to the sequences shown. Preparation of end-labeled double-stranded probes was performed as described (*4*).
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Specialized Role for a Murine Class I-b MHC Molecule in Prokaryotic Host Defenses

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Although nonclassical (class I-b) gene products represent the majority of murine major histocompatibility complex (MHC) genes, the role of these relatively nonpolymorphic molecules remains uncertain. Recently, one such protein, H-2M3 (formerly designated Hmt), was shown to bind and specifically present *N*-formylated peptides to cytotoxic T lymphocytes. Because *N*-formylation is characteristic of prokaryotic proteins, this MHC molecule may be especially adapted for a role in the mammalian defense against bacterial attack. The current studies demonstrate that an MHC molecule, indistinguishable from H-2M3, presents antigens derived from the intracellular pathogen *Listeria monocytogenes* to *Listeria*-specific CD8$^+$ cells.

Murine listeriosis has provided a model for studying the role of CD8$^+$ T cells in the host defense against intracellular pathogens (*1*). Although animals infected with *L. monocytogenes* generate a humoral response to infection, antibodies cannot damage invading bacteria because of their intracellular location. By contrast, peptide fragments derived from intracellular *L. monocytogenes* can be processed and presented in association with class I MHC products (*2, 3*), facilitating the lysis of infected cells by *Listeria*-specific CD8$^+$ cytotoxic T lymphocytes (CTLs). Because infected cells represent a major site for bacterial proliferation, this selective destruction can be an effective strategy by which the host can contain *L. monocytogenes* (and other intracellular pathogens) in vivo (*1, 4*).

The presentation of some prokaryotic antigens to mouse CD8$^+$ T cells is restricted by classical class I MHC products (H-2K, D, or L), implying that these molecules contribute to peptide recognition (*5*). However, a subpopulation of *Listeria*-specific CD8$^+$ T cells selectively lyses *Listeria*-treated allogeneic target cells lacking any shared K, D, or L allele (*6, 7*). Nonetheless, β_2-microglobulin is required for *Listeria* antigen recognition by these cells, implying that a class I MHC product is required in antigen presentation (*3*). This pattern suggests that these effectors recognize *Listeria*-derived peptides associated with a monomorphic, nonclassical class I-b MHC product (*8*) distinct from H-2K, D, or L.

The murine class I-b product H-2M3^a (*9*) is a particularly attractive candidate for *Listeria*-antigen presentation (*10*). This molecule, which is expressed by all common strains of laboratory mice, binds *N*-formyl peptides derived from the mitochondrially encoded reduced form of nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 1 (ND1) (*11*), forming the minor histocompatibility antigen Mta (maternally transmitted antigen). Using a competitive binding assay, we have demonstrated that this molecule preferentially binds peptides bearing an *N*-formyl group (*12*). By contrast, acetylated and unmodified peptides of identical sequence bind poorly or not at all to H-2M3^a. Because *L. monocytogenes* (and other prokaryotes) initiate protein synthesis with *N*-formylmethionine (*13*), we and others have speculated that H-2M3 may be adapted for the task of presenting microbial peptides to CD8$^+$ effectors (*11*). The current studies establish that a class I MHC product indistinguishable from H-2M3^a is responsible for the apparent "MHC-unrestricted" presentation *Listeria* antigens to CD8$^+$ T cells.

Listeria-specific CTLs derived from C57BL/6 (B6) mice (H-2K^b, D^b, H-2M3^a) have been characterized as MHC-unrestricted because they can lyse *Listeria*-treated target cells obtained both from syngeneic mice and from a range of allogeneic strains (*6, 7*). However, target cells from all strains previously tested express H-2M3^a (*10*). To examine whether *Listeria*-immune CD8 effectors are restricted by H-2M3^a, we measured the capacity of these effectors to lyse antigen-treated macrophages from B10.CAS2 mice. This mouse strain (produced by backcrossing a *Mus musculus castaneus* H-2 complex into a B10.BR, Mta$^+$ background) expresses H-2M3^b, a rare nonfunctional H-2M3 allele (*14*). Although the CTLs used in these experiments specifically lysed *Listeria*-treated macrophages from allogeneic BALB/c mice (Table 1), they could not destroy comparable targets prepared with B10.CAS2 macrophages (Table 1). The apparent absence of lysis of B10.CAS2 targets did not reflect intrinsic resistance to lysis by CTLs, because the same targets were readily lysed in the presence of an antibody to CD3, which induces antigen-independent, CTL-mediated lysis (*15*).

Table 1. Lysis of B6, BALB/c, and B10.CAS2 macrophages (M0) by *Listeria*-immune CD8 cells from B6 mice. Thioglycollate-induced peritoneal macrophages in experiment 1 from B6 (H-2K^b, D^b, H-2M3^a) and BALB/c (H-2K^d, D^d, H-2M3^a) or in experiment 2 from B6 and B10.CAS2 (H-2K^{cas2}, D^{cas2}, H-2M3^b) mice were plated at 3 × 10^4 cells per well in 96-well plates and allowed to adhere overnight at 37°C. Monolayers were then incubated with medium alone, with a partially purified *Listeria* membrane preparation (6 μg/ml) (*3*), or with an antibody to CD3 (2C11) (3 μg/ml) for 18 hours. At that time, monolayers were washed, treated with brefeldin (1 μg/ml) to block further antigen processing, and incubated with *Listeria*-immune CD8 cells (*3*), 9 × 10^4 cells per well, for an additional 6 hours at 37°C. *Listeria*-specific lysis of macrophage targets was quantitated with the use of a neutral red uptake technique (*3, 18*). The triplicate sets of numbers in experiment 2 represent the results of three separate, paired experiments.

M0s incubated with	Lymphocyte-mediated lysis (%) of M0 targets from		
	B6	BALB/c	B10.CAS2
	Experiment 1		
Medium	0	0	—
Listeria membrane antigen	43	50	—
	Experiment 2		
Medium	7, 11, 0	—	0, 0, 0
Listeria membrane antigen	54, 22, 52	—	0, 0, 0
Anti-CD3 antibody	61, 52, 68	—	69, 55, 47

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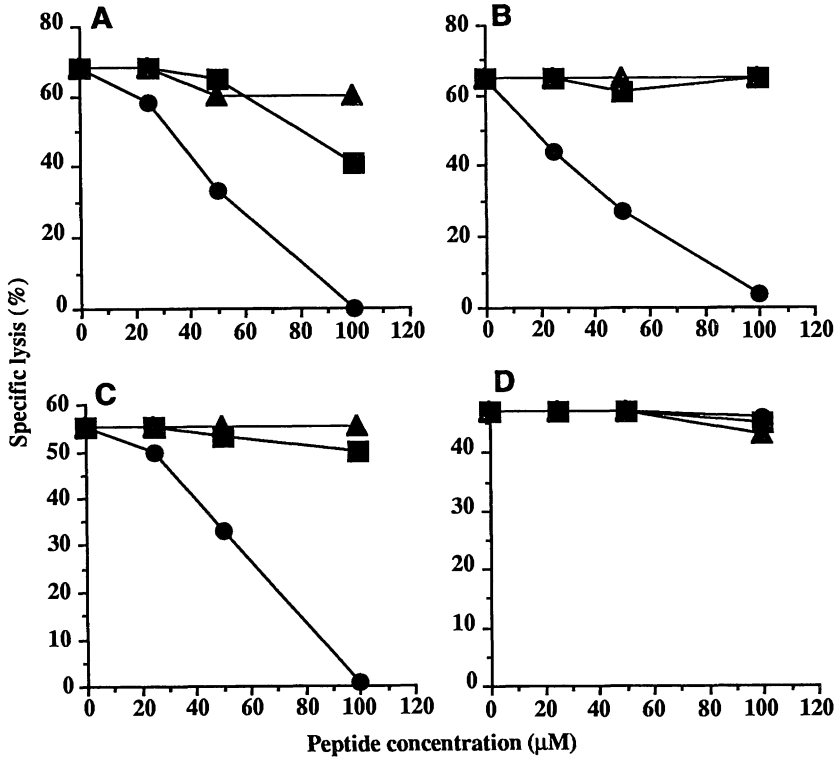

Fig. 1. Effects of *N*-formylated, *N*-acetylated, or nonsubstituted peptides on the presentation of *Listeria* antigen to CD8 cells. B6 macrophage monolayers were preincubated with formylated (circles), acetylated (squares), or nonsubstituted (triangles) peptides at 25 μM, 50 μM, and 100 μM doses. Peptides used for pretreatment included the following (*12*): (**A**) ND1 (MFFINI), (**B**) ND5 (MKVINIFTTSIL), (**C**) Bla-z (MFVLNKFF), or (**D**) VAMAP (MMFKFALYFI). One hour after peptide addition, *Listeria*-derived membrane antigen (6 μg/ml) was added to all monolayers. Eighteen hours later the monolayers were washed, treated with brefeldin to block further *Listeria* antigen processing, and then incubated with CD8 cells for 6 hours. We measured lysis with a neutral red assay as described in Table 1. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

The selective failure of the CTLs to recognize *Listeria*-treated B10.CAS2 cells suggests that *Listeria*-derived peptides are presented to CTL by H-2M3^a or a gene product closely linked to H-2M3^a.

Because H-2M3^a–mediated antigen binding can be competitively inhibited by some *N*-formylated peptides of mitochondrial and bacterial origin (*12*), we determined whether these peptides would also interfere with *Listeria* antigen presentation. B6 macrophages were incubated with *Listeria* antigen after preincubation with varying concentrations of several inhibitor peptides. Lysis of macrophage targets by *Listeria*-immune CTLs was then quantitated. *N*-formylated peptides derived from mitochondrial proteins ND1 and ND5 and from *Bacillus cereus* β-lactamase (Bla-z) bind to H-2M3^a and inhibit Mta-specific CTLs (*12*); each also blocked *Listeria*-specific target cell lysis (Fig. 1, A through C). By contrast, neither acetylated nor unmodified forms of these peptides demonstrated inhibitory activity. Inhibition was not observed if formyl peptides were added 18 hours after introduction of *Listeria* antigen. Thus, formyl peptides selectively inhibited the presentation of *Listeria* antigen and not the subsequent interaction of CTLs with antigen-bearing target cells. Unlike the peptides described above, *Vibrio anguillarum* membrane-associated protein (VAMAP) does not bind to H-2M3^a, despite its formylated state (*12*). Consistent with this, formylated VAMAP also failed to block *Listeria*-specific lysis (Fig. 1D). Thus, the antigen binding element responsible for presenting *Listeria*-derived antigens to CD8$^+$ T cells preferentially binds *N*-formyl peptides and does so with the same fine specificity pattern as H-2M3^a.

Some class I-b molecules have highly differentiated roles in the host defense. For example, MHC products within the Q and TL region can present viral peptides to αβ CTLs and heat-shock proteins to γδ T cells (*16*). Our results demonstrate involvement of an *N*-formyl peptide–dependent, class I-b MHC molecule, presumably H-2M3, in presentation of antigens from a prokaryotic, intracellular pathogen to CD8$^+$ CTLs. Immunofluorescent studies indicate that the responsible MHC-unrestricted *Listeria*-specific CD8 cell lines utilize an αβ T cell receptor in antigen recognition, as do Mta-specific CTLs (*17*). *Listeria*-specific CD8$^+$ T cells can adoptively transfer MHC-unrestricted, anti-*Listeria* immunity to naive mice (*7*), suggesting that this molecule not only presents antigen to CD8$^+$ T cells in vitro but also in vivo. Individual class I-b molecules may have evolved as highly specialized (monomorphic) tools for coping with recurrent challenges within the environment. Whereas H-2M3 is particularly adapted for binding *N*-formylated prokaryotic peptides, it is likely that other class I-b molecules may exploit other differences between mammalian and microbial antigens.

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