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Identification and Analysis of the Multigenic Control of T-Cells Responses to the 1-18 Peptide of the Hen Egg Lysozyme

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ABSTRACT

A genetic approach was used to investigate the factors that influence the T cell response to a synthetic peptide denoted hen egg lysozyme 1-18 (HEL 1-18). This peptide corresponds to a T cell epitope of the protein HEL that is presented by E^k class II MHC receptors. The level of response to HEL 1-18 varies among different inbred mouse strains. E^k expressing strains could be classified based on this criterion as either high responders (e.g., strain AKR/J and C57BR /cdJ) or low responders (e.g., strain B10.A, B10.BR, and B6.AKR). Analysis of the response of F1 hybrids between high and low responders indicated that this trait behaves in a codominant manner only in (AKR x B6.AKR) F1. We found that a variety of C57 series inbred strains, other than C57BR/cdJ (e.g., C57BL/6J, C57BL/10J, and C57/J), possess a dominant negative trait that attenuated the response of E^k–expressing strains to HEL 1-18. Further analysis of H2-congenic mice indicates that this dominant negative trait maps to MHC, as demonstrated by using the intra–H–2 recombinant congenic mouse strain, B10.A (4R) and B10.A (5R). Examination of recombinant progenies from intercrosses between AKR and C57L/J mice indicates that the dominant negative trait lies within the small region of MHC where overlap occurs. However, or one or more loci outside the MHC in the C57L/J mice may influence the response to the HEL 1-18 peptide. The effects of this trait are similar to those found in E^k – expressing low responder strains (B10.A, B10.BR and B6.AKR) suggesting that alleles for these immune response modifiers loci are shared between C57L, C57BL/6 and C57BL/10 strains.

Key words: MHC molecules, T cells, Immune response, Hen Egg, Lysosyme, Epitope

INTRODUCTION

Immune responses can be classified as either humoral or cell-mediated (Buoninfante and Cavaleri, 2025; Wang et al. 2020). Cell-mediated immunity is mediated by effector T cells generated specifically in response to antigens. Unlike humoral immunity, cell-mediated immunity can only be transferred by immune T cells and requires the recognition of peptides displayed together with molecules of the major histocompatibility complex (MHC) on a host cell surface (Pishesha et al. 2022; Wang et al. 2020). T cell receptors recognize either class I MHC molecules presented on all nucleated cells, or class II MHC molecules displayed on antigen-presenting cells (APC) (Pishesha et al. 2022). Each of these molecules plays a unique role in antigen presentation, ensuring that the immune system can recognize and respond to the different types of antigens encountered (Wang et al. 2020; Pishesha et al. 2022; Tang et al. 2025).

T cells, like B cells, arise from hematopoietic stem cells in the bone marrow. T cells migrate to the thymus gland to mature. During its maturation within the thymus, T cells come to express a unique antigen-binding receptor on their membrane, called the T-cell receptor (TCR) (Shah et al. 2021). T-cell receptors have an amino-terminal variable region with homology to the immunoglobulin V domain and a constant region with homology to the immunoglobulin C domain. T-cell receptors also have a short hinge region with a cysteine residue that forms the interchain disulfide bond. Each chain spans the lipid bilayer by a hydrophobic transmembrane domain whose notable feature is the presence of positively charged amino acids. These charged residues play an important part in the interaction of the T-cell receptor chains with the CD3 molecules (Sun et al. 2023). The variable region of the T cell receptor is derived from gene rearrangements in the V (variable), D (density), and J (joining) segments of the β chain, and the V and J segments of the α chain gene locus during T cell development in the thymus (Groettrup and von Boehmer 1993; Pfeffer and Mak 1994; Robey and Fowlkes 1994).

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The MHC molecules can be divided into two functionally and structurally distinct classes of cell surface receptor, class I and class II. The mouse has three different class I MHC genes (K, D, and L) that occupy different chromosomal positions within the complex (Molnarfi et al. 2013). Humans also have three separate MHC class I loci (A, B, and C) on chromosome 6. The molecules encoded by the human A, B, and C loci and the murine K, D, and L loci are highly similar (Kulski and Inoko 2003; Liu et al. 2024). Class II MHC molecules are comprised of two different polypeptides, called as α and β, both are encoded within the I region of the MHC. The mouse MHC class II region contains only two sets of genes, AE on chromosome 17, each coding for heterodimeric glycoproteins composed of α and β chains homologous to the human MHC class II molecules DP and DR, respectively (Malissen et al. 1983).

The earliest investigations into the regulation of T cell-mediated immune responses used a genetic approach, which led to the discovery of immune response genes that were later shown to encode MHC receptors (Abbas and Charles 2000; Shi et al. 2024). Cellular and biochemical studies have led to the recognition of antigen processing requirements and elucidated the antigen processing pathways and identified amino acid sequence motifs that distinguish presented epitopes. The initial goal of the current study was to identify inbred strains of mice that show variation in the immunodominance hierarchy of presented T cell epitopes within an intact protein antigen. The rationale was that if such strain variation in selection of dominant versus cryptic epitopes could be found among certain strains that possess the same MHC haplotype, we could establish a useful genetic approach to study mechanisms governing epitope selection. In the present study, we utilized a genetic approach to examine the relative T cell immune responses against HEL1-18, a T cell epitope of hen egg lysozyme (HEL). The basic strategy was to search for regions of well-defined T cell antigens that behaved as dominant epitopes in some strains, but as cryptic epitopes in other strains of the same H-2 haplotype. The HEL 1-18 peptide is one of nine peptides that can be derived from an intact HEL protein and are immunogenic in H-2a and H-2k mouse strains (i.e., strains that express both A^k and E^k class II MHC receptors) (Moudgil et al. 1997). HEL1-18, which binds to Ek, was chosen for this study because it has been reported to behave as a cryptic epitope in HEL 1-18 immunized mice of the B10.A and B10.BR strains (Gammon et al. 1987), but as a dominant epitope in AKR/J and C57BR/cdJ mice (Adorini et al. 1988).

The present study was an attempt to investigate the relative contribution of specific T cell epitopes in the overall response to intact antigen, which may lead to a more fundamental understanding of what influences the inherent immunogenicity of peptide antigens.

MATERIALS AND METHODS

Animals (Mice)

The following inbred strains were obtained from Jackson Laboratory (Bar Harbor, Maine): AKR/J, C57L/J, B10.A, C57BR/cdJ, B10.BR, and B6.AKR. The following F1 hybrid mice were bred at the University of Rochester Vivarium: (C57BR/cdJ × B10.A) F1, (C57BL/6J × AKR/J) F1, (C57BL/6J × C57BR/cdJ) F1, (AKR/J × B6.AKR) F1, and (AKR/J × C57L/J) F1.

Immunization

Mice were immunized as described previously by Alpert and Sprent (1982). Briefly, mice were injected in the hind foot pads with 50μL of emulsion. The emulsion consisted of 35μL antigen, 2I.5μL saline, and 25μL complete Freund's adjuvant (CFA). CFA and Hen Egg Lysozyme (HEL) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA); synthetic HEL peptides were purchased from Peptide Express (Fort Collins, CO, USA). The emulsion was made by repeatedly passing the mixture between two chromatograph syringes (Hamilton syringes) joined by a three-way stopcock. After immunization, the *in vivo* response was allowed to continue for eight days, at which time the mice were sacrificed and the draining popliteal lymph nodes were removed for *in vitro* proliferation analysis.

T-cell proliferation assay

The draining popliteal lymph nodes were removed and placed in a 10mL tube containing Hank's balanced salt solution (HBSS) on ice. Cells were released by gently grinding the isolated nodes between two frosted glass slides in the tissue culture dish that contained 10mL cold HBSS. The cell suspensions, which contain pieces of uncrushed lymph node, fat, and connective tissue, were transferred to a 15mL conical tube and placed on ice for 5 minutes to allow the debris to settle. Suspended cells were pipetted into a new 15mL conical tube and spun for 5 minutes at 4°C in a refrigerated centrifuge (1000rpm, 200 x g). The supernatant was aspirated, and the cell pellet was washed in 10mL HBSS and centrifuged a second time. The pellet of cells was resuspended in HL-1 media (using 2mL media for each pair of lymph nodes used in suspension) and kept on ice until all the cells were counted. Then 10μL of cell suspension was mixed with 90μL. Trypan blue dye, and the viable (non-blue) cell count was determined with a hemocytometer. The cell concentration of each tube was then adjusted to 5 x 10^6 cells/mL. Finally, 100μL of cells were placed into each well of a micro titer plate that contained 100μL of the antigen preparation and media and were placed in the incubator (37℃/5% CO2) for three days.

Assays were setup such that quadruplicate wells received an initial antigen concentration with subsequent sets of four wells receiving serial dilutions for the original antigen preparation. Quadruplicate wells were also set up containing 1.5μg/mL ConA and media alone, which served as positive and negative controls, respectively.

After three days of culture, 25μL of (1μCi) ^{3H}thymidine was added to each well, and incubated for another 18 hours. Plates were harvested on a Packard Micromate 196 cell harvester and the filters were read on a Packard Matrix 96 direct beta counter. Data was plotted as the mean [^{3}H] thymidine incorporation, comparing quadruplicate cultures, standard error of the mean (SEM).

Statistical analysis

Statistically significant variations ($P<0.05$) were detected using the two-sided Student's T test. Data were presented as the mean (± standard error of the mean).

Table 1: HEL-derived immunogenic A^k and E^k T cell epitopes

HEL Peptide	Sequence	MHC Molecule	Immunodominant Status
HEL1-18	KVFGRCELAAAMKRHGLD	E^K	Cryptic
HEL13-35	KRHGLDNYRGYSLGNWVCAAKFE	A^K	Dominant
HEL25-43	LGNWVCAAKFESNFNTQAT	E^K	Cryptic
HEL33-53	KFESNFNTQATNRNTDGSTDY	A^K	Subdominant
HEL46-61	NTDGSTDYGILQINSR	A^K	Dominant
HEL71-85	GSRNLCNIPCSALLS	A^K	Cryptic
HEL85-96	DITASVNCAK	E^K	Cryptic
HEL96-118	KKIVSDGDGMNAWVAWRNRCKGT	E^K	Cryptic
HEL116-129	KGTDVQAWIRGCRL	A^K	Subdominant

RESULTS

HEL 1-18 is a cryptic epitope in all A^k, E^k inbred strains

HEL-derived immunogenic A^k and E^k T cell epitopes are illustrated in Table 1 and Fig. 1. Experiments were set up initially to determine if these disparate observations regarding the behavior of HEL l-18 could be reproduced in a single laboratory. Mice of two inbred strains (AKR/J, C57BR/cdJ, B10.A, and B10.BR) were immunized with HEL 1-18 as described previously. Seven days later, draining lymph nodes were excised from sacrificed animals, and dissociated lymph node cells were tested for response to HEL 1-18. Results from representative experiments are shown in Fig. 2 and summarized in Table 2. The immunogenicity of HEL1-18 varies among different E^k expressing mouse strains.

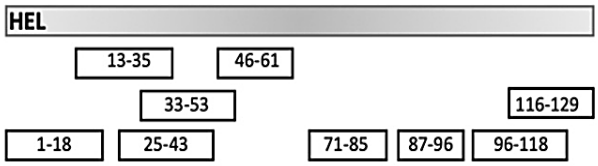

Fig. 1: Schematic showing epitopes of HEL.

Table 2: Immunological responses of various inbred strains to HEL 1-18

Strain	H-2 Haplotype	HEL 1-18
C57BR/cdJ	K	+++
AKR/j	K	+++
B10.A	A	-
B10.BR	K	-

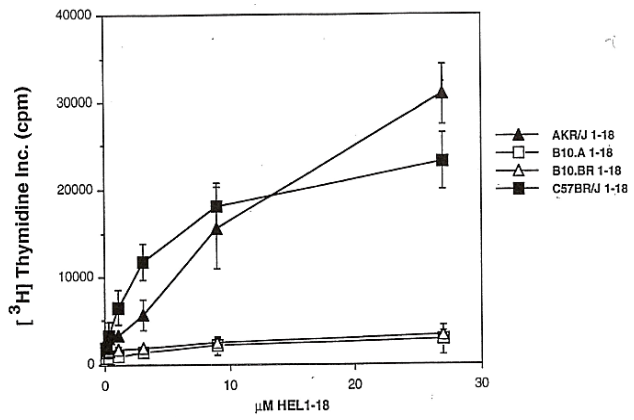

Fig. 2: Responses of AKR/j, B10.A, B10.BR and C57BR/cdJ strains to HEL 1-18. Mice were immunized with synthetic HEL 1-18 peptide, and draining lymph node cells were subsequently assayed for specific responses to HEL 1-18. Data were plotted as the mean of quadruplicate cultures (+/-) SEM.

AKR/J and C57BR/cdl mice demonstrated strong responses to HEL 1-18, whereas mice of B10.A and B10.BR strains responded poorly to this peptide. Thus, although HEL1-18 is clearly dominant in over ARK/J and C57BR mice, its status in the immunodominance hierarchy is more difficult to assess in B10.A and B10.BR mice, due to their relatively weak inherent immunogenicity.

Strength of the immunogenicity of HEL 1-18 among different E^k act dominant negative trait

In our previous publication, we observed that nonchromogenic F1 hybrid mice derived from breeding hemizygous TCR beta-chain transgenic mice (H-2^k) with C57Bl/6J mice were extremely poor responders to HEL 1-18 (Thatcher et al. 2000). As transgenic line used was established by successive breeding with C57BR/cdj mice. These results suggest that one or more genes within the C57BL/6 genome were capable of extinguishing the high responder phenotype of at least some E^k – expressing strains.

To test this hypothesis, F1 hybrid was generated between C57Bl/6J and ARK /J strains and tested for their responses to the HEL 1-18 peptide. As shown in Fig. 2, hybrids demonstrated extremely poor responses, even though both ARK and C57BR mice gave a strong response to this peptide. Thus, this finding is consistent with the concept that C57BL/6J mice contain one or more dominant negative regulatory genes of T cell response to HEL 1-18/ E^k. We also tested two additional inbred strains that are closely related to C57BL/6Jsj and C57BL/snSgL F1 hybrid between each of these other C57 strain and AKR/J mice were generated and used to investigate the strength of the same poor response to this antigen, even though the ARK/J mice were generated and used to investigate the strength of the HEL 1-18 E^k T cell response. As shown in Fig. 3 and 4, both (AKR x C57L) F1 and (AKR X B6) F1 mice exhibit the same poor response to this antigen, even though the AKR parental strain is a strong responder. These results indicate that all three C57 strains inbreed share a similar dominant negative effect on the HEL1-18 /E^k response, which likely depends on one or more common genes.

The dominant negative effect of C57 genes on AKR mice within the H-2^b haplotype

Among the genes that share allelic forms between C57BL/g and C57BL/10 are found in the H-2 complex. All three strains possess the H-2^b haplotype, and although it is not immediately apparent how MHC molecules could exert a dominant negative effect, the fact that they are an integral component of T cell antigen recognition makes them

candidates for further investigation. In support of this idea, it should be noted that B10.A mice were generated from the C57BL/10SnSgJ Strain, yet it does not confer this dominant negative trait (Date not Shown). The only difference between B.10 A and C57BL/10 SnSgJ strain is in their MHC haplotype. C57BL/10 with the H-2a haplotype of A/J (Lonai and McDevitt 1974).

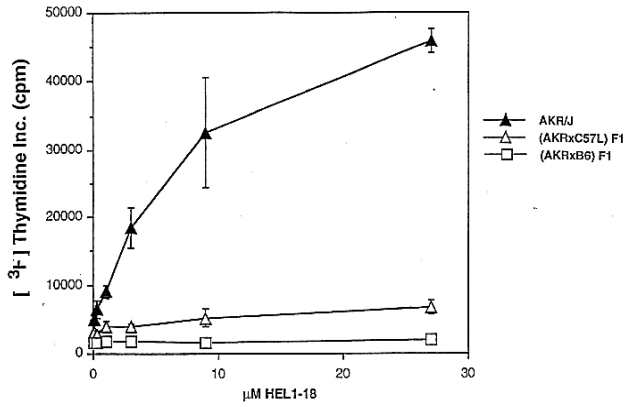

Fig. 3: Responses of AKR/j, (AKR x C57L) F1, and (AKR x B6) F1 strains to HEL 1-18. Mice were immunized with synthetic HEL 1-18 peptide and assayed for specific responses to HEL 1-18. Data were plotted as the mean of quadruplicate culture (+/-) SEM.

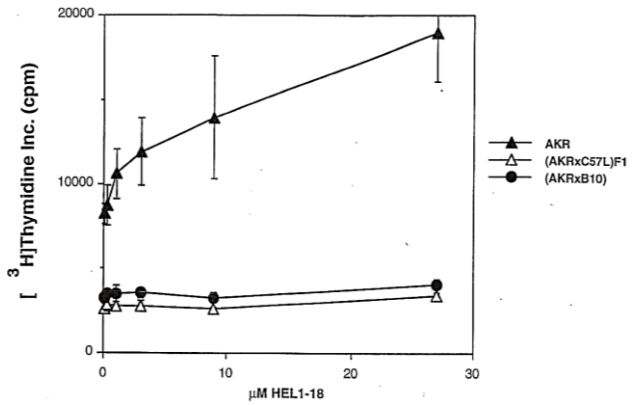

Fig. 4: Responses of AKR/J, (AKR x C57L) F1, and (AKR x BL/10) F1 strains to HEL 1-18. Mice were immunized with synthetic HEL 1-18 peptide and assayed for specific responses to HEL 1-18. Data were plotted as the mean of quadruplicate cultures (+/-) SEM.

Evidence in further support of this hypothesis comes from the analysis of the HEL1-18 response of another congenic strain, B6.AKR. This congenic inbred strain consists of mice with a C57BL/6 genetic background, except for the MHC, which comes from AKR ($H2^k$). Those congenic mice yield a poor response to HEL1-18 (Fig. 5), similar to that of the B10.A strain (Fig. 2). In addition, F1 hybrids between AKR and B6.AKR shows a modest response (Fig. 6). This result is consistent with the hypothesis that the dominant negative trait resides within H-2^b and not elsewhere in the C57BL/6 genetic background.

The dominant negative influence of H-2^b on the immunogenicity maps within the I-B sub region

To further localize the dominant negative trait within H-2^b, two independent intra-MHC recombinant congenic stains, B10.A (4R) and A10.A (5R) were analyzed. These two stains were produced from the intercrossing of the B10.A and C57BL/10 strains and the selection of recombinants within the H-2 complex. Table 3 listed the distribution of MHC alleles possessed by B10.A (4R) (Klein et al. 1981) and B10.A (5R) (Kobori et al. 1986; Simpson et al. 1986), as well as B10.A and C57BL/10 parental strains. Although B10.A (4R) and B10.A (5R) are independently derived, they represent reciprocal recombinant congenic strains. By comparing the HEL1-18 response of E^k expressing mice that possess an intact H-2^b region (AKR X C57BL/10) F1 with mice that possess either the right half of $H2^b$ (AKR X B10.A(4R)) F1 or the left half of $H2^b$ (AKR X B10.A(5R)) mice. Surprising, since it is expected that either (AKR X B.10.A (4R)) F1 (AKR X B10.A (5R) F1 will give a high response, all gave a poor response to HEL1-18 (Fig.7). This result suggests that either the dominant negative trait cannot be localized to a discrete region within the MHC, or it is confined to a small overlapping segment of the MHC-2B DNA shared by both B10.A (4R) and B10.A (5R) strains (Fig. 8).

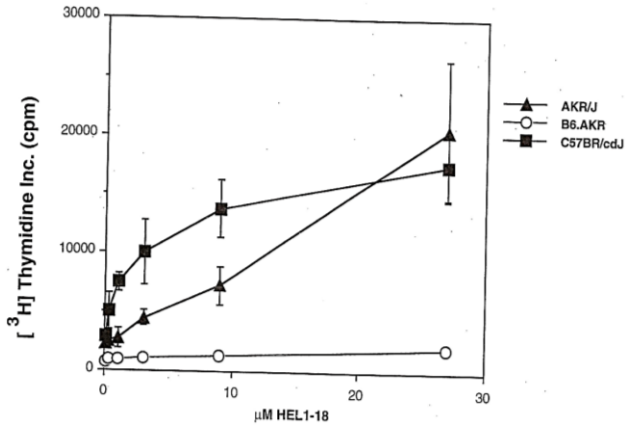

Fig. 5: Responses of AKR/J, B6.AKR and C57BR/cdJ F1 strains to HEL 1-18. Mice were immunized with synthetic HEL 1-18 peptide and assayed for specific responses to HEL 1-18. Data were plotted as the mean of quadruplicate cultures (+/-) SEM.

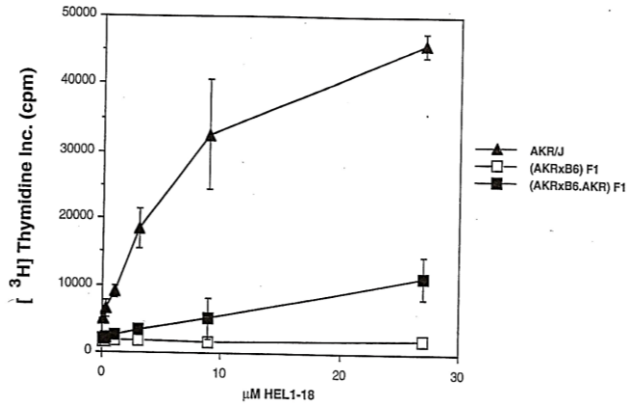

Fig. 6: Responses of AKR/J, (AKR/J x B6) F1, (AKR/J x B6.AKR) F1 strains to HEL 1-18. Mice were immunized with synthetic HEL 1-18 peptide and assayed for specific responses to HEL 1-18. Data were plotted as the mean of quadruplicate cultures (+/-) SEM.

Table 3: Distribution of alleles within the MHC of various inbred, congenic and intra -H2- recombinant congenic strains

Strain	MHC Alleles					
	K	Aα	Aβ	Eα	Eβ	D
AKR/J	K	K	K	K	K	K
C57BR/cdJ	K	K	K	K	K	K
B10.A	K	K	K	K	K	d
B10.BR	K	K	K	K	K	K
B10.A (4R)	K	K	K	K		b
B10.A (5R)	b	b	b	K/b	K	d
C57BL/10 (B10)	b	b	b	b/K		b

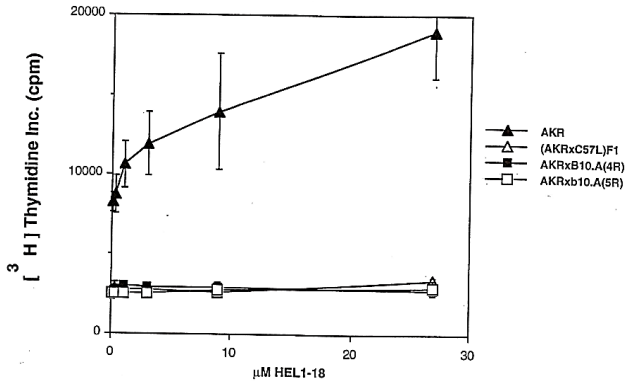

Fig. 7: Responses of AKR, (AKR x C57L) F1 strain and back cross mice (AKR x B10.A(4A)) F1 and (AKR x B10.A(5A)) F1 to HEL 1-18. Mice were immunized with synthetic HEL 1-18 peptide and assayed for specific responses to HEL 1-18. Data were plotted as the mean of quadruplicate cultures (+/-) SEM.

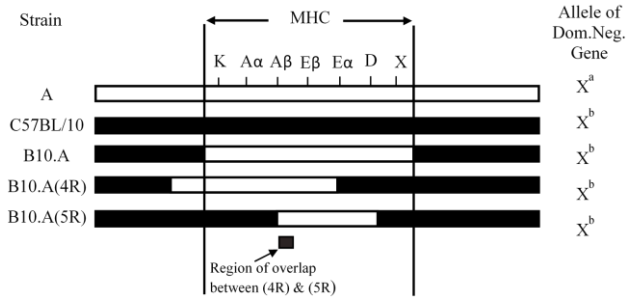

Fig. 8: The recombination boundaries of B10.A, B10.A (4R) and B10.A(5R) may differ at both ends. This a schematic representation of how the "K end "and "D end "boundaries of the three congenic strains may differ. Based on this scenario, it is possible to reconcile the apparently disparate data regarding the HEL 1-18/ E^k responses of B10.A, B10.A (4R) and B10.A(5R) mice. The gene that can carry the dominant negative trait is designated "X". X^b derived from C57BL/10, is the allele that possesses the dominant negative trait. X^a is an alternate allele from the A Strain that lacks this phenotype. The Vertical bars intersecting the five chromosome bars mark the MHC boundary defined in B10.A.

DISCUSSION

As a result of our initial analysis, we identified a slight variation in the immune responsiveness of the E^k-expressing strain to the HEL 1-18 peptide. This polymorphism resulted in the discrimination of high and low responder strains to HEL1-18 after immunization of mice with this peptide. The results show that F1 hybrids between high and low responding strains exhibit either low or intermediate levels of response. For example, the (AKR/J × C57BL/6) F1, (AKR × C57BL/10) F1, and (AKR/J × B6.AKR) F1 hybrids (as shown in Fig. 2, 4, and 6) all showed reduced or intermediate T cell responses. This suggests that the difference between high and low responding strains may be influenced by thymic education processes. In some F1 hybrids, the low response may be due to negative selection of HEL 1–18 specific T cells in the thymus. In hybrids showing intermediate responses, it appears that thymic selection—whether positive or negative—might not be the only factor involved. If the F1 hybrids had shown a high responder phenotype, it would have suggested that positive selection of HEL 1–18 specific T cells occurred. The impacts of the thymic selection on T cells responses to antigens were reviewed by Irla (2022) and Ashby and Hogquist (2024). Another factor that may contribute to low and intermediate responsiveness is the possibility that HEL 1–18 may specifically stimulate regulatory T cells resulting in inhibitory responses. A study by Adorini et al. (1979) demonstrated that the lack of responsiveness of B10 mice to HEL can be attributed to the activation of suppressor T cells by a small portion of the protein.

The demonstration of codominance implicates other elements of the immune response. As shown in Fig. 6, (AKR/J x B6.AKR) F1 strain for example has a particular TCR V gene segment that may normally dominate the HEL1-18/E^k response. In this scenario, a polymorphism in such a dominating V gene segment could alter the ability of TCRs using this V segment to recognize HEL1-18/E^k, resulting in a partial hole in the repertoire in Fl hybrids between high and low responder strains (Vessey et al. 1996). Future studies involving genetic mapping of this codominant trait, in conjunction with analysis of V gene usage by HEL 1-18/E^k-specific T cells, will allow this hypothesis to be tested. Alternatively, other candidate genes for controlling this high/low responder phenotype may influence the immune response through the regulation of T cell effector functions. It is possible that the strain variation observed resides in the preference for evoking Th1 versus Th2 responses to HEL1-18. Thus, strains that appear to be "low" responders to HEL1-18 may preferentially generate a Th2 response to this peptide and consequently perform poorly in a T cell proliferation assay. This explanation can be tested by measuring the frequency of Th1 and Th2 responder cells produced in different strains using ELISpot to discriminate between antigen-specific clones that produce Th1- vs. Th2-specific cytokines. The cytokines generated by antigen-presenting cells (APCs) in response to pathogens are the primary factor influencing segregation into TH1 or TH2 helper T cells (Gaudino and Kumar, 2019). It should be noted that other examples of inbred strain variation in the dominance of either Th1 or Th2 responses to particular antigens have been reported previously (Locksley et al. 1987; Scott et al. 1988), although these have involved the response to intact pathogens rather than single epitopes. Mice from backgrounds like BALB/c, A/J, and DBA/2 mice have been shown lean toward a TH2-predominant response while C57BL/6 mice have been shown to exhibit a TH1-type tendency to infections (Sellers et al. 2011). Finally, complementation tests using F1 hybrids between the various low responder strains will determine whether this trait is controlled by the same set of genes in each of these low responder strains.

The observation that several strains carry a dominant negative trait for responsiveness to HEL 1-18 represents a novel class of immune response gene effect. Three different lines of evidence indicate that this dominant negative phenotype resides within the H-2^b haplotype. First, this trait correlates with H-2^b among C57 series mouse strains. Three of four C57 strains tested, all possessing H-2^b (C57BL/6, C57BL/10 and C57L) carry the dominant negative trait. The one C57 strain that lacks this phenotype possesses the H-2^k haplotype (C57BR). Second, three independent C57 congenic strains which have had H-2^b replaced with another MHC haplotype (B10.A, B10.BR and B6'AKR) lack the dominant negative trait. This data strongly supports the contention that one or more genes within the H-2^b MHC actively attenuates the ability of E^k expressing mice from either presenting or otherwise responding to the HEL1-18 peptide.

It was expected that comparing the HEL 1-18 response of two set of F1 hybrids each between a high responder strain (AKR) and two complementary intra-I region recombinant congenic strains (B10.A(4R) and B10.A(5R) would allow an assignment of the dominant negative trait to one end of the MHC complex. Surprisingly, the results indicated that both intra-I region recombinant congenic strains possess this phenotype, preventing a simple mapping of the dominant negative trait. This result, if supported by additional experiments, leads to the following plausible explanations:

The boundaries of the A/J -derived congenic region differ between B10.A, B10.A (4R) and B10.A (5R)

One explanation is that the dominant negative gene is located immediately outside the limits of the classical H-2 complex. Furthermore, the boundaries of the congenic region differs between the three congenic strains, with the extent of the A/J derived region in B10.A includes the gene that influences the HEL 1-18 response (hence B10.A lacks the dominant negative trait), but in both B10.A (4R) and B10.A (5R) this gene is excluded from the A/J derived region (resulting in both of these strains carrying the dormant negative trait). An example of this scenario is shown in Fig. 2. Currently, there is no data that addresses the precise recombination compared to that of B10.A (4R) and (5R), however, as B10.A (4R) and (5R) independently arose early during the generation of the B10.A strain (Stimpfling and Richardson 1965). All the boundaries of the three strains likely differ.

Multiple independently acting dominant, negative loci exist within H-2^b

Another explanation for these results is that there are two or more dominant negative genes within the MHC complex, with one or more of these residing towards "K" the end and others located on the "D" end of the H-2 region. If true, these individual dominant negative genes would have to be capable of acting independently to affect the observed phenotype. Although formally possible, we believe that such a complicated *ad hoc* scenario is less plausible than some of the other possible explanations.

A single dominant negative locus exists within a small H-2^b-derived gerontic DNA region shared by B10.A(4R) and B10.A(5R)

Although B10.A(4R) and B10.A(5R) are considered to be functionally reciprocal recombinant congenic strains, they were independently derived and differ at the precise recombination point between H-2^b and H-2^k (Stimpfling and Richardson 1965). The recombination point for both have been shown to reside within the second intron of the Eβ gene, between β1 and β2 exons (Kobori et al. 1986; Zimmerer and Passmore 1991).

However, whereas the recombination point in B10.A(5R) has been localized to within a 1kb region at the 3`end of this intron (Kobori et al. 1986), the recombination point in B10.A(4R) been shown to be within a discreet 2.9 kb interval at the 5'end of the same intron (Zimmerer and Passmote 1991). These two recombination hotspots are separated by 192 base pairs, which is polymorphic between H-2_b and H-2^k (Kobori et al. 1986). Thus, a 192bp portion of the second intron of the Eβ gene differs between $E\beta^b$ and $E\beta^k$, and although the Eβ genes of B10.A(4R) and B10.A(5R) encode reciprocal hybrid proteins between $E\beta^b$ and $E\beta^k$, they share this 192bp intronic sequence derived from $E\beta^b$.

Therefore, the observed dominant negative trait could be associated with this 192bp polymorphic DNA segment (Zimmerer and Passmore 1991). Considering the small size of this polymorphic DNA region, it is highly unlikely that it encompasses a conventional structural gene. A small portion of a gene, such as an exon, could reside in this segment and be capable, via differential splicing, of being incorporated into a known gene within the MHC, such as Eβ. However, no evidence of incorporation of a "cryptic" exon has been reported for Eβ or any other MHC gene. An alternative explanation is that this 192bp DNA segment contains an exon that forms a part of an unknown gene that spans outside Eβ. A third possibility is that this genomic sequence contains a regulatory element that modulates the expression of one or more genes within the MHC.

The dominant negative effect is due to a complex interaction of "k" and "b" alleles of different class II MHC chains

The genetic data reported herein are reminiscent of results obtained from the detailed analysis of two immunogenetically defined I-sub regions, I-B and I-J (Kobori et al. 1986). Both I-J and I-B were mapped to the same relatively small DNA region within the EB gene as the dominant negative phenotype identified herein. In fact, our results are consistent with the localization of the dominant negative trait that we have identified with I-B, although the dominant negative aspect of this genetic trait is unlike any immune response effect ascribed to I-B (Simpson et al. 1986).

Nonetheless, a similar mechanism may be responsible for both the dominant negative and I-B immune response effects. In the case of I-B, Simpson and coworkers have proposed that one way of reconciling the apparently disparate immunological and genetic data is to propose that I-B effects are mediated by novel class II MHC molecules that are exclusively produced by H-2 hybrid and recombinant mice. This concept was formulated based upon the recognition during the 1980s that certain non-classical class II MHC molecules can be formed *in vivo* by pairing different class II chains in nonconventional combinations (Spencer and Kubo 1989; Ruberti et al. 1992; Spencer et al. 1993).

These pairings can comprise different allelic hybrid forms of standard class II molecules (e.g., $E\alpha^k$ and $E\beta^b$) or heterodimeric class II receptors (e.g., certain alleles of Eα together with certain alleles of Aβ). In situations where a given response depends upon certain hybrid class II molecules containing hybrid alleles, mapping studies would indicate the location of the "trait" to be in between the interacting genes (e.g., in between Eα and Eβ or Aβ using the above examples). Also, given that recombination within the MHC occurs predominantly within a limited number of recombinational hotspots (e.g., within the second intron of Eβ (Kobori et al. 1986; Zimmerer and Passmore 1991).

The locus responsible for the observed phenotype would map to the particular hotspot. This phenomenon has been used to explain many of the I-B region (k gene) effects (Simpson et al. 1986). In the case of the dominant negative trait considered here, such a hybrid MHC molecule scenario does not fit quite easily. This is because B10.A(4R) and B10.A(5R) are precisely reciprocal with regard to known MHC coding regions. Consequently, they share no class II allele, which is at variance with the observation that each strain conveys the dominant negative trait.

However, one way of explaining this problem would be to propose that the formation of hybrid $E\alpha^k$, $E\beta^b$ molecules in H-2^k/H-2^b hybrid mice exerts a dominant negative effect on the ability to form complexes between HEL 1-18 and $E\alpha^k$, $E\beta^b$. Furthermore, the dominant negative effect is carried by both the β1 and β2, exon of $E\beta^b$. Thus, both B10.A(4R), which expresses a $\beta1^k$ and $\beta2^b$ hybrid Eβ chain and B10.A(5R) which expresses the reciprocal $\beta1^b$ and $\beta2^k$ hybrid Eβ chain, carry the dominant negative trait.

In order to test these various possible explanations for the data we report, the most direct line of investigation would be to generate a series of transgenic mice on the H-2^k background containing different MHC genes derived from H-2^b mice. This would allow a direct test for associating the dominant negative trait with one H-2^b MHC gene. Further studies using "exon-shuffled' MHC genes could determine within which MHC domain this dominant negative trait resides.

Conclusion

A genetic approach was used in the current study to investigate the factors that influence the T cell response to the synthetic peptide HEL 1-18. T cells' specific responses against the HEL 1-18 peptide varied among different inbred mouse strains. Thus, Ek expressing strains could be classified as either high responders or low responders. Examination of recombinant progenies from intercrossing high and low responders' mice indicated that the dominant negative within the small region of MHC in which there is overlap with one or more loci located outside the MHC peptide binding groove, may influence the response to the HEL 1-18 peptide. Future studies may examine other factors negatively influencing the T cells response to HEL 1-18, starting with identifying the phenotypes of the HEL 1-18 responding T cells.

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