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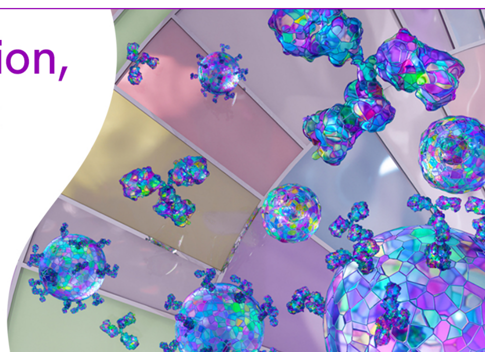

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A Peptide from Heat Shock Protein 60 Is the Dominant Peptide Bound To Qa-1 in the Absence of the MHC Class Ia Leader Sequence Peptide Qdm[1]

Adrian Davies,[2*] Suzanne Kalb,[2†] Bitao Liang,[‡] Carla J. Aldrich,[§] François A. Lemonnier,[¶] Hong Jiang,[‡] Robert Cotter,[†] and Mark J. Soloski[3*]

The MHC class Ib molecule Qa-1 binds specifically and predominantly to a single 9-aa peptide (AMAPRTLLL) derived from the leader sequence of many MHC class Ia proteins. This peptide is referred to as Qdm. In this study, we report the isolation and sequencing of a heat shock protein 60-derived peptide (GMKFDRGYI) from Qa-1. This peptide is the dominant peptide bound to Qa-1 in the absence of Qdm. A Qa-1-restricted CTL clone recognizes this heat shock protein 60 peptide, further verifying that it binds to Qa-1 and a peptide from the homologous *Salmonella typhimurium* protein GroEL (GMQFDRGYL). These observations have implications for how Qa-1 can influence NK cell and T cell effector function via the TCR and CD94/NKG2 family members, and how this effect can change under conditions that cause the peptides bound to Qa-1 to change. ***The Journal of Immunology,* 2003, 170: 5027–5033.**

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The murine MHC class Ib molecule Qa-1 preferentially associates with a peptide derived from aa 3–11 of most MHC class Ia D region-encoded molecules. This peptide has the sequence AMAPRTLLL and is termed Qdm (1–3). HLA-E, the human ortholog of Qa-1, also appears to primarily bind peptides derived from the leader sequence of many MHC class Ia molecules (4, 5). The precise molecular mechanism that results in the dominant and specific binding of such a small number of almost identical hydrophobic peptides is not clear. However, the crystal structure of the HLA-E leader peptide complex demonstrates that the binding properties of the groove of HLA-E may be important (6). HLA-E has the same basic structural framework as MHC class Ia molecules, but the side chains of the amino acid residues at positions P2, P3, P6, P7, and P9 of the peptide fall within well-defined pockets in the HLA-E-binding groove, and extensive hydrophobic interactions occur. Because of these unusual structural constraints, which exist along the length of the HLA-E-bound peptide, the binding site of HLA-E is highly specific and has minimal potential for promiscuous peptide binding. This situation is very different from that for MHC class Ia molecules, in which restrictions on the type of amino acid within the bound peptide only occur at two or three anchor residue positions that are located in deep pockets (7). Studies to define a minimal Qa-1-binding peptide on a polyglycine backbone demonstrate that peptides sharing five or six residues with Qdm do bind to Qa-1, but that their interaction is relatively weak. This indicates that multiple native residues within Qdm are required for strong binding (8). The candidate Qa-1 peptide-binding motif was determined via sequence analysis of peptides eluted from Qa-1 that had been folded in the presence of a random peptide library or pools of Qdm derivatives randomized at specific positions. These studies indicate that Qa-1 possesses the same anchor residue positions as HLA-E and that Qdm has an optimal sequence for binding. However, Qa-1 also has the capacity to bind a diverse repertoire of peptides, which are not necessarily derived from the leader sequences of proteins (9).

Qa-1 and HLA-E have been shown to act as ligands for CD94/NKG2 heterodimers, a family of lectin-like NK cell receptors (10–13). These receptors are expressed by NK cells, NKT cells, and a small fraction of $\alpha\beta$ and $\gamma\delta$ T cells (13–16). They represent one of several families of cell surface receptors expressed by NK cells (NK receptors), which regulate NK cell cytotoxic killing of infected, stressed, or transformed target cells (17–19). Specifically, CD94/NKG2 receptors, which can inhibit NK cell cytotoxicity, are thought to regulate NK cell-mediated killing of target cells that have defective class Ia expression or TAP function. CD94/NKG2 receptors are believed to modulate this process by surveying class Ia expression indirectly. They perform this function by monitoring the TAP-dependent presentation of the dominant leader peptide by Qa-1 and HLA-E (1, 20) in a peptide-specific fashion (9, 21–23). NK receptors, such as CD94/NKG2 receptors, are also thought to finely regulate the threshold for T cell activation (24, 25). In this situation, the level of T cell activation is determined by the integration of positive signaling events initiated by the TCR and negative signals mediated by other T cell surface proteins, such as NK receptors (26).

Although Qa-1 has been shown to bind Qdm as a single dominant peptide, several reports suggest that it can bind other peptides. Anti-Qa-1 Abs have been shown to block recognition of the copolymer Glu^{50}-Tyr^{50} by a $\gamma\delta$ T cell hybridoma (27). Tompkins et al. (28) demonstrated that $Qa\text{-}1^b$-restricted T cell hydribomas generated by immunizing low-responder $H\text{-}2^b$ mice with pork insulin recognize an insulin B chain determinant in a TAP-independent fashion. A peptide from the leader sequence of preproinsulin has

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been shown to bind to Qa-1^b and is recognized by Qa-1^b-restricted CTL (29). Qa-1-restricted CD8$^+$ T cells that specifically kill activated Vβ8$^+$ T cells have been reported by Jiang et al. (30, 31). These Qa-1-restricted T cells, which were induced by vaccination with Vβ8$^+$ T cells or staphylococcal enterotoxin B injection, presumably recognize Qa-1-presenting TCR-derived peptides. *Listeria monocytogenes*-specific CD8$^+$ T cells, which are Qa-1 restricted, have been identified (32, 33). The *Listeria* peptide(s) presented by Qa-1 remains to be identified. We previously demonstrated that a tryptic digest of *Mycobacteria bovis* heat shock protein 65 (hsp65)[4] can stabilize Qa-1 cell surface expression, suggesting that Qa-1 may be able to bind hsp-derived peptide(s) (34). Mass spectral analysis and Edman sequencing of naturally occurring peptides eluted from Qa-1 and from soluble Qa-1 refolded in the presence of various peptide libraries also indicate that peptides other than Qdm can bind to Qa-1 (2, 3, 9). More recent studies have demonstrated that an immunodominant epitope (GMQFDRGYL) from *Salmonella typhimurium* GroEL is presented by Qa-1 and recognized by CD8$^+$ T cells after natural infection. This epitope is conserved within many Gram-negative pathogens. Most of these GroEL/Qa-1-reactive T cells cross-react with a peptide derived from the homologous murine protein, hsp60 (GMKFDRGYI), in a Qa-1-restricted fashion (35, 36). These two peptides are not able to inhibit NK cell lysis of Qa-1-expressing target cells (21).

In this study, we report that the hsp60 peptide (GMKFDRGYI) is the dominant peptide bound by Qa-1 in the absence of Qdm. These results have implications for our understanding of how Qa-1 and the peptides it presents can influence NK and T cell effector function and how this effect can change under different conditions, such as infection, stress, and transformation.

Materials and Methods

Mouse strains

C57BL/6 (B6) mice were purchased from the National Cancer Institute (Frederick, MD). B6/β_2-microglobulin$^{-/-}$ (B6/β_2m$^{-/-}$) and B6/TAP$^{-/-}$ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B6/K$^{b-/-}$D$^{b-/-}$ mice were a kind gift to J. Foreman (University of Texas Southwestern Medical Center, Dallas, TX) from F. Lemonnier (Institut Pasteur, Paris, France).

Cell lines and transfectants

C1R cells and T2 cells expressing Qa-1^b were generated previously (J1 (1) and T2-g37 (13)). The Qa-1^b-restricted CTL clones D5D2 and 39.1D7X were described previously (1, 37). Clone D5D2 recognizes the Qdm peptide and other peptides that are very similar in sequence to it, including the D^k variant (AMVPRTLLL). Clone 39.1D7X recognizes the Qdm peptide.

Immunoprecipitation of peptides associated with Qa-1^b

C1R transfectants expressing Qa-1^b (C1R/Qa-1^b) (1×10^{10}) were lysed in Nonidet P-40 buffer (2% Nonidet P-40, 20 mM Tris, pH 7.2/150 mM NaCl, 1 mM 4-(2-aminoethyl)-benzenesulfonylfluoride, 0.001 mM pepstatin A) for 15 min at 4°C. Nuclei were removed by centrifugation at $20{,}000 \times g$ for 15 min. The supernatant obtained was passed through a 0.2-μm filter. MHC class I immunoprecipitates were obtained by applying the supernatant to an anti-human β_2m mAb BBM.1 (38)-coupled Hitrap affinity column (Amersham Pharmacia Biotech, Piscataway, NJ) at 4°C and recycling for 4 h. MHC class I/peptide complexes were eluted, and peptides were extracted in 0.1% trifluoroacetic acid (TFA; Pierce, Rockford, IL). Extracts were size fractionated using Amicon (Beverly, MA) Centriprep-10 membrane filters, and the low m.w. material was lyophilized. Lyophilized samples were resuspended in 1 ml 0.1% TFA/10% acetonitrile (J. Baker, Philipsburg, NJ) in water and fractionated by reverse-phase HPLC using a C18 column (Vydac, Hesperia, CA) integrated into a HPLC system (Waters, Milford, MA). Peptides were chromatographed with a 1.1% B/min gradient, as follows (solution A, 0.1% TFA/water; solution B, 0.1% TFA/ACN): 0–5 min at 100% A; 5–10 min to form a 4% B/min gradient to yield 80% A and 20% B; 10–45 min to form a 1.1% B/min gradient to yield 40% A and 60% B; 45–50 min at 40% A and 60% B; 50–55 min to form an 8% B/min gradient to yield 100% B; 55–60 min at 100% B; and 60–70 min to form a 10% A/min gradient to yield 100% A. All chromatography was at a flow rate of 1 ml/min, and 1-ml fractions were collected into siliconized tubes and frozen.

Mass spectrometry

Mass spectral analysis was performed using a Kratos Axima-Curved-Field Reflectron mass spectrometer (Manchester, U.K.). An aliquot of each HPLC fraction was lyophilized and resuspended at one-tenth the original volume in water. A total of 0.3 μl of the resulting solution was deposited on a sample site, and 0.3 μl of saturated ammonium sulfate and 0.3 μl of matrix (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% ethanol) were added. The mixture was air dried in a dark chamber at room temperature, and the sample was inserted into the mass spectrometer. The ions obtained were mass analyzed using time-of-flight mass spectrometry (1). Guanidination was performed by adding 1 μl of a specific lyophilized HPLC fraction, which had been resuspended at one-tenth the original volume in water, to 1 μl 6 M *O*-methylisourea at pH 11. This was incubated at 37°C for 2 h, then cleaned with C18 ziptips, using a standard protocol. The eluent obtained was deposited on a sample site, and 0.3 μl of matrix was added. The sample was then dried and mass analyzed.

Chromium release assay

Lysis of ^{51}Cr-labeled target cells was performed, as previously described (1). In brief, T2 transfectants expressing Qa-1^b (T2/Qa-1^b) were incubated at room temperature for ~15 h before use as target cells. Target cells were prepared from various mouse strains by culturing spleen cells in the presence of 1 μg/ml Con A for 3 days and live cells purified on a Ficoll gradient. All target cell types were labeled with ^{51}Cr (Amersham Pharmacia Biotech) for 1.5 h at room temperature. A total of 5000 labeled target cells/well was dispensed into round-bottom assay plates and incubated for 60–90 min at room temperature in the presence or absence of various synthetic peptides (10 μM final concentration) (Macromolecular Resources, Fort Collins, CO). CTL effector cells were added at the indicated E:T ratios and incubated for 4 h at 37°C before supernatants were collected and counted on a Microbeta instrument model 1450 (Wallac, Gaithersburg, MD). In Ab-blocking experiments using anti-T cell reagents, effector cells were preincubated with either 10 μl anti-Lyt-2.2 mAb 2.43 ascites (a gift from H. Levitsky, Johns Hopkins University School of Medicine, Baltimore, MD) or 20 μg anti-L3T4 mAb GK1.5 (BD PharMingen, San Diego, CA) per well for 1 h at room temperature before the addition of target cells. In all cases, the percent specific lysis at an indicated E:T ratio represents the mean of triplicate samples.

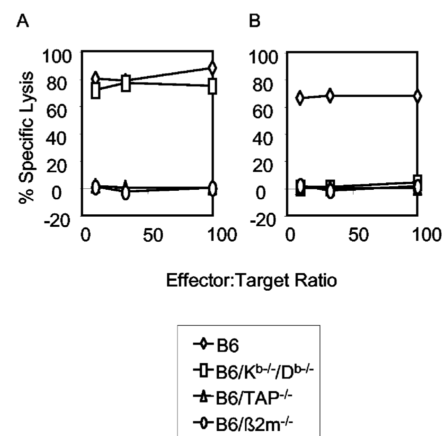

FIGURE 1. A Qa-1^b-specific CTL clone recognizes at least one non-Qdm Qa-1^b-binding peptide. The Qa-1^b-specific clones 39.1D7X (*A*) and D5D2 (*B*) were used as effector cells. Spleen cells from B6, B6/β_2m$^{-/-}$, B6/TAP$^{-/-}$, and B6/K$^{b-/-}$D$^{b-/-}$ mice were cultured for 3 days with Con A (1 μg/ml), labeled for 1.5 h at room temperature with ^{51}Cr, and used as targets in a standard 4-h ^{51}Cr release assay.

[4] Abbreviations used in this paper: hsp, heat shock protein; β_2m, β_2-microglobulin; MALDI, matrix-assisted laser desorption/ionization; TFA, trifluoroacetic acid.

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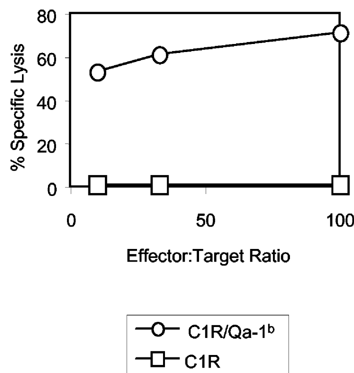

FIGURE 2. C1R cells contain at least one non-Qdm Qa-1^b-binding peptide. The Qa-1^b-specific clone 39.1D7X was used as an effector cell. C1R cells (C1R) and C1R cells expressing Qa-1^b (C1R/Qa-1^b) were labeled for 1.5 h at room temperature with ^{51}Cr, and used as targets in a standard 4-h ^{51}Cr release assay.

Results

A Qa-1^b-specific CTL that recognizes at least one non-Qdm Qa-1^b-binding peptide

To identify Qa-1^b-specific CTL that recognize Qa-1 when it is bound to peptides other than Qdm, we screened Qa-1^b-specific CTL for their ability to lyse lymphoblasts from B6/$K^{b-/-}D^{b-/-}$ mice. These mice were chosen because the only source of the dominant Qa-1^b-binding peptide Qdm in Con A-activated spleen cells from H-2^b mice is D^b (9, 39, 40). Despite the absence of Qdm, lymphoblasts from these animals express similar cell surface levels of Qa-1^b to wild-type animals (39, 41), and addition of Qdm peptide to lymphoblasts from B6/$K^{b-/-}D^{b-/-}$ mice does not significantly alter Qa-1^b cell surface levels. We reasoned that this level of Qa-1^b cell surface expression may be maintained by Qa-1 binding to other self peptides.

The Qa-1-restricted CTL clone 39.1D7X is a CTL clone that has been shown to recognize Qdm (37). 39.1D7X was able to lyse lymphoblasts from B6 or B6/$K^{b-/-}D^{b-/-}$ mice, but not B6/$\beta_2m^{-/-}$ or B6/TAP$^{-/-}$ mice, indicating that this clone must also recognize a TAP-dependent non-class Ia leader peptide (Fig. 1*A*). To reconfirm that lymphoblasts from B6/$K^{b-/-}D^{b-/-}$ mice contain no source of Qdm capable of binding to Qa-1^b, the Qa-1-restricted CTL clone D5D2 was used as an effector against the same set of target cells. D5D2 recognizes the Qdm peptide and other peptides that are very similar in sequence to it. As expected, this clone lysed cells from B6 mice, as they contain Qdm, but cells from B6/$K^{b-/-}D^{b-/-}$ mice were not recognized, verifying the absence of Qdm (Fig. 1*B*).

C1R cells contain at least one non-Qdm Qa-1^b-binding peptide that can be recognized by a Qa-1^b-specific CTL

It has previously been shown that C1R cells do not contain a source of Qdm peptide that can be presented by Qa-1^b; hence, Qa-1-restricted CTL clones that can only recognize Qa-1 when Qdm is bound cannot recognize C1R/Qa-1^b target cells (1). To determine whether there are other peptides present within C1R cells that can be presented to CTL by Qa-1^b, we tested the ability of the Qa-1-restricted CTL clone 39.1D7X to lyse C1R/Qa-1^b target cells. In the absence of exogenous peptide pulsing, C1R/Qa-1^b target cells were lysed, but untransfected targets were not (Fig. 2). These results suggest that there is at least one non-Qdm peptide found endogenously within C1R cells that can bind to Qa-1.

Identification of a peptide from hsp60 as the dominant Qa-1-binding peptide in the absence of Qdm

To identify the peptide or peptides bound by Qa-1^b in C1R cells, low m.w. peptides bound by Qa-1^b in C1R/Qa-1^b cells were isolated by immunoprecipitation with an anti-human β_2m mAb, followed by acid extraction and separation by reverse-phase HPLC. Two dominant peptide-containing fractions were isolated (Fig. 3*A*, fractions 17 and 18).

Matrix-assisted laser desorption/ionization (MALDI) mass spectroscopy of fraction 18 identified a unique protonated molecular ion (MH$^+$) with a mass/charge ratio of 1087.7 (Fig. 3). Qa-1 has previously been shown to present a peptide from *S. typhimurium* GroEL to CD8$^+$ CTLs. This peptide has the sequence

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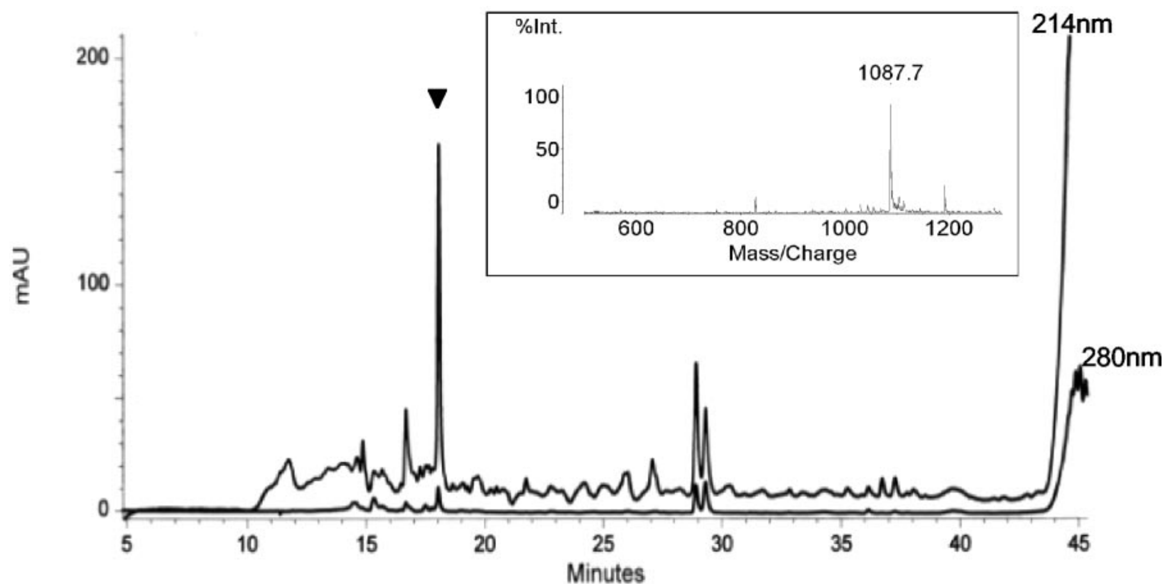

FIGURE 3. Isolation of the dominant Qa-1-binding peptide in the absence of Qdm. C1R cells expressing Qa-1^b (C1R/Qa-1^b) were lysed, and peptides were extracted from anti-β_2m immunoprecipitate. Peptides were separated by reverse-phase HPLC. Fractions 17 and 18 were the dominant peptide-containing fractions, designated by the inverted arrow. *Inset*, Displays the mass spectral profile obtained from MALDI time-of-flight analysis of fraction 18. Percent intensities were normalized to the most abundant peak in the region shown.

GMQFDRGYL and a molecular mass of 1086.2 Da. Most of these GroEL/Qa-1-reactive $CD8^+$ T cells cross-react with a homologous peptide, GMKFDRGYI, derived from murine hsp60, in a Qa-1-restricted fashion (35, 36). This hsp60 peptide is conserved between mice and humans. Postsource decay analysis on fraction 18 yielded a fragmentation pattern that was identical with that obtained from synthetic GMKFDRGYI and very similar to that obtained from synthetic GMQFDRGYL (Fig. 4, *A–C*). To confirm that the peptide was derived from human hsp60, we determined the number of lysines and terminal glycines present by guanidination of the peptides in question (42, 43). In these experiments, *O*-methylisourea reacts only with lysines and terminal glycines with a predicted 42-Da increase in molecular mass occurring for every diamidomethane group added. Guanidination of synthetic GroEL peptide caused a 42-Da increase in molecular mass, whereas guanidination of synthetic hsp60 peptide and fraction 18 caused a 84-Da increase in molecular mass, as assessed by MALDI mass spectroscopy (Fig. 4, *D–F*). Hence, the sequence of the dominant peptide in fraction 18 was deduced to be GMKFDRGYI. Database analysis confirmed that this peptide is derived from residues 216–224 of human hsp60.

MALDI mass spectroscopy analysis of the other HPLC fractions yielded complex spectra with no distinct peaks, except in the case of fractions 17, 21, and 22, in which three peptide peaks of 957, 1049, and 986 MH^+ units, respectively, were identified. These species, and other protonated molecular ions in fraction 18, were far less abundant than the hsp60 peptide, and it was not possible to obtain any information about their sequence. The Qdm peptide has a m.w. and hydrophobicity very similar to the 986 MH^+ peptide in fraction 22, but C1R cells do not contain this peptide (1).

Recognition of the peptides from GroEL and hsp60 by a Qa-1^b-specific CTL

To further verify that the peptides derived from GroEL and hsp60 can bind to $Qa\text{-}1^b$, we tested the ability of these peptides to sensitize T2/$Qa\text{-}1^b$ targets to lysis by the Qa-1-restricted CTL clone 39.1D7X. Preincubating with the GroEL and hsp60 peptides promoted target cell lysis, which could be completely blocked by anti-CD8 mAb (2.43) (Fig. 5), but not anti-CD4 mAb (GK1.5) (data not shown). Hence, the two peptides are effectively presented by $Qa\text{-}1^b$, and they facilitate TCR-mediated recognition by $Qa\text{-}1^b$-specific CTL.

Discussion

The data presented in this work demonstrate that in the absence of the dominant Qa-1-binding peptide Qdm, a peptide from hsp60

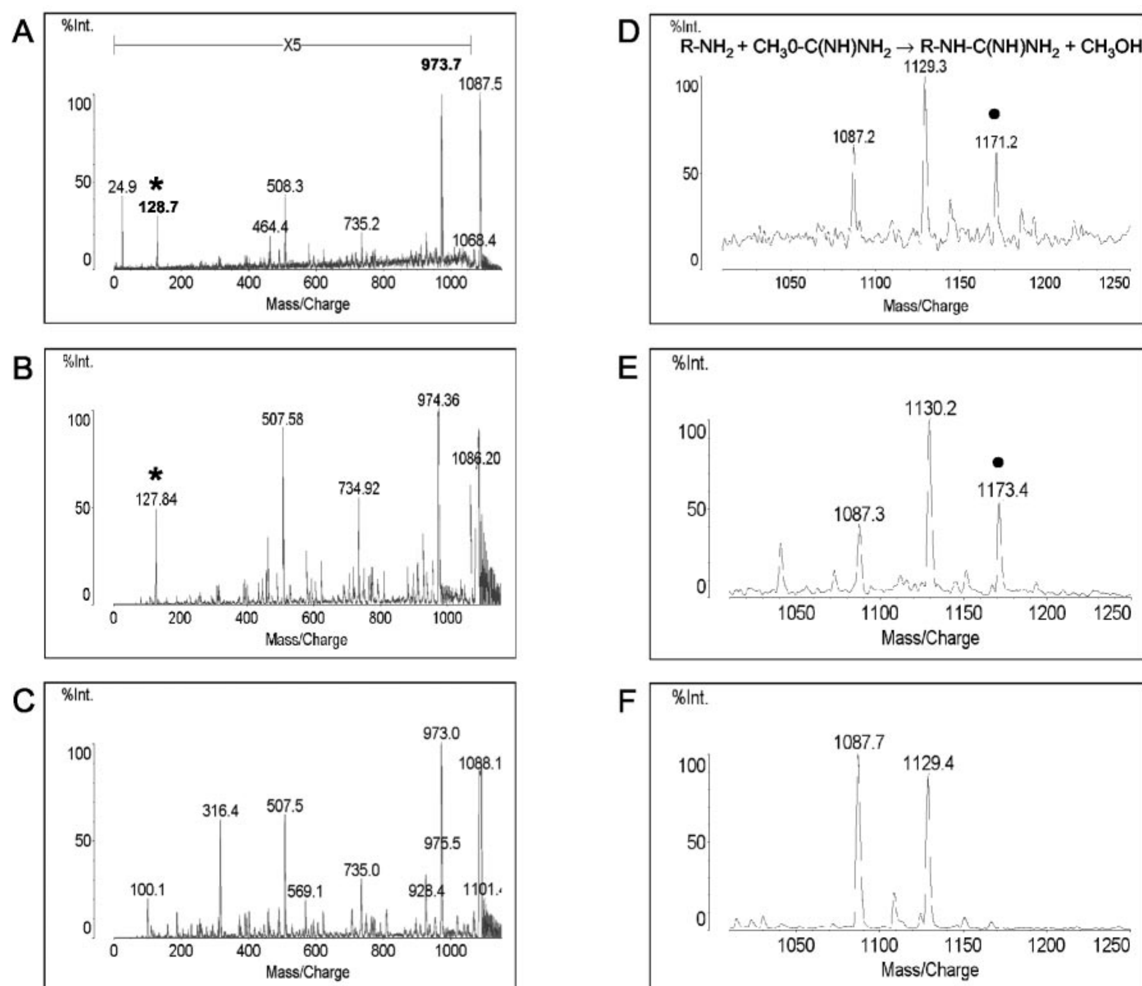

FIGURE 4. Sequencing of the naturally derived hsp60 peptide. *A–C*, Post source decay analysis of fraction 18 (*A*), synthetic hsp60 peptide (*B*), and synthetic GroEL peptide (*C*). The mass peak marked by an asterisk indicates an ion expected from the fragmentation of the hsp60 peptide, but not the GroEL peptide. *D–F*, To differentiate between K and Q at the third position, guanidination of fraction 18 (*D*), synthetic hsp60 peptide (*E*), and synthetic GroEL peptide (*F*) was performed, followed by mass spectrometry. The mass peak marked by a bullet designates the peptide species formed by the addition of two diaminomethane groups. Percent intensities were normalized to the most abundant peak in the region shown, unless otherwise indicated, as in *A*. *Inset*, Shows the chemical reaction occurring during guanidination.

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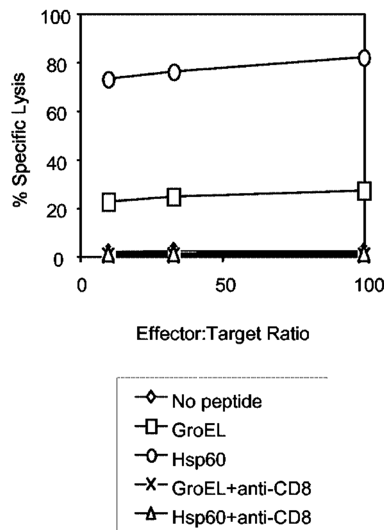

FIGURE 5. Peptides from GroEL and hsp60 are recognized by a Qa-1^b-specific CTL. T2 cells expressing Qa-1^b (T2/Qa-1^b) were labeled for 1.5 h at room temperature with ^{51}Cr, then incubated in the presence of the indicated peptide (10 μM) for 60–90 min. The Qa-1^b-specific clone 39.1D7X was then added to these targets in a standard 4-h ^{51}Cr release assay. A total of 10 μl anti-Lyt-2.2 mAb 2.43 (anti-CD8) ascites was included for Ab-blocking studies.

(GMKFDRGYI) is the major peptide bound by Qa-1. Although the cells used to identify this peptide, namely C1R cells, express very low levels of MHC class Ia molecules overall, they do express HLA-B35 at 60-fold reduced levels and HLA-Cw4 at normal levels (44), relative to the parental cell line. Peptides derived from the leader sequences of both of these proteins (VTAPRTVLL and VMAPRTLIL, respectively) have been shown to bind to Qa-1^b with a similar affinity to Qdm (8, 9, 21, 39). However, these two MHC class Ia leader peptides were not detected in the extracts from MHC class I immunoprecipitates. This indicates that the leader peptides derived from the HLA-B35 and HLA-Cw4 molecules are not efficiently processed and presented in C1R cells. Given that the leader peptides from HLA-B35 and HLA-Cw4 bind to Qa-1^b with similar affinity to Qdm, the hsp60 peptide should also be the dominant Qa-1-binding peptide in murine cells, which show a similar reduction in MHC class Ia molecule expression, processing, and presentation.

Previous studies have indicated that the hsp60 and GroEL peptides bind poorly to Qa-1 relative to the Qdm peptide (21). These studies, using CTLs that recognize Qa-1 leader peptide complexes, demonstrated that the hsp60 and GroEL peptides do not effectively compete with the Qdm peptide for binding and recognition. It is our hypothesis that, under circumstances in which either relevant MHC class Ia molecule expression is limiting or processing of MHC class Ia leader peptide is altered, resulting in leader peptide availability falling below a certain threshold, the hsp60 peptide could become the dominant peptide bound by Qa-1.

The amount of the hsp60 peptide available to bind Qa-1, relative to the Qdm peptide, could be altered under several conditions. The down-regulation or loss of MHC class Ia expression or alteration in MHC class Ia processing is often observed in transformed or infected cells (45–47); hence, the level of Qdm peptide available to bind Qa-1 may be altered in such situations. Under such circumstances, alternative Qa-1-binding peptides, even those with low affinity, may be presented. Alterations in the amount of hsp60 peptide available to bind Qa-1 may also occur. Hsp60 is constitutively expressed in normal cells, and its expression is up-regulated in response to various stress stimuli (48–51). Under stress conditions, hsp60 is sufficiently processed and presented by Qa-1 to accommodate target cell recognition by T cells. This is illustrated by experiments showing that stressed, but not normal unstressed murine target cells are lysed by Qa-1-restricted $CD8^+$ T cells, which recognize an epitope derived from *S. typhimurium* GroEL and cross-react with the hsp60 peptide (35, 36).

The relative expression levels of cell surface Qa-1 with MHC class Ia-derived leader or hsp60-derived peptide bound could influence NK cell recognition regulated by CD94/NKG2A. Cells expressing normal levels of MHC class Ia molecules would express high levels of Qa-1 with Qdm peptide bound and thus be resistant to lysis by NK cells. In cells in which MHC class Ia expression has been altered, corresponding low levels of Qa-1-Qdm complexes would be expressed and such targets would be more susceptible to NK cell lysis. This is supported by the following experimental evidence. First, the TAP-deficient human lymphoblastoid cell line T2, which cannot transport endogenous MHC class I leader peptides into the endoplasmic reticulum, when transfected with Qa-1 is more resistant to lysis by NK cells in a Qdm peptide-dependent fashion (13, 21). Second, the presence of the Qdm peptide results in decreased NK cell-mediated in vivo clearance of TAP-deficient RMA-S cells injected into C57BL/6 mice (52). Third, C1R cells, which have reduced levels of MHC class Ia molecules, when transfected with Qa-1 are also more resistant to lysis by Qa-1 $receptor^+$ NK cells when the Qdm peptide is present (21). Finally, NK cell lysis of lymphoblasts from MHC class Ia-deficient mice and the human MHC class Ia-deficient cell line 721.221, transfected with Qa-1, is inhibited by the Qdm peptide (9, 39). Therefore, CD94/NKG2A-dependent regulation of NK recognition and killing is exquisitely sensitive to the cell surface level of Qa-1-Qdm complexes.

Under conditions in which the amount of the hsp60 peptide bound to Qa-1 increases, relative to the Qdm peptide, the level of activation of CD94/NKG2-expressing NK cells could increase. Indeed, this appears to be the case because Qa-1-expressing C1R cells are effectively killed by CD94/NKG2A-expressing NK cells and exogenous Qdm peptide is required for inhibition (21). Hence, the levels of Qa-1 complexed with endogenous inhibitory MHC class Ia-derived leader peptides may be insufficient to adequately engage CD94/NKG2A. This is consistent with our inability to recover inhibitory MHC class Ia-derived leader sequence peptides from Qa-1 expressed in C1R cells and the dominance of the noninhibitory hsp60-derived peptide.

$CD8^+$ T cell activity could also be altered when the amount of hsp60 peptide bound to Qa-1 increases, relative to Qdm, as accumulating evidence suggests that NK receptors regulate the function of these cells. The expression of CD94/NKG2A by activated $CD8^+$ T cells is thought to modulate the T cell response by altering the TCR-triggering threshold (25, 53–57). CD94/NKG2A-mediated inhibition of human CTL Ag-specific effector function has been demonstrated both in vivo and ex vivo (58–61). Results from murine studies largely corroborate with the data obtained from human-based experiments. CD94/NKG2A partially inhibits murine allospecific CTL cytotoxicity and inhibits polyoma virus-specific CTL lysis, but not IFN-γ production (55, 56, 62). However, CD94/NKG2A does not inhibit OVA peptide (SIINFEKL)-specific or lymphocytic choriomeningitis virus-specific CTL effector function (56, 57). In transgenic mice expressing CD94 and NKG2A transgenes, a partial inhibition of staphylococcal enterotoxin B-mediated early T cell activation is observed (57). These results suggest CD94/NKG2A-mediated inhibition of T cells may be restricted to particular situations or may act in synergy with other receptors that are up-regulated in a similar manner (57).

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Hence, reducing the CD94/NKG2A inhibitory signal by increasing the amount of the hsp60 peptide bound to Qa-1, relative to the amount of Qdm peptide bound, could increase $CD8^+$ T cell activity. It is not known whether stimulatory NKG2 isoforms can modulate T cell function.

The activity of Qa-1-restricted $CD8^+$ T cells that recognize an epitope derived from *S. typhimurium* GroEL and cross-react with the hsp60 peptide (36) may also increase under conditions that cause the amount of the hsp60 peptide bound to Qa-1 to increase, due to increased TCR signaling. This is illustrated by the fact that stressed, but not normal unstressed murine target cells are lysed by these CTL (36). Such recognition of the hsp60 peptide, when presented by Qa-1, may also occur when the availability of the Qdm peptide is decreased and the hsp60 peptide is expressed at constitutive levels. This possibility is supported in this study by the finding that the hsp60 peptide is efficiently processed and presented by Qa-1 in C1R cells, which, like many other tumors, display reduced MHC class I expression. Thus, these Qa-1-expressing C1R cells are efficiently lysed by the hsp60-reactive Qa-1-restricted CTL clone 39.1D7X. Effector or memory CTL that have previously been stimulated by the GroEL or hsp60 peptide may be particularly sensitive to changes in the concentration of the hsp60 or Qdm peptides bound to Qa-1.

Therefore, changes in the level of the hsp60 peptide presented by Qa-1, relative to the amount of Qdm, as can occur under many conditions, such as infection, stress, and transformation, may result in the activation of Qa-1-restricted GroEL/hsp60-specific T cells via their TCR. Lysis of the target cell and potential autoimmune reactions may occur. More broadly, the activation level of many NK, NKT, and T cells may be increased via the interaction of Qa-1 with CD94/NKG2 receptors on their surface, when such changes in the composition of peptide bound to Qa-1 occur.

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