH as target cells for NK clones defining specificities 1, 2, 3, and 5. We have shown that all cells homozygous for an AH express either a group 1 or 2 determinant and that the phenotypes of heterozygous cells can be predicted from the results in the homozygous cells and a recessive model of inheritance for susceptibility to lysis. Homozygous cells

TABLE 3. Cytotoxicities of NK clones defining group 1 and 2 specificities against the 34 LCL target cells

Cell identification	AH present	NK reactivity[a] for indicated group		NK phenotype
		1	2	
Homozygous				
Q6/3975	7.1	85	10	1+, 2−
R6/12367	7.1	67	5	1+, 2−
R5/1518	8.1	65	2	1+, 2−
R5/843	8.1	90	6	1+, 2−
R9/52658	46.1	72	4	1+, 2−
R5/52658	46.1	75	ND	1, (2−)[b]
R7/4709	52.1	44	12	1+, 2−
R7/12580	52.1	32	2	1+, 2−
R8/15375	54.1	95	ND	1+, (2−)[b]
R6/12317	62.1	48	ND	1+, (2−)[b]
R6/12293	18.1	65	2	1+, 2−
R6/12383	60.3	61	0	1+, 2−
R6/12382	60.3	100	5	1+, 2−
R6/12336	44.2	14	ND	1+, (2−)[b]
R6/12303	18.2	4	42	1−, 2+
R5/5054	18.2	5	61	1−, 2+
R6/12337	57.1	3	88	1−, 2+
R0/9217	44.1	ND[d]	52	1−, 2+
R6/12333	13.1	11	54	1−, 2+
Q5/8086	35.1	2	ND	1−, (2+)[c]
R6/12327	35.4	8	ND	1−, (2+)[c]
Heterozygous				
R7/17219	7.1, 8.1	92	10	1+, 2−
Q9/20920	7.1, 8.1	70	0	1+, 2−
R0/23685	62.1, 8.1	63	3	1+, 2−
Q6/2731	62.1, 8.1	83	ND	1+, (2−)[b]
R5/10168	61.1, 7.1	70	ND	1+, (2−)[b]
R8/5618	60.3, 44.2	37	0	1+, 2−
Q5/7952	62.1, 44.2	27	ND	1+, (2−)[b]
R9/52661	46.1, 54.1	63	ND	1+, (2−)[b]
R5/13141	60.3, 57.1	0	ND	1−, 2−
Q6/8187	8.1, 57.1	5	0	1−, 2−
R9/52417	46.1, 13.1	4	2	1−, 2−
Q5/2711	7.1, 44.1	0	ND	1−, 2−
R0/22825	7.1, 44.1	0	0	1−, 2−

[a] Results are expressed as percent ^{51}Cr release.
[b] Group 2 negative based on positive reactivity with group 1.
[c] Group 2 positive based on negative group 1 reactivity.
[d] ND, not determined.

TABLE 4. HLA-C allele and epitope present on AH

NK-defined haplotype for indicated specificity				AH	Associated Cw allele	Putative epitope[a]
1	2	3	5			
+	−	+	+	7.1	Cw7	S--N
				8.1	Cw7	S--N
				60.1	Cw10	ND
−	+	+	−	57.1	Cw6	N--K
				18.2	Cw5	N--K
−	+	+	+	44.1	Cw5	N--K
+	−	ND[d]	ND	60.3	Cw3	S--N
				62.1	Cw3	S--N
				46.1	Cw1	S--N
				18.1	—	S--N
				44.2	—	S--N[b]
−	+	ND	ND	35.1	Cw4[c]	N--K
				35.4	Cw4[c]	N--K
				13.1	Cw6	N--K

[a] Putative epitope defined by amino acid residues 77 to 80 on HLA Cw α chain.
[b] As determined in this study.
[c] Based on sequence data submitted to GenBank (accession no. M84386).
[d] ND, not determined.

carry several NK-defined specificities, suggesting the presence of NK-defined haplotypes. Without exception, all examples of the same AH possessed the same group 1 or 2 specificity, and the more limited panel of cells tested possessed the same NK-defined haplotype, i.e, the NK-defined specificities are AH haplotypic. Therefore, the identification of AH will allow the identification of the associated NK specificities and provide a simple means of identifying the presence of these specificities in an individual. The NK-defined phenotype of an individual can be predicted based on the particular combination of AH present.

Whereas AH provide an excellent means of identifying the presence of a particular NK-defined haplotype, the relevant genes may be encoded anywhere along the haplotype. Elsewhere we have provided evidence that AH consist of several blocks of several hundred kilobases of DNA. Recombination occurs preferentially between these blocks but has not been observed within them. In fact, there appear to be at least four distinct blocks of polymorphism within the MHC interval, viz. (i) the α block, which carries HLA-A; (ii) the β block, which carries HLA-C, HLA-B, and CL (18); (iii) the γ block, which carries complementary component genes Cyp21 and Bf and C2 and C4; and (iv) the δ block, which carries the DR and DQ gene clusters. Mapping studies in both humans (12) and mice (41) and examination of several cells bearing recombinant AH suggest that the genes encoding group 1 and 2 NK-defined allospecificities are carried on the β block.

The nature of the target molecules for NK allorecognition has not been determined. It has been recently suggested that HLA class I molecules are the targets or ligands for NK receptors (32). HLA-Cw3 provides protection against lysis mediated by group 2-reactive clones. This resistance to lysis was inherited in a dominant manner and was specific for group 2 specificity. Two alternative epitopes defined by polymorphism of amino acid residues 77 and 80 on HLA-C have been shown to be associated with protection against group 1 and group 2 specificities (12). The present data support this hypothesis but also show that these epitopes are not associated with the group 3 or 5 specificity. HLA-C is included within the β block. In all cases for which data are available, the group 1- and 2-associated AH carry the predicted HLA-Cw epitope. However, cytotoxicity by group 1-reactive NK clones against the 44.2 AH homozygous cells are weak, and the two cells heterozygous for this AH exhibited considerably less cytotoxicity than did the other susceptible cells. We have shown that this AH carries a new HLA-C allele which may behave similarly to HLA-Cw*1401, as reported by Colonna et al. (16).

Two possible models to account for NK recognition have been suggested previously (35, 37). These involve either effector inhibition, during which an MHC class I molecule provides an inactivating signal that blocks the NK cell ability to lyse, or target interference, during which an appropriate class I molecule masks a putative self-epitope that is actually a molecular target for NK recognition leading to cell lysis. HLA-C may be one such class I molecule, but there is evidence that other class I molecules may also be involved (28, 45). The phenotype

conferred by HLA-Cw*1401 and the HLA-Cw allele carried on the 44.2 AH support the involvement of additional genes. Indeed, cellular responses are often controlled by the opposing actions of tyrosine kinases activating signaling and tyrosine phosphatases terminating signaling (46). For example, coligation of the immunoglobulin receptor and FcγRIIB on B cells stimulates the tyrosine kinases that phosphorylate the intracytoplasmic portion of FcγRIIB, which in turn recruits the SHP-1 phosphatase that terminates immunoglobulin signal transduction (19). Molecular analysis of several membrane receptors with inhibitory function revealed a common sequence, I/VxYxxL/V (the immune receptor tyrosine-based inhibitory motif [ITIM]), which binds the SHP-1 tyrosine phosphatase and halts positive signals transduced via other receptors (42). The two-immunoglobulin domain and three-immunoglobulin domain KIR isoforms with a long cytoplasmic tail possess two ITIMs, separated by 26 to 28 amino acids (18, 20, 48). Studies from several groups have recently demonstrated that activation of NK cells results in tyrosine phosphorylation of the KIR ITIMs, recruitment of SHP-1 and possibly SHP-2, and inhibition of NK cell-mediated cytotoxicity (7, 9, 10, 39). Like the way they function in NK cells, KIR can negatively regulate signals initiated in T cells via the T-cell receptor by recruitment of SHP-1 (26).

Our findings have important practical implications. NK allorecognition is likely to be involved in bone marrow graft rejection in humans, given the mouse model and the inadequacy of current matching. It has been shown that bone marrow from an HLA-A, -B, DR-, DQ-matched. MLC-nonreactive unrelated donor who was mismatched with the recipient for the NK-defined group 1 specificity was rejected (47). Also, there is evidence that mismatching within the β block, as with mismatching for the CL region, is associated with graft rejection (34). Matching for NK-defined allospecificities is therefore likely to be an important factor for successful bone marrow engraftment. Ultimately, the relevant target molecules need to be identified, and their genes must be mapped and characterized. It will be necessary to identify a marker specific for the MHC blocks associated with each of the approximately 40 AH present in each major racial group. Matching for these blocks will result in matching for all the NK allospecificities present within these blocks. We have data that the polymorphic CL region provides such a haplospecific marker for the β block. Cross-matching donor and recipient at CL would therefore match for the NK-defined specificities. Work is in progress to confirm the validity of this approach.

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RETRACTION

Alloreactivity and Association of Human Natural Killer Cells with the Major Histocompatibility Complex

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The publisher hereby retracts the above-entitled article (Clin. Diagn. Lab. Immunol. **6:**254–259, 1999). An investigation by the American Society for Microbiology Publications Board has determined that the paper plagiarized an earlier publication ("Human Natural Killer [NK] Alloreactivity and Its Association with the Major Histocompatibility Complex: Ancestral Haplotypes Encode Particular NK-Defined Haplotypes" by F. T. Christiansen, C. S. Witt, E. Ciccone, D. Townend, D. Pende, D. Viale, L. J. Abraham, R. L. Dawkins, and L. Moretta, J. Exp. Med. **178:**1033–1039, 1993). The paper therefore is being retracted by ASM, as it represents a clear violation of ASM's ethical standards.