Recognition of Class I Major Histocompatibility Complex Molecules by Ly-49: Specificities and Domain Interactions

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Summary

Ly-49 is a family of type II transmembrane proteins encoded by a gene cluster on murine chromosome 6. One member of this family, Ly-49A, is expressed by a natural killer (NK) cell subset, binds to class I major histocompatibility complex (MHC) molecules, and blocks the killing of target cells bearing the appropriate H-2 antigens. Here we show that another member of this family which is expressed by an NK cell subset, Ly-49C, recognizes H-2^b and H-2^d structures which are distinct from and overlapping with those recognized by Ly-49A. Interactions between Ly-49A and C and their class I ligands are entirely blocked by the antibodies 5E6, YE1/48, YE1/32, and A1, all of which were found to recognize epitopes contained within the carbohydrate recognition domain (CRD). However, cell–cell binding assays revealed that class I binding specificity is conferred by a combination of sequences within both the CRD and a 19–amino acid adjacent region. We also investigated the question of whether Ly-49A and C form dimers on cells which express both receptors. When coexpressed on COS cells, sequential immunoprecipitation demonstrated that these receptors pair exclusively as homodimers, with no evidence for heterodimeric structures. These observations provide insight into both the biochemical nature of the Ly-49 family as well as the receptor functions of Ly-49C on NK cells.

The recognition of certain class I MHC molecules by natural killer cells may result in the delivery of an inhibitory signal to the NK cell which prevents target cell lysis (1). This inhibition can be overcome by blocking with antibodies against either class I MHC or NK cell receptors. In humans, NK cell receptors for class I MHC include a novel family of molecules (NKAT/p58) whose members contain two or three external Ig domains (2–4). Individual members of this family are expressed by subpopulations of NK cells and have distinct specificities for either HLA-C (5, 6) or HLA-B alleles (7, 8). Although it belongs to the molecularly unrelated C-type lectin supergene family (9), CD94 also appears to function as an NK cell receptor for HLA-B (10). Antibodies against all of these receptors block the protection provided to a target cell by specific class I molecules.

In mice, the Ly-49 family has been implicated in class I recognition by NK cells. cDNAs encoding eight members of this family have been identified (Ly-49A-H) (11–15), and polymorphism of individual members has also been reported (Ly-49A, C, and G) (16–18). Studies using monoclonal antibodies which recognize specific members of this family have shown overlapping NK cell subset expression for Ly-49A (20%), Ly-49C (30%), and Ly-49G.2 (50%)

(15, 18–20). Ly-49A binds to H-2D^d and D^k (21, 22) and inhibits the lysis of target cells expressing these antigens (19). Ly-49G.2$^+$ NK cells appear to be inhibited by H-2^d antigens (possibly in a manner distinct from Ly-49A) (18), although a physical interaction between Ly-49G.2 and H-2 antigens has not been demonstrated.

Bone marrow transplantation studies have implicated Ly-49C$^+$ (5E6$^+$) NK cells in the recognition of class I MHC. This subset mediates the rejection of BALB/c (H-2^d), but not C57BL/6 (H-2^b), bone marrow when injected into lethally irradiated F$_1$ recipients (20). Cell–cell binding experiments in COS cells have demonstrated that Ly-49C binds to a wide range of cell lines, most likely through class I MHC (15). This binding appears to involve carbohydrates on class I MHC (23), a property also shared by Ly-49A (24). All Ly-49 molecules contain an extracellular region homologous to the carbohydrate recognition domain (CRD)[1] of C-type lectins (14, 15) which is thought responsible for their lectin activity.

In this study we have examined the specificity of the class I MHC ligands of Ly-49C, and have found these to be

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[1]Abbreviation used in this paper: CRD, carbohydrate recognition domain.

distinct from and overlapping with Ly-49A. Chimeras of Ly-49A and C have demonstrated that although anti-Ly-49 Abs recognize epitopes in the CRD and block cell–cell adhesion, ligand specificity is not entirely conferred by this domain. Additionally, these receptors exclusively form homodimers when simultaneously expressed in COS cells.

Materials and Methods

Ly-49A and C Chimeras. The Ly-49A cDNA (position 142-1079) was subcloned into the SstII site of plasmid BS KS+ and the Ly-49C cDNA (position 59-931) was subcloned into the EcoRI-HindIII sites of pBS KS+, and each were used as templates for all PCR reactions that amplified portions of the respective cDNAs. Chimeric constructs were generated by PCR amplification of regions of the Ly-49A and Ly-49C cDNAs using the oligonucleotides listed below, followed by the fusion of these amplified products via engineered restriction enzyme sites contained within the oligonucleotide sequence (small case letters refer to such sequences as well as linker sequences). The template source for each primer is indicated by its prefix (A-Ly-49A, C-Ly-49C); A1:ggaattctcgagTCCTTACAGCACACA; A2:ggaattctcgagAAC-AGTCTTGGTTTT; A3:ggggtaccTTAATACAGGAAACA; A4:ggaattctatcgatTTATTTTTCAGCAT; A5:ggaattctcgagTCCTcA-CcGgACACACAGGCAGAGGTGATAAA; C1:ggaattctcgagAAC-AGTCTTTGTTTT; C2:ggaattctcgagAACAGTCTTTGTTTT; C3:ggggTACCTTTAATCTGGT; C4:ggaattcaaatcgATAGATTG-TAGGCCAAGC; C5:ggaattctcgagTCCTtACaGcACACAGGC-AGAGGTGTTAAA.

The following primer combinations were used to amplify portions of Ly-49A and Ly-49C cDNAs (the position of the termini of the amplified products on the full-length Ly-49A cDNA [11] and Ly-49C cDNA [13] are in parenthesis): A5/A3 (586-1032); A1/A3 (567-1032); A2/pBS forward (142-567); A4/pBS forward (142-502); C5/C3 (511-912); C1/C3 (494-912); C4/C3 (415-912); C2/pBS reverse (59-494). All amplified products were subcloned into pBS KS+ and sequenced in their entirety to confirm that only specified mutations had occurred. The chimeric constructs were generated as follows, with the domain designations (see Fig. 6 *A*) in parenthesis: (*a*) A5/A3 (CRDA) was fused to C2/pBS reverse (NH₂C) using the engineered Xho1 site; (*b*) C5/C3 (CRDC) and C1/C3 (CRDC + IC) were fused to A2/pBS forward (NH₂A) using the engineered Xho1 site; and (*c*) C4/C3 (CRDC + IC + IIC) was fused to A4/pBS forward (NH₂A − IA − IIA) using the engineered Cla1 site.

Cell Lines and Transfections. The cell lines COS-1, A20, IC-21, R1.1, GM979, 20-8-4S, 34-2-12S, 34-5-8S, 34-1-2S, B8-24-3, K7-65, Y3, 28-14-8S, 28-11-5S, 3-83P were purchased from American Type Culture Collection (Rockville, MD) and were cultured in DMEM + 5% FCS. Ly-49 cDNAs were cloned into the vector pAX142 and expressed in COS cells by a DEAE-dextran method as previously described (15). The Ly-49C cDNA was originally obtained from a (C57BL/6 × CBA)F₁ library and the sequence was correctly submitted to GenBank, but its publication contained a typographical error (13). Nucleotide positions 195-196 were inadvertently printed as A-T, but are actually C-A. This clone has recently been demonstrated to be CBA-derived (16) and is identical to the Ly-49C allele found in BALB/c mice (17). The Ly-49D cDNA was obtained by screening a B10.D2 IL-2 activated NK cell cDNA library with an Ly-49A radiolabeled probe. The sequence of this clone differs from that previously reported for Ly-49D at two nucleotide positions, neither of

which result in amino acid changes (14). We have found an adenine and a guanine at nucleotides 65 and 79, respectively, whereas Smith et al. have reported a guanine and adenine at these positions (14).

Antibodies and Flow Cytometry. R-PE-conjugated 5E6 and FITC-conjugated A1 were purchased from PharMingen (San Diego, CA), and YE1/48, YE1/32, and 5E6 were purified from tissue culture supernatants using protein G–Sepharose 4 Fast Flow (Pharmacia LKB, Piscataway, NJ). The 5E6 hybridoma was kindly provided by Dr. Vinay Kumar (University of Texas Southwestern Medical Center, Dallas, TX). Cell staining procedures were carried out for 30 min at 4°C at concentrations of 10⁷ cells/ml, followed by two washes in PBS containing 2% FCS. Those samples stained with YE1/48 or YE1/32 required a secondary step with a goat anti-rat FITC Ab. Analysis was performed on a FACSort® (Becton Dickinson & Co., Mountain View, CA), and dead cells were stained with propidium iodide (1 μg/ml in final wash) and gated out.

Adhesion Assays. One d following transfection, COS cells were trypsinized and transferred to 6 cm dishes (3002; Falcon Labware, Oxnard, CA) at 2×10^5 cells/plate. 3 d post transfection, cells tested for adhesion to COS cells were labeled for 45 min with 1 μCi of $Na^{51}CrO_4/10^6$ cells, washed twice, incubated with the appropriate Ab for 30 min, and then plated (~5 × 10⁶ cells in 1.5 ml). Purified YE1/48, YE1/32, and 5E6 were added at a concentration of 5 μg/ml and all anti-class I MHC Abs were used as 1:10 dilutions of tissue culture supernatants. Adhesion assays were carried out for 2 h at 37°C, after which plates were washed three times with prewarmed media, bound cells were lysed with 10% Triton X-100, and radioactivity was determined.

Experiments which measure cpm bound were done in triplicate and values presented are the mean ± SD. Values expressed as percent control adhesion are relative to plates tested under identical conditions except for a preincubation step with media rather than an Ab. These numbers represent the mean ± SEM of at least three independent experiments.

Immunoprecipitation. COS cells were surface biotinylated as described (25) and lysed with lysis buffer (1% Triton X-100 in 10 mM Tris-Cl [pH 7.5], 150 mM NaCl). The cell lysates were centrifuged at the maximum speed in an Eppendorf microcentrifuge for 15 min at 4°C. The cleared cell lysates were incubated with anti–Ly-49-coupled beads (50 μl of 50% suspension in lysis buffer) for 1 h at 4°C with continuous mixing. The beads were then washed with the lysis buffer four times, and the bound proteins were eluted from the beads with 200 μl of 0.1 M Glycine-Cl buffer (pH 2.9) containing 1% Triton X-100 and 150 mM NaCl. The pH of the eluents was neutralized with a predetermined amount of 1 M Tris-Cl (pH 8.0). A portion of each eluent was subjected to Western blotting, and the rest was used for a second round of immunoprecipitation. The neutralized eluents were incubated with second set of antibody-coupled beads for 1 h at 4°C with continuous mixing. The beads were then washed with the lysis buffer, and the bound proteins were eluted with SDS-PAGE loading buffer. The eluted proteins were subjected to Western blotting and probed with peroxidase-conjugated streptavidin by the ECL chemiluminescence method (Amersham Corp., Arlington Heights, IL) according to the manufacturer's instructions.

Results

Domain Specificities of Anti-Ly-49 Antibodies. Several antibodies have been produced that recognize members of

Figure 1. Domain specificities of anti–Ly-49 antibodies. Chimeric Ly-49A/C cDNAs (see Materials and Methods) were transiently expressed in COS cells and tested for reactivity with YE1/48, YE1/32, A1, and 5E6. Domain abbreviations are as follows: cyt, cytoplasmic; TM, transmembrane; CRD, carbohydrate recognition domain. The numbers presented were obtained by flow cytometry and represent the mean fluorescence intensity following subtraction of background values (COS cells transfected with vector alone and stained with the specific Ab).

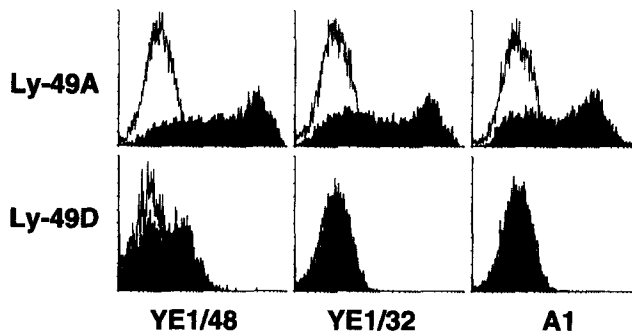|	Anti-Ly-49 Antibodies			
	YE1/48	YE1/32	A1	5E6
Ly-49A	344	404	418	0
Ly-49C	0	0	0	473
	0	0	0	459
	358	417	408	0

Figure 2. Cross-reactivity of anti–Ly-49A antibodies. Ly-49A and Ly-49D were expressed in COS cells and stained with YE1/48, YE1/32, and A1. Solid histograms represent cells transfected with Ly-49A or D, and empty histograms are cells transfected with vector alone.

the Ly-49 family (20, 26–28). In a previous study, we demonstrated that YE1/48 and YE1/32 recognize Ly-49A, and that 5E6 is specific for Ly-49C (15). No cross-reactions of these antibodies could be detected with any of the five Ly-49 molecules characterized at that time. To characterize the domains recognized by these antibodies (and the anti–Ly-49A antibody A1), we have constructed chimeras of Ly-49A and Ly-49C which swap the CRD of one molecule onto the amino terminus of the other (Fig. 1). Expression of these constructs in COS cells and subsequent FACS analysis has shown that all of the antibodies are specific for epitopes contained entirely within the CRD. Staining with all antibodies was identical to that of the parental molecule (Fig. 1).

Analysis of Ly-49 cDNAs which have recently been cloned has revealed a high degree of similarity between Ly-49A and Ly-49D in the carbohydrate recognition domain (90% identity) (14). Having shown that all anti–Ly-49 Abs are specific for CRD-epitopes, we therefore speculated that this high degree of similarity may result in cross-reactivity of Ly-49D with some or all anti–Ly-49A antibodies. When expressed in COS cells, however, Ly-49D showed only weak reactivity with YE1/48 when compared with Ly-49A, and no binding to YE1/32, A1, or 5E6 (Fig. 2).

Distinct Class I MHC Specificities of Members of the Ly-49 Family. COS cells expressing Ly-49A, C, and D were tested for their ability to bind cell lines representing four different haplotypes (H-2$^{d,k, b, and s}$). Each of the three Ly-49s was found to have a unique recognition pattern. Ly-49C bound all haplotypes tested, Ly-49A bound H-2$^{d and k}$, and Ly-49D bound weakly but consistently to H-2^k (Fig. 3).

Antibody blocking experiments with the H-2^d cell line demonstrated the specificity of Ly-49A for D^d, but not K^d (Fig. 4 *A*). As reported previously (19, 22), an Ab recognizing the α1/α2 domain (34-5-8S) entirely blocked adhesion, whereas an Ab against the α3 domain (34-2-12S) had no effect. Ly-49C–mediated adhesion was partially blocked by antibodies specific for either K^d (20-8-4S) or D^d (34-5-8S), and entirely blocked by the simultaneous addition of both antibodies (Fig. 4 *B*), suggesting that Ly-49C binds to both D^d and K^d. This was further supported by the total in-

hibition seen with an Ab recognizing both K^d and D^d (34-1-2S). As was seen with Ly-49A, the antibody directed against the α1/α2, but not α3, domain of D^d inhibited Ly-49C–mediated adhesion. Anti–Ly-49 antibodies inhibited the interaction of both Ly-49A (YE1/32 and YE1/48) and Ly-49C (5E6) with the H-2^d cell line (Fig. 4, *A* and *B*).

A panel of well characterized anti–H-2^b Abs has shown that Abs which recognize K^b entirely inhibit Ly-49C–mediated adhesion, whereas Abs against D^b are non-inhibitory (Fig. 4 *C*). Binding of an H-2^s cell line was also inhib-
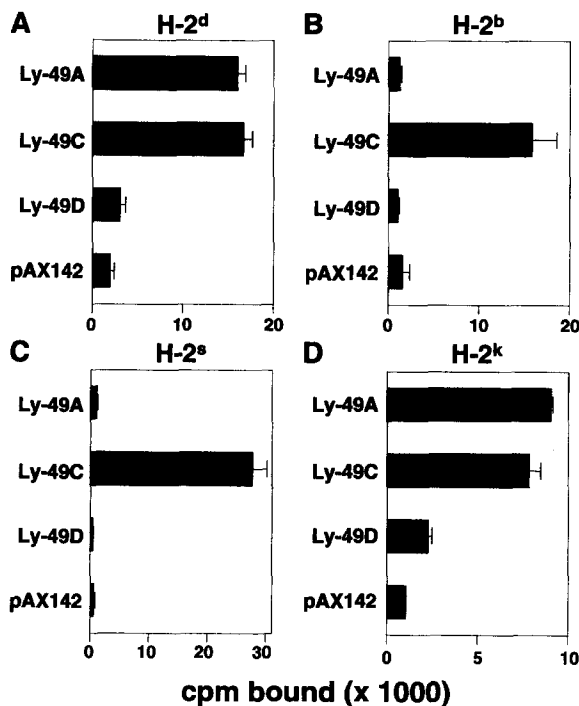

Figure 3. Cell-cell binding mediated by Ly-49A, C and D. Cell lines representing four haplotypes (H-2^d/A20, H-2^b/IC-21, H-2^s/GM979, H-2^k/R1.1) were labeled with ^{51}Cr and overlaid on COS cells transfected with an Ly-49 cDNA or vector alone (pAX142). Plates were washed after 2 h and radioactivity was determined. The numbers presented are the mean ± SD of triplicate plates.
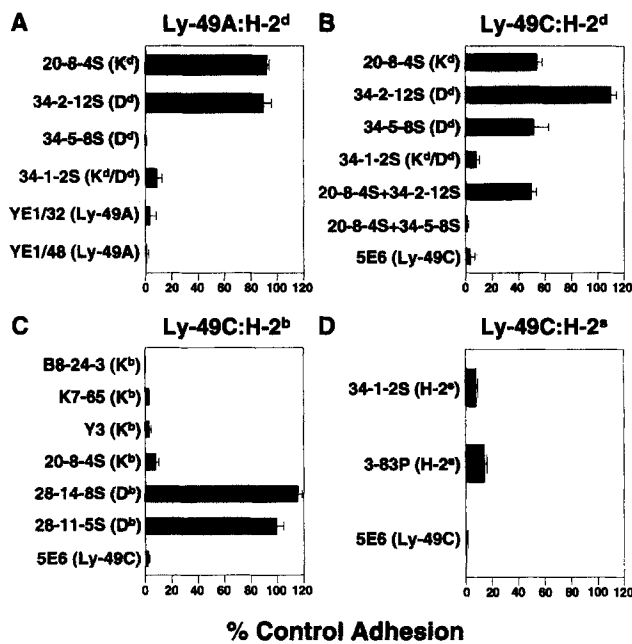
Figure 4. Distinct class I MHC specificities of Ly-49A and C. ^{51}Cr-labeled cell lines (H-2^d, H-2^b, H-2^s) were incubated for 2 h with Ly-49A– or C-transfected COS cells in the presence of antibodies against either class I MHC or Ly-49 (the antigenic specificities of each antibody is in parentheses next to the clone name). 100% control adhesion represents binding in the absence of antibody, and 0% adhesion is the level of the cell line binding to COS cells transfected with vector alone. Values presented are the mean ± SEM of at least three independent experiments.

Figure 5. Lack of molecular association between Ly-49A and C. (*A*) COS cells were transfected with either Ly-49A, Ly-49C, Ly-49A and C, or vector alone (pAX142). After 3 d, cells were stained with A1-FITC + 5E6-PE and analyzed by flow cytometry. (*B*) Transfected COS cells were surface biotinylated and Ly-49 was immunoprecipitated (*ip*) by either anti–Ly-49A (YE1/48) or anti–Ly-49C (5E6). Aliquots of the immunoprecipitates were either directly analyzed or subjected to a second round of immunoprecipitation using anti–Ly-49 as indicated. All of the immunoprecipitates were analyzed by Western blotting using peroxidase-conjugated streptavidin as a probe. The positions of the molecular mass standard are indicated (kD).

ited by Abs against class I MHC (Fig. 4 *D*), although the nature of the H-2^s structures recognized by these antibodies is not characterized. Additionally, 5E6 completely blocked adhesion to both the H-2^b and H-2^s cell lines (Fig. 4, *C* and *D*). All anti–H-2^k Abs tested were found to be ineffective at blocking the interaction between Ly-49A and D^k, and were therefore not tested with Ly-49C and Ly-49D (data not shown).

Ly-49A and C Form Homodimers but not Heterodimers. Approximately 5% of C57BL/6 (B6) NK cells express both Ly-49A and Ly-49C (15). Each of these receptors have been characterized as disulfide-linked dimeric cell surface antigens of similar size and highly related amino acid sequences (11–13, 20). We therefore evaluated the ability of these related molecules to form heterodimers when simultaneously expressed in COS cells. COS cells cotransfected with Ly-49A and C cDNAs expressed both Ly-49 molecules on the cell surface as shown by flow cytometric analysis (Fig. 5 *A*). Ly-49 on these cells were immunoprecipitated by either anti–Ly-49A (YE1/48) or anti–Ly-49C (5E6), and the resultant immunoprecipitates were subjected to a second round of immunoprecipitation with anti–Ly-49 antibodies. If Ly-49A and C formed heterodimers on the transfected COS cells, Ly-49 precipitated by anti–Ly-49A in the first round of immunoprecipitation should have been reprecipitated by anti–Ly-49C in the second round of immunoprecipitation and vice versa. As shown in Fig. 5 *B*,
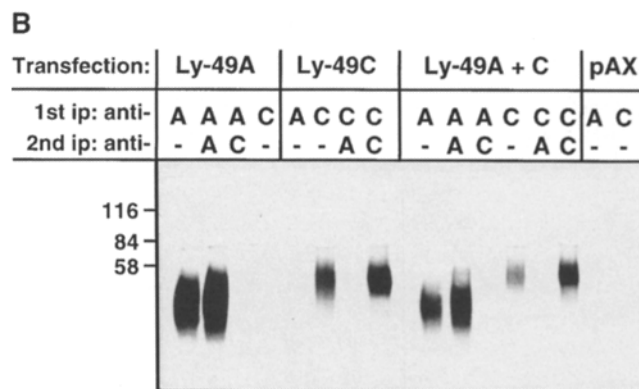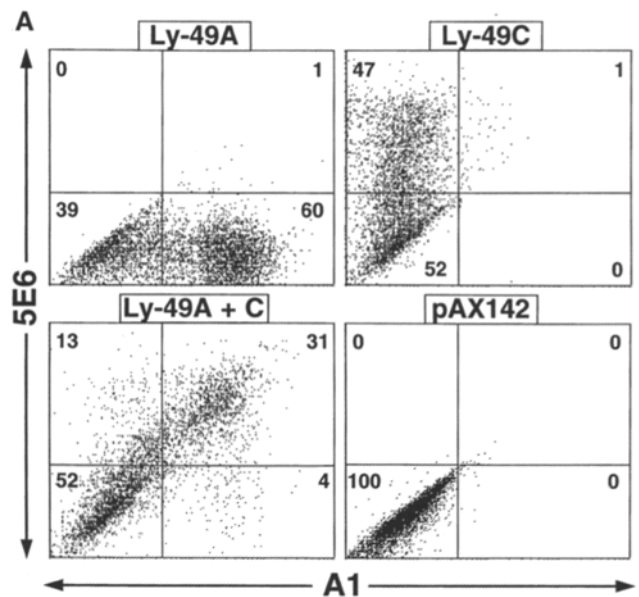
the Ly-49 dimers precipitated by anti–Ly-49 antibodies in the first round of immunoprecipitation were reprecipitated by the same antibodies in the second round but not by different anti–Ly-49 antibodies. An extended exposure of the same blot shown in Fig. 5 *B* also gave no indication of reprecipitation with a different antibody in the second round (data not shown), indicating that heterodimers were either not present or that their formation was a rare occurrence compared to that expected for random dimerization of the two polypeptide chains.

Ligand Binding Domains of Ly-49. Ly-49A and C each contain an extracellular CRD of ~125 amino acids which accounts for 60% of the extracellular portion of the mole-

cule (11–13). Domain swapping experiments have demonstrated that all anti–Ly-49 Abs tested are reactive with epitopes found within the CRD. This same set of chimeras was also tested in cell–cell binding assays to localize the region of Ly-49s responsible for ligand recognition. It was found that when either the CRD of Ly-49C is transplanted onto the amino terminus of Ly-49A, or vice versa, neither of the chimeric molecules bound the same wide range of cell lines as does Ly-49C (Fig. 6 A). However, both chimeras bound to the H-2^d ^{and} ^{k} cell lines which are normally bound by the parental molecules.

An extension of this analysis has shown that H-2^b and H-2^s binding is recovered in a chimera that includes an additional 32, but not 6, Ly-49C–specific amino acids in the region immediately adjacent to the CRD (Fig. 6, A and B). Residue(s) required for Ly-49C–specific binding may therefore be narrowed to a 26–amino acid area. Exclusion of those residues shared by Ly-49A and C at the carboxy and amino ends of this region allows for a greater refinement to a 19–amino acid segment required for H-2^b and H-2^s binding. This region is characterized by 10 amino acid differences between the two molecules as well as a stretch of four amino acids deleted in Ly-49A. Ly-49D is the only other of the eight Ly-49 cDNAs that has this same deletion (14).

Discussion

The results presented above demonstrate that Ly-49C binds a range of class I MHC molecules, including K^d, D^d, and K^b, but not D^b. In addition, Ly-49D also weakly binds to an H-2^k cell line. These findings support the hypothesis that Ly-49 is a family of NK cell receptors with related, but distinct, functions. Ly-49A and Ly-49C differ in both their pattern of expression by NK cell subsets, as well as their specificities for class I molecules. Interestingly, both of these properties overlap between the two molecules, in that they both bind to H-2D^d and ~5% of B6 NK cells coexpress both receptors (15).

The related sequence and disulfide-linked dimeric structure of Ly-49A and C suggests that these molecules may form heterodimers in cells that express both receptors. However, when both Ly-49A and C are simultaneously expressed in COS cells and sequentially immunoprecipitated with antibodies against Ly-49A followed by Ly-49C, or vice versa, only homodimeric structures are detected. This suggests that Ly-49A$^+$C$^+$ NK cells possess two sets of distinct Ly-49 homodimers, and no Ly-49A/C heterodimers. It is therefore likely that such double positive NK cells recognize the full range of class I molecules recognized by Ly-49A and C, rather than a potentially novel specificity recognized by a heterodimer. Allelic exclusion
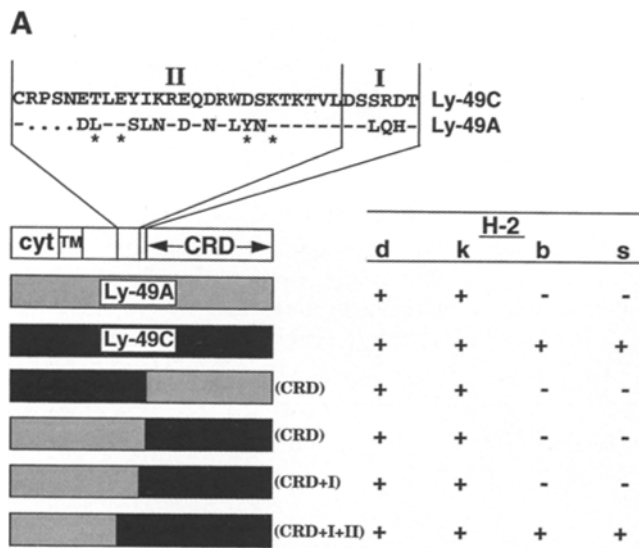
A

Figure 6. Ligand binding domains of Ly-49. (A) Chimeric Ly-49A/C cDNAs (see materials and methods) were transiently expressed in COS cells and tested for binding (+/−) to H-2^d (A20), H-2^k (R1.1), H-2^b (IC-21), and H-2^s (GM979) cell lines. Regions representing Ly-49A and Ly-49C sequence are in gray and black, respectively. Amino acid sequence comparison is shown for a portion of the extracellular region immediately adjacent to the CRD (——— identity; • gap). TM indicates the location of the transmembrane segment, cyt designates the cytoplasmic domain, and * identifies amino acid residues differing between Ly-49C^{BALB} and Ly-49C^{B6} (16, 17). (B) Binding of Ly-49A/C chimeras to an H-2^b cell line (IC-21). After a 2-h coincubation with transfected COS cells at 37°C, unbound cells were washed away and plates were photographed. (1) Ly-49C; (2) Ly-49C (CRD)/Ly-49A (amino terminus); (3) Ly-49C (CRD + I + II)/Ly-49A (amino terminus).

B
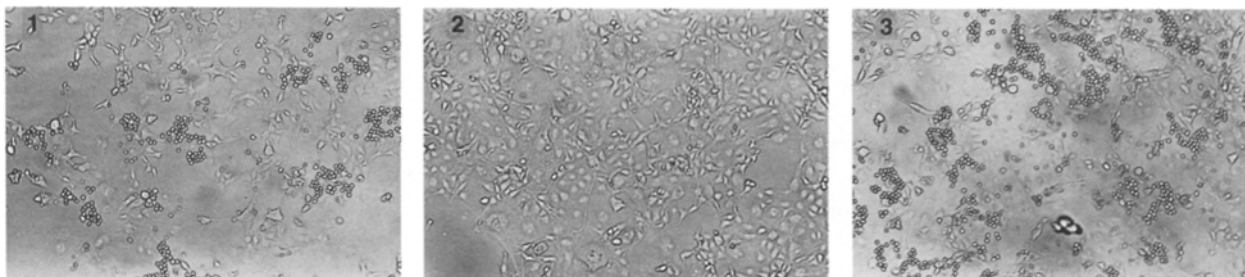
appears to be an additional means of selecting against Ly-49 heterodimers within a single NK cell (16). Such a mechanism prevents the potential pairing of the highly similar alloforms of Ly-49A and C (92–98% amino acid identity) in a heterozygous animal and thereby maintains a single receptor specificity.

By constructing chimeras between Ly-49A and C we have characterized domains essential for ligand specificity. In a cell–cell adhesion assay, Ly-49C binds to cell lines of H-2^b and ^{s} haplotypes, whereas Ly-49A does not. We found that the CRD of Ly-49C spliced onto the amino terminus of Ly-49A was unable to confer the binding characteristics of the entire Ly-49C polypeptide. Additional Ly-49A/C chimeras revealed a 19–amino acid stretch adjacent to the CRD required for Ly-49C–specific binding. Although this demonstrates that sequences in this area are essential in determining the ligand specificity of Ly-49C, this region is clearly not the sole determinant. Chimera with the CRD of Ly-49A spliced onto the stem of Ly-49C do not bind to H-2^b and H-2^s cell lines. These data therefore demonstrate that a combination of sequences within the CRD as well as in the adjacent stem region are essential determinants of Ly-49 ligand specificity.

We have previously shown that Ly-49C binds some carbohydrates and that carbohydrates on class I MHC seem to be important for interactions with Ly-49C (23). Our results reported here, combined with these previous observations, suggest that this binding may be mediated by the carbohydrates as well as the peptide backbone of class I MHC simultaneously interacting with distinct domains of Ly-49. Such a model predicts that not only the level of class I MHC on a target cell, but also its glycosylation pattern, may regulate its sensitivity to NK cells. An alternate explanation of these chimeric Ly-49 findings is that binding to class I MHC is mediated by either the CRD alone or the stem alone, but the specificity may be altered by structural changes in an adjacent region. Further studies are required to test these hypotheses.

Ly-49C$^+$ NK cells derived from (C57BL/6 × BALB/c)F$_1$ (CB6F$_1$) mice lyse parental BALB/c (H-2^d), but not B6 (H-2^b), Con A blasts (29). Ly-49C has been proposed to transmit negative signals upon interaction with K^b. This is supported by the observation that K^b bearing targets are lysed following preincubation with anti-5E6 (Fab')$_2$, implying that the delivery of a negative signal through Ly-49C is being blocked (29). Additionally, Ly-49C$^+$ NK cells derived from a homozygous BALB/c mouse are unable to lyse BALB/c blasts, and preincubation with anti-5E6 results in lysis of these targets as well (29). The suggestion that Ly-49C interacts with class I and delivers negative signals is supported by our binding studies that have demonstrated that Ly-49C binds to H-2^b (K^b, but not D^b) and H-2^d (K^d and D^d) structures. However, the lysis of BALB/c blasts by CB6F$_1$ Ly-49C$^+$ NK cells indicates that H-2^d class I molecules cannot, at least under some circumstances, provide the proper protective signal to Ly-49C$^+$ NK cells.

One explanation for this apparent paradox is that allelic forms of Ly-49C may have different class I specificities and or affinities. The Ly-49C used in these binding studies is the BALB/c allelic form, and differs from the recently described B6 receptor by 14 extracellular amino acids (16, 17). Four of these differences (indicated by ★ in Fig. 6 A) are in the 19–amino acid stem region which has been implicated in the determination of ligand specificity. The allelic exclusion that has been demonstrated for Ly-49A and C (16) predicts that a CB6F$_1$ mouse has two subsets of Ly-49C$^+$ NK cells: Ly-49C^{BALB} and Ly-49C^{B6}. The Ly-49C^{B6} fraction may therefore be responsible for reactivity against BALB/c targets, whereas both the Ly-49C^{BALB} and Ly-49C^{B6} fractions appear to be inhibited by H-2^b antigens (20, 29). Alternatively, the education process in an F$_1$ mouse may calibrate Ly-49C receptor levels by a mechanism similar to that demonstrated for Ly-49A (30), resulting in the reactivity of Ly-49C$^+$ NK cells against homozygous H-2^d targets. Resolution of these questions will require an evaluation of the binding properties of the Ly-49C^{B6} receptor as well as a more precise characterization of the means by which class I MHC affects lysis by Ly-49C$^+$ NK cells.

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