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Lessons from *H2–M3*$^{-/-}$ (M3°) Mice: M3 Restricted T Cells Initiate the Primary Immune Response Against *Listeria* Infection

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$CD8^+$ T cells which recognize pathogenic peptides derived from MHC class I contribute significantly in host defense against intracellular bacteria, viruses and some parasites. In particular, the role of $CD8^+$ T cells during microbial infection is well defined in experimental listeriosis.

Listeria monocytogenes is a Gram-positive intracellular pathogen that infects wide ranges of cell types including macrophages, hepatocytes, and endothelial cells. During *Listeria* infection, $CD8^+$ T cells have been shown to play an important role in clearing infection (Sasaki *et al.*, 1990). Interestingly, the primary response against *Listeria* in mice was independent of the polymorphic class Ia loci (D'Orazio *et al.*, 2003). This observation suggested that $CD8^+$ T cells which recognize *Listeria* epitopes presented by an MHC class Ib molecule can effectively clear *Listeria*. Subsequently, cytotoxic T lymphocytes (CTLs) recognized *Listeria* epitopes via MHC class Ib molecules H2-M3 (M3) and Qa-1 are identified (Seaman *et al.*, 1999).

MHC class Ib molecules are structurally similar to MHC class Ia molecules, but binds each unique antigen rather than conventional nonameric peptides. M3, one of them, is a highly specialized antigen presenting molecule which has the high affinity to *N*-formylated peptides in mice (Lindahl *et al.*, 1997). Binding of the NADH dehydrognase subunit I (ND1) peptide to M3 is dependent on the presence of the *N*-formyl group that forms a tight association with the B pocket in the hydrophobic peptide-binding groove (Lindahl *et al.*, 1997). Due to this unique binding property, the affinity of M3 for nonformylated peptides is 100-1,000 fold lower (Lindahl *et al.*, 1997). Since the murine class Ia molecules K and D do not bind *N*-formylated peptides properly and all prokaryotes initiate protein synthesis with *N*-formyl methionine, M3 may have been selected in evolution for the specialized presentation of conserved structures, *N*-formyl peptides, derived from microbial antigens. Subsequently it has been shown that *N*-formyl methionine peptides derived from *L. monocytogenes* and *Mycobacterium tuberculosis* can be presented by H2-M3 (Gulden *et al.*, 1996; Chun *et al.*, 2001).

The finding that M3 can present multiple *Listeria*-derived epitopes to $CD8^+$ T cells suggests that M3-restricted effector T cells may play a key role in the clearance of infection. Recently, the kinetics of the clonal expansion of ^{f}MIGWIIA-specific M3-restricted or LLO 91-99-specific H2-K^d (K^d)-restricted $CD8^+$ T cells following natural infection with *Listeria* was examined using peptide-loaded

class Ia and class Ib tetramers (Kerksiek *et al.*, 1999). Surprisingly, the M3-restricted T cell response preceded the K^d-restricted response (peak at 5-7 vs 7-9 days) and the M3-restricted T cells were 3-4 folds more abundant. Although the generality of these observations needs to be examined using other M3-restricted epitopes, this would imply that distinct class I families influence different stages of the $CD8^+$ T cell response to infection, with M3-restricted T cells playing a key role in controlling the early stages of *Listeria* infection.

In this study, we assessed the role of M3 in primary infection as well as secondary infection with *L. monocytogenes* using *H2-M3*$^{-/-}$ (M3°) mice. We found that higher bacterial burden in these mice during an early stage of primary *Listeria* infection. This is due to the lack of effective CTL generation as well as a defective NK cell activity and nitric oxide secretion. In later stage, M3° mice show a diminished size of total $CD8^+$ T cells. Thus, this result indicates that the role of M3 restricted T cells are important to initiate the primary immune response against *Listeria* infection during an early stage of infection and also affect the population of total $CD8^+$ T cells during later stage.

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