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The natural killer cell receptor Ly-49A recognizes a peptide-induced conformational determinant on its major histocompatibility complex class I ligand

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ABSTRACT **Natural killer (NK) cells are inhibited from killing cellular targets by major histocompatibility complex (MHC) class I molecules. In the mouse, this can be mediated by the Ly-49A NK cell receptor that specifically binds the H-2D^d MHC class I molecule, then inhibits NK cell activity. Previous experiments have indicated that Ly-49A recognizes the $\alpha1/\alpha2$ domains of MHC class I and that no specific MHC-bound peptide appeared to be involved. We demonstrate here that alanine-substituted peptides, having only the minimal anchor motifs, stabilized H-2D^d expression and provided resistance to H-2D^d-transfected, transporter associated with processing (TAP)-deficient cells from lysis by Ly-49A$^+$ NK cells. Peptide-induced resistance was blocked only by an mAb that binds a conformational determinant on H-2D^d. Moreover, stabilization of "empty" H-2D^d heavy chains by exogenous β_2-microglobulin did not confer resistance. In contrast to data for MHC class I-restricted T cells that are specific for peptides displayed by MHC molecules, these data indicate that NK cells are specific for a peptide-induced conformational determinant, independent of specific peptide. This fundamental distinction between NK cells and T cells further implies that NK cells are sensitive only to global changes in MHC class I conformation or expression, rather than to specific pathogen-encoded peptides. This is consistent with the "missing self" hypothesis, which postulates that NK cells survey tissues for normal expression of MHC class I.**

Natural killer (NK) cells lyse cellular targets that may include certain transformed tumor cells or cells infected with intracellular pathogens (1). Since NK cells do not generally kill normal cells, they must possess mechanisms to discriminate among their targets. Major histocompatibility complex (MHC) class I molecules, almost ubiquitously expressed on normal tissues, provide one means by which NK cells discriminate among targets; tumor cells expressing MHC class I are more resistant to lysis by NK cells than derivatives that specifically lack MHC class I expression (2–5). To explain this phenomenon, it has been hypothesized that NK cells survey tissues for the normal expression of MHC class I (6). In the absence of MHC class I or under conditions of its dysregulated expression, NK cells are somehow released from its inhibitory influence and kill the target. Thus, in accord with the "missing self" hypothesis (6, 7), NK cells can distinguish between normal (self) and aberrant or absent (nonself) MHC class I molecules on potential cellular targets.

Recent studies indicate that this MHC class I-dependent resistance to NK lysis is mediated by surface NK cell receptors that engage target cell MHC class I (8–12). As a result of this interaction, NK cells are inhibited from killing targets. In the mouse, the Ly-49A NK cell receptor, a member of a family of highly related molecules, is such a receptor (13). We previously demonstrated that the engagement of Ly-49A by H-2D^d led to global inability of Ly-49A$^+$ NK cells to kill targets *in vitro* and to decreased expression of Ly-49A *in vivo* (14–16). Previous analysis suggested that Ly-49A recognizes the $\alpha1/\alpha2$ domains of H-2D^d because (*i*) an anti-$\alpha1/\alpha2$-domain-specific mAb but not an anti-$\alpha3$-domain-specific mAb blocked the interaction in cytotoxicity and cell binding experiments (14, 15); and (*ii*) Ly-49A was down-regulated in B10.D2^{dm1} mice (16) that express the mutant chimeric dm1 MHC class I molecule consisting of only the $\alpha1$ and NH_2-terminal half of the $\alpha2$ domains of H-2D^d (with the remainder of the MHC class I heavy chain derived from H-2L^d; ref. 17). Since H-2L^d does not interact with Ly-49A as measured in cytotoxicity or cell binding assays (14, 15), this localizes Ly-49A binding to the $\alpha1$ and NH_2-terminal half of the $\alpha2$ domain of H-2D^d, consistent with the mAb blocking data.

With respect to the region of the MHC class I molecule critical to its interaction, Ly-49A therefore resembles the polymorphic MHC class I-restricted antigen-specific receptors on T cells (TCRs) that recognize complexes of peptide bound to the polymorphic $\alpha1/\alpha2$ domains of MHC class I molecules (18), in contrast to monomorphic CD8 "accessory" molecules that bind less polymorphic $\alpha3$ domains (19). Indeed, several studies in human and mouse systems suggest that peptides may play a role in MHC class I-associated resistance (20–22). However, some of these studies used experimental systems involving bulk NK cell populations and targets that expressed MHC class I normally. Exogenous peptides could confer resistance, but it was unclear whether sufficient displacement of endogenous peptides would occur and whether specific NK cell receptors, such as Ly-49A, were involved in such effects. Thus, the role of MHC class I-associated peptides required further investigation.

Some clarification has come from recent examination of this issue by Correa and Raulet, who performed important experiments with the transporter associated with processing (TAP)-2-deficient cell line RMA-S, transfected with H-2D^d (23). Although this cell does not express H-2D^d normally on the cell surface, H-2D^d expression could be stabilized by exogenous addition of H-2D^d-binding peptides. This led to target resistance to lysis by Ly-49A$^+$ NK cells. Five of six H-2D^d peptides induced resistance; the sixth was less capable, but it also did not effectively stabilize H-2D^d expression, suggesting that H-2D^d confers resistance regardless of the peptide bound. This study effectively ruled out the possibility that Ly-49A binds glycosylated peptides, as suggested by its homology to the C-type lectins (24). Moreover, no specific endogenous peptide was

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Abbreviations: NK, natural killer; MHC, major histocompatibility complex; TCR, T-cell antigen receptor; E/T ratio, effector-to-target cell ratio; β_2-m, β_2-microglobulin; KIR, killer cell inhibitory receptor; TAP, transporter associated with processing.

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found to be better than any other in conferring resistance (23). This study strongly suggested that peptides play a more generic role, but it remained to be determined what is specifically recognized on MHC class I molecules by Ly-49A. There are several possibilities. (*i*) Ly-49A may bind a peptide-independent linear determinant on the $\alpha1/\alpha2$ domain. The peptide requirement would be explained because they are generally needed for normal MHC class I expression at reasonable levels. (*ii*) Inasmuch as bound peptides are known to confer conformational changes in MHC class I molecules (25–28), Ly-49A may instead bind a generic peptide-induced conformational determinant on H-2D^d. These first two possibilities contrast interactions of the TCR with MHC. (*iii*) Analogous to TCR reactivity, Ly-49A could interact with specific determinants on the peptides themselves or to specific conformational determinants on H-2D^d induced only by certain peptides (18). This possibility predicts that only certain peptides may induce resistance or binding, inconsistent with previous studies of a small group of peptides. Further investigation was therefore warranted because NK cell receptors may recognize MHC class I-peptide complexes in a manner distinct from MHC class I-restricted TCR.

In this study, we have determined that Ly-49A can recognize H-2D^d molecules stabilized by alanine-substituted peptides containing the minimal anchor residues necessary for binding MHC class I. Moreover, when H-2D^d was expressed in an "empty" configuration, at levels equivalent to peptide-loaded H-2D^d molecules, it was not recognized by Ly-49A. Thus, Ly-49A recognizes a peptide-stabilized conformational determinant on MHC class I, independent of bound peptide, indicating a fundamental distinction between MHC class I-specific receptors on NK cells and T cells.

MATERIALS AND METHODS

Cell Lines and Antibodies. The LKD8 cell line (generously provided by Elizabeth Bikoff, Harvard University) is a TAP-deficient, H-2^k-derived, embryonic stem cell transfected with a construct encoding the H-2D^d molecule (28, 29). Hybridomas producing mAbs 28-8-6 (anti-H-2D^d), 34-4-20 (anti-H-2D^d), 34-1-2 (anti-H-2D^d and -H-2K^d), 34-5-8S (anti-H-2D^d-$\alpha1/\alpha2$), 34-2-12S (anti H-2D^d-$\alpha3$), and SF1-1.1.1 (anti-K^d) were obtained from American Type Culture Collection.

Peptides. Peptides used in these experiments (Table 1) were either based on sequences of peptides eluted and purified from native H-2D^d molecules or previously identified viral or antigenic peptides (30–33). Peptides pD239 and pD2359 contain alanine residues at every position except requisite anchor residues. All peptides had been previously synthesized and tested for binding to H-2D^d molecules (30). Peptides were stored lyophilized, reconstituted in PBS, and sterile-filtered for use.

Table 1. Peptides used in this report

Peptide	Sequence 1	2	3	4	5	6	7	8	9	10	11	H-2 specificity
	-	**G**	**P**	-**R**/**K**-		-	-	**L**				D^d
P18-I10	R	**G**	**P**	G	**R**	A	F	V	T	I		D^d
pD36	V	**G**	**P**	Q	**K**	N	E	N	**L**			D^d
pD38A	A	**G**	**P**	D	**R**	T	E	K	**L**			D^d
pD39	K	**G**	**P**	D	**K**	G	N	E	F			D^d
pD44	D	**G**	**P**	V	**R**	G	I	S	I			D^d
pTum$^-$35B	N	**G**	**P**	P	H	S	N	N	F	G	Y	D^d
pD2359	A	**G**	**P**	A	**R**	A	A	A	**L**			D^d
pD239	A	**G**	**P**	A	A	A	A	A	**L**			D^d
pMCMV	Y	P	H	F	M	P	I	N	L			L^d
pOVA$_{257-264}$	S	I	I	N	F	E	K	L				K^d
pL29	A	P	A	A	A	A	A	A	**L**			L^d
PEIA$_{234-243}$S2	S	S	**P**	S	N	T	P	P	E	I		D^b

The amino acid sequences of the indicated synthetic peptides are shown with their specificity for MHC class I molecules (30). Peptides pD239 and pD2359 contain the minimal anchor residues required for binding H-2D^d, and the remaining residues consist of alanine residues. All peptide preparations have been shown to bind H-2D^d and tested in capacity to stimulate H-2D^d-restricted T cells (30–33).

NK Cells. Ly-49A$^+$ and Ly-49A$^-$ NK cells were prepared from interleukin 2-activated NK cells derived from C57BL/6 mice as previously described (14). Briefly, nylon wool nonadherent spleen cells were cultured in recombinant interleukin 2 (800 units/ml) for 3 days. Adherent cells were harvested, depleted of T cells, then cultured for 3 more days. The Ly-49A subsets were isolated by panning with anti-Ly-49A, or complement depletion, as described. After an additional 3 days in culture, the cells were used in cytotoxicity assays.

Cytotoxicity Experiments. LKD8 targets were cultured for at least 2 days in RPMI 1640 medium supplemented with L-glutamine (300 μg/ml), 2-mercaptoethanol (5×10^{-5} M), penicillin (100 units/ml), streptomycin (100 μg/ml), and 2% heat-inactivated fetal calf serum at 37°C. [Under these conditions, MHC class I expression was minimal (data not shown).] LKD8 targets were labeled with sodium [^{51}Cr]chromate in RPMI 1640 medium without fetal calf serum for 1 hr at 37°C, washed, then resuspended in RPMI 1640 medium with 2% fetal calf serum. Labeled targets (10^5) were then distributed to individual wells of 96-well round bottom plates, so as to use a single preparation of labeled targets. (This eliminated variability in target labeling that would have occurred if targets were preincubated with peptide and then each loaded target was separately labeled.) As indicated, peptides or human β_2-microglobulin (β_2-m; Calbiochem) were added, then the wells were incubated at 37°C for 1 hr. [Preliminary experiments with addition of exogenous peptides demonstrated that a 1-hr incubation with peptide stabilized MHC class I expression, as determined by flow cytometric experiments with mAbs specific for H-2D^d, to approximately the same level as overnight incubation (data not shown).] Effector cells were harvested, washed, resuspended in RPMI 1640 medium containing 2% fetal calf serum, and added to the assay wells that were then incubated at 37°C for 4 hr. Cell-free supernatants were harvested, and radioactivity was assessed by gamma counter. The percentage of specific cytotoxicity was determined by a standard formula (14).

RESULTS

To analyze further the role of MHC class I-bound peptides in resistance to NK cells, we used LKD8 cells, a mouse embryonic cell line that manifests a TAP deficiency and has an MHC class I-deficient phenotype resembling RMA-S (28). Importantly, it was previously transfected with H-2D^d and we found that it was extremely susceptible to natural killing (Fig. 1*A*). However, when a peptide that stabilized H-2D^d was added, the target became resistant to lysis only by Ly-49A$^+$ NK cells (Fig. 1*A*). The target remained susceptible to killing by Ly-49A$^-$ NK cells, and peptides specific for H-2D^b or H-2L^d did not confer resistance. All peptides known to bind H-2D^d were also effective in stabilizing H-2D^d expression on LKD8. In addition, these conferred resistance to lysis by Ly-49A$^+$ NK cells (Fig. 1*B*). We also tested a synthetic peptide derived from the pTum$^-$ 35B peptide, identified by Szikora *et al.* (34), taking into account the H-2D^d motif. In contrast to an NH_2-terminal truncation previously studied (23), we found that our pTum$^-$ 35B-derived peptide functioned well in assays of H-2D^d stabilization and inhibition of cytolysis. Thus, all peptides capable of stabilizing H-2D^d expression on LKD8 also produced resistance to lysis by Ly-49A$^+$ NK cells.

To evaluate the possibility that Ly-49A recognition could be sensitive to alterations at any one of the amino acid positions,

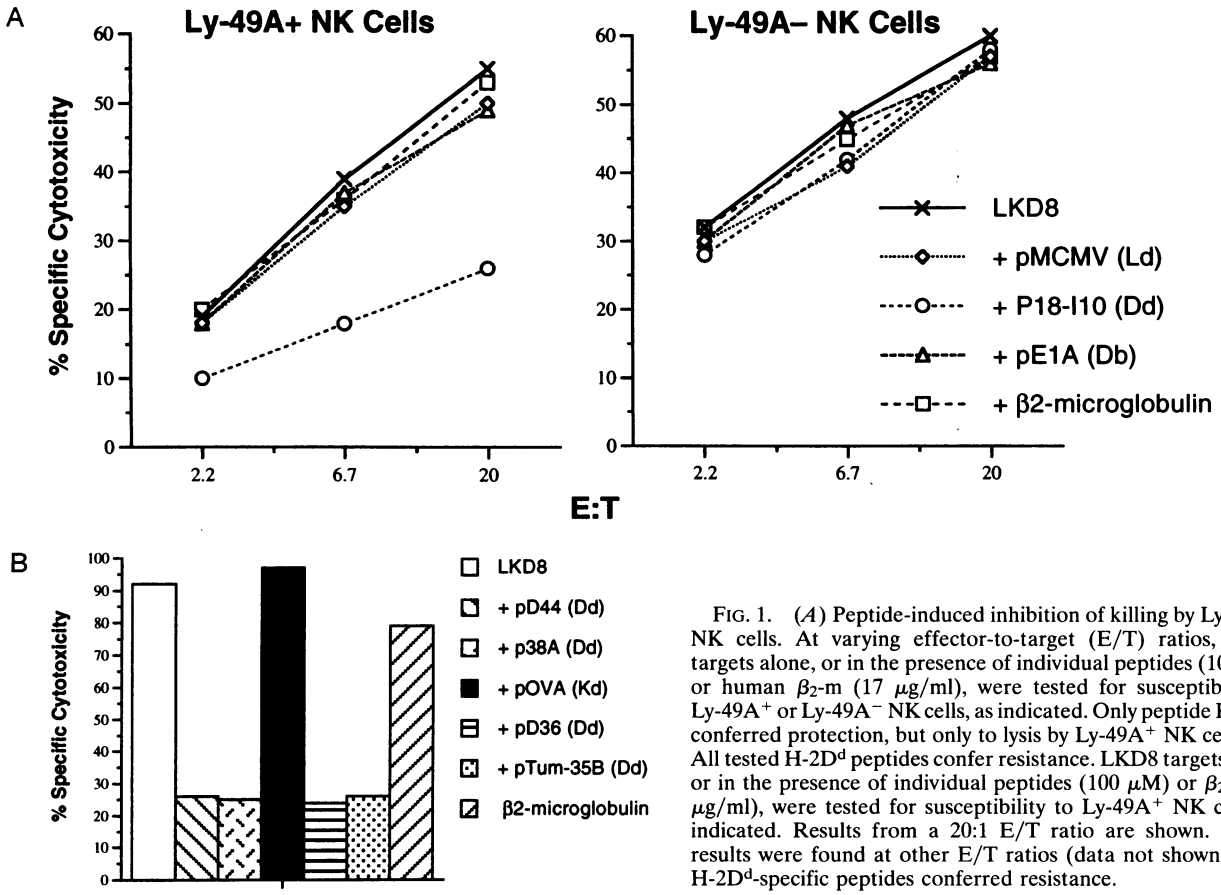

FIG. 1. (*A*) Peptide-induced inhibition of killing by Ly-49A+ NK cells. At varying effector-to-target (E/T) ratios, LKD8 targets alone, or in the presence of individual peptides (100 μM) or human β_2-m (17 μg/ml), were tested for susceptibility to Ly-49A$^+$ or Ly-49A$^-$ NK cells, as indicated. Only peptide P18-I10 conferred protection, but only to lysis by Ly-49A$^+$ NK cells. (*B*) All tested H-2D^d peptides confer resistance. LKD8 targets alone, or in the presence of individual peptides (100 μM) or β_2-m (17 μg/ml), were tested for susceptibility to Ly-49A$^+$ NK cells, as indicated. Results from a 20:1 E/T ratio are shown. Similar results were found at other E/T ratios (data not shown). Only H-2D^d-specific peptides conferred resistance.

we used alanine-substituted peptides that contained the minimum anchor residues required for H-2D^d binding. At saturating concentrations, these peptides conferred essentially equivalent stability of H-2D^d as assessed by reactivity with mAbs 34-2-12S and 34-5-8S, specific for a determinant on α3 or a conformational determinant on α1/α2, respectively (data not shown). However, H-2D^d molecules loaded with the alanine-substituted peptides did not display the epitope recognized by mAb 28-8-6, suggesting that this mAb recognizes an epitope dependent on specific peptide residues (data not shown). This observation is consistent with the finding that only a proportion of normal H-2D^d molecules bind to mAb 28-8-6S (35). Nevertheless, the alanine-substituted peptides were capable of inducing resistance to Ly-49A$^+$ NK cells (Fig. 2). Moreover, the alanine-substituted peptide was just as efficient as native peptide in conferring resistance, as indicated by dose-response experiments (Fig. 3), even though the relative affinity of the alanine-substituted peptide for H-2D^d is lower than the native peptide (30). In contrast to the data for TCRs, therefore, these data strongly suggest that Ly-49A recognition of H-2D^d does not involve direct interaction with peptide.

To evaluate further the epitope on peptide-loaded H-2D^d recognized by Ly-49A, we attempted to block the interaction between these molecules with different mAbs (Fig. 4). Successful blockade leads to reversal of resistance, i.e., restoration of killing (14). Although epitopes for all mAbs were reconstituted by peptide (data not shown), only mAb 34-5-8S was capable of completely restoring lysis, consistent with our prior observations on native H-2D^d molecules (14). Moreover, because mAb 34-5-8S is specific for a private epitope on H-2D^d, this result rules out a surreptitious effect from endogenous H-2^k class I molecules. The mAb 28-8-6 did not have any effect, even though it bound to H-2D^d molecules loaded with native peptides, consistent with the alanine-substituted peptide analysis. These data suggested that Ly-49A may recognize a determinant that is similar to the epitope bound by mAb 34-5-8S.

Inasmuch as mAb 34-5-8S binds a peptide-induced conformational epitope on the α1/α2 domain of H-2D^d (28), we sought to determine if Ly-49A binds a similar determinant. As previously reported (28), exogenous human β_2-m stabilized H-2D^d on the cell surface, as indicated by staining with mAb specific for the α3 domain of H-2D^d in flow cytometric analysis (Fig. 5 *A* and *B*). This level was essentially equivalent to staining of peptide-stabilized H-2D^d. However, β_2-m was relatively less efficient at inducing the epitope recognized by mAb 34-5-8S (Fig. 5 *C* and *D*). Because this occurred without added peptide, this β_2-m stabilization is likely to be of "empty" H-2D^d molecules on the cell surface, as described (28). Most

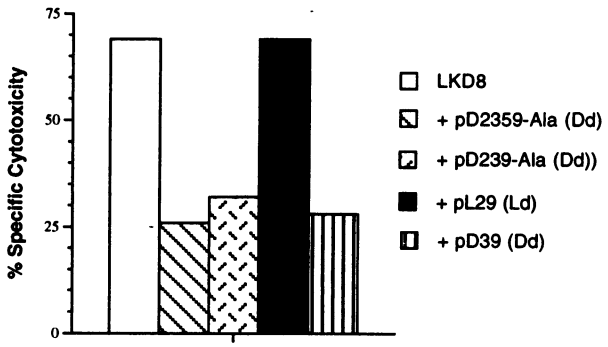

FIG. 2. Alanine-substituted peptides confer resistance. LKD8 targets alone, or in the presence of individual peptides (100 μM) representing native peptides or alanine-substituted peptides, were tested for susceptibility to Ly-49A$^+$ NK cells, as indicated. Results from a 6.7:1 E/T ratio are shown. Similar results were found at other E/T ratios (data not shown).

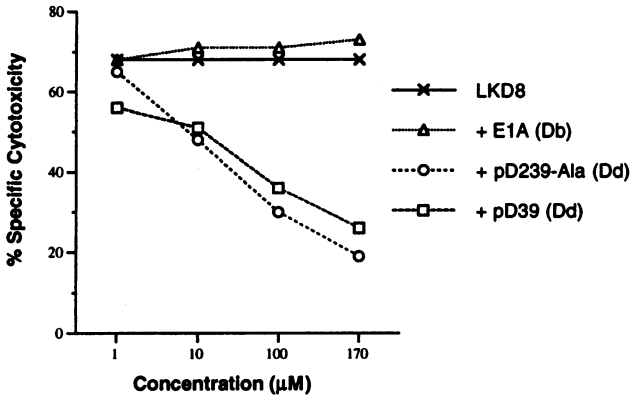

FIG. 3. Alanine-substituted peptides confer resistance similar to native peptide. LKD8 targets alone, or in the presence of individual peptides representing native peptide (pD39) or alanine-substituted peptide (pD2359), were tested for susceptibility to Ly-49A$^+$ NK cells at varying peptide concentrations, as indicated. Results from a 6.7:1 E/T ratio are shown. Similar results were found at other E/T ratios (data not shown).

significantly, peptides did confer resistance to Ly-49A$^+$ NK cells but β_2-m did not (Fig. 5 *E* and *F*). Thus, these data indicated that the level of H-2D^d on the cell surface is not the sole determinant of protection but that H-2D^d molecules must have bound peptide.

DISCUSSION

Our studies indicate that a number of H-2D^d-binding peptides, including synthetic peptides representing the H-2D^d-binding motif, contribute to changes in the "empty" H-2D^d molecules expressed on TAP-deficient cells. Such changes apparently result in a structure that interacts with Ly 49A that then delivers signals to inhibit cytolysis by NK cells. Protection is sensitive to the level of peptide-bound H-2D^d, and the amount of peptide required for protection is significantly higher than that observed for TCR activation. In contrast, molecules stabilized by β_2-m are incapable of delivering the inhibitory signal, indicating that the level of H-2D^d alone (without peptide) is insufficient to confer protection. H-2D^d molecules, stabilized with β_2-m, remain less reactive with mAb 34-5-8S, indicating that the $\alpha1/\alpha2$ domain unit is not folded in a native configuration. Taken together, these findings indicate that Ly-49A appears to recognize a conformational determinant on

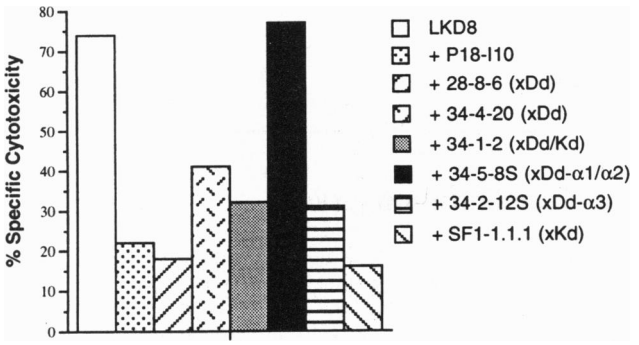

FIG. 4. Reversal of peptide-induced inhibition by an mAb specific for a peptide-induced conformational determinant on H-2D^d. LKD8 targets alone, or in the presence of peptide p18-I10 alone (100 μM) or peptide with different mAbs (10 μg/ml), were tested for susceptibility to Ly-49A$^+$ NK cells, as indicated. Results from a 6.7:1 E/T ratio are shown. Similar results were found at other E/T ratios (data not shown). Only mAb 34-5-8S totally restored killing of peptide-loaded LKD8 targets to the level of unloaded LKD8 cells. mAbs alone (without peptide) had no effect on killing (data not shown).

H-2D^d conferred by the binding of apparently any peptide and that reasonably high levels are needed to achieve protection.

Ly-49A, therefore, may be considered as a monitor of the general "health" of MHC class I molecules on the cell surface. Ly-49A does not appear to be sensitive to replacement of endogenous peptides by foreign peptides; antigenic or viral peptides used here also conferred protection. In contrast, Ly-49A is apparently sensitive to mechanisms that would globally disrupt target cell expression of appropriately loaded MHC class I molecules or interrupt normal MHC class I expression. Inasmuch as Ly-49A is specific for only certain MHC class I molecules, these mechanisms could include such defects as mutations in $\alpha1/\alpha2$ domain of MHC class I heavy chains. Alternatively, such defects could globally affect peptide binding or its conformation, including mutations affecting association of MHC class I heavy chains with β_2-m or, as described here and by others, TAP deficiencies (23, 36). This is consistent with a basic tenet in the "missing self" hypothesis. Moreover, these findings are in striking contrast to the exquisite peptide specificity of TCRs that can distinguish single amino acid substitutions in antigenic peptides, resulting in antagonistic responses or no activation (37). Thus, this is a fundamental distinction between the Ly-49 family of NK cell receptors and TCR in the recognition of MHC class I–peptide complexes.

Our studies also permit a tentative localization of the Ly-49A contact site on peptide-loaded H-2D^d molecules. Because different anti-H-2D^d mAbs bind to distinct regions of H-2D^d molecules, it is of interest that only mAb 34-5-8S was capable of reversing the inhibition of cytolysis conferred by peptide binding to H-2D^d. As expected, mAb 34-2-12S, which recognized the $\alpha3$ domain of H-2D^d, had little or no effect on NK lysis, but surprisingly, mAbs 28-8-6S, 34-4-20S, and 34-1-2S, all of which bind the $\alpha1/\alpha2$ domain (37–40), failed to show the same effect as mAb 34-5-8S. mAb 28-8-6S binds only a subset of H-2D^d molecules, apparently related to the particular self-peptides complexed with MHC, supported by our studies demonstrating that mAb 28-8-6S does not react with H-2D^d molecules loaded with alanine-substituted motif peptides. It also appears to largely interact with a contact surface consisting of the left side of the $\alpha1/\alpha2$-helices and bound peptide as indicated by mutagenic epitope mapping studies and cross-reactivity of mAb 28-8-6S on H-2K^b but not H-2K^{bm10} (which has natural polymorphisms in the left-hand side of the $\alpha2$-helix; ref 41). Clearly, this region is not critical for the interaction of H-2D^d with Ly-49A. On the other hand, mutagenic studies also suggest that 34-5-8S binds to a region of H-2D^d dependent on polymorphisms in the NH_2-terminal part of the $\alpha2$ domain (31). Thus, Ly-49A may interact with this portion of peptide-loaded H-2D^d molecules, in contrast to human killer cell inhibitory receptors (KIR; i.e., p58, NKAT, and NKB1) that interact with the right-hand side of the $\alpha1$-helix (42, 43).

Previous reports of human NK cell specificity also suggest that human KIR molecules can recognize specific peptide–MHC complexes (44). In addition, when Storkus *et al.* analyzed protection against bulk populations of human NK cells, they were able to localize HLA class I-associated resistance to a single residue (Asp-74) in the C pocket of the peptide binding cleft (20), suggesting specific involvement of bound peptides. Other studies in the mouse have suggested that bound peptides, such as those derived from viral infection, may confer NK cell specificity (22). In contrast to the structurally distinct NK receptors, Ly-49A recognition seems to be unaffected by any specific peptide fractions eluted from H-2D^d-bearing cells (23). Although this could represent differences when bulk NK cell populations are used, the human KIR and mouse Ly-49A results may represent differences among receptor systems (8–12). The systems could complement each other because human KIR are structurally distinct from the Ly-49 receptor

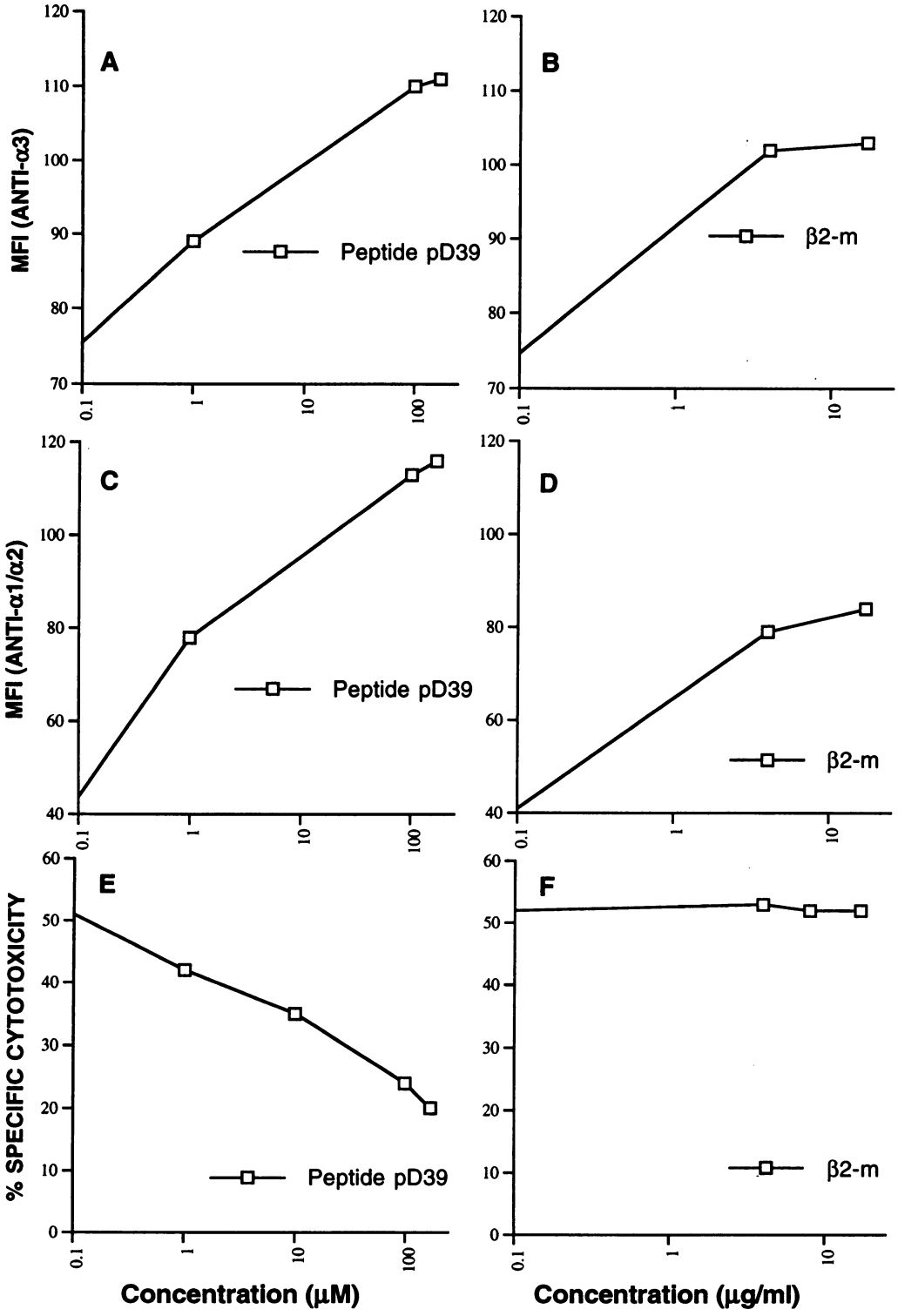

FIG. 5. Ly-49A recognizes a peptide-induced conformational determinant on H-2D^d molecules. LKD8 targets alone, or in the presence of varying concentrations of peptide pD39 (*A*, *C*, and *E*) or human β_2-m (*B*, *D*, and *F*), were analyzed by flow cytometry for reactivity with mAb specific for α3 domain of H-2D^d (mAb 34-2-12S; *A* and *B*) or peptide-induced conformational determinant on α1/α2 domain (mAb 34-5-8S; *C* and *D*), as indicated. Cells incubated in parallel were tested for susceptibility to lysis by Ly-49A$^+$ NK cells (*E* and *F*). Results from a 6.7:1 E/T ratio are shown. Similar results were found at other E/T ratios (data not shown). On the log scale, the lowest concentration in all panels represented no addition and is on the axis. β_2-m stabilized H-2D^d molecules as indicated by anti-α3 staining but poorly conferred the epitope recognized by the peptide-conformation-specific anti-α1/α2 mAb, as previously reported (28). Even at high concentrations, β_2-m did not confer resistance compared with resistance induced by low concentrations of peptide pD39.

family. Alternatively, it is possible that bulky residues in bound peptides could block recognition of folded MHC class I molecules. For Ly-49A, we think this is unlikely, because the synthetic peptides that have NK inhibitory activity include those with different bulky and charged residues at a variety of positions that would be expected to be exposed upon binding to H-2D^d. Therefore, it is possible that the human KIR and mouse Ly-49A receptor systems could be complementary in an

as yet undefined manner, and that further examination of human and mouse homologues of these receptor systems may clarify these differences.

Peptide loading of MHC class I molecules for NK cell inhibition requires significantly higher peptide concentrations than that required for activation of MHC class I-restricted T-cell clones (45). This suggests that there may be threshold differences in activation versus inhibition. Alternatively, this could be reflected by lower affinities of NK cell receptors for MHC class I when compared with TCR affinity or by intrinsic differences in signaling pathways. Nevertheless, the data suggest that MHC class I inhibition would not be subject to minor variations in normal expression, preventing killing of otherwise normal cells.

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