DIFFERENT STRUCTURAL CONSTRAINTS FOR RECOGNITION OF MOUSE H-2K^d AND -K^k ANTIGENS BY ALLOIMMUNE CYTOLYTIC T LYMPHOCYTES

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Class I genes of the mouse MHC encode cell surface glycoproteins that are crucial for the cellular immune response. These antigens, called H-2K, -D, and -L, consist of an H chain (mol wt 45,000) noncovalently associated with β2-microglobulin (mol wt 12,000; reviewed in reference 1). The H chain is integrated in the plasma membrane, with the largest part exposed on the cell surface and ~30–40 amino acids protruding on the cytoplasmic side. The extracellular part can be divided into three domains of similar size (α1, α2, and α3) each comprising ~90 amino acids (2–4).

Several H-2 class I genes have been cloned and characterized: the L^d gene (5, 6), the K^d gene (7), the K^b gene (8), the K^k gene (9), and the D^d gene (10). These genes all have eight exons, which correlate with the domains of the corresponding protein. The first exon encodes the signal sequence (11); exons 2, 3, and 4 correspond to the three outer domains α1, α2, and α3; the fifth exon encodes the transmembrane domain; and the remaining exons (6, 7, and 8) encode the cytoplasmic domain and the 3' noncoding region (reviewed in reference 12).

It has been shown (13) that during viral infection, H-2 antigens play an important role in the T lymphocyte response. CTL have the ability to recognize and selectively kill the infected cells. The specificity of the CTL is not directed against the viral antigen per se, but rather requires in addition self-determinants present on H-2 class I antigens. This phenomenon is known as H-2 restriction (13).

Several of the cloned H-2 class I genes have been reintroduced into cells by transfection. These genes are transcribed and translated, and their protein products can function as restricting elements in CTL assays (14–16). By using genetically altered H-2 genes we and others (17–21) have identified the regions...
of the H-2 antigens recognized by CTL, both during virus infection and in an allogeneic response.

Recently, we have analyzed (19) a large number of individual T cell clones directed against either the H-2Kd or -Kk. That study confirmed that very few CTL recognize individual domains on the H-2 molecule (19). In the present report we describe a new series of H-2 hybrid antigens that have the COOH-terminal half of the a2 domain (a2B) exchanged between H-2Kd, -Kk, and -Kb. Our results show that the a1 domain and the NH2-terminal half of the a2 domain (a2A) are required for recognition by CTL directed against the H-2Kk antigen. In contrast, for the H-2Kd antigen, the a2B domain plays a crucial role in T cell recognition.

Materials and Methods

Cell Culture and DNA Transfection of Cells. The recipient cell line used in this study was 1T 22-6 (H-2b). Cells were grown in DMEM containing 10% FCS serum. Cells were transfected by a modification of the method of Wigler et al. (22), which has been described earlier (9). The neophosphotransferase gene (23) was used as the selectable marker, together with the antibiotic G-418 (Gibco Laboratories, Grand Island, NY).

FACS Analysis and Immunoprecipitation. For these techniques we have used the mAb H100-27.55 (24) to detect the H-2Kk antigen, as well as the products of the hybrid genes pJ2 and pJ5. For the analyses of the H-2Kd antigen and the hybrid gene products pJ1, pJ3, and pJ4, the mAbs K9-18 and 20-8-4S (Hammerling, G., unpublished results and reference 25) were used. The method for labeling cells with these antibodies has been outlined before (26), and the cells (1–2 × 10⁵) were analyzed on a FACS II flow cytometer gated to exclude nonviable cells (27).

Construction of Hybrid Genes. The hybrid genes pJ1–pJ5 were constructed as described in the text and shown in the legend to Fig. 1. The gene pJ5 was constructed by using an endogenous Nco I site in the intron separating exons 3 and 4. The generation of the different subclones was done according to Frischhauf et al. (28), and has been described in detail (9).

Generation and Assay of CTL.CTL from normal and alloimmune mice were generated as described in detail previously (29). Briefly, 25 × 10⁶ spleen cells from normal mice (C3H or BALB/c) or alloimmune mice (A/J or BALB/c immunized intraperitoneally 3–4 wk previously with 10⁸ viable BALB/c or A/J spleen cells, respectively) were cultured for 5 d with an equal number of irradiated (2,000 rad) stimulator spleen cells in 20 ml of DMEM containing additional amino acids, 5% FCS, and 5 × 10⁻⁵ M 2-ME. Recovered viable cells were assayed for cytotoxicity at various E/T cell ratios in a 3-h assay using ⁵¹Cr-labeled transfected fibroblasts as targets. Percent specific ⁵¹Cr release was calculated as described (29). Spontaneous ⁵¹Cr release (in the absence of effector cells) was 10–20% of total incorporated radioactivity.

Other Materials. Restriction enzymes and other DNA-modifying enzymes were from Boehringer Mannheim, Schwalbach, Federal Republic of Germany; New England Biolabs, Beverly, MA; or Bethesda Research Laboratories, Bethesda, MD. Geneticin (G-418) was obtained from Gibco Laboratories. Radioactive isotopes were from Amersham Corp., Amersham, United Kingdom.

Results

Construction and Expression of H-2K Hybrid Genes. We have used the same method as described previously (28) to construct five new hybrid H-2K genes. To construct the hybrid gene pJ1, we used a subclone of a deletion in the 5' to 3' direction of the H-2Kd gene (9). This subclone, pKd-6b was digested with the restriction enzymes Eco RI and Cla I (Fig. 1). This generates a DNA fragment
FIGURE 1. Construction of the H-2K hybrid genes pJ1-pJ5. (A) A subclone of the pKd plasmid, pKd-6b, was digested with Eco RI and Cla I restriction enzymes to generate a 8,900-bp fragment. This fragment contains exons 4–8 of the H-2Kd gene, as well as the entire pBR322 plasmid except for 23 bp between the Eco RI and Cla I sites. The pKd plasmid was partially digested with Bcl I, followed by complete cut with Eco RI. This generates a 2,900-bp fragment containing the entire two first exons and part of the third of the Kd gene (a2A region). The 391-bp fragment containing the a2B region of H-2Kd origin was generated by digestion of subclone pKd-8h (9) with Cla I and Bcl I. The three fragments were ligated together to yield pJ1. (B) We constructed plasmids pJ2, pJ3, pJ4, and pJ5 in much the same way as in A. (A and B) Filled boxes denote sequences (exons) of H-2Kd origin, whereas open boxes denote H-2Kk exons. The hatched box is the 3' noncoding region. The plasmid pJ5 has its a2B region of H-2Kd origin (indicated by dots). The exon encoding the signal sequence is indicated by an S; exons 2, 3, and 4 are called a1, a2, and a3; the membrane spanning sequence is denoted by an M and the three cytoplasmic exons are called I1, I2, and I3. The 3' noncoding region is denoted NC. Restriction enzymes were: R, Eco RI; C, Cla I; SI, Sal I; H, Hind III; and N, Nco I. Allele-specific sequences are indicated. See text for further details.

with the length of ~8,900 bp, and it contains exons 4–8 of the H-2Kd gene. In addition, it has almost the entire pBR322 plasmid sequences retained between the Hind III and Eco RI sites, and can therefore be used as a vector fragment for replication in Escherichia coli. The second fragment was generated from a
complete Eco RI cut of the pKd plasmid, which had previously undergone partial digestion with the Bcl I enzyme. This 2,900 bp fragment contains the complete exons 1 and 2 and part of the third exon (the a2 domain). The break point in the third exon is at the Bcl I site at codon 142 of the mature H-2Kd antigen. The second half of the a2 domain (COOH-terminal end) was isolated from a subclone of the H-2Kk gene, pKk-8h, and is a Bcl I/Cla I fragment with a length of 391 bp. The three fragments were ligated together and we isolated recombinant plasmids. We determined the DNA sequences of the recombination sites and surrounding sequences. No unexpected rearrangements were found. The recombinant plasmid pJl carries H-2Kd sequences, except for the COOH-terminal half of the a2 domain (codons 142–182), which is of H-2Kk origin. Fig. 1A shows a schematic outline of the procedure.

In a similar way we have constructed the plasmids pJ2, pJ3, pJ4, and pJ5 (Fig. 1B and Materials and Methods). The hybrid genes were introduced into 1T 22-6 cells (H-2d), together with the neoprophotransferase gene as a selectable marker. Cell clones resistant to the antibiotic G-418 were further examined for their expression of hybrid H-2K antigens. This was done by using two different techniques. First, the cells were analyzed in a FACS and, secondly, the molecular weights of the hybrid antigens were determined by SDS-PAGE. For both methods, we used mAbs against either H-2Kd or -Kk. The first approach verified cell surface expression of H-2 hybrid gene products for all five gene constructs (Fig. 2). The second method revealed that the hybrid antigens had a correct molecular weight (data not shown). Thus, no major rearrangements had occurred in those regions of the gene that were not sequenced after the construction had been finished. From these experiments we conclude that H-2K hybrid molecules are expressed on the surface of the recipient cell line 1T 22-6 in amounts similar to the parental H-2Kk and -Kd antigens (Fig. 2, compare B with C and D; compare F with G, H, and J).

Recognition of the Hybrid H-2K Antigens by CTL. When the entire a2 domain of either H-2Kk or -Kd antigens is nonsyngeneic with the α1 domain, the cytolytic response is almost totally abolished for both influenza A-specific and allogeneic T cells (9, 19). To more precisely localize the region(s) that is crucial for CTL recognition, we have analyzed alloreactive T cells for their ability to lyse transfected cells expressing the new hybrid antigens.

Mice of the C3H strain (H-2k) were immunized in vitro with BALB/c (H-2d) splenocytes to generate CTL against the H-2Kd, -Dd, and -Ld antigens. Similarly, BALB/c mice were immunized with C3H cells to generate CTL against H-2Kk and Dk. These two sets of primary CTL were analyzed for their ability to lyse 51Cr-labeled target cells expressing the hybrid antigens.

Target cells expressing the pJ2 gene (J2-21 cells) were as well recognized by CTL directed against H-2d as were those expressing the parental Kd antigen (Fig. 3A). Control 1T 22-6 cells and cells expressing the pJ1 gene (J1-13 cells) (see Fig. 1B) were not recognized by these CTL, whereas cells expressing the H-2Kd antigen were lysed slightly more than background. The CTL directed against H-2k specifically lysed Kk-expressing target cells and J2-21 cells (Fig. 3B). Thus, the J2-21 cells were lysed by T cells directed against both H-2Kk and Kk, despite the fact that only the COOH-terminal half of the a2 domain (α2B) was of Kd origin.
anti-K^k | anti-K^d

\[ \text{RELATIVE FLUORESCENCE INTENSITY (A.U.)} \]

**FIGURE 2.** Cell surface expression of hybrid genes. Transfected IT 22-6 cells were stained by indirect immunofluorescence using mAbs H100-27.55 (A-D) or 20-8-4S and K9-18 (E-J), and were analyzed by flow microfluorometry. Cell clones examined are indicated. The values given in brackets correspond to the mean fluorescence intensity.

origin. J1-13 cells, IT 22-6 cells, and cells expressing the H-2K^d antigen were not lysed by the anti-H-2K^k CTL.

As these CTL were raised in mouse strain combinations that differed also in the H-2D gene product, we could not exclude the possibility that anti-H-2D CTL crossreacted with the hybrid antigens. To rule out this possibility we raised additional CTL specific only for H-2K antigens (i.e., A/J anti-BALB/c [H-2K^k, D^d anti-H-2K^d/D^d] and BALB/c anti-A/J). In this experiment we included the J3-28 and J4-23 cells expressing the pJ3 and pJ4 genes, respectively, as additional controls. Also, to increase assay sensitivity, the T cells were primed in vivo before being restimulated in vitro.

The results clearly showed that J2-21 cells were lysed by anti-H-2K^d CTL
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FIGURE 3. CTL recognition of cells expressing hybrid antigens. (A) Splenocytes from C3H mice immunized in vitro with irradiated BALB/c stimulator cells were assayed for cytotoxicity against the following targets cells: Kd-19 (filled circle); Kk-44 (open circle); J1-13 (filled triangle); J2-21 (open triangle); 1T-22-6 (filled square). (B) Splenocytes from BALB/c mice immunized as above with C3H cells, were assayed as in A. Symbols are the same as in A.

FIGURE 4. Analysis of cell lines expressing H-2K hybrid antigens with H-2Kd- and -Kk-specific CTL. (A) Splenocytes from A/J alloimmune mice were restimulated in vitro with BALB/c cells and were analyzed for their ability to lyse target cells expressing hybrid H-2K antigens. Target cells were: Kd-19 (filled circle); Kk-44 (open circle); J1-13 (filled triangle); J2-21 (open triangle); J4-23 (open square); J3-28 (X); 1T22-6 (filled square). (B) Spleen cells from BALB/c alloimmune mice were restimulated with A/J cells and analyzed as above. Symbols are the same as in A. See text for further details.

almost as efficiently as Kd-expressing cells (Fig. 4A). Furthermore, the J3-28 cells were lysed significantly over background. These cells express the pJ3 gene that contains the same α2B domain as the pJ2 gene (see Fig. 1B). The control 1T 22-6 cells, as well as J4-23 cells and Kk-expressing cells, were not lysed (Fig. 4A). Thus, this experiment excludes the participation of immunization to H-2Dd antigens as an explanation for the lysis of J2-21 cells by anti-H-2d CTL. In a reciprocal experiment (Fig. 4B), CTL raised against H-2Kk lysed both J2-21 and J3-28 cells (although to a lesser extent than Kk-expressing cells), while control 1T 22-6 cells and Kd-expressing cells were not lysed.

These results indicate two important structural differences between the Kd
and K\(^{\text{k}}\) antigens: (a) the \(\alpha 1\) and \(\alpha 2\text{A}\) domains of the H-2K\(^{\text{k}}\) antigen can constitute a target molecule for anti-K\(^{\text{k}}\) CTL; and (b) the \(\alpha 2\text{B}\) domain of the H-2K\(^{\text{d}}\) antigen seems to play an essential role for recognition by anti-H-2K\(^{\text{d}}\) CTL.

**Role of the \(\alpha 2\text{B}\) Domain for Anti-H-2K\(^{\text{k}}\) CTL.** The results described above suggest that the \(\alpha 2\text{B}\) region of the second domain of the H-2K\(^{\text{k}}\) antigen is of little importance for recognition by anti-H-2K\(^{\text{k}}\) CTL. We decided to examine this further by analyzing J5-07 target cells expressing the pJ5 gene (Fig. 1B) where this region of the \(K^{\text{k}}\) gene has been replaced by the homologous region from the H-2K\(^{\text{b}}\) gene.

Interestingly, these target cells were lysed as efficiently as cells expressing the parental H-2K\(^{\text{k}}\) gene by CTL directed against K\(^{\text{k}}\) (Fig. 5). J2-21 cells were also lysed but to a lesser extent. 1T 22-6 cells and K\(^{\text{d}}\)-expressing cells were not lysed. This result indicates that the \(\alpha 2\text{B}\) region of the \(\alpha 2\) domain is not of crucial importance for recognition by K\(^{\text{k}}\)-specific CTL or that the H-2K\(^{\text{k}}\) and -K\(^{\text{b}}\) antigens share considerable homology in this region. This will be discussed further below.

**Discussion**

In this paper we describe the construction of a novel series of hybrid genes involving H-2K\(^{\text{d}}\) and -K\(^{\text{b}}\). The site of recombination is situated in the middle of the third exon encoding the \(\alpha 2\) domain (Fig. 1, A and B). We have arbitrarily called the NH\(_2\) terminal half of the \(\alpha 2\) domain, \(\alpha 2\text{A}\), whereas the COOH-terminal half is called \(\alpha 2\text{B}\). We have introduced these new genes into the genome of 1T 22-6 cells (H-2\(^{\text{d}}\)) and established cell clones that stably express the corresponding H-2K hybrid antigens on their surface (Fig. 2).

Our aim in constructing the hybrid genes was to be able to analyze in greater detail the structural requirements for H-2K antigens to constitute target molecules for CTL. Therefore we examined cells expressing the hybrid antigens for their susceptibility to lysis by both anti-H-2K\(^{\text{d}}\) and anti-H-2K\(^{\text{k}}\) CTL. Surprisingly, J2-21 cells, which express the hybrid gene pJ2 and have H-2K\(^{\text{d}}\) sequences only in the \(\alpha 2\text{B}\) region of the \(\alpha 2\) domain, were lysed by both types of CTL (Figs. 3 and 4).
We draw the following conclusions from these results: (a) the α1 and α2A domains of the H-2K^d molecule are important for recognition by alloreactive T cells, whereas the α2B domain seems of less importance; (b) the same domains (α1 and α2A) of the H-2K^d molecule do not seem crucial for recognition by anti-K^d T cells. This does not mean that these regions can be deleted or are not participating in structural domains recognized, but rather reflects a flexibility of the α2B region of the K^d antigen to accept a high degree of variability in the α1 and α2A domains; and (c) the α2B domain of the H-2K^d antigen is of crucial importance for alloreactive CTL directed against the K^d antigen. Cells expressing the pJ1 gene (Figs. 1B, 3A, and 4A) are not recognized by anti-K^d CTL, thus confirming the importance of the α2B domain. Similarly, J4-23 cells, which express the pJ4 gene, are not recognized by either set of T cells.

It is surprising that the product of the pJ2 gene is so readily recognized by anti-K^d CTL, as our previously described gene, PC35 (9, 19), which is of H-2K^d origin in the entire α2 domain, is not. This probably indicates that the pJ2 gene product has undergone a conformational change due to the α2A region being of H-2K^k origin. Such a change presumably does not alter the overall three-dimensional structure of the molecule, as evidenced by the fact that it is still recognized by T cells, but rather may expose structures of crucial importance for T cell recognition. We believe that further analyses of the product of the pJ2 gene might eventually lead to a better understanding of what T cells do recognize.

The α2B region of H-2K^k influences the recognition by alloreactive CTL only to a limited degree (Figs. 3B and 4B). Furthermore, by substituting the homologous segment of the H-2K^b gene for this region in J5-07 cells, lysis by anti-H-2K^k CTL was not affected (Fig. 5). In contrast, J2-21 cells are lysed (three to four times) less efficiently than J5-07 cells by anti-K^k CTL (Fig. 5). A careful analysis of the amino acid sequence for the three H-2K alleles in the α2B region reveals that H-2K^b and -K^k share several residues that are different from the H-2K^d antigen (Fig. 6; residues 144, 145, 155, 163, and 177). Three of these residues (positions 155, 163, and 177) represent nonconservative shifts and include charged amino acids. We consider it likely that one or several of these amino acids are involved either directly or indirectly (via a conformational change) in the different recognition pattern seen for the anti-K^k CTL in Fig. 5 (c.f., J2-21 cells and J5-07 cells). Interestingly, one of these residues (position 155) has been shown to be responsible for T cells being able to distinguish between H-2K^b and its mutant K^bml (30–32). The total number of amino acid differences between K^b and K^k in the α2B region is five, of which at least four are nonconservative changes (Fig. 6; positions 152, 156, 173, and 174). The corresponding number for an H-2K^d/K^k comparison is eight, with at least four involving charged amino acids (Fig. 6; positions 155, 156, 163, and 177). Given this limited number of possibilities, we hope that site-directed mutagenesis of some of these amino acids will allow us to identify the residue(s) responsible for allospecific T cell recognition in this system.
**Figure 6.** Amino acid comparison of the α2B domain of three H-2K alleles. Nucleotide and deduced amino acid sequences of the H-2K^d^, -K^k^, and -K^b^ antigens were compared for residues 142–182. Amino acid substitutions are indicated above the nucleotide sequences, whereas dashes denote identity with the H-2K^d^ sequence. Single nucleotide changes are also indicated.
We have constructed a new series of hybrid genes among the H-2K\textsuperscript{d}, -K\textsuperscript{k}, and -K\textsuperscript{b}. The site of recombination occurs in the third exon, encoding the a2 domain, and divides this domain into two parts, a2A and a2B. The novel genes differ only in the COOH-terminal half of the a2 domain, i.e., the a2B region. This region, comprising residues 142–182, contains a limited number of amino acid differences between the three alleles. The hybrid genes have been introduced into 1T 22-6 cells (H-2\textsuperscript{a}), and cell surface expression of hybrid antigens was verified. Cells expressing different types of hybrid antigens have been examined for their susceptibility to lysis by cytotoxic T lymphocytes directed either against the H-2K\textsuperscript{d} antigen or the H-2K\textsuperscript{k} antigen. Our results show that the a1 and a2A domains of the H-2K\textsuperscript{k} antigen can constitute target molecules for alloimmune anti-K\textsuperscript{k} T cells, whereas the a2B region, when exchanged for K\textsuperscript{d} or K\textsuperscript{b} sequences, plays only a limited role. In contrast, the a1 and a2A domains of K\textsuperscript{d} are not sufficient to be recognized by alloimmune anti-K\textsuperscript{d} T cells. In this instance, the a2B domain seems to play an essential role. This region has undergone several amino acid substitutions involving charged residues.

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References

1. Klein, J. 1975. Biology of the Mouse Histocompatibility Complex. Springer-Verlag, Heidelberg, Federal Republic of Germany. 620 pp.
2. Coligan, J. E., T. J. Kindt, H. Uehara, J. Martiniko, and S. G. Nathenson. 1981. Primary structure of a murine transplantation antigen. Nature (Lond.). 291:35.
3. Klein, J. 1979. The major histocompatibility complex of the mouse. Science (Wash. DC). 203:516.
4. Ploegh, H. L., H. T. Orr, and J. L. Strominger. 1981. Major histocompatibility antigens: the human (HLA-A, -B, -C) and murine (H-2K, H-2D) class I molecules. Cell. 24:287.
5. Evans, G. A., D. H. Margulies, R D. Camerini-Otero, K. Ozato, and J. G. Seidman. 1982. Structure and expression of a mouse major histocompatibility antigen gene, H-2L\textsuperscript{a}. Proc. Natl. Acad. Sci. USA. 79:1994.
6. Moore, K. W., B. T. Sher, Y. H. Sun, K. A. Eakle, and L. Hood. 1982. DNA sequence of a gene encoding a BALB/c mouse L\textsuperscript{d} transplantation antigen. Science (Wash. DC). 215:679.
7. Kvist, S., L. Roberts, and B. Dobberstein. 1983. Mouse histocompatibility genes: structure and organisation of a K\textsuperscript{d} gene. EMBO (Eur. Mol. Biol. Organ.) J. 2:245.
8. Weiss, E., L. Golden, R. Zakut, A. Mellor, K. Fahrner, S. Kvist, and R. A. Flavell. 1983. The DNA sequence of the H-2K\textsuperscript{k} gene: evidence for gene conversion as a mechanism for the generation of polymorphism in histocompatibility antigens. EMBO (Eur. Mol. Biol. Organ.) J. 2:453.
9. Arnold, B., H.-G. Burgert, U. Hamann, G. Hämerling, U. Kees, and S. Kvist. 1984. Cytolytic T cells recognize the two amino-terminal domains of H-2 K antigens in tandem in influenza A infected cells. Cell. 38:79.
10. Sher, B. T., Nairn, R., Coligan, J. E., and Hood, L. E. (1985). DNA sequence of the mouse H-2D\textsuperscript{d} transplantation antigen gene. *Proc. Natl. Acad. Sci. USA.* 82:1175.

11. Blobel, G., and B. Dobberstein. 1975. Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* 67:835.

12. Steinmetz, M., and L. Hood. 1983. Genes of the major histocompatibility complex in mouse and man. *Science (Wash. DC)* 222:727.

13. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. *Adv. Immunol.* 27:51.

14. Goodenow, R. S., M. McMillan, A. Oern, M. Nicolson, N. Davidson, A. J. Frelinger, and L. Hood. 1982a. Identification of a BALB/c H-2L\textsuperscript{d} gene by DNA-mediated gene transfer. *Science (Wash. DC).* 215:677.

15. Mellor, A. L., L. Golden, E. Weiss, H. Bullman, J. Hurst, E. Simpson, R. F. L. James, A. R. M. Townsend, P. M. Taylor, W. Schmidt, J. Ferluga, L. Leben, M. Santamaria, G. Atfield, H. Festenstein, and R. A. Flavell. 1982. Expression of murine H-2K\textsuperscript{b} histocompatibility antigen in cells transformed with cloned H-2 genes. *Nature (Lond).* 298:529.

16. Margulies, D. H., G. A. Evans, K. Ozato, D. Camerini-Otero, K. Tanaka, E. Appella, and J. G. Seidman. 1983. Expression of H-2K\textsuperscript{d} and H-2L\textsuperscript{d} mouse major histocompatibility antigen genes in L cells after DNA-mediated gene transfer. *J. Immunol.* 130:463.

17. Ozato, K., G. A. Evans, B. Shykoff, D. Margulies, and J. G. Seidman. 1983. Hybrid H-2 histocompatibility gene products assign domains recognized by alloreactive T cells. *Proc. Natl. Acad. Sci. USA.* 80:2040.

18. Reiss, C. S., G. A. Evans, D. H. Margulies, J. G. Seidman, and S. J. Burakoff. 1983. Allospecific and virus-specific cytolytic T lymphocytes are restricted to the N or C1 domain of H-2 antigens expressed on L cells after DNA-mediated gene transfer. *Proc. Natl. Acad. Sci. USA.* 80:2709.

19. Arnold, B., U. Horstmann, W. Kuon, H.-G. Burgert, G. J. Hämerling, and S. Kvist. 1985. Alloreactive cytolytic T-cell clones preferentially recognize conformational determinants on histocompatibility antigens: analysis with genetically engineered hybrid antigens. *Proc. Natl. Acad. Sci. USA.* 87:7030.

20. Allen, H., D. Wraith, P. Pala, B. Askonas, and R. A. Flavell. 1984. Domain interactions of H-2 class I antigens after cytotoxic T cell recognition sites. *Nature (Lond.)* 309:279.

21. Bluestone, J. A., M. Foo, H. Allen, D. Segal, and R. A. Flavell. 1985. Allospecific cytolytic T lymphocytes recognize conformational determinants on hybrid mouse transplantation antigens. *J. Exp. Med.* 162:268.

22. Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, and L. Chasin. 1979. DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells. *Proc. Natl. Acad. Sci. USA.* 76:1373.

23. Southern, P. J., and P. Berg. 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J. Mol. Appl. Genet.* 1:327.

24. Lemke, H., G. J. Hämerling, and U. Hämerling. 1979. Fine specificity analysis with monoclonal antibodies of antigens controlled by the major histocompatibility complex and by the Qa/T1 region in mice. *Immunol. Rev.* 47:175.

25. Ozato, K., and D. H. Sachs. 1981. Monoclonal antibodies to mouse MHC antigens.
III. Hybridoma antibodies reacting to antigens of the H-2b haplotype reveal genetic control of isotype expression. *J. Immunol.* 126:317.

26. Burgert, H.-G., and S. Kvist. 1985. An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. *Cell.* 41:987.

27. MacDonald, H. R., and P. Zaech. 1982. Light scatter analysis and sorting of cells activated in mixed leukocyte culture. *Cytometry.* 3:55.

28. Frischauf, A. M., H. Garoff, and H. Lehrach. 1980. A subcloning strategy for DNA sequence analysis. *Nucleic Acids Res.* 8:5541.

29. Cerottini, J.-C., H. Engers, H. R. MacDonald, and K. T. Brunner. 1974. Generation of cytotoxic T lymphocytes in vitro. I. Response of normal and immune spleen cells in mixed leukocyte cultures. *J. Exp. Med.* 140:703.

30. Pease, L. R., D. H. Schulze, G. M. Pfaffenhbach, and S. G. Nathenson. 1983. Spontaneous H-2 mutants provide evidence that a copy mechanism analogous to gene conversion generates polymorphism in the major histocompatibility complex. *Proc. Natl. Acad. Sci. USA.* 80:242.

31. Schulze, D. H., L. R. Pease, S. S. Geir, A. A. Reyes, L. A. Sarmiento, R. B. Wallace, and S. G. Nathenson. 1983. Comparison of the cloned H-2K<sup>b</sup> variant gene with the H-2K<sup>b</sup> gene shows a cluster of seven nucleotide differences. *Proc. Natl. Acad. Sci. USA.* 80:2007.

32. Weiss, E. H., A. Mellor, L. Golden, K. Fahrner, E. Simpson, J. Hurst, and R. A. Flavell. 1983. The structure of a mutant H-2 gene suggests that the generation of polymorphism in H-2 genes may occur by gene conversion-like events. *Nature (Lond.)* 301:671.