REGULATION OF T-CELL-MEDIATED LYMPHOLYSIS BY THE MURINE MAJOR HISTOCOMPATIBILITY COMPLEX

II. Control of Cytotoxic Responses to Trinitrophenyl-K and -D Self Products by H-2K- and H-2D-Region Genes

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Genetic regulation of cell-mediated lympholysis (CML) responses to antigenic determinants that are recognized in association with H-2K- and H-2D-coded self structures have been reported for trinitrophenyl (TNP)-modified cells (TNP-self) (1) and viral-infected (2) cells, as well as to cells expressing the sex-linked H-Y antigen (3). Multi-gene (Ir gene) control of CML to the H-Y antigen has been demonstrated to map within the I-region of H-2 (4, 5). Other Ir-like genetic effects have been reported in which preferential CML responses have been observed against TNP-self cells and viral-infected cells. In this type of immune regulation, the haptenic or viral determinants are recognized predominantly in association with either the K- or the D-region products (6). Similar findings have been recently observed in the CML response against influenza virus-infected human leukocytes (7).

Recent investigations involving the generation of cytotoxic responses to TNP-self syngeneic cells have demonstrated the uniqueness of K k products in this system. First, preferential cytotoxic responses against K k-TNP antigens were observed, regardless of the accompanying D-region allele present on the stimulating population (6, 8, 9). Second, only strains that express the K k alleles have been shown to generate CTL responses against stimulator cells treated with low concentrations of trinitrobenzene sulfonate (low-dose TNP-self) (10). Mapping studies have been reported for the preferential recognition of K k over D d in the CML to TNP-self products (11) as well as that to vaccinia virus (12). However, it has not been established whether the k haplotype associated with CTL responses to low-dose TNP-self maps to the K or I regions (10). The present study was designed to provide more precise mapping of the influence of the H-2 k haplotype on the CML response to TNP-self. For this purpose we have utilized the recently derived K,I-A congeneric, recombinant mouse strain, B10.MBR (13). In this report, we demonstrate that the ability to generate a CTL response to low-dose TNP-self maps to the left of the I-A subregion. We also show that CML responses are not detected to TNP-self in association with H-2D q. The role of the H-2K- and H-2D-region gene products are discussed with respect to their importance in the regulation of CML responses involving H-2-restricted antigenic systems.

Materials and Methods

Mice. The C57BL/10 (K b I b S b D b) B10.BR (K b I b S b D b), and DBA/1 (K b I b S b D b) mouse strains were purchased from The Jackson Laboratory, Bar Harbor, Maine. The B10.AKM
Fig. 1. Cell-mediated cytotoxic responses of spleen cells from B10.AKM (K<sup>k</sup>k<sup>k</sup> D<sup>k</sup>) (●), C57BL/10 (K<sup>b</sup>b<sup>b</sup> D<sup>b</sup>) (▲), and B10.MBR (K<sup=q</sup>q<sup=q</sup> D<sup=q</sup>) (∇) mice sensitized in vitro to autologous spleen cells modified with 5, 0.5, or 0.1 mM TNBS. Effector cells were assayed on unmodified and TNBS-modified (5 mM) B10.BR (K<sup>k</sup>k<sup>k</sup> D<sup>k</sup>) (A-C), C57BL/10 (K<sup>b</sup>b<sup>b</sup> D<sup>b</sup>) (D-F), and DBA/1 (K<sup=q</sup>q<sup=q</sup> D<sup=q</sup>) (G-I) PHA-stimulated splenic blasts. Spontaneous lysis was 29.6%, 30.4%, and 27.3% for modified B10.BR, C57BL/10, and DBA/1 targets, respectively. Maximum lysis detected on unmodified targets was 2.8%. Standard errors of the means were <3% and have been omitted from the figures.

Results and Discussion

Previous studies have demonstrated that spleen cells from H-2<sup>k</sup> and H-2<sup>b</sup> but not H-2<sup>b</sup> mouse strains generated CML responses to low-dose TNP-self (10). These results are confirmed in Fig. 1A–F. B10.AKM spleen cells generated strong cytotoxic responses against 5 mM TNBS-treated stimulating cells (high dose TNP-self) and 0.5 and 0.1 mM TNBS-treated stimulating cells (low-dose TNP-self) (Fig. 1A–C). However, C57BL/10 spleen cells failed to generate cytotoxic T-lymphocytes (CTL) against autologous stimulating cells treated with 0.5 or 0.1 mM TNBS (Fig. 1D–F). In fact, C57BL/10 mice are poorer responders to high-dose TNP-self than B10.BR mice (compare Fig. 1A and D; [10]). The results of Fig. 1A–C, together with studies using the B10. A strain (10) indicate that splenic lymphocytes from mice expressing the k haplotype in the K and I regions of H-2 generate CML responses to low-dose TNP-self. To determine whether CML response potential to low-dose TNP-self was
associated with the \( k \) haplotype in the \( K \) or \( I \) regions, the B10.MBR recombinant strain was studied. As shown in Fig. 1 D–F, B10.MBR spleen cells generated CTL when stimulated with autologous cells treated with 5 but not with 0.5 or 0.1 mM TNBS. The introduction of \( k \) alleles throughout the \( I \) region did not result in ability to respond to low-dose TNP in association with \( H-2K^k \) products. It should be noted that the preference of \( K^k \) over \( D^d \) self products in CML responses to vaccinia virus (12) as well as to TNP-self (11) have been mapped to the left of \( I-A \), presumably to the \( K \) region. The results of the present study, which would appear to involve a different \( h \)-like CML function, i.e., ability to respond to low-dose TNP-self, is also associated with the \( k \) haplotype and requires \( k \) alleles in the \( K \) region. Thus, the control of this response must involve \( K \) region genes. Our findings have not addressed the question of whether \( I \)-region genes also play a role in this response, since a \( K^kI^b \) recombinant mouse strain has not been found.

It has been previously demonstrated that the presence of the \( K^k \) allele in the responding and stimulating cell populations results in a strong CTL response to \( K^k \)-TNP, but a weak response to TNP-modified \( D \)-region products (6, 8, 9). The B10.AKM and the B10.MBR recombinant strains express the \( k \) and \( b \) haplotypes, respectively, in the \( K \) region, but are identical at \( I \) and \( D \left( I^kD^q \right) \) (13). Thus, these two strains provide an opportunity to test whether there is preference of \( K^k \) over \( D^q \) in the CML response to TNP-self, and whether introduction of the \( b \) allele in \( K \) region would result in a CML response to \( D^q \)-TNP. The results shown in Fig. 1 G–I, indicate that C57BL/10 TNP-CTL and B10.MBR TNP-CTL mediate low but equivalent levels of cytotoxicity against \( H-2^q \) target cells. The observation that the B10.AKM TNP-CTL failed to lyse \( H-2^q \) targets suggests that the marginal level of lysis detected on \( H-2^q \) targets is a result of cross-reactive TNP-CTL (1, 14). In contrast to the CML responses to \( D^k \)-TNP and \( D^d \)-TNP, which can be generated when \( K^k \) is not present in the responding and stimulating populations, the absence of the \( K^k \) allele in the B10.MBR did not result in a CML response to \( D^q \)-TNP. Notably, the finding that B10.SQR (\( K^1I^bD^q \)) (data to be published elsewhere) spleen cells also failed to generate detectable CTL against \( D^q \)-TNP products makes it likely that the presence of \( k \) alleles in the \( I \) region of B10.AKM and B10.MBR are not associated with the poor response to TNP-\( H-2D^q \).

Our findings are similar to those reported for CTL responses against vaccinia and Sendai virus (12). It was suggested that the failure to generate CML responses against vaccinia and Sendai virus recognized in association with \( D^k \) self products may be attributable to \( h \) genes mapping to the \( H-2D \) region (12).

The findings discussed above indicate that the \( K \)- and \( D \)-region gene products are involved in the regulation of cytotoxic responses against self products recognized in association with viral and haptenic determinants. These observations can be accounted for by at least two models. It is possible that certain genes (\( h \) genes) for CML that are distinct from the structural genes coding for self-recognition products map within the \( K \) and \( D \) regions (12). Alternatively, it is possible that the lack of or magnitude of a CTL response is primarily attributable to the antigenic properties of each particular \( K \)- and \( D \)-region-coded molecule. In this model it would not be necessary to postulate separate regulatory (\( h \)) genes which also map to the \( K \) and \( D \) regions. Instead, a regulatory function and the antigenic structure involved in the generation of restricted CTL would both be coded for by the same gene. These models
have not considered those regulatory aspects of CTL responses which map to the I
region, and which may be associated with helper cell function (4, 5). The lack of CTL
against $D^q$-TNP suggests that $H-2D^q$ products lack self-determinants that are
immunogenic in association with TNP. It is, therefore, possible that any self antigen
with a similar structure would likewise be ineffective in generating a TNP-self CML
response. Recent structural studies utilizing N-terminal amino acid sequencing (15)
and serological (16) analyses have demonstrated a marked similarity between $L^d$ and
$D^q$ but not between $L^d$ and $D^d$ molecules. It is noteworthy that strong TNP-specific
CTL responses are generated against $D^d$ self products. However, the two structurally
similar ($L^d$ and $D^q$) molecules both fail to induce detectable cytotoxic responses to
TNP (17, 18). The inability to generate CTL against vaccinia and Sendai virus in
association with self $H-2D^q$ could also be attributed to a poor immunogenic structure
between these viruses and this particular H-2 molecule (12).

The findings of this report demonstrate that CTL responses against TNP in
association with K or D self products are controlled by genes mapping within the H-
2K and H-2D regions. These observations are consistent with the possibility that the
potential to respond to a particular antigenic determinant recognized in association
with K or D products is a function of the structure of those K and D antigens. This
does not exclude the possibility that a defect in the T-cell repertoire is involved, which
could be influenced by those same structural gene products. However, some major
histocompatibility complex (MHC)-linked Ir gene effects might be accounted for by
the failure of a specific foreign antigenic determinant to be immunogenic in association
with a particular MHC-coded self product (12). Self products could be coded for by
$H-2K$ or $H-2D$ for activation of cytotoxic precursors, and by $H-2I$ (4) for activation of
helper (or suppressor) cell precursors. In such a model it would not be necessary to
postulate the existence of regulatory (Ir) genes in addition to the structural genes that
code for K, D, and I self antigens.

Summary

An $H-2K/IA$ recombinant mouse strain was used to map the genes within the H-2
complex which determine the ability to respond in cell-mediated lympholysis (CML)
to low doses of trinitrophenyl-self (TNP-self). It was found that gene(s) which map to
the K region are involved in regulation of CML response to low-dose TNP-self. It was
also found that CML response to TNP recognized in association with $H-2D^q$ was not
detectable in this recombinant. These findings are discussed with respect to the
involvement of the $H-2K$ and $H-2D$ regions by structural and/or regulator gene
functions.

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