Recognition of Virus-infected Cells by Natural Killer Cell Clones Is Controlled by Polymorphic Target Cell Elements

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Summary

Natural killer (NK) cells provide a first line of defense against viral infections. The mechanisms by which NK cells recognize and eliminate infected cells are still largely unknown. To test whether target cell elements contribute to NK cell recognition of virus-infected cells, human NK cells were cloned from two unrelated donors and assayed for their ability to kill normal autologous or allogeneic cells before and after infection by human herpesvirus 6 (HHV-6), a T-lymphotropic herpesvirus. Of 132 NK clones isolated from donor 1, all displayed strong cytolytic activity against the NK-sensitive cell line K562, none killed uninfected autologous T cells, and 65 (49%) killed autologous T cells infected with HHV-6. A panel of representative NK clones from donors 1 and 2 was tested on targets obtained from four donors. A wide heterogeneity was observed in the specificity of lysis of infected target cells among the NK clones. Some clones killed none, some killed only one, and others killed more than one of the different HHV-6-infected target cells. Killing of infected targets was not due to complete absence of class I molecules because class I surface levels were only partially affected by HHV-6 infection. Thus, target cell recognition is not controlled by the effector NK cell alone, but also by polymorphic elements on the target cell that restrict NK cell recognition. Furthermore, NK clones from different donors display a variable range of specificities in their recognition of infected target cells.

NK cells are a distinct subset of lymphocytes, expressing a $CD3^-CD56^+$ phenotype, that can recognize and lyse tumor cell lines and virus-infected cells in vitro without MHC restriction or prior sensitization (1, 2). In vivo models have shown that NK cells accumulate and proliferate at the site of active virus replication (3). In *scid* mice, deficient in T and B lymphocytes, depletion of NK cells resulted in higher virus titers and reduced survival rate of infected animals (4). The absence of a documented memory response in NK cells and of complete virus clearance in the absence of T cells suggested that NK cells serve as a first line of defense.

The detection of NK activity within unstimulated, unfractionated PBMC, and the lack of MHC restriction suggested that NK cells may be relatively homogeneous in their target cell specificity. The first evidence for heterogeneity in target cell recognition by NK cells came from the analysis of NK clones and their ability to recognize alloantigens expressed on normal PBL (5). In addition, human NK cells have been divided into subsets according to the surface expression of molecules defined by the mAbs GL183 (6) and EB6 (7). These two mAbs recognize related molecules with a relative molecular mass of ~58,000 and can regulate cytolytic activity of NK cells that express these molecules. The surface phenotype of NK cells based on the combined use of mAbs GL183 and EB6 correlates with NK-defined allospecificities (7). Another group of NK cell–specific surface molecules may be involved in target cell recognition. These molecules are type II transmembrane proteins with homology to C-type lectins and belong to at least two families of genes located in a region of mouse chromosome 6, called the NK complex (8–10). A subset of mouse NK cells expressing Ly-49, a member of the NK complex expressed as a homodimer with a relative molecular mass of ~85,000, failed to lyse cells expressing certain MHC class I alleles (11). Thus, NK activation and NK-mediated target cell recognition may be controlled by multiple surface NK molecules that provide either positive or negative signals to the lytic machinery.

Sensitivity to NK allospecific lysis is a recessively inherited trait that maps to the class I region of the MHC (12–14). This finding is consistent with evidence that MHC class I molecules can dominantly protect against NK lysis (11, 15–17, reviewed in 18). While NK allorecognition must be controlled by polymorphic target cell elements, it is not yet known whether such elements play a role in NK recognition of normal cells after virus infection.

Since the major biological function of NK cells appears to be elimination of autologous cells that have been infected or transformed, we investigated whether autologous PHA-induced T cell blasts, known to be resistant to lysis by autologous NK cells, would become susceptible to lysis upon infection by human herpesvirus 6 (HHV-6).[1] HHV-6 was used because it infects with high efficiency normal activated T cells and, under appropriate culture conditions, yields a homogeneous population of viable infected target cells (19). Lysis of HHV-6-infected cells was observed with autologous NK clones, but this property was confined to a subset of such clones. In addition, we demonstrate for the first time that NK recognition of untransformed cells infected by a virus is controlled by polymorphic elements expressed by the target cell.

Materials and Methods

Antibodies and Immunostaining. For the characterization of NK cells, the following mAbs were used: Leu4 (CD3), Leu2a (CD8), Leu19 (CD56) (Becton Dickinson & Co., San Jose, CA), KD1 (CD16; reference 6), GL183 (6), and EB6 (7). 13D6 is a mAb to an HHV-6 envelope protein (20), anti-HLA-A, -B, -C is a commercial mAb to the class I heavy chain (Olympus, Lake Success, NY), W6.32 is a mAb to class I heavy chain and β_2-microglobulin complex (American Type Culture Collection, Rockville, MD), BB7.2 is a mAb specific for HLA-A2 (21), B1–23 is a mAb specific for HLA-B and -C molecules (22), B2.62 is a mAb specific for β_2-microglobulin (a gift from J.-C. Chermann [23]). mAbs SA24.23 and F4.326, specific for HLA-B and HLA-C, were gifts from S. Y. Yang (Memorial Sloan-Kettering Cancer Center, New York, NY). NK clones were incubated with mAb for 30 min at 4°C, washed and stained with a goat anti–mouse IgG serum conjugated with FITC (Caltag, San Francisco, CA) for 30 min at 4°C, washed, and analyzed with a FACScan® cytofluorimeter (Becton Dickinson & Co.). Cultures of infected and uninfected PHA-activated T cell blasts grown in the absence of exogenous lymphokines were washed and stained as described above.

Isolation of $CD3^-CD56^+$ Cells and Clones. PBMC from normal healthy volunteers were obtained by separation on a Ficoll-Hypaque gradient followed by incubation at 37°C for 1 h to remove monocytes by plastic adherence. The cells were subsequently washed in ice-cold medium (Iscove's modified essential medium; Biofluids, Rockville, MD) containing 2% FCS, resuspended at 10^7 cells/ml (in 2 ml), and immunostained for 1 h with 200 μg Leu4 mAb. Cells were washed twice to remove excess antibody and incubated for 45 min with magnetic beads coupled with anti-mouse Ig antibodies (Advanced Magnetics, Cambridge, MA) under continuous rotation. The enrichment was repeated twice to reach a level of >90% $CD3^-CD56^+$ cells as assessed by cytofluorimetric analysis. The enriched population was tested directly in a lysis assay or seeded at 10^5 cells/well on 2×10^5 irradiated (4,000 rad) PBMC and expanded in 96-well plates in complete medium consisting of Iscove's modified essential medium with 10% human serum, 2 mM glutamine, 100 U/ml rIL-2 (gift of Hoffmann-La Roche, Nutley, NJ), and 10% of a solution of purified human IL-2 (6011; Schiapparelli ENI Diagnostic, Fairfield, NJ). After 5–7 d, cells were harvested and enriched again as described above to reach >95% $CD3^-CD56^+$ cells. NK clones were established by limiting dilution cloning in 96-well plates of freshly enriched $CD3^-$ lymphocytes in the presence of 2×10^5 irradiated PBMC/well (4,000 rad). Cells were seeded in a medium consisting of Iscove's modified essential medium with 10% human serum, 2 mM glutamine, 100 U/ml rIL-2, and 0.2 μg/ml PHA (Sigma Chemical Co., St. Louis, MO). After 48 h 100 μl of medium/well was replaced with complete medium. After 5–6 d an additional 100 μl was replaced with 100 μl of complete medium containing 10^5 irradiated PBMC. 1 wk later, growing microcultures were expanded in several wells of 96-well plates. Homogeneity of cell surface staining (>95%) with mAbs Leu2 (CD8), Leu4 (CD3), Leu 19 (CD56), KD1 (CD16), GL183, and EB6 of each microculture, as well as a calculation of cloning efficiency (24), were used to assess clonality.

HHV-6 Infection. HHV-6 strain GS (25) was propagated in freshly activated umbilical cord blood mononuclear cells as described (19). In vitro infection was carried out with supernatant from infected cord blood cultures. The virus titer was determined by serial 10-fold dilutions of the supernatant on activated cord blood mononuclear cells. PBMC were activated with 1 μg/ml PHA for 48 h in culture medium (RPMI 1640 supplemented with 10% FCS, 2 mM glutamine), washed three times, and resuspended in 2 ml of HHV-6 stock virus (10^6 50% tissue culture infectious dose/ml) at the concentration of 5×10^6 cells/ml for 2 h. The culture was subsequently diluted with prewarmed culture medium to 10^6 cells/ml, and incubated at 37°C in humidified 5% CO_2 for 5–7 d. The infected cultures were collected when >50% of cells exhibited the typical enlarged, homogeneously rounded morphology, and dead cells were removed by centrifugation through a Nycoprep 1.068 gradient (Nicomed, Oslo, Norway). Cell viability after Nycoprep treatment was >90%, as assessed by trypan blue exclusion.

Cytotoxicity Assays. The NK-sensitive target cell line K562 was maintained in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine. It was maintained free of mycoplasma contamination, as determined by the GenProbe mycoplasma contamination kit (Gen-Probe Inc., San Diego, CA). Freshly derived PBL and NK populations obtained either without in vitro activation or after cultivation in complete medium for 1 wk were mixed with target cells at different E/T ratios in V-bottomed 96-well plates. NK clones were tested against different target cells at several E/T ratios in triplicate wells. Although experiments always included titrations of effector cells, some of the data are presented at a given E/T ratio, as indicated in the figure legends. Target cells ($0.5–1 \times 10^6$) were washed, resuspended in 200 μl of RPMI 1640 supplemented with 50% FCS, labeled with sodium ^{51}chromate (100 μCi; Amersham Corp., Arlington Heights, IL) for 2 h, washed twice, counted, and resuspended at a final concentration of 5×10^4 cells/ml. 100 μl of the suspension was added to the effector cells in a 96-well V-bottomed plate. The plate was centrifuged at 600 g for 2 min and incubated at 37°C for 4 h. The supernatant was harvested (Skatron, Inc., Sterling, VA) and counted in a gamma counter (Packard Instr., Meriden, CT). Maximum chromium release was determined by lysing target cells in PBS + 1% Triton X-100. Specific release was calculated as described (24). Target cell recognition by NK clones was considered positive when ⩾20% specific lysis was achieved. Each NK clone was assigned a "specificity" type according to the

[1] *Abbreviation used in this paper:* HHV-6, human herpesvirus 6.

pattern of lysis of HHV-6-infected target cells from four donors. The 16 possible patterns of lysis of the 4 infected target cells were called A–P, as detailed in the legend to Fig. 4.

Results

Lysis of HHV-6-infected Cells by Autologous NK Cells. To test whether normal PBMC infected by HHV-6 could be lysed by autologous NK cells, PHA blasts, either uninfected or infected, were incubated with freshly derived autologous PBL (Fig. 1 *A*) or with enriched unstimulated CD3$^-$ cells (Fig. 1 *B*). A pure population of CD3$^-$ cells expanded in IL-2 was also tested (Fig. 1 *C*). A strong lytic activity specific for the infected cells was clearly detectable in the IL-2 expanded CD3$^-$CD56$^+$ cells (Fig. 1 *C*), as well as with NK cells that had not been stimulated with IL-2 in vitro (Fig. 1 *B*). A much lower level of lysis of infected cells was observed with unfractionated PBL (Fig. 1 *A*). The level of lysis obtained with HHV-6-infected target cells was lower than that obtained with the classical NK target cell line K562, suggesting that virus-infected autologous cells may be inefficiently lysed by individual NK cells, or that only a fraction of NK cells may recognize autologous infected targets.

To investigate these two possibilities, a total of 132 NK clones isolated from the same donor (no. 1) in five independent cloning experiments were tested for their ability to lyse K562 or autologous PHA blasts, either uninfected or infected with HHV-6. While none lysed autologous uninfected cells, and all lysed K562 efficiently, approximately half of the clones (65/132) were able to specifically lyse infected cells ($\geq$20% specific lysis at an E/T ratio of 8). In addition, there was a noticeable and completely reproducible variation between NK clones in the magnitude of the cytolytic activity. The frequency of lytic clones was not related to the cloning

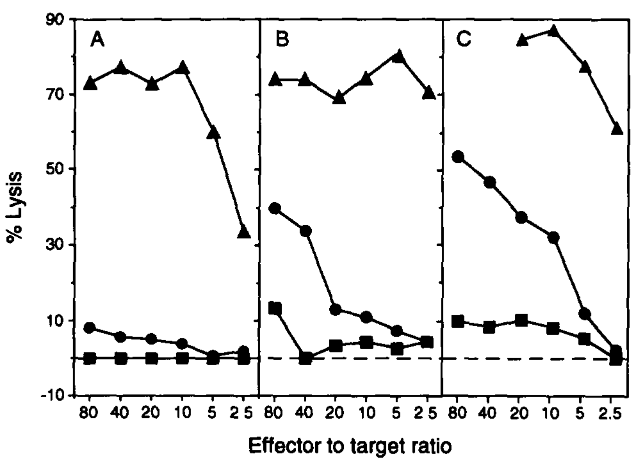

Figure 1. Purified CD3$^-$CD56$^+$ cell populations recognize HHV-6-infected autologous cells. Freshly isolated PBL (*A*), a freshly isolated and enriched CD3$^-$CD56$^+$ cell population (*B*), or a purified CD3$^-$CD56$^+$ cell population expanded in IL-2 (*C*) were tested for their ability to kill autologous PHA blasts that were either uninfected (*squares*) or infected with HHV-6 (*circles*). Killing of the NK target cell line K562 (*triangles*) was also measured.

efficiency (which varied between 1 and 10%), and did not change in different cloning experiments. Results obtained with 30 clones are shown in Table 1. The lysis level of autologous infected cells by each NK clone was highly reproducible in separate experiments. These clones belonged to three distinct subpopulations of NK cells according to the expression of the surface molecules defined by mAbs GL183 and EB6 (Table 1).

NK clones themselves can be infected by HHV-6, provided that such NK clones are unable to kill HHV-6-infected autologous target cells (26). The ability of NK clones to kill autologous HHV-6-infected cells was the same whether lysis was assayed with uncloned PHA blasts or cloned NK cells as targets (data not shown). Therefore, the observed heterogeneity in the ability of different NK clones to lyse autologous infected targets is not due to a heterogeneous sensitivity to lysis of the target cells.

Surface Expression of Class I MHC Molecules on HHV-6-infected Cells. Several viruses have been suggested to evade immune surveillance by downregulating the expression of MHC class I molecules in the infected cells (27). It has also been reported that absence of self-class I molecules can render hematopoietic cells sensitive to lysis by NK cells (15, 16, 28, 29). However, HHV-6 infection of umbilical cord lymphocytes did not affect cell surface levels of MHC class I molecules (P. Lusso, unpublished observation). Several antibodies were used to test whether surface levels of class I molecules were affected on HHV-6-infected PHA blasts (Fig. 2). Activated T cells infected for 6 d with HHV-6 were compared with the same uninfected cells incubated for 6 d under the same conditions. An antibody to an HHV-6 envelope protein revealed that virtually all cells in the culture were productively infected (Fig. 2 *a*). The surface level of class I heavy chains, detected with a mAb to a nonconformational epitope of class I, was unaffected by HHV-6 infection (Fig. 2 *b*). On the other hand, the surface levels of class I/β_2-microglobulin complexes, HLA-A2 molecules (the only detectable A allele on donor 4), and HLA-B + HLA-C molecules were somewhat reduced (to ~75, ~85, and ~55% of control levels, respectively) on infected cells (Fig. 2, *c–e*). Similarly, the level of β_2-microglobulin was slightly reduced in infected cells (Fig. 2 *f*). Two additional antibodies (SA24.23 and F4.326), reacting preferentially with HLA-B and HLA-C molecules, showed a similar reduction (not shown). The data suggest that on infected cells surface levels of heavy chains complexed with β_2-microglobulin may be slightly lower than on uninfected cells.

Heterogeneous Ability of NK Clones to Lyse Autologous and Allogeneic HHV-6-infected Cells. The observation that only half of the NK clones exceeded 20% lysis of autologous HHV-6-infected cells raised the question of whether the ability to kill an infected target is the sole property of the effector cell clone, or whether it is also controlled by target cell elements. To specifically address this question, a panel of 32 clones derived from donor 1 and of 13 clones from donor 2 were assayed with autologous targets, as well as with 3 unrelated allogeneic targets that were either uninfected or infected with

Table 1. *Lysis of HHV-6-infected Cells by Autologous NK Clones*

Clone	Phenotype		Percent lysis*		
	GL183	EB6	Uninfected PHA blasts	HHV-6-infected PHA blasts	K562
3S46R	–	+	1.1	41.8	80.1
19S46R	–	+	0.0	14.7	75.3
12S46P	–	+	0.0	5.7	82.7
15S46P	–	+	0.0	4.1	77.0
12S/S	–	+	0.0	3.5	77.7
A18/6	–	+	0.0	25.3	90.4
25D5/1	–	+	0.0	8.3	84.2
A2/3	–	+	0.0	7.7	79.5
A36/6	–	+	0.0	10.8	93.0‡
17D3/6	–	+	0.0	23.7	81.0‡
14D5/1	–	+	0.7	23.8	81.7
A27/6	–	+	0.0	24.2	76.0
24S46P	+	+	0.0	3.6	80.7
23S/S	+	+	0.0	13.0	78.8
14S46P	+	+	0.0	9.3	88.6‡
7BIS	+	+	0.5	4.9	77.6‡
A3/3	+	+	0.0	7.8	73.7
22D3/3	+	+	0.0	1.8	73.7
A31/6	+	+	1.0	31.6	73.4
9D5/1	+	+	0.0	41.1	84.8‡
6D5/3	+	+	0.0	0.0	69.2
31-A§	+	+	0.0	0.0	99.3‡
7	–	–	3.8	28.7	99.9‡
19	–	–	0.0	86.4	91.0‡
63	–	–	6.9	66.5	94.0‡
01-A§	–	–	5.3	50.4	67.3‡
07-A§	–	–	0.0	84.2	93.3‡
9S46P	–	–	0.0	30.6	85.2‡
13S/S	–	–	0.7	43.6	87.2
A4/3	–	–	0.0	36.9	74.7‡

* Average from two or three separate experiments of specific lysis measured at an E/T ratio of 8. The variability between experiments in the level of lysis by each clone was very small. Out of the 77 average values shown, 66 were derived from experimental values with a deviation from the mean of ≤5%, 9 with a deviation from the mean of ≤10%, and 2 with a deviation from the mean of >10% (clone 24S46P on target K562 with 86.6, 88.5, and 67.2% lysis; clone 19 on HHV-6-infected targets with 72.9 and 100%).
‡ These 13 clones were tested only once on the target K562.
§ Measured at an E/T ratio of 5.

HHV-6. A few clones displayed allospecific lysis of uninfected targets. In contrast, a great heterogeneity among NK clones was seen in their ability to lyse infected targets from different individuals, as illustrated in Fig. 3. For example, in repeated experiments, clone 25D5/1 only lysed one of the allogeneic infected targets (Fig. 3 *A*), clone 17D3/6 lysed infected targets to varying degrees (Fig. 3 *B*), and clone A3/3 did not reach 20% lysis with any of the infected targets (Fig. 3 *C*). Similarly, different specificities were represented among the NK clones from donor 2, three of which are displayed in Fig. 3, *D–F*. Testing the clones over a wide range of E/T ratios revealed that nonkiller phenotypes were not reversed at higher concentrations of effectors, and that all the clones had very similar lytic activities on the cell line K562 (Fig. 3). Thus,

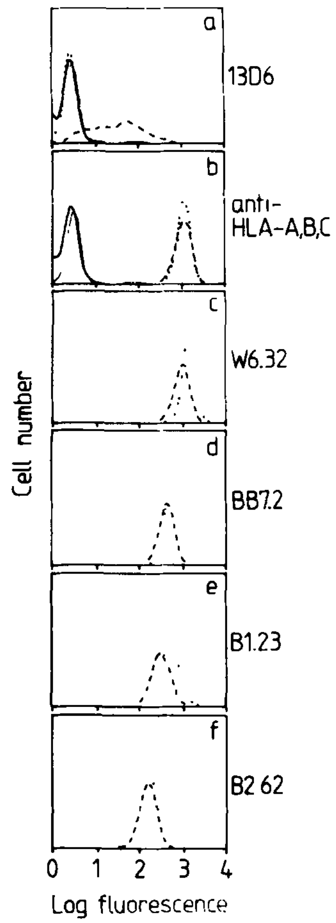

Figure 2. HLA class I expression on HHV-6-infected cells. Infected and uninfected PHA blasts from donor 4 cultured for 6 d were stained with the following antibodies: (*a*) 13D6, mAb to an HHV-6 envelope glycoprotein; (*b*) anti-HLA-A, -B, -C, mAb to class I heavy chains; (*c*) W6.32, mAb to class I heavy chain and β_2-microglobulin complex; (*d*) BB7.2 mAb specific for HLA-A2; (*e*) B1.23, mAb specific for HLA-B and -C molecules; and (*f*) B2.62, mAb specific for β_2-microglobulin. Uninfected cells are represented by the dotted line, infected cells by the dashed line. *a* and *b* also show cells stained with the second reagent only as control for background fluorescence (*heavy solid line*, uninfected; *light solid line*, infected cells).

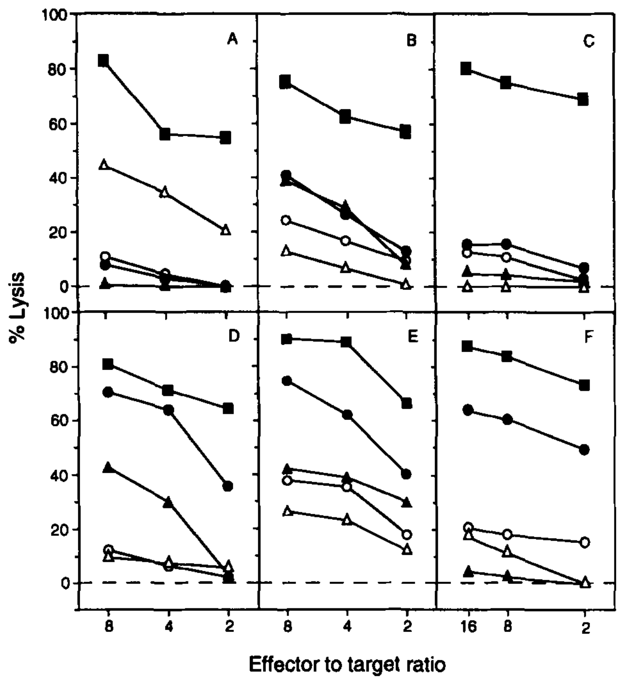

Figure 3. Heterogeneous recognition of autologous and allogeneic HHV-6-infected cells by NK clones. Three representative clones derived from donor 1: 25D5/1 (*A*), 17D3/6 (*B*), and A3/3 (*C*), and three clones from donor 2: 12wA-7 (*D*), 12w53-10 (*E*), and 12wA-14 (*F*) were tested for their ability to lyse K562 cells (*squares*), and HHV-6-infected PHA blasts from donor 1 (*open circles*), 2 (*filled circles*), 3 (*filled triangles*), and 4 (*open triangles*). Killing of uninfected PHA blasts, either autologous or allogeneic, was at background levels (see Fig. 4).

target cell elements control the ability of NK clones to lyse HHV-6-infected cells.

A complete analysis of 16 representative NK clones from donor 1 and 12 clones from donor 2 is displayed in Fig. 4. For each clone, the level of lysis achieved with uninfected and HHV-6-infected cells from four donors was a stable and reproducible property. Out of the 16 possible types of specificities for lysis of 4 infected targets, 9 were represented among NK clones from donor 1 (only 6 are included in Fig. 4), and 8 types were represented among the clones from donor 2.

One of the allogeneic targets (donor 4) carried the NK-1 allospecificity previously described (18, 30). As expected, allogeneic activity against uninfected target 4 was detected primarily within the GL183$^-$EB6$^+$ subset of NK clones (Fig. 3 *C*). A total of 28 of 40 GL183$^-$EB6$^+$ clones from donor 1 lysed the NK-1 allospecific target 4 (only three are shown in Fig. 4). Infection of target 4 did not alter its sensitivity to allospecific NK clones (Fig. 3 *H*).

Some correlations between the surface phenotype of the clones and their type of target cell lysis specificity were noted. For example, all five GL183$^-$EB6$^-$ clones of donor 1 (three are shown in Fig. 4) killed all the infected targets, but this property was shared by only 3 of 16 GL183$^+$EB6$^+$ clones (one is shown in Fig. 4) and by none of 8 GL183$^-$EB6$^+$ clones. Each phenotypic group of NK clones from the same donor contained specificity types that had little overlap with other phenotypic groups. Of the nine specificity types found in clones from donor 1, only two (types A and B) were shared between phenotypic groups, and none of the eight specificity types in clones from donor 2 overlapped between phenotypic groups.

Distinct Specificities in Target Cell Recognition between NK Clones from Two Unrelated Donors. Because of the polymorphic nature of the cellular elements that control resistance or susceptibility to lysis by NK clones (Figs. 3 and 4), NK cells from different donors may display different abilities to lyse target cells after virus infection. A comparison between NK clones from donors 1 and 2 revealed differences in their target cell specificities, even between clones belonging to the same phenotypic subgroup (Fig. 4). For example, in donor 1 autologous infected target cells were killed mainly by GL183$^-$EB6$^-$ clones, whereas in donor 2 this property was shared by GL183$^-$EB6$^-$ and GL183$^+$EB6$^+$ clones (Table 1 and Fig. 4). Similar differences were observed when the various groups of clones were tested against allogeneic infected target cells. For example, of the eight specificity types displayed by GL183$^+$EB6$^+$ clones only one (type M) was shared by clones from both donors.

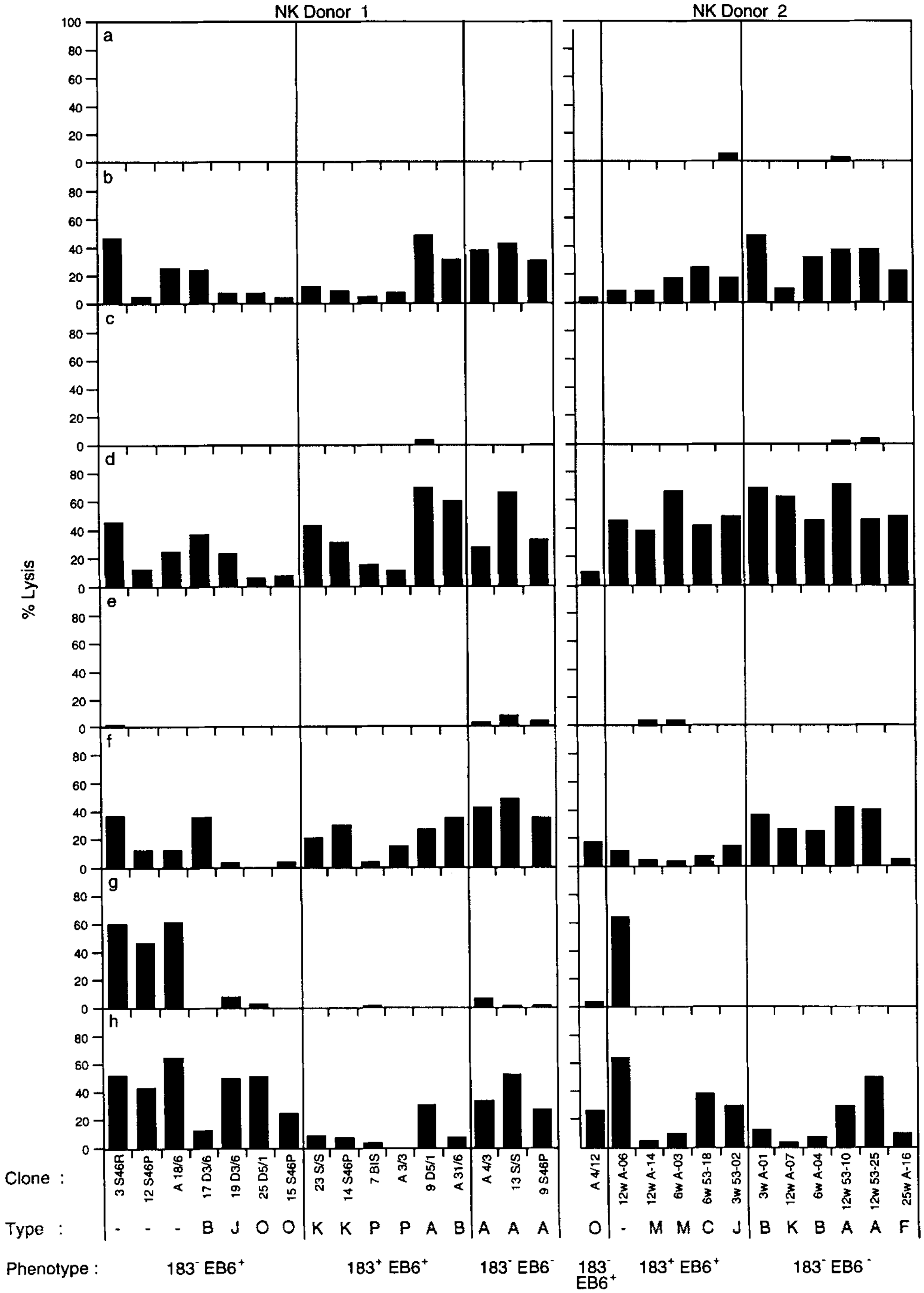

Figure 4. Restricted recognition of HHV-6-infected cells by NK clones. 16 clones from donor 1 (*left*) and 12 clones from donor 2 (*right*) were tested against the following PHA blast target cells: uninfected no. 1 (*a*), HHV-6-infected no. 1 (*b*), uninfected no. 2 (*c*), HHV-6-infected no. 2 (*d*), uninfected no. 3 (*e*), HHV-6-infected no. 3 (*f*), uninfected no. 4 (*g*), and HHV-6-infected no. 4 (*h*). Data represent averages from at least two experiments. Out of the 224 average values shown, 199 were derived from experimental values with a deviation from the mean of ≤5%, 23 with a deviation from the mean of ≤10%, and 2 with a deviation from the mean of >10% (clone A31.6 in *d* with 54.9, 87.7, and 39.5% lysis; clone 12wA-06 in *g* with 51.0 and 81.3% lysis). The type of specificity in the lysis of HHV-6-infected targets is indicated for each clone as follows: lysis (≥20% at an E/T ratio of 8) of all four targets (*A*), all but no. 4 (*B*), all but no. 3 (*C*), only nos. 1 and 2 (*F*), only nos. 2 and 4 (*J*), only nos. 2 and 3 (*K*), only no. 2 (*M*), only no. 4 (*O*), or none (*P*). Clones displaying allospecific lysis of uninfected target no. 4 (–) were not included in this classification. NK clones have been grouped according to their phenotype, as indicated.

Discussion

Several new conclusions can be drawn from the results of this study. First, normal, untransformed cells infected with HHV-6 can be specifically recognized by autologous NK cell clones. This experimental system is an improvement over previous studies that have relied mostly on bulk populations of NK cells tested on nonautologous transformed cell lines. Second, recognition of virus-infected target cells does not require in vitro activation of the NK cells. The role of NK cells in the control of virus infection in humans has not been directly assessed. However, the occurrence of multiple and severe herpetic infections in patients congenitally lacking NK cells (31, 32) suggests that NK cells provide an important primary defense against viruses belonging to the *Herpesviridae* family. Our finding of a consistent lysis against HHV-6-infected autologous cells without the need of in vitro priming supports the view of a direct role for NK cells in the control of HHV-6 infections. Specificity at the level of individual NK clones was evident in that only a subset of clones lysed autologous HHV-6-infected cells. This distinction into killers and nonkillers of autologous HHV-6-infected cells correlated with the susceptibility of NK cells themselves to infection by this virus (26). Interestingly, HHV-6 infection seems to be reactivated in the chronic fatigue syndrome (33, 34), and may act as a cofactor in AIDS (35, 36). Both syndromes are associated with a reduced NK activity (37–40).

The third and most important conclusion is that lysis of infected cells by NK clones is controlled by target cell elements. HHV-6-infected PHA blasts from a particular donor may be lysed by some NK clones but not by others. In turn, NK clones display different specificities of lysis when tested with infected targets derived from several unrelated donors. This form of restricted target cell recognition by NK clones is quite different from MHC-restricted T cell recognition because most of the NK clones are not limited to lysis of autologous infected cells. Some NK clones that did not lyse infected autologous target cells were able to lyse allogeneic infected targets.

The fourth conclusion was derived from the analysis of a large panel of NK clones from two donors that were tested for lysis of HHV-6-infected PHA blasts from four individuals: a great heterogeneity exists in target cell specificities among NK clones from a single donor. Furthermore, the direct comparison between NK clones from donors 1 and 2 revealed differences in their specificities of target cell lysis. Even clones belonging to the same phenotypic subgroup, as defined by the mAbs GL183 and EB6, displayed different specificities in the two donors. Although these data are consistent with the existence of distinct NK repertoires in different individuals, it remains possible that different specificities in donors 1 and 2 were somehow selected by the cloning procedure. Different repertoires of NK clones may develop in individuals because of the requirement for NK cells to tolerate uninfected autologous cells, and because of the polymorphic nature of the cellular elements that control resistance or susceptibility to lysis by NK clones. To explain the observed heterogeneity in the specificities of NK clones, HHV-6 infection must have a selective influence on the expression or structure of the different allelic forms of these target cell elements. It is unlikely that simple downregulation of cell surface MHC class I molecules caused by HHV-6 can account for the observed sensitivity to NK lysis. First, surface levels of HLA class I molecules were only partially reduced on HHV-6-infected cells. Second, simple absence of class I alleles cannot explain the vast heterogeneity in the specificities of NK clones.

The heterogeneity displayed by NK clones tested with HHV-6-infected PHA blasts appears more complex than that described so far for NK clones able to kill normal uninfected allogeneic cells. However, three characteristics described for allorecognition by NK clones belonging to different phenotypic subgroups are also applicable to the present study of NK activity against virus-infected cells, namely: (*a*) a given target cell can be susceptible to lysis by NK clones recognizing different specificities (complex haplotype); (*b*) the same phenotypic subset may be directed towards different allospecificities in different donors; and (*c*) clones displaying different specificities may be confined to the same subset (30).

Two models have been proposed to account for target cell recognition by NK cells. According to the masking hypothesis (41), class I molecules mask a putative target structure recognized by NK cells. Recognition by NK cells occurs when the target structure is unmasked, as a consequence of dissociation or absence of class I molecules. This model is difficult to reconcile with the allospecific recognition by NK cells, unless a polygenic system of target structures, expressed in all individuals, is postulated, some of which are masked to provide self-tolerance, while others, which are not complexed with self-class I molecules, provide targets for allorecognition.

Another model, derived from the "missing self" hypothesis, suggests that NK cells receive a negative signal when self-class I is recognized (42). The absence of self, or the presence of modified self, would fail to turn off NK cells, and lysis would take place. Combined with recent data suggesting an involvement of peptides bound to class I molecules in target cell recognition by NK cells (43–45), the "missing self" hypothesis is compatible with the data presented here. Recognition of class I/peptide complexes by NK cells may be mediated by a group of receptor molecules that are selectively expressed on different NK clones. NK recognition of class I/peptide complexes could be disrupted in virus-infected cells due to occupancy of class I molecules by viral peptides. The restricted recognition of different target cells by the same NK clone observed in this study could be explained by the fact that each NK receptor molecule recognizes a group of related class I molecules (as in the case of recognition of $H\text{-}2^d$ or $H\text{-}2^k$ by Ly-49; reference 11) that display different affinities for viral peptides. For example, certain autologous class I/peptide complexes may not be affected by virus infection, resulting in resistance to lysis by some autologous NK clones, even though the same clones may recognize allogeneic infected cells in which a related class I molecule was affected by infection. The complex patterns of target cell recognition by NK clones observed here probably do not result from a simple downregulation of specific class I alleles in HHV-6-infected cells, but rather from a differential effect on self-epitopes expressed in the context of class I molecules. However, the putative role of MHC class I molecules in the recognition of virus-infected cells by NK effectors remains to be established.

The data presented here clearly demonstrate that, in the natural situation of virus infection, polymorphic elements expressed on the host cells dictate whether lysis by specific NK cells will occur and, further, that a wide range of target cell specificities is exhibited by NK clones isolated from a single individual.

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