FINE MAPPING OF EPITOPES BY INTRADOMAIN K^d/D^d RECOMBINANTS

By JEAN-PIERRE ABASTADO, CHRISTIAN JAULIN, MARIE-PAULE SCHUTZE, PIERRE LANGLADE-DEMOYEN, FERNANDO PLATA, KEIKO OZATO,* and PHILIPPE KOURILSKY

*From the Unité de Biologie Moléculaire du Gène, Unit 277, Institut National de la Santé et de la Recherche Médicale, Unité Associée 535, Centre National de la Recherche Scientifique, Institut Pasteur, 75724 Paris, Cédex 15, France; and the *Laboratory of Developmental and Molecular Immunity, National Institute of Child and Human Development, National Institutes of Health, Bethesda, Maryland 20892*

The mouse *H-2K*, *D*, and *L* genes of the MHC encode highly polymorphic antigens expressed at the cell surface. While their polymorphism is reflected in graft rejections, these class I MHC antigens mediate recognition processes involving cells of the immune system (1).

A large number of mAbs have been raised against these polymorphic molecules (2–6). They have been used to analyze in details the expression of different H-2 antigens in different tissues and to approach questions dealing with the interaction between the T cell receptor and foreign antigens presented in association with MHC molecules. Better knowledge of the portion of the H-2 antigen recognized by the different mAbs would be helpful in delineating epitope structures.

Three external domains of MHC class I genes are encoded in separate exons of the gene. Several groups have taken advantage of this fact to generate hybrid antigens in which given domains have been replaced by the corresponding ones of another antigen (7–18). These exon-shuffling experiments have also been used to produce interspecies HLA/H-2 (11, 19) as well as class II/class I hybrids (20). In several instances, this has permitted the assignment of particular alloantigenic determinants recognized by mAbs or by allospecific or virus-specific CTLs to a given domain.

To refine such studies and more precisely map epitopes recognized by mAbs, it is necessary to obtain recombinants within the domains. However, it is often difficult to construct the appropriate recombinants because convenient restriction endonuclease sites are generally lacking. Site-directed mutagenesis can be used, but requires a new construction for each new mutant sequence. Weber and Weissmann (21) have used in vivo recombination in *Escherichia coli* to produce chimeric IFN sequences. We have improved this technique in such a way as to obtain many recombinants in which the recombination point can now be easily predicted and detected. In the previous study (22), we analyzed several

Address correspondence to J.-P. Abastado, Unité de Biologie Moléculaire du Gène, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris, Cédex 15, France.

parameters involved in the rate of recombination and the characteristics of recombinants produced. The procedure can be applied to any pair of homologous sequences displaying up to 10% of mismatched nucleotides and, perhaps, more. In this way, dozens of chimeric H-2 sequences differing by as few as one amino acid can be easily produced in one experiment.

Since previous studies (7, 10, 12, 15, 18) have assigned most alloantigenic determinants to the two first external domains of H-2 antigens (N and C1), we have predominantly constructed chimeric H-2 antigens with recombination points located within these two domains. 11 different chimeric H-2 sequences have been obtained in which the 5′ part is derived from *H-2K*^{d} and the 3′ part from *H-2D*^{d}. These new H-2 sequences, introduced into a eukaryotic expression vector, were expressed in Ltk$^-$ cells after transfection. The expressed chimeric antigens were tested with a panel of 61 mAbs specific for either H-2K^d or H-2D^d. This has allowed us to assign small peptidic sequences (2–6 amino acids) as part of the antigenic determinants recognized by 59 out of 61 mAbs.

Materials and Methods

DNA Sequencing. Recombinants were sequenced by the Maxam and Gilbert procedure (23) after end labeling with T4 polynucleotide kinase or terminal deoxynucleotidyl transferase.

Subcloning into VcDNA. For expression in transfected cells, we used a vector (VcDNA) constructed and kindly provided by Dr. R. Breathnach and described in reference 24. For each recombinant, the 1,169-bp Eco RI–Nru I fragment was purified by electrophoresis in low-gelling agarose and ligated, without purifying the DNA from agarose, into VcDNA cut by Eco RI and Hpa I restriction endonucleases.

Monoclonal Antibodies. The mAbs used in this work have been previously described (2–6). Culture supernatants of hybridoma cells were used at a dilution of 1:10, ascites fluids at a dilution of 1:100, and protein A–Sepharose–purified Ig at a dilution of 1:200 to 1:800.

Transfections. 10 μg of recombinant plasmid were coprecipitated by ethanol with 300 ng of pAG0 containing the HSV1 thymidine kinase (*tk*) gene (25). The pellet was resuspended and transfected with calcium phosphate into 10^6 freshly plated Ltk$^-$ cells. After 2 d, transfected cells were selected in HAT medium. 2 wk later, the cells were trypsinized, allowed to react with mAb 34-1-2 in ice for 30 min, washed, stained with FITC-conjugated goat anti-mouse Ig serum under the same conditions, and washed. The 10% brightest cells were collected under sterile conditions by flow cytometry.

Immunofluorescence Analyses. Transformed cells (10^6) were incubated with 50 μl of mAb in ice for 30 min and then washed with HBSS supplemented with 5% heat inactivated FCS and 0.1% azide. The cells were then stained with FITC-conjugated goat F(ab′)$_2$ anti-mouse F(ab′)$_2$ and goat F(ab′)$_2$ anti-mouse Fc (Cappel Laboratories, Cochranville, PA) in ice for 30 min and washed as mentioned above. Stained cells were fixed in HBSS containing 1% paraformaldehyde. Typically, 2×10^4 cells were analyzed by flow cytometry.

Flow Cytometry Analyses and Sorting. FITC-labeled cell flow cytometry was performed using a cytofluorograf 50HH (Ortho Diagnostic Systems Inc., Westwood, MA) with the 488 nm line from an argon ion laser (lexel 95-4) at 200 mW power. The low-angle light scattering was used for gating, thus eliminating the contribution of debris from the fluorescence histograms. The exciting line of the laser was excluded by a dichroic filter and a narrow pass filter (514–540 nm). Analyses and sorting were monitored by a microprocessor system 2150 (Ortho Diagnostic Systems Inc.).

Results

Construction of Chimeric H-2 Sequences. The technique used to obtain chimeric H-2 sequences is depicted in Fig. 1. It has been described in detail elsewhere

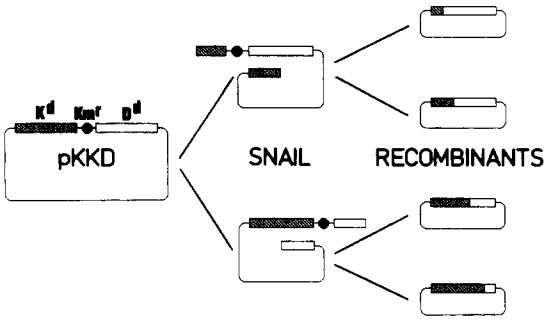

FIGURE 1. Production of chimeric H-2 sequences by the snail technique. The plasmid pKKD carries, between the *H-2K*^{d} and the *H-2D*^{d} sequences, the kanamycin resistance gene (*Km*^{r}). Linearization within one of the two H-2 sequences yields a snail that, upon transformation into *E. coli*, can recircularize by intramolecular recombination within the regions of homology. Recombinants have lost the *Km*^{r} marker.

(22), as part of an analysis of the molecular mechanism of intramolecular recombination. Briefly, the plasmid pKKD carried the *H-2K*^{d} sequence derived from a cDNA isolated in our laboratory (26). Downstream and in the same orientation it also carried the *H-2D*^{d} sequence derived from the cDNA pAG64 (27). In between, we inserted the kanamycin resistance gene (Km^r) of the Tn5 transposon. To obtain recombinants, pKKD DNA was cut in one of the two H-2 sequences which, after transformation into *E. coli*, led to intramolecular homologous recombination at high frequency. 10 ng of linearized pKKD were usually sufficient to produce 100–200 recombinants. Recircularization by end-to-end ligation was sometimes observed but yielded <10–20% of the transformants. Actual recombinants lost the *Km*^{r} marker, and were easily scored as kanamycin sensitive. Intramolecular recombination between the two H-2 sequences usually occurred at some distance from the site where pKKD had been cut. We found that the frequency of recombination decreases gradually with the distance from this cut and we used this property to isolate recombinants all along the H-2 sequence. Oligonucleotide probes (14-mers) specific for *H-2K*^{d} or *H-2D*^{d} have been used to map the recombination point by colony hybridization on the transformants.

Due to the order of the K^d and D^d sequences on the initial plasmid pKKD, all recombinants obtained had the 5′ end of *H-2K*^{d} and the 3′ end of *H-2D*^{d}. Therefore, despite the fact that pAG64 is not a complete cDNA copy of *H-2D*^{d} and lacks its first 40 amino acids, all recombinants potentially encoded complete H-2 heavy chains, of which, the signal sequence and the 40 first amino acid were always derived from H-2K^d.

Because we were interested in mapping alloantigenic determinants recognized by mAbs and since previous studies (7, 10, 12, 15, 18) have shown that these were predominantly located in the first and second external domains (N and C1), we chose 22 recombinants out of which 18 were recombined within those two domains. We sequenced those 22 recombinants and found 11 different sequences. None of the recombinants had suffered deletions, insertions, or point mutations. The positions of the 11 recombination points are indicated in Fig. 2 (R1 to R11). Four (R1 to R4) are recombined within the first domain, five (R5 to R9) within the second domain, and two (R10 and R11) within the NH_2-terminal part of the third domain.

Expression of the Chimeric H-2 Sequences. The 11 recombinant H-2 sequences were subcloned into the VcDNA vector (24). This plasmid had been devised to

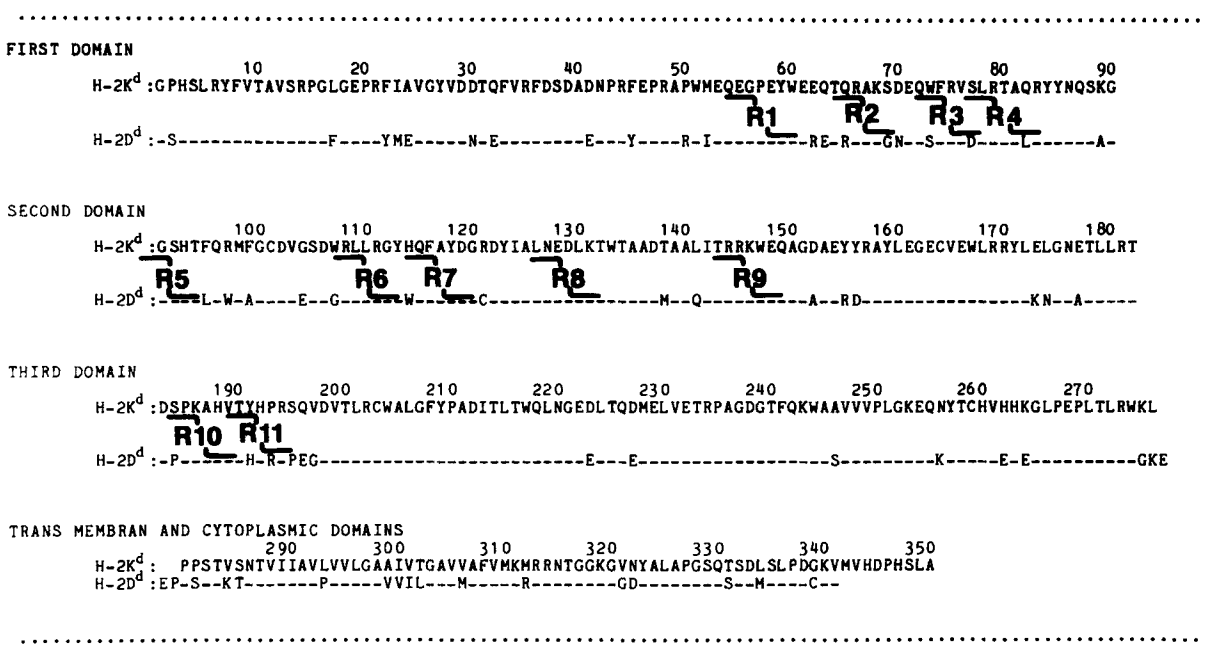

FIGURE 2. Amino acid sequences of the chimeric H-2 antigens. The H-2K^d and H-2D^d sequences have been aligned. Dashes indicate identical residues. The nucleotide sequence of each recombinant has been determined and the corresponding predicted amino acid sequence is presented. One-letter amino acid code.

TABLE I
Expression of Chimeric H-2 in L Cells

Cells	Mean of fluorescence	
	34-1-2	TO122
L-K^d	81	185
L-D^d	519	541
L	5	13
R1	63	166
R2	289	251
R3	272	507
R4	329	534
R5	232	391
R6	54	128
R7	232	252
R8	151	188
R9	145	173
R10	369	505
R11	118	268

express cDNA sequences under control of the SV40 early T antigen promoter. It has been previously used (24) to express a full-length *H-2K*^{d} cDNA sequence to a significant level in L cells. After cotransfection with the HSV1 *tk* gene into mouse Ltk$^-$ cells (H-2^k), TK$^+$ transformants were selected in HAT medium. The bulk of transformants were analyzed by flow cytometry using the mAb 34-1-2 specific for both H-2K^d and H-2D^d (2). This antibody gave no background on Ltk$^-$ cells. The percentage of positive cells and the level of expression was found to vary from one cell line to the other. For each recombinant, we isolated, by flow cytometry, cells giving comparable levels of fluorescence after staining with this mAb. This level was in the same order of fluorescence as that obtained with BALB/c 3T3 cells (H-2^d). The mean fluorescein value obtained with 34-1-2 or T0122 (another K^d- and D^d-specific mAb) was at least 10 times higher than that observed for untransformed cells (Table I), although some transformants required numerous cycles of cell sorting to achieve this result.

Reactivity of H-2-specific mAbs on Cell Lines Expressing Chimeric H-2 Antigens. A total of 77 H-2-specific mAbs were tested on the 11 cell lines expressing chimeric H-2 antigens by indirect immunofluorescence using a flow cytometer. All antibodies were negative on L cells. 58 were specific for H-2D^d but negative for H-2K^d, 3 were specific for H-2K^d but not for H-2D^d, 2 were positive with both antigens, and 14 were negative with both antigens. An example of the reactivity of a D^d-specific mAb (H147) on the different cell lines is shown in Fig. 3 and a summary of the results obtained with K^d- and/or D^d-specific mAbs is presented in Fig. 4.

15 mAbs (Fig. 4, group *A*) were found to recognize D^d but none of the recombinants. 11 mAbs (group *B*) were found to recognize D^d and R1 but none of the other recombinants. By comparing the sequences of R1 and R2 (Fig. 2), three amino acid residues of D^d are identified that differ from K^d residues; they are R62, E63, and R65 and are thus likely to be critical for the recognition by these 11 mAbs. This indicates that at least one of these residues belong to the

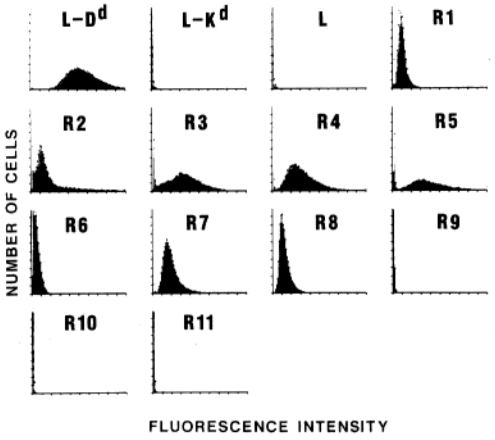

FIGURE 3. Flow cytometry analysis of cells expressing the different chimeric H-2 antigens after staining with mAb H-147 (3). Cells were stained as described in Material and Methods section. 2×10^4 cells were analyzed. Abscissa, relative fluorescence intensity with linear amplification in arbitrary units; ordinate, number of cells. L, untransfected cells; L-D^d, L cells transfected with H-2D^d; L-K^d, L cells transfected with H-2K^d; R1–R11, L cells transfected with the corresponding K^d/D^d recombinant.

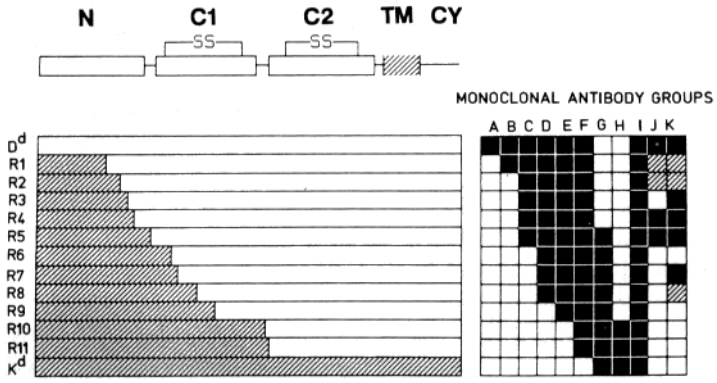

FIGURE 4. Reactivity of mAbs on L cells expressing chimeric H-2 antigens. (*Left*) Schematic drawing of the parental and chimeric H-2 antigens. The hatched region corresponds to the K^d-derived portion of the antigens. (*Right*) Summary of the flow cytometry analysis of the different cell lines expressing these antigens after staining with K^d- and/or D^d-specific mAbs. mAbs yielding the same pattern have been grouped. A black square indicates that the recombinant is recognized while a white square indicates that it is not. Hatched squares correspond to dull staining. List of the mAbs grouped according to the pattern of reactivity on the chimeric H-2 antigens: (*A*) T0107, T0111, T0116, T0119, T0120, T0128, T0129, T0131, T0133, T0137, T0138, T0139, T0142, T0143, 66-2-4; (*B*) T0124, T0127, T0132, T0134, T0136, T0141, T0144, 34-421, 27-11-13, 28-8-6, T14c; (*C*) T0112, T0113, T0114, T0115, T0117, T0121, T0123, T0126, T0130, 28-11-5, 34-420, 34-5-8, 66-13-6, H81-199-1, T148; (*D*) T0102, T0103, T0104, T0108, T0109, T0110, T0118, T0125, 66-4-8, 66-13-5, 66-13-19, H147; (*E*) T0105, T0106; (*F*) 34-2-12; (*G*) 20-8-4, F35-119-18; (*H*) 31-3-4; (*I*) 34-1-2, T0122; (*J*) T0145; (*K*) 23A-5-21.

epitope(s) recognized by group B mAbs. Residues are given in the one-letter code and their positions are numbered as in Fig. 2.

As shown in Fig. 4, group *C*, 15 mAbs recognized D^d, R1, R2, R3, R4, and R5 at comparable levels, but not R6, R7, R8, R9, R10, R11, nor K^d. Comparing the sequences of R5 and R6 (Fig. 2) allows us to assign at least part of the epitope(s) recognized by group C mAbs to residues L95, W97, A99, E104, and G107. 12 mAbs (group *D*) recognized D^d, and R1 to R8 at comparable levels but not R9, R10, R11, nor K^d (see Fig. 3 as an example). By comparing R8 and R9, we deduced that residues M138, and Q141 belong to the epitope(s) recognized

by these mAbs. 2 mAbs (group *E*) recognized D^d and R1 to R9, but not R10, R11, nor K^d. Consequently, the recognized epitope(s) contains residues A152, R155, D156, K173, N174, A177, and/or P184.

As shown in Fig. 4, group *F*, 1 mAb (34-2-12) was positive on D^d and all recombinants but negative on K^d. Its epitope was thus mapped in the third domain, downstream from position R193.

Out of the K^d-specific mAbs, 2 were found negative on D^d, R1, R2, R3, and R4, but positive on R5 to R11 as well as K^d (group *G*). Therefore, residues Q82, and K89 are part of the epitope(s) recognized by these mAbs.

1 K^d-specific mAb (31-3-4) was found to react with R10, R11, and K^d, but not with R1 to R9 nor D^d (group *H*). Thus its epitope contains residues D152, Y155, Y156, E173, L174, E177, and/or S184.

2 D^d-specific mAbs (T0145 and 23-5-2D) gave a more complex pattern (groups *J* and *K*). For example, T0145 was positive on D^d, R4, and R5, dull on R1 and R2, but negative on R3, R6 to R11, and K^d.

The 14 mAbs negative with both K^d and D^d, but positive with other mouse MHC class I antigens (23-10-1, 28-14-8, 30-5-7, 28-13-3, 66-3-5, 64-3-7, H-154, H-118, H-117, H-149, H-122, H-148, H-121, and H-108) were negative on all recombinants (data not shown), while the 2 mAbs positive with K^d and D^d were positive on all recombinants (group *I*).

Discussion

We have used in vivo recombination in E. coli to produce chimeric H-2 antigens. This method, depicted in Fig. 1 and described in detail elsewhere (22), is derived from the original method of Weber and Weissmann (21) with major improvements. It is based on the recircularization of a linear DNA molecule carrying homologous sequences. By cutting the parental plasmid in one of the two H-2 sequences at different sites, any type of recombinant can be obtained (22). Oligonucleotide probes specific for one or the other parental sequence allow rapid mapping of the recombination point.

Of 22 sequenced recombinants, 11 had different sequences. None of them had suffered rearrangement, nor point mutation. These 11 chimeric sequences were subcloned in an expression vector and transfected in Ltk^- cells. Transfected cells were screened by indirect immunofluorescence, using the mAb 34-1-2 recognizing both parental sequences H-$2K^d$ and H-$2D^d$. For all recombinants, positive cells were detected. This result implies that all recombinants are expressed on the cell surface. Thus, all signals necessary for the translation, the posttranslational modification, the transport to the cell surface, and the insertion into the membrane, as well as the correct folding and proper recognition by the antibody, are functional in the hybrid molecules. Indeed, sequence elements involved in these processes probably exist in homologous positions in the two parental sequences and may be conserved upon recombination. For example, both H-$2K^d$ and H-$2D^d$ antigens have cystein residues at positions 100 and 163 involved in a disulphide bridge known to be important for the folding of the second external domain (28). As such, all recombinants would be expected to and do have cystein residues at these positions. Similarly, it is possible that many characteristics of the tertiary structure, as well as characteristics of the primary

structure, are conserved within the parental molecules and recombinants derived from them. Superposition of the crystallographic structure of three different IgS has revealed a high degree of coincidences in atomic positions (29). If the same holds true for H-2 class I molecules, recombinant molecules may thus all share the same basic regular structure as the parental ones. Several lines of evidences indicate that the H-2 multigene family has evolved by different kinds of homologous recombination (unequal crossing-over, intra-, and interallelic conversion). The latter may create a very large diversity (30), and our results suggest that many of the different H-2 sequences thus generated may be functional. This argument, however, must be taken with caution, since it was found that $Q7/K^d$ and $Q7/L^d$ (14), as well as some combinations of class I/class II (20) exon-shuffled recombinants, are not expressed on the cell surface.

We have tested 77 mAbs on these chimeric H-2 antigens. Most of them (75) gave a simple pattern of reactivity: mAbs recognizing both parental sequences recognized all recombinants; mAbs recognizing neither K^d nor D^d recognized none of the recombinants. D^d-specific mAbs recognized the most 5′ recombinants, whereas K^d-specific mAbs recognized the most 3′ recombinants. Only two mAbs presented a more complex pattern. This pattern, observed reproducibly with different batches of mAbs, is difficult to explain. It likely indicates that the structure recognized by these mAbs was perturbed in some recombinants. The fact that this situation was infrequent *a posteriori* shows the method can be utilized to define the epitopes recognized.

The fact that a given mAb recognizes all recombinants harboring a certain residue but none of those lacking this residue indicates that this residue is necessary for the recognition, although this alone may not be sufficient. Therefore, residues thus identified are likely to be only part of the epitope recognized by the mAb.

When two recombinants differing at multiple amino acid positions present a distinct pattern of reactivity with the same mAb, it would mean that at least one of these residues belongs to (or influences) the epitope recognized by the mAb. When those multiple changes are in neighboring positions and particularly if they involve charged amino acids, several of these residues may influence the epitope.

Some of the mAbs analyzed in the present study have been previously characterized by exon shuffling and/or site-directed mutagenesis. T0107, T0119, T0120, T0129, T0131, T0133, and T0137 (Fig. 4, group *A*) were previously mapped to the N domain by L^d/D^d (15) and D^P/D^d (18) recombinants by exon shuffling. Our data (Fig. 4) confirm these previous assignments and refine the recognized portion of the molecule to residues 1–52. In addition, we deduce that these mAbs recognize positions where the D^d sequence differs from that of L^d, D^P, and K^d. As shown in Fig. 5, the only residues between 1 and 52 that satisfy this criterion are residues S2, F17, and I52.

T0124, T0136 (Fig. 4, group *B*) were previously mapped to the N domain by L^d/D^d and D^P/D^d exon-shuffled recombinants, whereas 34-421 and 28-8-6 (group *B*) were mapped to the N domain by an L^d/D^d exon-shuffled recombinant (15, 18). Moreover, the former three mAbs were shown to recognize a mutated L^d in which residues I63, Q65, and I66 were replaced with the corresponding D^d

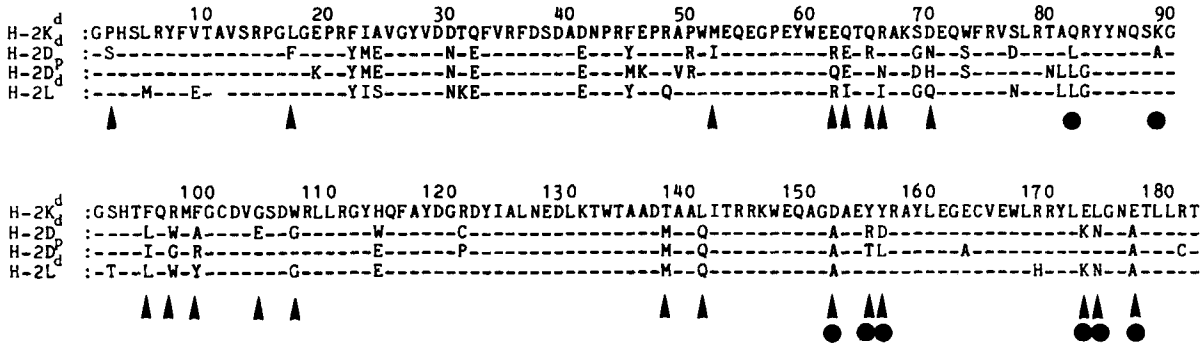

FIGURE 5. Comparison of amino acid sequences of the N and C1 domains of H-2K^d, H-2D^d, H-2D^p, and H-2L^d. Residues indicated with an arrowhead are those potentially recognized by D^d-specific mAbs. Those indicated with a filled circle are those potentially recognized by K^d-specific mAbs. References: H-2K^d (26), H-2D^d (35), H-2D^p (36) and H-2L^d (37).

amino acids (31). As shown by our data (Fig. 4), these mAbs recognize residues R62, E63, and/or R65. Since residue 62 is identical between L^d and D^d (R) and residue 66 is identical between K^d and D^d (R), results obtained by different approaches are in full agreement.

We extended such refinements to eight other mAbs, five of which had been mapped to the N domain by L^d/D^d exon-shuffled recombinants (T0127, T0132, T0134, T0141, and T0144) (15). Nevertheless, a possible contribution of C1 had been suggested by a D^P/D^d exon-shuffled recombinant (18). In addition, T0127 and T0144 were shown to recognize the mutated L^d with residues 63, 65, and 66 from D^d while T0132, T0134 recognized another mutated L^d with residues 63, 65, 66, and 70 of D^d type (31). Consistent with this observation, our results (Fig. 4) map these mAbs to residues R62, E63, and R65. The possible contribution of the C1 domain to the epitope cannot be further assessed since R1 and R2 have a C1 domain derived from D^d.

mAbs 34-420 and 34-5-8 were previously mapped to C1 by L^d/D^d exon-shuffled recombinants (8) and L^d/D^d and D^P/D^d recombinants (8, 18). We refine this mapping to residues L95, W97, A99, E104, and/or G107 (Fig. 4). Moreover, residues L95, W97, and G107 are excluded since they are identical between L^d and D^d (Fig. 5). Thus, these mAbs are more likely to recognize positions A99 and/or E104. Similarly, the mAb 28-11-5 was previously mapped in C1 by K^b/D^b exon-shuffled recombinants (9). Since K^b and D^b have identical residues at positions 99, 104, and 107, it is likely to recognize the L95 and/or the W97. From the remaining group *C* mAbs, six (T0112, T0113, T0114, T0117, T0121, T0123) had previously been mapped by D^P/D^d exon-shuffled recombinants to the C1 domain (18), in agreement with the present data (Fig. 4).

mAb 20-8-4 was previously mapped to N by K^d/K^k (13) and by K^b/D^b (10) exon-shuffled recombinants. In agreement with this mapping, we assigned residues Q82 and K99 to its epitope (Fig. 4).

mAb 34-2-12 (group *F*) was mapped in C1 downstream from the R193 (Fig. 4) in agreement with previous analyses suing L^d/D^d (7, 8), $Q7/D^d$ (14), or HLA/H-2 (11) exon-shuffled recombinants as well as truncated H-2 (20).

12 mAbs (group *D*) were mapped between R8 and R9 that differ only at positions 138 and 141 (Fig. 4). Five of them (T0103, T0104, T0108, T0109, and T0110) were previously mapped to the N domain by L^d/D^d exon-shuffled recombinants, while three (T0104, T0108, and T0109) were assigned to the N and/or C1 domain(s) by D^P/D^d recombinants (18). This apparent discrepancy is readily explained if one notes (Fig. 5) that L^d, D^P, and D^d have identical residues at positions 138 and 141 (M and Q). It is thus sufficient to assume that part of the epitope is contributed by amino acid 138, 141 and part by residues in the N domain (Fig. 5). At these positions, the sequence of D^d and that of K^d should both differ from those of L^d and D^P. Four positions satisfy this criterion in the N domain (66, 70, 81, 83). In agreement with this interpretation, four mAbs belonging to group *D* (T0108, T0109, T0110, and T0118) recognize the mutated L^d in which positions 63, 65, 66, and 70 are D^d type (31). Taken together, these data suggest a physical interaction or proximity of residues 66–70 to residues 138–141, inasmuch as they belong to the same epitopes.

Two D^d-specific (group *E*, T0105 and T0106) and one K^d-specific (group *H*,

TABLE II

Tentative Assignment of Polymorphic Residues in K^d and D^d Recognized by Specific mAbs

Monoclonal antibodies	Residues
D^d-specific	
T0107, T0119, T0120, T0129, T0131, T0133, T0137	S2, F17, I52
T0111, T0116, T0128, T0138, T0139, T0142, T0143, 66-2-4	2–52
T0124, T0127, T0136, T0141, T0144, 34-421, 27-11-13, 28-8-6, T14C	R62, E63, R65
T0132, T0134	R62, E63, R65, R66, N70
34-420, 34-5-8	A99, E104
28-11-5	L95, W99
T0112, T0113, T0114, T0115, T0117, T0121, T0123, T0126, T0130, 66-13-6, H81-199-1, T148	L95, W97, A99, E104, G107
T0104, T0108, T0109	R66, N70, M138, Q141
T0102, T0103, T0110, T0118, T0125, 66-4-8, 66-13-5, 66-13-19, H147	M138, Q141
T0105, T0106	A152, R155, D156, K173, N174, A177
34-2-12	193–276
K^d-specific	
20-8-4, F35-119-18	Q82, K89
31-3-4	D152, Y155, Y156, E173, L174, E177

31-3-4) mAbs were mapped between R9 and R10 (Fig. 4) between residues 152 and 184 (Fig. 2), in accordance with the previous mapping of these mAbs to the C1 domain using D^p/D^d (18) or K^d/K^k (13) exon-shuffled recombinants.

Our assignments for alloantigenic epitopes recognized by mAbs are summarized in Table II. For 61 mAbs specific for the K^d or D^d molecules, we have been able to assign a small number of residues to the recognized epitope. By comparison with previous exon-shuffling studies, we have, in many cases, identified two or three critical residues.

As previously published by Darsley et al. (18), the matching of chimeric antigens with a panel of mAbs occasionally reveals interdomain interactions. Because a pair of class I antigens may share the same residues at a number of polymorphic positions noncontinuous epitopes may appear continuous in experiments using recombinants made from a single pair of class I antigens. The use of the intradomain recombinants allowed us to identify additional noncontinuous epitopes which are possibly involved in interdomain interactions.

Amino acids residues assigned here for alloantigenic determinants may, in combination with neighboring residues, produce characteristic secondary/tertiary structures for which we have no information at the moment. The three-dimensional structure of class I molecules should help in interpreting these observations. Crystallography data analysis is near completion and reveals close interactions between N and C1 giving rise to a platform β strand on top of which are two α helices connected by a loop (32; Wiley, D., unpublished observations). The region 152-182 would be one of these alpha helices. Interestingly, this region also contains the epitopes recognized by allogeneic CTLs specific for the

mutant H-2K^{bm1} (33) or for H-2K^d (16) and the homologous region of HLA-A3 contains the determinant of influenza specific HLA-A3 restricted and allogeneic CTLs specific for HLA-A3 (34).

The available techniques, including those described here, now permit to readily generate a large number of chimeric H-2 molecules. It appears likely that the use of several pairs of H-2 antigens would produce a large set of detailed serological data, possibly pinpointing single amino acid residues participating in various epitopes.

Summary

11 intradomain recombinants between H-2K^d and H-2D^d were produced using an original technique based on in vivo recombination in *Escherichia coli*. After transfection into mouse L cells, all these recombinants were expressed at high levels on the cell surface. The specificities of 77 mAbs were examined on these cell lines. mAbs could be organized in 12 groups. In each group, a small number of amino acids participating in the recognized epitope(s) were identified. In a few instances, noncontinuous epitopes comprising amino acids belonging to different domains of the antigen were found. The data thus obtained are compatible with those produced in previous exon-shuffling experiments, but permit a much more precise definition of recognized epitope(s).

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