Evidence of a Natural Killer (NK) Cell Repertoire for (Allo) Antigen Recognition: Definition of Five Distinct NK-determined Allospecificities in Humans

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Summary

Previous studies indicated that $CD3^-CD16^+$ natural killer (NK) cells are capable of specific alloantigen recognition. Thus, alloreactive NK clones lysed normal allogeneic target cells (phytohemagglutinin [PHA] blasts) bearing the stimulating alloantigen but did not lyse autologous cells or the majority of unrelated allogeneic cells. In this study we investigated whether NK cells isolated from single individuals could exhibit different allospecificities. To this end, we derived large numbers of $CD3^-CD16^+$ clones (in the presence of PHA) from fresh $CD3^-$ peripheral blood lymphocytes. Cloning efficiencies ranged between 5 and 10%. The resulting $CD3^-CD16^+$ clones were tested for their reactivity against a panel of allogeneic PHA blasts (derived from six donors). In a given individual (A), four distinct groups of clones could be identified according to their pattern of reactivity (over 400 clones have been analyzed). Clones that could be assigned to one or another group of specificity represented 36% of all clones derived from this donor. The remaining clones did not display cytolytic activity against any of the allogeneic target cells used in the panel. None of the clones lysed autologous (A) PHA blasts, yet, these cells were lysed by the representative clones G10 and H12 specific for donor A. Clones displaying a cytolytic pattern of reactivity identical to that defined for donor A were present in other individuals studied, however not all groups of allospecific clones were necessarily represented in different individuals. Allospecific clones belonging to the various groups were homogeneous in the expression of EB6/GL183–triggering surface molecules, and could thus be assigned to one or another of the previously defined subsets of NK cells. Genetic analysis of the new NK-defined alloantigens was performed in representative families. The corresponding characters were found to segregate independently and, at least for three of them, an autosomic recessive type of inheritance could be demonstrated. Moreover, the comparative analysis of the segregation of the major histocompatibility complex haplotypes and the recessive or dominant alleles of the genes governing the five specificities analyzed indicated that there is no independent sampling between the two genetic traits, thus suggesting that the genes regulating the NK-defined specificities are carried by chromosome 6. Finally, some donors expressed more than one specificity, thus providing evidence for an NK-defined complex haplotype.

It is a common notion that NK cells do not express known receptors for antigen and lyse tumor cells through a non-MHC–restricted type of recognition (1, 2). However, recent evidence in our and in other laboratories indicated that peripheral blood–derived $CD3^-CD16^+$ NK cells were capable of specific recognition of allogeneic cells (3–5). Moreover, this ability was clonally distributed, and in no instances could lysis of autologous cells be observed (3, 6). In the case of the first NK-defined specificity, the character of "susceptibility to lysis" appeared to be inherited in an autosomic recessive manner, and the gene governing susceptibility or resistance to lysis appeared to be carried by chromosome 6 (6, 7). In addition, the specific lysis of allogeneic cells could be distinguished from the non-MHC–restricted lysis of tumor cells (6).

In the present study, we show the existence of at least five NK-defined allospecificities. More importantly, NK clones derived from single individuals were found to recognize sev-

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eral distinct allospecificities, supporting the notion of the existence of an NK cell repertoire. In addition, all clones expressing a given allospecificity were phenotypically homogeneous with respect to the expression of GL183- and/or EB6-triggering surface molecules. Genetic analysis showed that all the NK-defined specificities segregated independently. The lack of independent sampling between MHC haplotypes and the recessive or dominant alleles governing the various specificities was consistent with their localization on chromosome 6. Finally, at least three specificities were found to be inherited in an autosomic recessive manner.

Materials and Methods

Isolation and Cloning of CD3$^-$CD16$^+$ Lymphocytes and Evaluation of Cytolytic Activity. PBL derived from normal donors were isolated on Ficoll-Hypaque gradients and cells were then incubated with a mixture of anti-CD3 (OKT3; Ortho Pharmaceutical, Raritan, NJ), anti-CD4 (HP26), and anti-CD8 (B9.4) mAbs, followed by treatment with rabbit complement for 1 h at 37°C as previously described (3, 6).

Viable cells were isolated by Ficoll-Hypaque gradients and cloned under limiting dilution conditions in the presence of irradiated feeder cells, 0.1% PHA (Gibco Ltd., Paisley, Scotland), and a source of exogenous IL-2 (rIL-2; Cetus Corp., Emeryville, CA), as previously described for both T and NK cell cloning (3, 8, 9).

The cytolytic activity of cloned cells was tested in a 4-h ^{51}Cr-release assay in which effector cells were tested against PHA blasts derived from different allogeneic (or autologous) donors. PHA blasts were obtained by culturing PBL for 4 d with 0.5% PHA (vol/vol) in the presence of rIL-2 (100 U/ml). Target cells were used at 5 $\times$ 10^3/well, for a final E/T cell ratio of 5:1. Percent specific lysis was determined as previously described (3, 6, 8, 9).

Flow Cytofluorometric Analysis. 10^5 cells were stained with the appropriate mAb followed by fluoresceinated goat anti-mouse Ig. Control aliquots were stained with the fluorescent reagent alone. All samples were then analyzed on a flow cytometer (FACS STAR®; Becton Dickinson & Co., Mountain View, CA) gated to exclude nonviable cells. The percentage of positive cells was calculated on histograms displaying $\log_{10}$ of fluorescence (in arbitrary units) vs. number of cells. An electronic gate was positioned on the basis of 99% of autofluorescent negative cells. Fluorescent cells trespassing the gate were considered as positive. The mAbs used in these studies were represented by anti-Leu-4 (anti-CD3, Becton Dickinson & Co.), KD1 (10) (anti-CD16), GL183 (11), and EB6 (12).

HLA Typing. PBMC from normal donors were HLA typed using the standard National Institutes of Health (Bethesda, MD) complement-mediated microcytotoxicity assay.

Statistical Analysis. For each alloreactive NK clone a logistic model was fitted to the data (13) (i.e., the percent specific ^{51}Cr release detected in cytolytic assays against different allogeneic target cells at the E/T ratio of 5:1). In a first model, the grand mean of data obtained was calculated. Successively, in a second model, a dummy variable was introduced. Thus the data were divided in two subpopulations (susceptible or resistant to lysis). The deviance (i.e., goodness of fit measure) for the two different models was calculated.

The difference of deviances between the two models is χ^2_1. Data were analyzed with GLIM statistical package (14).

We have considered as informative for susceptibility or resistance to lysis only clones in which a χ^2_1 value was >3.84 (i.e., $p < 0.05$).

Results

In a previous study we showed that CD3$^-$CD16$^+$ NK cells isolated from a given donor (termed 1) and cultured in MLC with irradiated allogeneic cells from the individual A were able to specifically recognize and lyse PHA blasts derived from individual A (6). Moreover, specific NK cells also could be generated in the reverse combination in which anti-1 effector cells were derived from donor A. In the present study, to investigate whether NK cells from a given individual could exhibit different patterns of reactivity against allogeneic cells, we analyzed large numbers of CD3$^-$CD16$^+$ clones obtained from donor A. In this series of experiments, NK clones were derived directly from CD3$^-$ lymphocytes isolated from peripheral blood and cultured under limiting dilution conditions in the presence of PHA and irradiated feeder cells, without a preliminary selection in MLC. Under these culture conditions, CD3$^-$CD16$^+$ NK clones proliferated independently on their ability to recognize given alloantigens. The cloning efficiencies in four different experiments were 5–10%. All the clones obtained were analyzed for their cytolytic activity against a panel of normal target cells (PHA blasts) derived from different allogeneic donors. 149 (36%) of 415 clones analyzed displayed cytolytic activity against one or more of the allogeneic target cells of the panel. In particular, clones displaying >20% cytolytic activity at 5:1 E/T ratio were arbitrarily considered positive and clones displaying <10% cytolytic activity at the same E/T ratio were scored negative. Table 1 shows the cytolytic activity of 17 representative alloreactive clones. As with our previous reports (3, 6), none of the clones derived from donor A lysed autologous PHA blasts. On the other hand, PHA blasts from donor A could be efficiently lysed by the control clones H12 and G10 derived from donor 1, which were previously shown to display anti-A-specific cytolytic activity (6). The allospecificity identified by clones H12 and G10 will be defined as specificity 1, and clones displaying this specificity will be referred to as group 1 clones. Contrary to group 1 clones, all other clones shown in Table 1 were derived from donor A. These clones were characterized by their different patterns of cytolytic activity against the panel of allogeneic target cells analyzed. On these bases, different groups of NK clones derived from donor A could be identified. Thus, a group of NK clones lysed donors 1, 2, 3, and 5, but failed to lyse donors 4 and 6 (group 2). Another group lysed donors 1, 2, 4, and 6, but failed to lyse donors 3 and 5 (group 3). Clones from group 4 lysed donors 1–4, but not donors 5 and 6. Finally, clones from group 5 lysed only donor 6. The clones belonging to group 2 represented 18% (74/415) of all NK clones derived from donor A, whereas clones of group 3 were 12% (51/415). Those of group 4 and group 5 were 5% (19/415) and 1% (5/415), respectively. It should be stressed that, as indicated above, >60% of all NK clones derived from donor A did not display cytolytic activity against any of the allogeneic target cells analyzed in the panel. Taken together, these data indicate that distinct NK clones derived from a single individual are able to recognize different allospecificities expressed by normal allogeneic target cells. Thus, four new different patterns of NK

Table 1. *CD3⁻CD16⁺ NK Clones Displaying Different Patterns of Alloreactivity Can Be Generated from Donor A*

Clones	Normal target cells (PHA blasts) from donor							GL183/EB6 surface phenotype§	
	A*	1	2	3	4	5	6	GL183	EB6
Group 1									
H12	**58**‡	1	1	2	5	3	**52**	–	+
G10	**65**	3	2	4	6	1	**70**	–	+
Group 2									
AN4	0	**26**	**24**	**24**	2	**44**	0	+	+
ALE 5	0	**51**	**54**	**42**	0	**30**	2	+	+
AP27	0	**41**	**36**	**43**	2	**35**	0	+	+
N25.5	2	**42**	**38**	**35**	0	**40**	2	+	+
Group 3									
T50.4	0	**54**	**37**	8	**36**	5	**59**	–	–
AGT.2	8	**22**	**27**	9	**25**	7	**44**	–	–
AGT.11	4	**34**	**40**	8	**42**	8	**43**	–	–
AGD.5	4	**44**	**35**	9	**47**	6	**46**	–	–
Group 4									
N25.5	0	**42**	**39**	**35**	**32**	6	0	+	+
AGD.17	0	**52**	**39**	**24**	**27**	4	9	+	+
AGD.27	0	**37**	**33**	**36**	**39**	8	5	+	+
AGD.31	0	**47**	**43**	**40**	**38**	9	5	+	+
Group 5									
AGD.22	2	9	1	0	1	2	**25**	–	+
AGD.48	1	0	0	0	2	1	**47**	–	+
6.3	0	2	0	0	0	ND	**65**	–	+
50.42	0	2	2	0	0	0	**51**	–	+
25.11	0	ND	0	0	2	2	**33**	–	+

Group 1 clones were derived from MLC in which responder cells were derived from donor 1 and stimulating cells from donor A. Clones of groups 2–5 were derived from donor A directly from freshly isolated CD3⁻ PBL, in the presence of PHA, without preliminary selection in MLC.

* In this test target cells were labeled with ^{51}Cr.

‡ Results are expressed as percent of ^{51}Cr-release at E/T ratio of 5:1.

§ Surface antigen expression has been determined by indirect immunofluorescence and FACS® analysis, as described in Materials and Methods and illustrated in reference 10.

cell reactivity could be identified. For the sake of simplicity, we define as specificities 1, 2, 3, 4, and 5 those recognized by clones belonging to groups 1, 2, 3, 4, and 5, respectively.

Donor AO was susceptible to lysis by group 1 clones, as was donor A, and has been analyzed in detail. As shown in Table 2, PHA blasts from donor AO were susceptible to lysis by the NK clones H12 and G10 (group 1). Among the NK clones derived from donor AO, those reactive with allogeneic cells included in the panel represented as much as 70% of all clones (107/153). However, in this donor, only two groups of alloreactive clones could be identified. It is notable that these two groups of alloreactive clones were undistinguishable from groups 2 and 5 clones derived from donor A. Table 2 shows eight representative clones derived from donor AO. It is interesting that clones displaying specificity 2 were largely predominant, representing 68% of all clones (104/153).

Correlation between NK-defined Allospecificities and EB6/GL183 mAb-defined NK Subsets. We previously demonstrated that GL183 and EB6 mAbs recognize two triggering molecules which share biochemical and functional properties (11, 12) on the surface of human NK cells. Moreover, according to the reactivity of NK cells with GL183 and/or EB6 mAbs, four different, phenotypically stable subsets have been identified (GL183⁺EB6⁺; GL183⁻EB6⁺; GL183⁺EB6⁻; and GL183⁻

Table 2. *$CD3^-CD16^+$ Alloreactive NK Clones Derived from Donor AO*

Clones	Normal target cells (PHA blasts) from donor: AO*	A	1	2	3	4	5	6	GL183/EB6 Surface phenotype GL183	EB6
Group 1										
H12	63	58	1	1	2	5	3	52	−	+
G10	57	65	3	2	4	6	1	70	−	+
Group 2										
AM7	0‡	0	53	46	75	0	41	0	+	+
AM23	0	0	35	41	44	2	24	0	+	+
16.23	0	0	83	66	81	5	85	3	+	+
AM21	3	0	58	78	77	2	32	2	+	+
AM29	2	2	40	63	60	4	44	0	+	+
16.20	0	0	35	54	51	0	38	0	+	+
MARY25	0	2	38	44	45	0	37	2	+	+
Group 5										
A016	5	7	2	3	0	0	5	47	−	+

Group 1 clones were derived from MLC in which responder cells were derived from donor 1 and stimulating cells from donor A. Clones of groups 2 and 5 were derived from donor AO directly from purified $CD3^-$ PBL cultured in limiting dilution analysis in presence of irradiated feeder cells and PHA.

* Eight different ^{51}Cr-labeled target cells were analyzed. Target cells were represented by PHA-induced blasts derived from donor AO (autologous target cells), by PHA blasts derived from donor A who, similar to donor AO, is susceptible to lysis by group 1 clones and by PHA blasts derived from six different random donors.

‡ Results are expressed as percent of ^{51}Cr-release at E/T ratio of 5:1.

$EB6^-$) (12). It is significant that a precise correlation has previously been found between the ability to recognize the allospecificity 1 (group 1 clones) and the expression of the $GL183^-EB6^+$ surface phenotype (12). In the present study, we further investigated whether the expression of given GL183/EB6 surface phenotypes correlated with other groups of alloreactive clones. To this end, the various groups of alloreactive clones were analyzed for the surface expression of

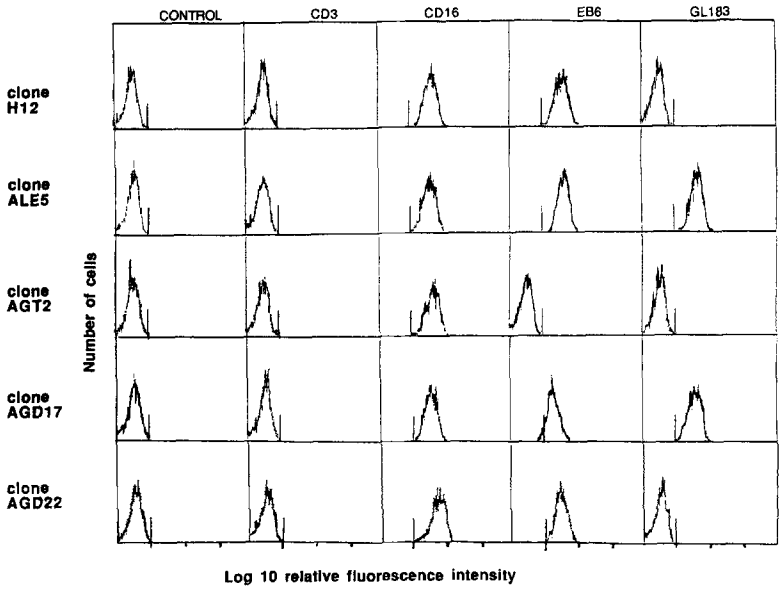

Figure 1. Surface phenotype of representative alloreactive NK clones recognizing different specificities (see Table 1). The mAbs used in this study were represented by anti-Leu-4 (anti-CD3), KD1 (anti-CD16), GL183, and EB6.

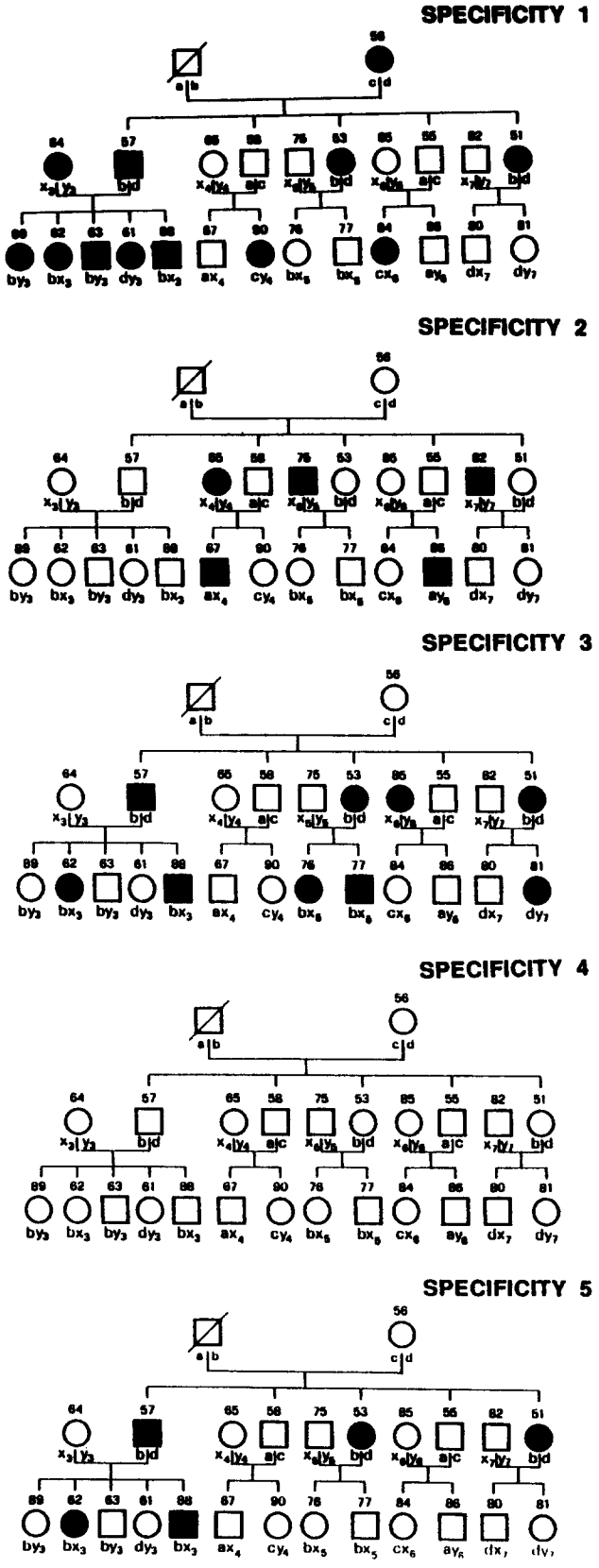

Figure 2. Mode of inheritance in family A, of the character susceptibility to lysis by NK clones recognizing the various specificities. For specificity 1, clone H12 has been used. Statistical analysis of cytotoxicity data for susceptible versus resistant targets revealed for this clone a χ_1^2 value of 43.5 ($p < 0.001$). Clone ALE5 (χ_1^2 37.3, $p < 0.001$), and clone Mary25 (χ_1^2 35.8, $p < 0.001$) have been used for specificity 2. For specificity 3, clone AGD.5 (χ_1^2 11.48, $p < 0.001$), clone AGT.11 (χ_1^2 8.56 $p < 0.01$), and clone AGT.2 (χ_1^2 5.45 $p < 0.025$) have been used. For the specificity 4, clone AGD.17 (χ_1^2 3.97, $p < 0.05$) and clone AGD.31 (χ_1^2 4.23, $p < 0.05$) have been used. For specificity 5, clone AGD.48 (χ_1^2 7.6, $p < 0.01$) and clone AO16 (χ_1^2 10.51, $p < 0.01$) have been used. Filled symbols represent susceptible donors, and open symbols represent resistant donors. Circles indicate female donors and squares indicate male donors. Small letters identify serologically-defined MHC haplotypes (for details, see reference 6). The family analyzed in this figure has been described in a previous report (6) for the pattern of inheritance of specificity 1.

GL183 and EB6 molecules. As shown in Tables 1 and 2, all clones recognizing specificity 2 (derived either from donor A or donor AO) were characterized by the double-positive ($GL183^+EB6^+$) surface phenotype. It is worth noting that in donor AO, all 104 double-positive clones recognized specificity 2. In contrast, in donor A, 74/128 of the double-positive clones analyzed recognized specificity 2, whereas 19 recognized specificity 4. In both donors, all of the infrequent $GL183^-EB6^+$ clones (five clones derived from donor A and three from donor AO) recognized specificity 5. Group 3 clones were isolated from donor A, but not from donor AO. Thus, in donor A all the clones recognizing this specificity were homogeneously represented by double-negative ($GL183^-EB6^-$) clones. In this individual, 51/279 of double-negative clones recognized specificity 3. On the contrary, none of 41 double-negative ($GL183^-EB6^-$) clones derived from donor AO displayed cytolytic activity against allogeneic target cells of the panel. In Fig. 1, the FACS® profiles of five representative clones belonging to each of the groups of alloreactive clones are shown.

Segregation Analysis of the NK-defined Alloantigens. The previously defined allospecificity recognized by group 1 NK clones has been shown to be controlled by a gene locus termed EC1 (6). The EC1 locus is inherited in an autosomic recessive manner and is carried on chromosome 6 in the human MHC region (7). Having identified new NK-defined allospecificities, we analyzed their mode of inheritance in representative families. To this end, a series of clones belonging to each group has been selected. These clones were first analyzed for their ability to lyse PHA blasts derived from a large group of random allogeneic donors to define the parameters used to discriminate among susceptible and resistant donors in a ^{51}Cr-release assay. Having introduced a dummy variable to distinguish between susceptible and resistant donors (susceptible >20% lysis at 5:1 ratio and resistant <10% lysis), we performed a statistical analysis for each of these clones (see Materials and Methods). All of the clones tested displayed a χ_1^2 value >3.84 (i.e., $p < 0.05$) and were thus used for the genetic analysis. As shown in Fig. 2, in family A, clones recognizing different allospecificities lysed different members of this family (it is noteworthy that clones recognizing specificity 4 failed to lyse all of the family members). For example, individual 64, homozygous for the recessive allele of the EC1 locus ($EC1^-/^-$) (6), was susceptible to lysis by clone H12

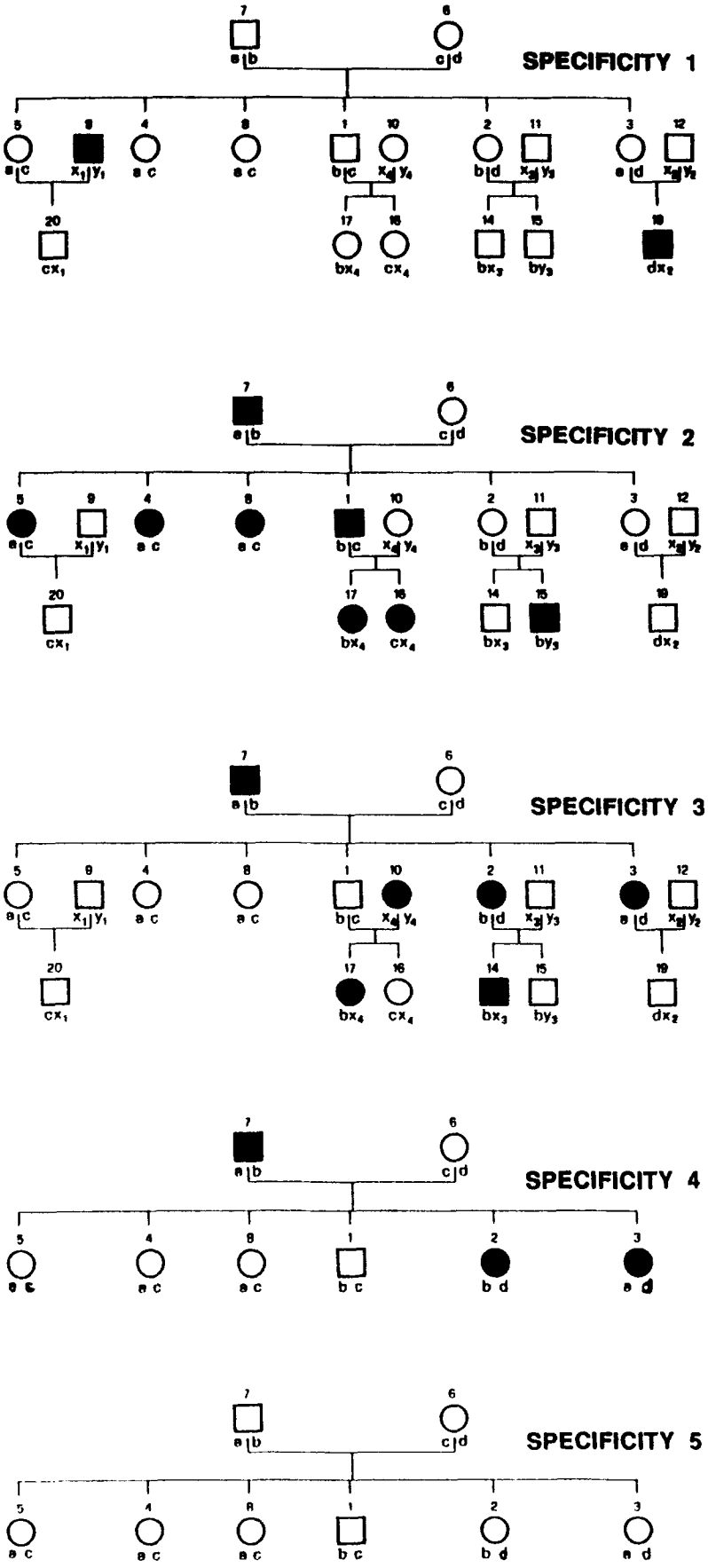

Figure 3. Mode of inheritance in family N, of the character susceptibility to lysis by NK clones displaying various specificities. Small letters indicate serologically defined MHC haplotypes. The clones used to define the various specificities are described in the Fig. 2 legend. The third generation of this family was characterized only by clones recognizing specificity 1, 2, and 3.

(recognizing specificity 1) but was resistant to lysis by clones ALE5 (specificity 2), AGD.5 (specificity 3), AGD.17 (specificity 4), or AGD.48 (specificity 5). Conversely, her mate (individual 57), equally sensitive to clone H12 (specificity 1), was susceptible to lysis by clones recognizing specificity 3 or 5, but not by clones defining specificities 2 and 4.

We next analyzed the pattern of segregation of the various specificities. Regarding the specificity 2, Fig. 2 shows that mating between two individuals (donor 85 and 55), both resistant to lysis by group 2 clones (such as clone ALE5), generated siblings that were either resistant (donor 84) or susceptible (donor 86) to lysis by the same group of clones. The recessive mode of inheritance of specificity 2 was confirmed in two other families shown in Figs. 3 and 4. Thus, for example, in Fig. 3 (family N) the mating between the heterozygous donors 2 and 11 generated progenies that were either resistant or susceptible to lysis. In addition, in Fig. 4 (family M), the mating between donors M. M. and A. M. generated a sibling susceptible (L. M.) and a sibling resistant (Al. M.) to lysis. The comparative analysis of the segregation of the MHC haplotypes and the recessive or dominant alleles governing specificity 2 indicated that there is no independent sampling of the two genetic traits. Thus, in family A (Fig. 2) the recessive allele of specificity 2 segregated with the MHC haplotype a, whereas the dominant allele segregated in all donors with the haplotypes b, c, and d. Similarly, in family N (Fig. 3) the recessive allele segregated with the haplotypes a, b, and c while the dominant allele was associated with the haplotype d. As a consequence, it appears that, as with the previously identified specificity 1, the gene(s) governing the expression of specificity 2 would be present on chromosome 6.

Regarding specificity 3, the formal proof of the recessive inheritance was obtained in family M, in which both parents were resistant to lysis by group 3 clones, and the two siblings were resistant (Al. M.) and susceptible (L. M.) to lysis, respectively. In addition, the comparative analysis of the pattern of segregation of specificity 3 and of the HLA haplotypes indicated that the locus governing susceptibility or resistance to lysis by anti-3 clones is also carried by chromosome 6. Indeed, as shown in Fig. 2, all three donors of family A carrying the MHC haplotype b and d were susceptible to lysis, thus suggesting that these two haplotypes segregated with the recessive allele of specificity 3. In addition, the recessive type of inheritance was suggested by mating between donor 55 (carrying the haplotype a and c and resistant to lysis) and donor 85 (carrying the haplotype x6 and y6 and susceptible to lysis). These donors generated two siblings, 84 (cx6) and 86 (ay6), who were both resistant to lysis. These findings suggest that the dominant allele for specificity 3 cosegregates with a and c haplotypes. The pattern of segregation in family N provides further information that is consistent with the concept that specificity 3 is also recessively inherited, and that genes controlling the expression of this specificity are carried on chromosome 6.

The analysis of the segregation of specificities 4 and 5 has been hampered, at least in part, by the fact that only a few members of the families analyzed carried these specificities,

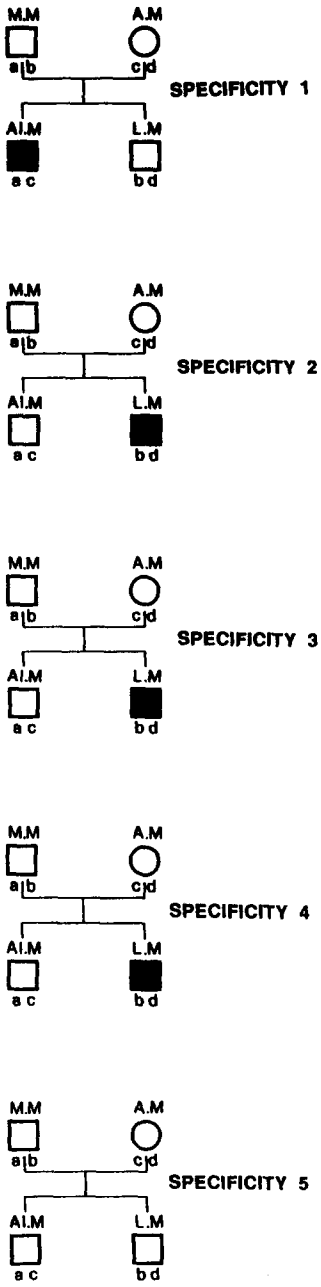

Figure 4. Mode of inheritance in family M, of the character susceptibility to lysis by NK clones displaying various specificities. Capital letters identify the various donors, and small letters indicate serologically defined MHC haplotypes. The clones used to define the various specificities are described in the Fig. 2 legend.

and by the low number of available clones recognizing them. Nevertheless, the recessivity of specificity 4 is suggested by the analysis of family M (Fig. 4); the analysis of family N (Fig. 3) suggested that the MHC haplotypes a, b, and d cosegregated with the recessive allele of specificity 4; and the dominant allele segregated with the c haplotype. Regarding specificity 5, the lack of suitable combinations of heterozygous parents did not allow precise conclusions on its mode of inheritance. However, the finding that donors 51, 53, and 57 carrying the b and d haplotypes were all susceptible to lysis by group 5 clones is consistent with the concept that the gene controlling the expression of specificity 5 is carried by chromosome 6. Moreover, the segregation of this character

(specificity 5) in only two of five siblings of the mating between individuals 64 and 57 is consistent with a recessive type of inheritance of the character. On the basis of this interpretation, donor 64 could be considered as heterozygous, carrying the recessive allele associated with MHC haplotype x3 and the dominant allele with y3.

An important piece of information derived from the analysis of the three families is that some individuals expressed more than one specificity (i.e., are susceptible to lysis by different groups of clones). For example, donors 53, 57, and 51 of the family A were lysed by clones of group 1, 3, and 5. On the other hand, donor 7 of family N and donor L. M. of family M were lysed by clones of group 2, 3, and 4. These data suggest the existence of complex haplotypes that uniquely characterize each individual with respect to susceptibility to lysis by NK clones recognizing different allospecificities.

Discussion

In the present study, by analysis of alloreactive NK clones, we provide direct evidence for the existence of at least five NK-defined specificities and thus of an NK cell repertoire. All NK clones that recognize a given allospecificity were found to express a homogeneous surface phenotype, as defined by the expression of GL183- and/or EB6-triggering molecules. Genetic analysis of the various NK-defined allospecificities showed that all the corresponding characters segregated independently. In addition, at least three of these characters were inherited in an autosomic recessive manner, and the lack of an independent sampling between MHC haplotypes and the dominant or recessive alleles of the genes controlling the various specificities suggested that they were carried by chromosome 6. Moreover, some donors carried more than one specificity, thus suggesting the existence of a complex haplotype, with respect to the NK-defined alloantigens.

Our present finding that cloned NK cells derived from single individuals recognize different allospecificities indicates that, NK cells, much like T or B lymphocytes, express a repertoire for (allo)antigen recognition. It should be stressed that only a fraction of clones derived from a given individual reacted with the panel of allogeneic target cells. Thus, for example, NK clones reactive with at least one of the six allogeneic target cells used in the panel represented <40% of all NK clones derived from donor A. Therefore, it is possible that at least some of the remaining clones may recognize other still undefined allospecificities (i.e., not included in the panel).

The existence of groups of clones displaying unique patterns of alloreactivities supports the notion that allogeneic target cells express different alloantigens that represent the target molecules recognized by NK cells. To obtain further information on these alloantigens, we analyzed their mode of inheritance in representative families. We have previously shown that the locus EC1, governing susceptibility or resistance to lysis by group 1 NK clones, is located on chromosome 6 in the MHC region (6, 7). In addition, the character susceptibility to lysis by group 1 clones appeared to be recessively inherited. Our present study is consistent with the idea that a similar mode of inheritance and chromosomal location is valid also for the other NK-defined specificities. However, this conclusion can be firmly drawn only for specificities 2 and 3 (on the basis of the large number of data). Regarding specificities 4 and 5, it should be stressed that all of the available data are not in contrast with a recessive type of inheritance or with the location of the corresponding genes on chromosome 6. It is important that the various characters displayed an independent segregation. These data further support the notion of the existence of different alloantigens. It is notable that several individuals expressed more than one alloantigen. For example, donors 51, 53, and 57 of the family A (Fig. 2) expressed specificities 1, 3, and 5; donor L. M. (family M, Fig. 4) and donor 7 (family N, Fig. 3) expressed specificities 2, 3, and 4. Similarly, the random donors 1 and 2 (Table 1) coexpressed specificities 2, 3, and 4. Given the independent segregation of these specificities, our data suggest the existence of multiple determinants, each specifying a different alloantigen. It is notable that in murine hybrid resistance phenomenon studies as well, a complex haplotype for Hh-1 system has been described (15, 16).

What could be the nature of the alloantigens specifically recognized by NK cells, and how can their recessive mode of inheritance be explained? We previously hypothesized that the recessivity may reflect the existence of (*trans*-acting) regulatory genes on chromosome 6, mapping in the MHC region (7). An alternative explanation would be the ability of NK cells to specifically recognize self-epitopes, perhaps carried by MHC class I antigens (17). If this is the case, the recognition of the self-epitope(s) would induce an inhibitory signal resulting in inhibition of cytolysis. Thus, only allogeneic donors who lack the genes coding for the epitope that provides the inhibitory signal in both chromosomes would be susceptible to lysis by a given NK clone. In agreement with this hypothesis, several reports suggest an involvement of class I molecules in the NK cell recognition (18, 19). Thus, tumor cells sensitive to NK cell–mediated killing are often (but not invariably) low in MHC class I antigen expression. In addition, transfection of NK-sensitive target cells with different MHC class I genes often protects them from NK cell–mediated lysis (20). In accordance with the hypothesis that MHC class I represents the target molecules for NK cell recognition, the existence of an NK cell repertoire could be interpreted as the ability of different NK cells to recognize different class I self-epitopes. The absence (or the masking) on tumor cells of (one or more of) these epitopes would determine the susceptibility to lysis by appropriate NK clones. This interpretation would be consistent with the previously observed heterogeneity of the NK-mediated lysis of tumor cells.

We have previously shown that NK cells express clonally distributed triggering surface molecules (GL183 and EB6) that define four subsets of $CD3^-CD16^+$ NK cells (12). More importantly, analysis of possible relationships existing between the expression of GL183/EB6 molecules and the first defined specificity (specificity 1) showed a striking correlation with one of these subsets. Thus, all MLC-derived NK clones displaying specificity 1 were characterized by the $EB6^+GL183^-$ surface phenotype. In addition, essentially all $EB6^+GL183^-$ clones derived directly from PBL (i.e., with-

out preselection in MLC) were found to display specificity 1 (12). In the present study, we have further investigated whether clones recognizing other specificities could be assigned to one or another of the phenotypically defined subsets. We found that all clones recognizing a given specificity could be assigned to one or another subset. For example, clones with a specificity of 2 or 4 were double positive ($GL183^+$ $EB6^+$), and clones displaying specificity 3 were double negative. All of the infrequent clones with specificity 5 (which could be isolated only in donors susceptible to lysis by group 1 clones) were $EB6^+GL183^-$. All together, these data are consistent with the idea that molecules belonging to the GL183/EB6 family may be part of a receptor structure involved in alloantigen recognition and in the specific lysis of normal allogeneic cells. These data are reminiscent of a recent report in mice in which a mAb-defined subset of NK cells mediated the rejection of incompatible Hh-1-d bone marrow grafts (21).

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