Structural similarity (molecular mimicry) between viral epitopes and self-peptides can lead to the induction of autoaggressive CD8\(^+\) T cell responses. Based on the flexibility of T cell receptor/antigen-major histocompatibility complex recognition, it has been proposed that a self-peptide could replace a viral epitope for T cell recognition and therefore participate in pathophysiological processes in which T cells are involved. To address this issue, we used, as a molecular model of viral antigen, the H-2D\(^b\)-restricted immunodominant epitope NP-(396–404) (FQPQNGQFI) of lymphocytic choriomeningitis virus (LCMV). We identified peptide sequences from murine self-proteins that share structural and functional homology with LCMV NP-(396–404) and that bound to H-2Db with high affinity. Structural and functional homology with LCMV NP-tide sequences from murine self-proteins that share nucleoprotein (NP)-(396–404) (FQPQNGQFI) of lymphocytic choriomeningitis virus (LCMV). We identified peptide sequences from murine self-proteins that share structural and functional homology with LCMV NP-(396–404) and that bound to H-2D\(^b\) with high affinity. One of these self-peptides, derived from tumor necrosis factor receptor I (FQPSNWHFM, amino acids 302–310), maintained LCMV-specific CD8\(^+\) T cells in an active state as observed both in vitro in cytotoxic assays and in vivo in a model of virus-induced autoimmune diabetes, the rat insulin promoter-LCMV NP transgenic mouse. The natural occurrence and molecular concentration at the surface of H-2\(^b\)-positive spleen cells of tumor necrosis factor receptor I (302–310) were determined by on-line \(\mu\)-high pressure liquid chromatography/mass spectrometry and supported its biological relevance.

Molecular mimicry, a process in which potentially autoreactive T cells are activated in the periphery by major histocompatibility complex (MHC)\(^1\)-restricted cross-reactive self-peptides, is suspected to be a possible mechanism triggering pathological (versus normal) immunity, particularly virus-induced autoimmune disease (1). Although the recognition of a viral antigenic peptide by the T cell receptor (TCR) is a most specific process, structurally altered antigenic variants or mimic peptides, which can be unrelated in terms of primary sequence to the viral antigen (2–4), can still be recognized by CD4\(^+\) or CD8\(^+\) T cells (5, 6). The functional properties of these peptides depend on their structure, and their interaction with the TCR can lead to either full or partial T cell activation or antagonism (5). Such cross-reactive peptides are thought to play a role in pathophysiological situations such as T cell selection (7, 8), anergy (9), viral escape (10, 11), and autoimmune disorders (12).

Although the development of autoimmune disease has been commonly associated with CD4-bearing T cells (4), there are now both clinical and experimental observations that strongly suggest that autoreactive CD8\(^+\) T cells may also be involved (13, 14). However, the molecular mechanisms by which autoreactive CD8\(^+\) T cells are activated or maintained in a functional state remain mostly unknown. In this study, we explored the possible role of MHC class I-restricted self-peptides in these mechanisms because of their potential importance in clinical disease. Our aim was (i) to identify putative self-molecular mimics of a viral antigen, (ii) to characterize their structural and functional properties, and (iii) to determine their biological relevance. For this, we used the H-2D\(^b\)-restricted immunodominant epitope located in nucleoprotein (NP)-(396–404) (FQPQNGQFI) of lymphocytic choriomeningitis virus (LCMV), a model of choice for dissecting the molecular or cellular mechanisms involved in an autoreactive CD8\(^+\) T cell response. Indeed, the H-2D\(^b\)-restricted CD8\(^+\) T cell response mounted against the LCMV NP is directed toward the immunodominant epitope sequence NP-(396–404) (15–17). The LCMV-infected mouse typically undergoes activation and massive expansion of CD8\(^+\) T cells, which consistently stay at a high level throughout the animal’s life span, even in the absence of detectable virus and/or viral antigen (18–20). These cytotoxic T cells (CTLs) represent a potential source of autoreactivity, particularly if their cytolytic function remains intact. The H-2\(^b\)-transgenic mice, which express LCMV NP under the control of the rat insulin promoter (RIP), represent an in vivo model of autoimmune disease (21, 22) in which LCMV NP-(396–404) can be the target of autoreactive CTLs. After LCMV infection or adop-

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The abbreviations used are: MHC, major histocompatibility complex; TCR, T cell receptor; NP, nucleoprotein; LCMV, lymphocytic choriomeningitis virus; CTL, cytotoxic T lymphocyte; RIP, rat insulin promoter; IDDM, insulin-dependent diabetes mellitus; TNFR, tumor necrosis factor receptor; HPLC, high pressure liquid chromatography; ESI-MS, electrospray ionization mass spectrometry; IFN-\(\gamma\), interferon-\(\gamma\).
tive transfer of anti-LCMV CTLs, these RIP-LCMV NP mice develop insulin-dependent diabetes mellitus (IDDM), a virus-induced autoimmune disease in which both CD4+ and CD8+ T cells are involved (23). In these mice, the destruction of β cells and ensuing IDDM have been attributed to MHC class I-restricted CD8+ T cells (22, 24, 25). Furthermore, the finding that IDDM does not develop in MHC class I-deficient mice or CD8-depleted mice (24) provides additional support for the involvement of CD8+ T cells and, consequently, of MHC class I-restricted self-peptides.

Here, we first identified a set of six nonameric sequences from endogenous proteins sharing structural and functional homology with LCMV NP-(396–404) (26). Five of these endogenous peptides bound with high affinity to H-2Dd and generally acted as antagonists of lysis by LCMV-specific CTLs. In the presence of H-2Dd pulsed with three of these peptides, LCMV-specific CTLs were maintained in long-term culture. We then selected one of these peptides, tumor necrosis factor receptor I (TNFR)-302–310, which activated LCMV NP-specific CTLs, allowing them to kill LCMV-infected cells in a peptide-specific, MHC-restricted manner. Adoptive transfer of TNFR-(302–310)-activated CTLs into RIP-LCMV NP × RIP-B7.1 transgenic mice provoked a specific destruction of β cells of the islets of Langerhans and caused IDDM. Using on-line μ-high pressure liquid chromatography (HPLC)/electrospray ionization mass spectrometry (ESI-MS), we demonstrated the presence of TNFR-(302–310) at the surface of H-2Dd spleen cells, a finding supporting its biological relevance.

EXPERIMENTAL PROCEDURES

Cell Lines, Mice, and CTLs—The T2 human mutant cell line transfected with H-2Dd (T2-Dd) (26) was used in binding experiments. The murine H-2Dd cell lines MC57 and RMA and the H-2Dd cell line BALB/c (H-2d) were obtained from the breeders. Geneticin (400 μg/ml) or LCMV-infected macrophages.

In Vitro Cytotoxic Assays—LCMV-infected or peptide-pulsed uninfected target cells were incubated for 1 h at 37 °C with 3HCr and washed, and LCMV-specific CTLs were added at the indicated effector/target ratio. Target and effector cells were incubated at 37 °C in a final volume of 200 μl. After a 5-h incubation period, fractions (100 μl) were removed and counted for 3HCr activity. The percent specific lysis was calculated as 100× (cpm experiment−cpm spontaneous release)/cpm (total release−cpm spontaneous release). Total release and spontaneous release were determined by incubating the labeled cells with 1% Nonidet P-40 and culture medium, respectively. In all experiments, samples were run in triplicate, and the mean values are given. Assay was assayed in a 5-h 3HCr release assay as described above, except that RMA cells were preincubated with a suboptimal concentration of LCMV NP-(396–404) (giving 30–40% lysis) during 3HCr labeling, extensively washed, and incubated with a 1, 10, or 100 μM concentration of the indicated peptides for 30 min at 37 °C before CTLs were added at an effector/target ratio of 5:1.

IFN-γ Production—RMA cells (3 × 106 cells/well) were incubated for 1 h at 37 °C in the presence of the indicated peptides (1 × 10−10 to 1 × 10−11 M) and then restimulated with 10 units/ml IFN-γ. The level of IFN-γ production was then measured by using enzyme-linked immunosorbent assay using R4-642 and biotinylated XMG1.2, followed by incubation with α-phenylendiamine hydrochloride (Sigma). The color was read at 490 nm using an enzyme-linked immunosorbent assay reader (TiterTek Multiskan Plus MKII, EFLAB, Finland) and normalized to the values obtained for a standard curve of recombinant murine IFN-γ (Sigma).

Extraction of Endogenously Presented Peptides from Spleen Cells—Peptides were acid-extracted from the cell surface as previously described (32). Briefly, splenocytes (1–2 × 109) were washed three times with Hanks' balanced salt solution and then suspended in 0.5 ml of 0.5% triton X-100, 0.1 M citric acid and 0.066 mM Na2HPO4 at pH 3.0 for 2 min. The eluted material was desalted on Waters Sep-Pak column according to the manufacturer's instructions, vacuum-concentrated, and centrifuged in 1% trifluoroacetic acid on a Centricon 3 (3-kDa cutoff; Amicon, Inc.) at 3800 × g for 90 min at 4 °C. The filtered material was vacuum-concentrated and resuspended in 100 μl of 0.08% trifluoroacetic acid. Peptide fractions were separated on a reversed-phase C18 column (Aquapore, 7 μm, 0.3 × 100 mm; Brownlee) using a Waters 6000 controller system. Samples (25 μl) were injected and separated using a system gradient of 5–36% solvent B for a 60-min period at a flow rate of 200 μl/min. Solvent A was 0.08% trifluoroacetic acid in H2O, and solvent B was 0.08% trifluoroacetic acid in CH3CN. Fractions (200 μl) were collected, lyophilized, and stored at −80 °C until analysis by on-line μ-HPLC/ESI-MS.

Structural Identification of Naturally Occurring Peptides by On-line μ-HPLC/ESI-MS—The collected HPLC fractions were injected on a reversed-phase C18 microcolumn (PepMapTM, 3 μm, 0.3 × 100 mm; LC Packings). Elution was performed with a gradient of 5–55% solvent B in 25 min. Solvent A was 0.05% acetic acid in 95.5 (v/v) H2O/CH3CN, and solvent B was 0.05% acetic acid in 20.0% (v/v) H2O/CH3CN. The flow rate was 0.4 ml/min, and the column temperature was maintained at 40 °C. UV monitoring and MS data were recorded on a Finnigan MAT TSQ700 triple quadrupole mass spectrometer equipped with an electrospray source either by scanning the range of masses corresponding to m/z values between 300 and 1800 every 3 s or by using the “single ion monitoring” mode, where the quadrupole is set to transmit only one particular m/z value, corresponding to an ion of interest, centered in a 1 m/z unit window. Low
energy collision-activated dissociation MS/MS experiments were conducted in the “selected reaction monitoring” mode, where the first quadrupole is set to transmit the precursor ion of interest and the third quadrupole is scanned over a 10- m/z unit mass range centered on the m/z value of the fragment ion of interest. Argon was used as the collision gas at a collision pressure of 1.2 millitorr. The collision energy was 28 eV in the laboratory frame of reference.

Molecular Modeling of H-2D<sup>b</sup>Peptide Interactions—The H-2D<sup>b</sup> structure was obtained from crystallographic data of H-2D<sup>b</sup> with influenza virus peptide NP-(366–374) (33). The LCMV NP-(396–404) and TNFR-(302–310) nonamers were introduced manually using the program O. Side chain conformation was based on the most probable rotamer and the probable hydrogen bonds. After transfer of H-2D<sup>b</sup> data (10<sup>12</sup> to 10<sup>6</sup> m) were tested in a classical CTL assay by measuring the lysis of peptide-coated MC57 target cells by NP-specific CTL clone NP18 (effector/target ratio of 5:1). EC<sub>50</sub> values represent the peptide concentrations inducing half of the maximal lysis effect.

Peptide energy collision-activated dissociation MS/MS experiments were conducted in the “selected reaction monitoring” mode, where the first quadrupole is set to transmit the precursor ion of interest and the third quadrupole is scanned over a 10- m/z unit mass range centered on the m/z value of the fragment ion of interest. Argon was used as the collision gas at a collision pressure of 1.2 millitorr. The collision energy was 28 eV in the laboratory frame of reference.

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RESULTS

Structural and Functional Anatomy of the LCMV NP-(396–404) Epitope—We first identified the residues of LCMV NP-(396–404) involved in MHC binding and those serving as TCR contacts. Binding assays of monoalanine-substituted analogs (Table I) confirmed Asn<sup>400</sup> at P5 and Ile<sup>404</sup> at the C terminus as the weak MHC-binding properties of the analogs. Similar results obtained with the NP-(396–404)-specific CTL clone NP18 (35) are shown in Table I. The strongest effect was observed at position 8, where substitution of Ala for Phe yielded a peptide totally unable to sensitize target cells even at the highest peptide concentration tested, indicating that Phe<sup>403</sup> is the critical (main) TCR contact. CTL sensitization properties were also profoundly altered, but not abolished, after substitution of Phe<sup>396</sup> at P1 and Gln<sup>399</sup> at P4, indicating that these two residues also play a role, although of lesser importance, as TCR (auxiliary) contacts. Substitution of residues at positions 2 (Gln, 6 Gly), 7 (Gln), and 9 (Phe) had little effect. The effects observed at positions 3 and 5 were expected as a consequence of the weak MHC-binding properties of the analogs. Similar results were obtained with polyclonal populations of CTLs obtained from C57BL/6 mice either infected with LCMV Arm or immunized with synthetic NP-(396–404) (data not shown). These three main (Phe<sup>403</sup>) and auxiliary (Phe<sup>396</sup> and Gln<sup>399</sup>) TCR contacts identified in the NP-(396–404) sequence undoubtedly represent the overall CTL response against this antigen. A summary of MHC binding and TCR interactions is illustrated in Fig. 1A.

Search for Murine Self-peptides Sharing Minimal Functional Homology with LCMV NP-(396–404)A. anatomy of the viral epitope. Residues implicated in MHC binding or in TCR interactions were identified using the monoalanine scanning approach. The main and auxiliary interactions are shown as black and stippled arrows, respectively. B, search criteria for nonameric self-peptide sequences sharing minimal functional homology with LCMV NP-(396–404) (FQPQNGQFI). We searched the Swiss Protein Database by (i) fixing the required functional amino acids at P5, P8, and P9; (ii) imposing or excluding some amino acids known to play a positive (at P2 and P3) or negative (at P1) role in H-2D<sup>b</sup> binding (31); and (iii) allowing any of the 20 natural amino acids at P4, P6, and P7.
TABLE II

| Peptide         | Sequence     | Homology | Origin of protein | Cellular location       | Tissue distribution | IC₅₀ for H-2Db affinity |
|-----------------|--------------|----------|-------------------|-------------------------|---------------------|-------------------------|
| NP-(396–404)    | FQPQNGQFIM   | —        | Viral nucleoprotein| Cytosol                | Infected cells, broad | 5.7 ± 1.4               |
| TNFR-(302–310)  | FGPNSNWHFM   | 5/9 (56%)| TNF-R            | Membrane               | Tumor, lymphoid     | 0.65 ± 0.34             |
| LAP-(177–185)   | RSIQNAQFL    | 5/9 (56%)| Lysosomal acid phosphatase | Lysosome               | Ubiquitous         | 8.7 ± 1.8               |
| PKC-(351–359)   | FGDNKFUL     | 4/9 (44%)| Protein kinase Cₖ | Cytosol, membrane      | Skin, lung          | 0.12 ± 0.04             |
| IgVH-(91–99)    | DNPKNTLFL    | 4/9 (44%)| Ig heavy chain, V region | Cytosol               | β cells, extracellular fluids | 1200 ± 208             |
| NCad-(569–577)  | NNYNATFLL    | 3/9 (33%)| N-cadherin        | Membrane               | Neurons             | 29.3 ± 1.3              |
| LDH-(282–290)   | YGIENEVFL    | 3/9 (33%)| Lactate dehydrogenase, heavy chain | Cytosol | Heart muscle | 1.5 ± 0.2 |

**A** | cytolysis | a | lysis (%) | 0 | 10 | 20 | 30 | 40 | 50 |
|------|------------|---|------------|---|---|---|---|---|---|
| NP   | 396–404    |   | 0 | 0 | 0 | 0 | 0 | 50 | 50 |
| NCad | 569–577    |   | 0 | 0 | 0 | 0 | 0 | 50 | 50 |
| LAP  | 177–185    |   | 0 | 0 | 0 | 0 | 0 | 50 | 50 |
| LDH  | 282–290    |   | 0 | 0 | 0 | 0 | 0 | 50 | 50 |
| PKC  | 351–359    |   | 0 | 0 | 0 | 0 | 0 | 50 | 50 |
| TNFR | 302–310    |   | 0 | 0 | 0 | 0 | 0 | 50 | 50 |

**B** | IFN-γ production | no peptide | NP 396-404 | NCad 569-577 | LAP 177-185 | LDH 282-290 | PKC 351-359 | TNFR 302-310 |
|------|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 0    | 5                   | 10          | 15          | 20          | 25          | 30          | 35          |
| IFN-γ (IU/ml) | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 |

**C** | proliferation | no peptide | NP 396-404 | NCad 569-577 | LAP 177-185 | LDH 282-290 | PKC 351-359 | TNFR 302-310 |
|------|---------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 0    | 5                   | 10          | 15          | 20          | 25          | 30          | 35          | 40 |
| CPM x 10⁴ | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 |

**D** | T cell antagonism | peptide (µM) | GP 276-286 | NCad 569-577 | LAP 177-185 | LDH 282-290 | PKC 351-359 | TNFR 302-310 |
|------|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 0    | 5                   | 10          | 15          | 20          | 25          | 30          | 35          | 40 |
| % lysis | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 |

Fig. 2. Functional properties of murine self-peptides upon reacting with H-2Db class I-restricted LCMV NP-(396–404)-specific CTLs. A, sensitization of target cells to lysis by CTL clone NP18 (panel a) or polyclonal CTLs (panel b) specific for LCMV NP-(396–404) was analyzed in a ⁵¹Cr release assay as described under “Experimental Procedures.” B, IFN-γ production. Enzyme-linked immunosorbent assays were carried out as described for the cytotoxicity assays, except that supernatants were collected after 24 h and tested for IFN-γ content. Results are expressed as international units/ml and are representative of two independent experiments. C, proliferation. T cells (1 × 10⁴) were mixed with γ-irradiated, peptide-pulsed RMA cells (5 × 10⁴) and incubated for 72 h at 37 °C. [³H]Thymidine was added during the last 16 h, and incorporation was determined by scintillation counting. D, T cell antagonism. The antagonist properties of the self-peptides were assessed in cytotoxicity assays. RMA cells were loaded with a suboptimal concentration (giving ~50% maximal lysis) of LCMV NP-(396–404); washed; and incubated with a 1, 10, or 100 µM concentration of the indicated peptide before addition of CTLs. Results are from one representative experiment among three. NCad, N-cadherin; LAF, lysosomal acid phosphatase; LDH, lactate dehydrogenase; PKC, protein kinase C; GP, glycoprotein.
2B) and T cell proliferation (Fig. 2C), as expected. In contrast, none of the self-peptides incited either IFN-γ production or measurable T cell proliferation in vitro. Similar results were obtained when Ca²⁺ mobilization was analyzed (data not shown).

The Murine Self-peptides Homologous to LCMV NP-(396–404) Are Antagonists of the NP-specific TCR—Since structural modification can alter TCR recognition by provoking partial agonism or antagonism (36–39), we next determined if the self-peptides could behave as antagonists. Fig. 2D depicts the reduced lytic function of the NP-specific CTLs by the murine self-peptides with minimal homology to NP-(396–404). Lysis of labeled target cells coated with suboptimal concentrations of NP-(396–404) was inhibited in the presence of increasing concentrations of synthetic self-peptides, i.e. 50–90% inhibition was reached at the highest concentration tested (100 μM). In negative control experiments, no antagonism was detected with the two other H-2Db-restricted LCMV immunodominant epitopes: glycoprotein-(276–286), which binds to H-2Db with a high affinity, similar to that of NP-(396–404) (15) (Fig. 2), and glycoprotein-(33–41) (data not shown).

Long-term Maintenance of LCMV-specific CTLs by Mimicry Peptides—To determine whether mimicry peptides could maintain anti-LCMV CTLs in long-term culture, we used single suspensions of spleen cells from C57BL/6 mice 45–60 days after LCMV infection. Such splenocytes contain numerous anti-LCMV and particularly anti-NP-(396–404) memory CTLs (17). These CTLs were cultured with macrophages coated with peptide, infected with LCMV, or left untreated. The three peptides selected for this study, N-cadherin-(569–577), protein kinase C-(351–359), and TNFR-(302–310), shared three, four, and five functional residues with LCMV NP-(396–404), respectively (see Table II). As summarized in Table III, in the absence of stimulatory signals, T cells died in culture within 2 weeks. Specifically was demonstrated when H-2b-restricted activity. We used the self-peptide TNFR-(302–310) for negative control experiments, no antagonism was detected (data not shown).

We then determined whether the TNFR-(302–310)-stimulated CTLs had preserved cytoytic activity in vivo. For this purpose, such CTLs were adoptively transferred into double RIP-LCMV NP × RIP-B7.1 transgenic mice. In such mice, either LCMV infection or adoptive transfer of anti-NP-(396–404) CTLs causes CTL infiltration into the islets of Langherans, insulitis, and IDDM as measured by the elevation of blood glucose and reduction of pancreatic insulin (21, 22, 40). As shown in Table V, insulitis and elevated blood glucose levels indicating IDDM were observed in recipients of adoptively transferred LCMV-specific CTLs previously co-cultured with TNFR-(302–310) for >2 months. Immunohistochemical analysis confirmed CTL infiltration into the pancreases of mice (data not shown). Specificity was demonstrated when H-2d transgenic mice failed to develop insulitis or IDDM after adoptive transfer of H-2Dd-restricted CTLs primed by TNFR-(302–310)-coated or LCMV-infected macrophages.

The TNFR-(302–310) Peptide Is Naturally Presented at the Surface of H-2b Spleen Cells—The final and important step was to document that such a self-peptide with the ability to maintain functional virus-specific CTLs was present at the cell surface. To do so, peptides were extracted from the surface of splenocytes, separated by HPLC, collected into fractions of interest, and then analyzed by on-line μ-HPLC/EIS-MS. Because the TNFR-(302–310) peptide contained a methionine residue, its chemical modification was expected during the extraction and analysis process. Indeed, as a control, the synthetic TNFR-(302–310) peptide eluted in two distinct peaks, fractions 52 and 58 (Fig. 3, STEP-1, F52 and F58). As assessed by MS, fraction 58 corresponded to the unmodified form of the synthetic peptide (M + 2H)²⁺, m/z 561.9, and fraction 52.
corresponded to the chemically modified form bearing an oxidized C-terminal methionine ((M\(^{2+}\)H\(^2\))\(^{+}\), m/z 569.9; as shown in Fig. 3, STEP-1, panel a, inset). When analyzed at different concentrations (10–1000-fold dilutions), we noted that the susceptibility of TNFR-(302–310) to oxidation both under sample storage conditions and during analysis increased with peptide dilution (data not shown). Peptides extracted from C57BL/6 (H-2b) or Dba/2 (H-2d) splenocytes were then separated, and their respective fractions 52 were collected and analyzed by on-line \(\mu\)-HPLC/ESI-MS (Fig. 3, STEP-2). An ion whose mass and retention time were identical to those of the oxidized form of the synthetic TNFR-(302–310) peptide was clearly detected in fraction 52 of H-2b (but not H-2d) spleen extracts. Identification of this natural form of TNFR-(302–310) was unambiguously confirmed by (i) overloading experiments, which gave a perfectly superimposable coelution of the natural product and the overloaded synthetic oxidized form; and (ii) MS/MS experiments, which produced a fragment ion specific for the oxidized TNFR-(302–310) peptide upon collision, as detected by selected reaction monitoring (Fig. 3, STEP-2, right panels).
NP-(396–404) and TNFR-(302–310) in the H-2D\textsuperscript{b}-binding groove. The \(\alpha\)- and \(\alpha\)-MHC domains are shown as gray ribbons. The peptides are shown as blue (LCMV NP-(396–404), FGQPNGGQFI) or green (TNFR-(302–310), FGPSNWFM) sticks. The model shows the perfect superimposition of the two peptide backbones and of the side chains of the shared residues Phe\textsuperscript{B}, Pro\textsuperscript{B}, Asn\textsuperscript{B}, and Phe\textsuperscript{B}. The model also illustrates the steric hindrance of the bulky side chain of Trp\textsuperscript{B} of TNFR-(302–310), which points out of H2-D\textsuperscript{b} and likely results in altered TCR/peptide/MHC interactions.

panels. Based on the measured ionic current intensity, the amount of the TNFR-(302–310) peptide extracted from \(10^8\) H-2\textsuperscript{b} spleen cells was \(\sim 120\) fmol. Given the yield of the extraction procedure (10–15\%), the amount of TNFR-(302–310) detected on the cell surface was estimated at 1 pmol/10\(^8\) cells, which represents an approximate number of 7200 molecules/cell.

**DISCUSSION**

Structural similarity (molecular mimicry) between viral epitopes and self-peptides can lead to the induction of MHC class II-restricted autoimmune CD\textsuperscript{4} T cells (4) as well as MHC class I CD\textsuperscript{8} T cell responses (13, 14, 41, 42). Both clinical and experimental evidence continues to accumulate that infectious agents encoding proteins that cross-react with host self-proteins can play a role in molecular mimicry (43, 44).

Data base search for peptides sharing structural homology with a given antigen may be successfully employed to identify potential cross-reactive endogenous ligands (1, 4, 42). Using this strategy, we were able to identify at least five murine self-peptides with predictable high H-2D\textsuperscript{b}-binding affinity that share with the LCMV NP-(396–404) antigen its main TCR contact residue (Phe at P8). Among these five peptides, three also shared one of the two auxiliary TCR contact residues, either Phe at P1 (protein kinase C-(351–359), TNFR-(302–310)) or Gln at P4 (lysosomal acid phosphatase-(177–185)). In addition, the TNFR-(302–310) peptide had a third residue in common with LCMV NP-(396–404) (Pro at P3), resulting in a marked functional homology between the viral epitope and the self-peptide (three out of three MHC-binding residues and two out of three TCR contact residues). Yet, we failed to find a murine self-peptide that bore the three TCR contacts of the viral antigen. One may consider that the number of peptides identified by our method was unexpectedly small. However, the small set of self-peptides we found (<10) resembled that of other D\textsuperscript{b}-restricted (7) or K\textsuperscript{b}-bound (8) peptides implicated in T cell-positive selection and identified using different approaches. Also, a search for mimicry peptides able to interact with MHC class II-restricted autoreactive T cell clones specific for the myelin basic protein produced a similarly small group (4). These previous results validate our approach, which is further strengthened by the identification of one of these peptides (TNFR-(302–310)) in the material eluted from the surfaces of H-2\textsuperscript{b} cells.

By which molecular mechanism(s) can the naturally occurring TNFR-(302–310) peptide and the other self-peptides mainta...
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self-peptides and hypothesized on their possible role in these mechanisms. Because of the potential importance of these self-peptides in clinical disease, the findings presented here may have important consequences, particularly in terms of therapeutic application, by helping to design peptidic or (better) non-peptidic molecular mimics to target and/or inhibit potentially autoreactive T cells.

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