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A Novel Class II-Binding Motif Selects Peptides That Mediate Organ-Specific Autoimmune Disease in SWXJ, SJL/J, and SWR/J Mice1

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Idiopathic dilated cardiomyopathy (DCM) is a syndrome characterized by dilatation of the ventricular chambers on one or both sides of the heart. Accompanying this dilatation is a concomitant loss of myocyte contractility that often leads to decreased cardiac output and large-scale multiorgan failure (1). DCM may be induced experimentally by several methods, including immunization with protein (2) or protein fragments (3–5) derived from cardiac myosin or infection with cardiotropic viruses (6–8).

Recent work has shown that immunization with xenotypic porcine cardiac myosin with 90% homology with murine cardiac myosin results in experimental autoimmune myocarditis (EAMC) and DCM in SWXJ mice expressing the H-2q,s hybrid haplotype of the SWR/J (H-2q) and SJL/J (H-2s) parental strains (9).

In the current study, we have identified disease-inducing peptides of cardiac myosin by using a novel sequence-binding motif for I-Aβ and I-Aα class II molecules. We focused our attention on cardiac α-myosin heavy chain (CAMHC) because it is the predominant protein expressed in the adult murine heart (10, 11) and because several studies have shown that the α-chain is more immunogenic than the highly homologous cardiac β-myosin heavy chain (CBMHC) (12, 13). However, the H-2q,s motif may be particularly useful in implicating previously overlooked proteins as autoimmune targets and in facilitating the development of new organ-specific autoimmune mouse models for human diseases. The Journal of Immunology, 2002, 169: 6507–6514.
used autoimmune susceptible SJL/J, SWR/J, and SWXJ mouse strains.

**Materials and Methods**

**Mice and immunization**

SWXJ (H-2k) mice were generated by mating SJL/J (H-2b) males with SWR/J (H-2d) females at The Jackson Laboratory (Bar Harbor, ME). At 6–10 wk of age, mice were injected s.c. in the abdominal flank with 200 μg of peptide and 400 μg *Mycobacteria tuberculosis* H37RA (Difco, Detroit, MI) in 200 μl of an emulsion of equal volumes of water and IFA (Difco). In experiments involving disease induction, each mouse also received an i.v. injection on day 0 with 2.0 μg of purified Bordetella pertussis toxin (List Biological Labs, Campbell, CA). Mice were euthanized by asphyxiation with CO₂, followed by cervical dislocation. All protocols were approved by the Institutional Animal Care and Use Committee in compliance with the Public Health Service Policy on humane care and use of laboratory animals.

**Peptides**

All peptides were synthesized by the Molecular Biotechnology Core Facility of the Lerner Research Institute using standard solid-phase methodology and 9-fluorenylmethoxycarbonyl side chain-protected amino acids. Peptides were purified >97% by reverse-phase HPLC, and amino acid composition was confirmed through mass spectroscopy. Synthesized peptides were derived from the published sequence of murine cardiac α- and β-myosin heavy chain isoforms (14, 15).

**Cell culture and proliferation assays**

To determine peptide immunogenicity, lymphocytes were isolated from lymph nodes (LN) 10 days after immunization. Draining inguinal and axillary LN were removed and teased into single cell suspensions, washed three times in HBSS (Life Technologies, Grand Island, NY), and cultured in 96-well flat-bottom microtiter Falcon plates (BD Labware, Franklin Lakes, NJ) at 3 × 10⁵ cells/well in DMEM (Mediatech Cellgro, Herndon, VA) supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 1% HEPES buffer, 2% 1-glutamine, and 1% penicillin/streptomycin (Life Technologies). Synthetic peptides were added in serial 10-fold dilutions to triplicate wells with positive control wells containing 2 μg/ml anti-mouse CD3 (BD PharMingen, San Diego, CA) or 20 μg/ml *M. tuberculosis* H37RA. Negative control wells contained no Ag or various doses of peptide p139–151 of myelin proteolipid protein 139–151, an immunogenic peptide that induces experimental autoimmune encephalomyelitis (EAE) in SJL/J mice (19). Cells were cultured at a final volume of 200 μl/well. To measure recall responses to immunogenic peptides, spleens were removed 8–9 wk after immunization, and lymphocytes were enriched by centrifugation for 15 min at 2400 rpm on a Ficoll gradient containing 14% Ficoll (Ficoll, Eurobio, Paris, France) and 90% (w/v) sucrose (Gibco, Grand Island, NY). Cells were washed twice in HBSS, and cultured in 96-well flat-bottom Falcon plates, as described above. All cell cultures were incubated at 37°C in humidified air containing 5% CO₂. After 96 h of culture, wells were pulsed with methyl[3H]thymidine (1.0 μCi/well, sp. act. 6.7 Ci/mmol; New England Nuclear, Boston, MA). Sixteen hours after pulsing, cultures were harvested by aspiration onto glass fiber filters. Levels of incorporated radioactivity were determined by scintillation spectrometry. Results are expressed as mean cpm of triplicate experimental cultures with Ag divided by mean cpm of cultures without Ag (stimulation index).

**T cell proliferation**

Ten days after immunization with either pe406–425 or pe1631–1650, CD4⁺ and CD8⁺ T cells were positively purified from whole LN cells (LNC) by magnetic bead separation on a MidiMACS cell separator (Miltenyi Biotech, Auburn, CA), according to manufacturer’s specifications. The enriched fractions were determined by analysis on a FACScan flow cytometer using CellQuest software (BD Biosciences, San Jose, CA) and was consistently found to be >95%.

**MHC restriction**

MHC restriction was determined by several methods. Primed CD4⁺ and CD8⁺ T cells were purified from LNC 10 days after immunization of SWXJ mice with either pe406–425 or pe1631–1650. The purified cells were activated with peptide at 100 μg/ml in cultures containing 2.5 × 10⁵ cells/microtiter well and 5 × 10⁴ gamma-irradiated (2500 rad) splenocyte feeders from SWXJ mice and from parental SJL/J and SWR/J mice. Ag-specific proliferation was determined, as described above. Class II restriction was also performed by measuring proliferation of purified CD4⁺ T cells from primed LN in the presence of 10 μg/ml of anti-I-A⁺ (clone 10-3.6) or anti-I-A⁺ (clone 25-9-17) purchased commercially (BD PharMingen).

**Purification of mouse cardiac myosin**

Mouse cardiac myosin was purified from Swiss Webster white mouse hearts purchased commercially (Pel-Freez, Roger, AR). Myosin purification was performed, as previously described (20). Briefly, hearts were minced and homogenized in Heselbach-Schneider solution containing 0.3 M KCl, 0.15 M KH₂PO₄, 0.01 M Na₃PO₄, and 1.0 mM MgCl₂ at pH 6.8. The solution was stirred and centrifuged, and the supernatant was precipitated overnight in a 20-fold volume excess of H₂O. The myosin was collected by centrifugation at 12,000 × g for 10 min; resuspended in 0.3 M KCl, 0.01 M imidazole, 5.0 mM MgCl₂, and 5.0 mM Na₃ATP buffer at pH 6.8; and recentrifuged to remove actin. The myosin protein was precipitated by the addition of an 8-fold volume excess of H₂O, and the precipitate was resuspended in 0.3 M KCl, 0.01 M imidazole buffer at pH 6.8. After centrifugation, the supernatant was diluted with a 6.5-fold volume excess of H₂O, allowed to stand for 1 h, and centrifuged. The pellet was dissolved in 0.5 M KCl, 0.01 M imidazole buffer at pH 6.8, and the purity of each myosin preparation was determined by gel analysis using 7.5% SDS-PAGE and Coomassie brilliant blue.

**Passive transfer of EAMC**

Male SWXJ mice 6–8 wk old were immunized with either pe406–425 or pe1631–1650, as described above, but without *Bordetella pertussis* adjuvant. Ten days later, inguinal and axillary LN were cultured with 20 μg/ml Ag at 5 × 10⁵ cells/ml in 24-well flat-bottom Falcon plates (BD Biosciences) in a total volume of 2.0 ml/well in DMEM supplemented as described above. After 4 days of activation with Ag, cells were washed thoroughly, and 2–4 × 10⁶ cells were injected into the tail veins of naive gamma-irradiated (450 rad) SWXJ recipients. At 8–9 wk following transfer, hearts were examined for histopathologic changes, and recall responses to priming immunogens were assessed by proliferation and cytokine ELISAs.

**Histopathology**

Thirty minutes before euthanasia, mice were injected i.p. with 100 μl Heparin (Elkins-Sinn, Cherry Hill, NJ) to prevent postmortem clotting. After euthanasia, hearts were removed, perfused with PBS, and fixed in Formalin overnight. After paraffin embedding, two to three serial 5-μm cross sections were obtained from the apex of the heart, thereby showing cross sections of the left and right ventricles. Heart sections were stained with either H&E or Masson’s trichrome and examined by light microscopy for the presence of inflammation or fibrosis, respectively. The percentage of heart tissue involved in inflammation was determined in a blinded manner by an investigator who was unaware of the tissue source at the time of analysis. Criteria for the presence of dilated cardiomyopathy included: 1) presence of fibrotic tissue; 2) marked ventricular dilatation, i.e., an increase in the radius of one or both ventricular chambers without a proportionate increase in mural wall thickness; and 3) increase in the heart weight:body weight ratio calculated by dividing the heart weight in milligrams by the body weight in grams. Sections taken from brain, liver, and soleus skeletal muscle were similarly processed and evaluated for the presence of inflammation and fibrosis.

**Enzyme-linked immunosorbent assay**

Cytokine concentrations were determined by ELISA measurement of supernatants of primed lymphocytes cultured in supplemented DMEM for 48 h at a concentration of 5 × 10⁵ cells/well in 24-well flat-bottom Falcon plates (BD Biosciences). Stimulating Ag was added at a concentration of 20 μg/ml in a final volume of 2.0 ml/well. Purified capture/detection Ab pairs and recombinant cytokines were obtained commercially (BD Pharmingen). Capture/detection Ab pairs included anti-mouse IFN-γ (R4-6A2 and biotin XMG1.2, anti-mouse IL-2 (JES6-1A2 and biotin JES6-5H4), anti-mouse IL-4 (BVD4-1D11 and biotin BVD6-24G2), anti-mouse IL-5 (TRFK5 and biotin TRFK4), and anti-mouse IL-10 (JES5-2A5 and biotin SXC-1). Absorbance was measured at 405 nm using a model 550 ELISA microplate reader (Bio-Rad, Hercules, CA). Standard values were plotted as absorbance vs cytokine concentration, and sample cytokine concentrations were determined as values within the linear part of the standard curve established using known concentrations of each cytokine.
Results

Immunogenicity of CAMHC peptides containing the -KXXS-MHC class II-binding motif

We addressed the issue of identifying disease-inducing sequences of cardiac myosin. We focused on CAMHC because of its predominance in the adult murine heart (10, 11) and its high immunogenicity (12, 13). However, conventional epitope mapping with overlapping peptides seemed rather formidable because ∼400 20mers would have to be synthesized to represent the entire CAMHC primary sequence of 1939 aa (14, 15). We were able to bypass conventional epitope mapping by observing that a number of class II-restricted T cell epitopes activating CD4+ T cells from SJL/J and/or SWR/J mice contained a 4-aa -KXXS- sequence motif in which 2 irrelevant aa separate a lysine or conservatively substituted arginine residue for a serine residue (16–18) (Table I). Thus, we postulated that -KXXS- or -RXXS- sequences may represent a binding motif for MHC class II molecules expressed in SWXJ mice, namely I-A\(^\beta\) and I-A\(^\alpha\).

We next synthesized four peptides from two regions of mouse CAMHC that contained the proposed binding motif and also had complementary sequences in CBMHC that did not contain the binding motif and/or had substantial sequence disparity (Table II). Individual SWXJ female mice were immunized with each of the four selected peptides, and the primed LNC were tested for their ability to proliferate in response to each immunogen. We found that substantial recall responses were elicited from primed LNC in response to po406–425 and po1631–1650, but not in response to the overlapping peptides po628–647 and po630–649 (Fig. 1a). Analysis of 48-h supernatants from peptide-activated cultures showed that the response to po406–425 and po1631–1650 was characterized by a predominant Th1-like phenotype with high production of IFN-\(\gamma\) and IL-2 and minimal production of IL-4 and IL-5 (Fig. 1b).

Active and passive induction of EAMC and DCM with po406–425 and po1631–1650

To determine whether the immunogenic po406–425 and po1631–1650 peptides were capable of inducing EAMC and DCM, we actively immunized male SWXJ mice with each peptide and assessed disease induction by gross examination and histologic analysis of hearts 8 wk later. In addition, the ability to passively transfer disease was evaluated by adoptive transfer of peptide-activated LNC from primed mice into naive recipients. Our results showed that po406–425 induced EAMC and DCM with combined active and passive incidences of 75 and 63%, respectively, whereas po1631–1650 induced EAMC and DCM with somewhat lower combined incidences of 57 and 40%, respectively (Table III; Fig. 2).

Affected hearts showed an overall increase in size, as indicated by significant increases in heart weight-body weight ratios whether disease was actively or passively induced with either peptide. Hearts showed gross white discoloration due to the presence of inflammatory foci along the pericardium (Fig. 2, e and f), and dissection of the myocardium revealed delicate, fluffy myofibers due to right-sided ventricular dilatation (Fig. 2, b and c). Affected mice showed hepatomegaly and splenomegaly, with histologic evidence of centrilobular congestion indicative of right-sided cardiac impairment.

The histopathologic findings correlated strongly with the observed clinical signs in affected mice. Cross sections through heart ventricles showed clusters of inflammatory foci occurring most frequently in the pericardium surrounding the right ventricle (Fig. 2, e and f). Inflammatory infiltrates were readily apparent and were specific for cardiac tissue because infiltration was not evident in multiple sections examined from brain, liver, and soleus skeletal muscle (data not shown). The lack of skeletal muscle infiltration is not surprising in light of the substantial sequence disparity between the immunogenic CAMHC peptides and their homologous skeletal myosin sequences (21). DCM was readily apparent in 10 of 16 mice with po406–425-induced EAMC and in 6 of 15 mice with po1631–1650-induced disease (Table III; Fig. 2, b and c). Cross sections of ventricles showed marked dilatation (Fig. 2, b and c) and fibrotic tissue damage (Fig. 2, h and i). Taken together, the gross and histologic findings are consistent with an overall appearance of autoimmune-mediated myocarditis and uncompensated ventricular dilatation.

Recall responses to po406–425 and po1631–1650 in mice with EAMC and DCM

We characterized the memory response of mice with EAMC by ex vivo analysis of splenocyte proliferation to po406–425 and po1631–1650 8–9 wk after immunization of male SWXJ mice. In each case, splenocytes from EAMC mice elicited recall proliferative responses to the priming immunogen (Fig. 3a). ELISA analysis of culture supernatants showed that responses were consistent with a Th1-like phenotype with elevated recall production of IFN-\(\gamma\) and IL-2, but not IL-4 and IL-5 (Fig. 3b). Similarly, 8–9 wk after adoptive transfer of 2×10\(^7\) peptide-activated LNC, splenocytes from EAMC mice showed immunogen-specific proliferative responses (Fig. 3c) and a Th1-like cytokine phenotype (Fig. 3d).

Crypicity of po406–425 and po1631–1650

We assessed the relative immunodominance of po406–425 and po1631–1650. Immunodominant peptides elicit recall responses following immunization with whole protein, and when used as immunogens induce recall responses to the intact protein. In contrast, cryptic peptides do not elicit recall responses following immunization with the whole protein, and when used as immunogens do not induce recall responses to the intact protein (22). To determine the immunogenic features of po406–425 and po1631–1650,
we immunized female SWXJ mice with each peptide and with mouse cardiac myosin and then measured the proliferative responses to the peptide immunogens and to the intact protein 10 days later. LNC from mice primed with either p406–425 (Fig. 4a) or p1631–1650 (Fig. 4b) responded to each immunogen, but were unable to respond to intact mouse cardiac myosin. LNC from mice primed to whole cardiac myosin showed recall responses to cardiac myosin, but were unresponsive to each peptide (Fig. 4c). Thus, our results show that p406–425 and p1631–1650 are nondominant, cryptic peptide Ags of mouse cardiac myosin.

**Dual parental I-A<sup>a</sup> and I-A<sup>q</sup> restriction of CD4<sup>+</sup> T cell responses to p406–425 and p1631–1650**

We next characterized the MHC restriction elements involved in responses to p406–425 and p1631–1650. Ten days after immunization of female SWXJ mice with either p406–425 or p1631–1650, CD4<sup>+</sup> and CD8<sup>+</sup> T cells were positively purified from whole LNC by magnetic bead separation (>95%) and tested for reactivity to each peptide immunogen at 100 μg/ml. Compared with whole LNC, the CD4<sup>+</sup>–enriched population showed enhanced proliferation to each peptide, whereas CD8<sup>+</sup>–enriched cells showed markedly diminished autoreactivity (Fig. 5a). Treatment at initiation of peptide-activated cultures with 10 μg/ml of Ab specific for I-A<sup>a</sup> or I-A<sup>q</sup> resulted in diminished responsiveness to each peptide, indicating that primed CD4<sup>+</sup> T cells responded to both peptides in a dual I-A<sup>a</sup>- and I-A<sup>q</sup>-restricted manner (Fig. 5b). The dual MHC restriction was confirmed by testing responses of parental SJL/J and SWR/J mice primed to each peptide. Ten days after peptide immunization, splenocytes from parental SJL/J and SWR/J mice responded to each peptide in a manner that was similar to responses obtained from SWXJ mice (Fig. 5c). Although responses appeared to be somewhat enhanced in the SWR/J (H-2<sup>q</sup>) strain compared with the SJL/J (H-2<sup>s</sup>) mouse (Fig. 5, b and c), these differences were not significant in our study. Final verification of the dual parental I-A<sup>a</sup> and I-A<sup>q</sup> restriction for each peptide occurred when we found that each peptide induced EAMC and DCM in both parental SJL/J and SWR/J mouse strains (data not shown).

**An intact -KXXX- motif is required for peptide antigenicity**

We next examined the motif’s sequence requirements for antigenicity. Ten days after immunization of female SWXJ mice with p406–425, LNC were tested for responsiveness to the native p406–425 peptide containing the intact -KQQS- sequence as well as to peptide variants containing substitutions for the terminal lysine and serine residues. As shown in Fig. 6, recall responses were elicited when the -KQQS- sequence remained intact or was replaced by -RGQC- representing two conservative amino acid substitutions, arginine for lysine and cysteine for serine. However, recall responses were not elicited by a peptide containing the non-conservative -GGQQ- double substitutions or when the p406–425 β-chain homologue containing the -KGQN- nonconservative single substitution was used to test recall responses. Our results indicate that peptide antigenicity requires an intact -KXXX- sequence or a sequence containing conservative amino acid substitutions for the terminal lysine and serine residues.

### Table III. CAMHC peptides induce EAMC and DCM in SWXJ mice

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Type of Induction</th>
<th>Incidence of EAMC</th>
<th>Mean % of Heart Showing Inflammation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Heart Wt: Body Wt Ratio&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Incidence of DCM&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>p406–425</td>
<td>Active</td>
<td>6/8</td>
<td>3.38 ± 1.75</td>
<td>5.1 ± 0.2</td>
<td>5/8</td>
</tr>
<tr>
<td></td>
<td>Passive</td>
<td>6/8</td>
<td>4.88 ± 2.29</td>
<td>5.3 ± 0.2</td>
<td>5/8</td>
</tr>
<tr>
<td>p1631–1650</td>
<td>Active</td>
<td>4/8</td>
<td>3.21 ± 1.12</td>
<td>5.0 ± 0.2</td>
<td>3/8</td>
</tr>
<tr>
<td></td>
<td>Passive</td>
<td>4/7</td>
<td>3.52 ± 1.20</td>
<td>5.3 ± 0.3</td>
<td>3/7</td>
</tr>
<tr>
<td>PBS control</td>
<td>None</td>
<td>0/4</td>
<td>0</td>
<td>4.0 ± 0.6</td>
<td>0/4</td>
</tr>
</tbody>
</table>

<sup>a</sup> The percentage of heart tissue involved in inflammation was determined in a blinded manner, as described in Materials and Methods.

<sup>b</sup> Heart weight:body weight ratios were determined by dividing heart weight (mg) by body weight (g).

<sup>c</sup> DCM was determined by the presence of fibrotic tissue, an increase in the radius of one or both ventricular chambers without a proportionate increase in mural wall thickness, and an increase in the heart weight:body weight ratio.
In the present study, we identified an MHC class II-binding motif capable of predicting CAMHC peptides that target severe cardiac-specific autoimmune disease in SWXJ mice and in the parental SJL/J and SWR/J mouse strains. Application of the -KXXS- motif successfully pinpointed p/H9251\textsuperscript{406–425} and p/H9251\textsuperscript{1631–1650} from a total of four peptides selected from the large 1939-aa sequence of CAMHC. The importance of this finding lies in the fact that a simple 4-aa peptide motif may be used to identify immunogenic peptides of self proteins in strains of mice widely used in autoimmune studies.

SJL/J and SWR/J mice are particularly susceptible to EAE, an animal model for multiple sclerosis, and peptides from several myelin proteins have been shown to induce EAE (23). In at least two cases, a single peptide has been shown to induce EAE in both SJL/J and SWR/J mice. Myelin basic protein 87–99 VHFF KNIVTPRTP and proteolipid protein 104–117 KTTICGKGLS ATVT are encephalitogenic determinants in SJL/J and SWR/J mice as well as in their SWXJ F\textsubscript{1} hybrid strain (16, 24–26). Thus, the dual parental immunoreactivity observed in the current study in response to p\textsubscript{406–425} and p\textsubscript{1631–1650} may be common in SJL/J and SWR/J mice and may provide enhanced responsiveness to a given immunogen in SWXJ hybrids, perhaps as a result of the combined responses of parental I-A\textsuperscript{a}- and I-A\textsuperscript{b}-restricted T cell repertoires, as has been shown (16).

The nondominant, cryptic nature of the p\textsubscript{406–425} and p\textsubscript{1631–1650} peptides is a common trait of self Ags, presumably because it is more difficult to achieve tolerance to nondominant determinants compared with immunodominant epitopes (27), and because there is an efficient thymic deletion of the high affinity autoreactive T cell repertoire responding to immunodominant peptides (28, 29). Indeed, positive selection of autoreactive T cells responding to immunodominant self determinants is often the apparent result of unusual circumstances such as failed thymic capture of immunodominant peptides having extremely low affinities for MHC molecules (30) or failed thymic expression of protein isoforms containing immunodominant peptides (31, 32). It is widely believed that the relatively high immunogenicity of CAMHC compared with CBMHC may be due to the fact that the CAMHC \alpha-isoform is predominantly expressed postnataally, thereby avoiding the ontogenic window of thymic medullary negative selection that begins at about day 11 of gestation in mice (4). In contrast, the CBMHC \beta-isoform is expressed predominantly during embryogenesis (10, 11), coincident with thymic negative

**FIGURE 2.** Active and passive induction of EAMC and DCM with p/H9251\textsuperscript{406–425} and p/H9251\textsuperscript{1631–1650}. Hearts from normal 14-wk-old male SWXJ mice show no signs of ventricular dilatation (a), inflammatory infiltrates (d), or fibrosis (g). In contrast, 8 wk after immunization of male SWXJ mice with p\textsubscript{406–425}, hearts showed ventricular dilatation (b) (*), parenchymal infiltration of mononuclear cells (e), and fibrotic damage (h). Similarly, 8–9 wk after adoptive transfer of 2–4 \times 10\textsuperscript{7} activated ps1631–1650-specific LNC, hearts showed ventricular dilatation (c), inflammation (f), and fibrotic changes (i). Sections are representative of similar tissue damage observed following active or passive induction of disease with either immunogenic peptide. Magnification = \times 40 for a–c; \times 100 for d, e, g, and h; and \times 160 for f and i. Ventricular dilatation and inflammation were assessed in sections stained with H&E, whereas the presence of tissue fibrosis was determined in trichrome-stained sections. Inflammatory infiltrates or fibrotic changes were not observed in multiple sections examined from brain, liver, and soleus skeletal muscle (data not shown).
Nevertheless, the cryptic nature of the \( p_{H9251}^{406-425} \) and \( p_{H9251}^{1631-1650} \) peptides does not prevent them in any way from inducing a high incidence of active and passive EAMC and DCM, thereby providing further support for the importance and perhaps predominance of self crypticity in autoimmunity. Indeed, recent studies by Brehm et al. (33) indicate that cryptic determinants from one virus may become immunodominant when engaged following prior exposure to another virus containing a distinct, but cross-reactive cryptic epitope. Therefore, it is quite conceivable that ordinarily cryptic cardiac myosin epitopes may become immunodominant after repertoire shaping by environmental or age-related events. In addition, others have shown that progression of autoimmune disease may be associated with the accumulation of cryptic self recognition (34). Thus, crypticity may provide the basis for initiation of autoimmunity following postviral repertoire shaping and may serve as a way to maintain chronic inflammation directed against self during progression of autoimmune disease.

Prior models for EAMC and DCM have been developed primarily in C3H or A/J mice expressing the H-2k haplotype or BALB/c mice expressing the H-2d haplotype. Thus, the current study provides a new murine model for EAMC and DCM in a genetic background previously unexplored for this disease. Moreover,
Although the -KXXS- motif is not de
it is abundantly and exclusively expressed in the heart (10, 11) and because it is highly immunogenic (12, 13). However, there is rea-
son to believe that the pathogenesis of EAMC and DCM may involve other less abundant heart proteins, as has been shown in
EAE studies in which the minor myelin constituent myelin oligo-
dendrocyte glycoprotein serves as the apparent immunodominant
protein target in C57BL/6 mice (41) and is highly encephalitogenic
in SJL/J mice (17). Thus, the -KXXS- motif may be particu-
larly useful in identifying disease-inducing peptides of other proteins
expressed predominantly in the heart, such as cardiac troponin T
(42), phospholamban (43), and cardiac laminin (44, 45).

Finally, the -KXXS- motif may be applied for developing mouse
models for putative human autoimmune disease that currently have
no satisfactory murine model. This approach may be particu-
larly useful in situations in which there is a limited availability of organ-
specific proteins. This appears to be the case in a number of dis-
cases, such as idiopathic granulomatous orchitis (46), chronic
abacterial prostatitis (47), and autoimmune sensorineural hearing
loss (48). Indeed, we have recently developed an organ-specific
autoimmune mouse model for the latter disease using immuno-
genic -KXXS- containing peptides derived from inner ear-specific
proteins (data not shown). Thus, the broad applicability of the
-KXXS- motif may be particularly useful in implicating previously
overlooked proteins as autoimmune targets and in developing new
organ-specific autoimmune mouse models for human diseases.

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