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Mycobacterium tuberculosis Heat Shock Fusion Protein Enhances Class I MHC Cross-Processing and -Presentation by B Lymphocytes[1]

Aaron A. R. Tobian,* Clifford V. Harding,[2,3]* and David H. Canaday[2,3†]

Exogenous heat shock protein (HSP):peptide complexes are processed for cross-presentation of HSP-chaperoned peptides on class I MHC (MHC-I) molecules. Fusion proteins containing HSP and Ag sequences facilitate MHC-I cross-presentation of linked antigenic epitopes. Processing of HSP-associated Ag has been attributed to dendritic cells and macrophages. We now provide the first evidence to show processing of HSP-associated Ag for MHC-I cross-presentation by B lymphocytes. Fusion of OVA sequence (rOVA, containing $OVA_{230-359}$ sequence) to *Mycobacterium tuberculosis* HSP70 greatly enhanced rOVA processing and MHC-I cross-presentation of $OVA_{257-264}$:K^b complexes by B cells. Enhanced processing was dependent on linkage of rOVA sequence to HSP70. *M. tuberculosis* HSP70-OVA fusion protein enhanced cross-processing by a CD91-dependent process that was independent of TLR4 and MyD88. The enhancement occurred through a post-Golgi, proteasome-independent mechanism. These results indicate that HSPs enhance delivery and cross-processing of HSP-linked Ag by B cells, which could provide a novel contribution to the generation of $CD8^+$ T cell responses. HSP fusion proteins have potential advantages for use in vaccines to enhance priming of $CD8^+$ T cell responses. ***The Journal of Immunology,*** **2005, 174: 5209–5214.**

Heat shock proteins (HSPs)[4] are molecular chaperones expressed by prokaryotes and eukaryotes that bind polypeptide chains, prevent aggregation, and support protein folding (1). The expression of many HSPs is increased with stress (e.g., heat, anoxia, and glucose starvation). Members of the HSP70 family are constitutively expressed in eukaryotic and prokaryotic cells. This family includes *Mycobacterium tuberculosis* (MTB) HSP70. These HSPs bind hydrophobic regions of nascent polypeptides and unfold or disaggregate misfolded proteins to yield productive folding intermediates.

HSPs have immunological functions (2). Mammalian HSPs, e.g., HSP70 (3–6), HSP90 (4, 7), gp96 (4, 7–10), calreticulin (11, 12), and HSP110 (13), form highly immunogenic complexes with chaperoned peptides. After processing of HSP:peptide complexes by APCs, these peptides, which may derive from tumor Ags (2–4, 11, 13, 14), viral Ags (6), or other sources, are presented on class I MHC (MHC-I) molecules. We demonstrated recently that bacterial HSPs, e.g., *Escherichia coli* DnaK and MTB HSP70, enhance processing and MHC-I presentation of chaperoned peptides (15). Dendritic cells and macrophages process exogenous HSP: peptide complexes by alternate MHC-I Ag processing (cross-processing) mechanisms, resulting in cross-presentation of exogenous HSP-chaperoned peptides to $CD8^+$ T cells (5, 15, 16). Bacterial and mammalian host HSPs may also contribute to MHC-II Ag processing (17–19).

HSPs may enhance cross-processing by cytosolic or vacuolar mechanisms. Some researchers suggest that endocytosed mammalian HSPs enhance processing by vacuolar mechanisms (8, 20), and in some cases such processing is TAP independent (21). Others propose that mammalian HSPs enhance cytosolic processing because their effect appears TAP-dependent and inhibited by brefeldin A (an inhibitor of anterograde Golgi transport) and lactacystin (a proteasome inhibitor) (16, 22). One caveat is that these inhibitors and the TAP-deficient state decrease post-Golgi MHC-I levels and thereby inhibit vacuolar as well as cytosolic alternate MHC-I Ag-processing mechanisms (23–25). Recently, we have shown that the extent to which vacuolar and cytosolic processing mechanisms contribute to cross-processing of HSP-chaperoned peptides is dependent on the type of APC. We observed that cytosolic mechanisms are used more by dendritic cells, and vacuolar mechanisms are used more by macrophages (15).

Recombinant HSP fusion proteins (with antigenic sequences fused to the N or C terminus of the HSP) have been shown to elicit $CD8^+$ T cell and Ab responses (26–31). Although native HSPs form noncovalent complexes with chaperoned peptides that may dissociate, recombinant HSP fusion proteins can stably incorporate antigenic polypeptide sequences that are covalently linked to the HSP. Moreover, antigenic sequences incorporated in HSP fusion proteins can be significantly longer than antigenic peptides typically included in noncovalent HSP:peptide complexes. These features potentially allow stable incorporation of multiple antigenic epitopes in a single HSP fusion protein for use in vaccination.

Processing of HSP-associated Ags for MHC-I Ag presentation has been studied extensively with dendritic cells and macrophages. Cross-processing of HSP fusion proteins by B cells, however, has not been studied with either mammalian or bacterial HSPs. B cells

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[4] Abbreviations used in this paper: HSP, heat shock protein; ER, endoplasmic reticulum; HSP-OVA, heat shock OVA fusion protein; MHC-I, class I MHC; MTB, *Mycobacterium tuberculosis*; ODN, oligodeoxynucleotide.

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cross-present exogenous CpG DNA-linked Ag to $CD8^+$ T cells and may contribute to priming of $CD8^+$ T cell responses (32). We considered the hypothesis that B cells cross-process HSP-associated Ags for MHC-I presentation, potentially contributing to the generation of $CD8^+$ T cell responses.

This study reveals that fusion of a sequence from OVA (rOVA, containing $OVA_{230-359}$ sequence) to MTB HSP70 sequence enhances rOVA cross-processing and MHC-I presentation by B cells. MTB HSP70-OVA fusion protein enhanced cross-processing primarily by a CD91-dependent process. The major enhancement of MHC-I Ag processing was not dependent on TLR2, TLR4, CD40, or MyD88. HSP enhancement of cross-processing by B cells could provide a novel contribution to the generation of $CD8^+$ T cell responses and provide a basis for the use of HSP fusion proteins in vaccines to enhance the priming of $CD8^+$ T cell responses.

Materials and Methods

Cells and media

Unless otherwise specified, incubations were performed at 37°C in 5% CO_2 in standard medium consisting of DMEM (Invitrogen Life Technologies), 10% heat-inactivated FCS (HyClone), 50 μM 2-ME, 1 mM sodium pyruvate, 10 mM HEPES buffer, and antibiotics. B6D2F1/J, C57BL/6, C57BL/10ScN, C57BL/10ScSn, and B6.129P2-$TNFrsf^{-/-}$ ($CD40^{-/-}$ on C57BL/6 background) female mice were obtained from The Jackson Laboratory. HLA-A2/K^b transgenic mice (expressing an MHC-I fusion protein consisting of the α-1 and α-2 domains of HLA-A2.1 and the α-3 domain of H-2 D^d) (33) were a gift from V. Engelhard (University of Virginia, Charlottesville, VA). $TLR2^{-/-}$ mice (34) and $MyD88^{-/-}$ mice (35) were provided by O. Takeuchi and S. Akira (Osaka University, Osaka, Japan) and were bred onto the C57BL/6 background for six to eight generations. B6D2F1/J mice were used for all experiments except those involving knockout models, which used appropriate C57BL/6 or C57BL/10ScSn mice for controls. Because CD43 is expressed on all murine splenocytes except resting conventional (B-2) B cells, naive splenic B cells were isolated from whole splenocytes by negative selection with anti-CD43 microbeads (Miltenyi Biotec) according to the manufacturer's instructions (36). Flow cytometry showed that CD43-negative cells lacked expression of TCR β-chain, CD11c, and MAC-1, and >95% expressed the B cell marker, B220.

HSP fusion proteins and reagents

MTB HSP70 with a C-terminal His tag in pET-23 (Novagen) was provided through the Tuberculosis Research Materials and Vaccine Testing Contract (Colorado State University, Fort Collins, CO) and prepared as previously described (17). To create His-tagged MTB HSP70-OVA fusion protein, the truncated OVA sequence (amino acid residues 230–359; cDNA from R. Young, Massachusetts Institute of Technology, Cambridge, MA) was cloned between HSP70 and a C-terminal His tag (26). *E. coli* BL-21 (Novagen) was induced with isopropyl-β-D-thiogalactoside for 4 h and lysed with BugBuster (Novagen). His-tagged HSP70 and His-tagged HSP70-OVA were purified under native conditions with nickel columns (Novagen) (17). To create control His-tagged rOVA not associated with HSP70, the identical $OVA_{230-259}$ sequence was cloned in pET21 encoding a C-terminal His tag (Novagen). The rOVA was prepared under native conditions (17) or from inclusion bodies under denaturing conditions. Inclusion bodies were pelleted from bacterial lysate by centrifugation and were incubated in 6 M guanidine; rOVA was prepared by affinity chromatography with nickel columns and dialysis against PBS to allow refolding. Recombinant HSP70 preparations retained residual LPS contamination (chromatography with immobilized polymixin or hydrophobic binding columns resulted in complete loss of HSP protein, and detergent treatment for removal of endotoxin abolished HSP activity). *Limulus* amebocyte LPS assays (QCL1000 kit; BioWhittaker) detected maximum experimental LPS concentrations of 0.3 μg/ml in HSP70, HSP70-OVA, and rOVA prepared under nondenaturing conditions. The LPS content of rOVA preparations prepared from inclusion bodies under denaturing conditions was <0.01 μg/ml. Control experiments showed that addition of LPS (from *E. coli* O127:B8; Difco) at concentrations in this range or higher (3.0 μg/ml) did not replicate the effects of HSPs, allowing us to discriminate HSP-specific effects. CpG oligodeoxynucleotide (ODN) 1826 (TCCATGACGTTCCTGACGTT; phosphorothioate modified to resist nuclease degradation) was provided by Coley Pharmaceutical Group (Wellesley, MA) and dissolved in 10 mM Tris and 1 mM EDTA (<1 ng LPS/μg DNA). The maximum concentration of ODN in cultures was 2 μg/ml, resulting in maximum possible LPS contamination of <1 pg/ml from this source in experiments. Influenza virus strain A/PR/8/34 allantoic fluid was obtained from Charles River Laboratories. Anti-CD91 mAb (5A6) was purchased from Biodesign International, and isotype control IgG2b was obtained from Zymed Laboratories.

Ag processing and presentation assays

Naive splenic B cells were plated in 96-well, U-bottom plates at 2×10^5 cells/well, incubated with Ag for 18–24 h, washed, and incubated for 24 h with CD8OVA1.3 T hybridoma cells (10^5 cells/well), which are specific for $OVA_{257-264}$:K^b complexes (37). For Ag-processing inhibitor experiments, B cells were incubated with brefeldin A or lactacystin for 10 min before Ag loading, incubated with Ag and inhibitors for 10 h, fixed with 0.5% paraformaldehyde, and incubated with T cell hybridoma cells. In some cases, B cells were incubated overnight with CpG ODN 1826 (2 μg/ml) before addition of inhibitors and Ag to insure a fully activated B cell phenotype. Control experiments with influenza virus used 4VA1 T cell hybridoma cells, specific for influenza matrix M1 peptide (GILGFVFTL) presented by HLA-A2 (38). Supernatants (100 μl) were frozen, thawed, and assessed for IL-2 using a colorimetric CTLL-2 bioassay (39). CTLL-2 cells (5×10^3/well) were incubated with supernatants for 24 h at 37°C, Alamar Blue (Trek Diagnostic Systems) was added (15 μl/well) for 24 h, and Alamar Blue reduction was determined by the difference in OD at 550 and 595 nm using a Bio-Rad model 550 microplate spectrophotometer.

Results

MTB HSP70 fusion protein promotes MHC-I cross-processing and -presentation of an associated antigenic epitope by B cells

We hypothesized that MTB HSP70-OVA fusion proteins enhance alternate MHC-I processing and presentation of OVA by B cells. To test this hypothesis, we prepared naive splenic B cells and studied their ability to process MTB HSP70-OVA fusion protein, composed of full-length MTB HSP70 with C-terminal fusion of the $OVA_{230-359}$ sequence. In comparison, we also studied processing of a recombinant OVA fragment (rOVA) containing the same $OVA_{230-359}$ sequence. By comparing the processing and presentation of HSP70-OVA and rOVA, we determined the ability of B cells to process exogenous HSP-associated Ags and identify the specific role of the HSP.

To assess processing of HSP70-OVA and rOVA for MHC-I cross-presentation, naive splenic B cells were incubated for 24 h with rOVA or HSP70-OVA. The cells were then washed and incubated with CD8OVA1.3 T hybridoma cells to detect $OVA_{257-264}$:K^b complexes. The results (Fig. 1*A*) demonstrated effective cross-processing of HSP70-OVA by B lymphocytes. Moreover, HSP70-OVA was processed for presentation of the $OVA_{257-264}$ epitope with significantly greater efficiency than rOVA (Fig. 1*A*), indicating that HSP70 enhanced cross-processing of the linked antigenic sequence.

To determine the kinetics of cross-processing, B cells were incubated with MTB HSP70-OVA or rOVA for 2, 6, or 24 h (Fig. 2). The cells were then fixed and incubated with CD8OVA1.3 T hybridoma cells to detect SIINFEKL:K^b complexes. Little or no SIINFEKL:K^b presentation was detected at 2 h, and HSP-enhanced presentation was observed between 6 and 24 h of processing. Maximum presentation of SIINFEKL:K^b complexes was only achieved after 24 h.

To distinguish generalized enhancement of Ag processing by HSP signaling vs specific enhancement of processing of HSP-linked Ag, B cells were also incubated with HSP70 and rOVA (not linked). Incubation with HSP70 slightly enhanced processing of rOVA, but did not achieve the much higher processing efficiency seen with HSP70-OVA (Fig. 1*A*). These data demonstrate that HSP70 fusion protein can deliver a fused antigenic sequence to substantially enhance its cross-processing and with only slight enhancement of general cross-processing function (i.e., for Ag not linked to the HSP).

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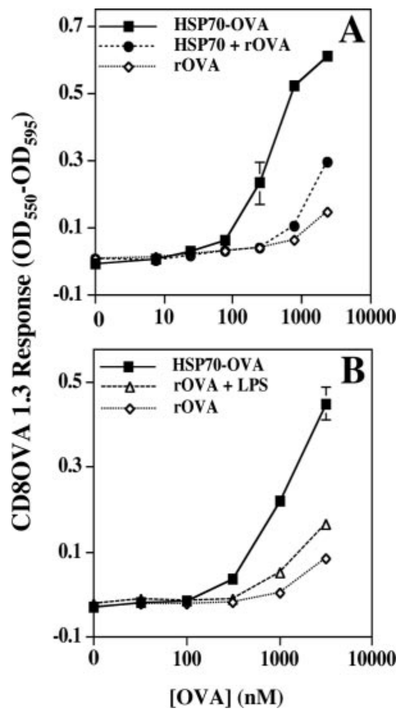

FIGURE 1. MTB-HSP fusion proteins enhance alternate MHC-I Ag processing in naive B cells. B cells were incubated for 24 h with HSP70-OVA, HSP-70, and rOVA or with rOVA alone. The cells were washed and incubated with MHC-I-restricted CD8OVA1.3 T hybridoma cells that recognize $OVA_{257-264}$:K^b complexes. Supernatants were assessed for IL-2 using a colorimetric CTLL-2 bioassay. *A*, HSP70-OVA is processed more efficiently than HSP70 (50 μg/ml) plus rOVA (HSP70 + rOVA) or rOVA alone. *B*, HSP70-OVA is processed with greater efficiency than rOVA with 0.3 μg/ml LPS (maximal LPS concentration in HSP70-OVA, 0.3 μg/ml) or rOVA alone. The results in each panel are representative of at least three independent experiments. When error bars are not visible, they are smaller than the symbol width. Data points represent the mean of triplicate samples with SD.

Because residual LPS contamination persisted in preparations of HSP70-OVA and HSP70, we performed control experiments to determine whether LPS contributed to modulation of B cell Ag processing and presentation. B cell processing of rOVA was examined with or without the addition of LPS at levels (0.3 μg/ml) exceeding the maximum amount of LPS from HSP preparations. Under these conditions, LPS produced a slight enhancement of rOVA processing, but did not produce the substantially more efficient processing observed with HSP70-OVA (Fig. 1*B*). Higher LPS concentrations (3.0 μg/ml) also produced only a similar slight enhancement of rOVA processing (data not shown). Thus, the substantial enhancement of MHC-I Ag processing and presentation by HSP70 fusion protein was not produced by LPS.

MTB HSP70-OVA enhances alternate MHC-I Ag processing through vacuolar mechanisms in B cells

HSPs may enhance alternate MHC-I Ag processing by cytosolic or vacuolar mechanisms. To determine the relative roles of vacuolar and cytosolic mechanisms, B cells were incubated with HSP70-OVA in the presence of brefeldin A or lactacystin, fixed, and incubated with CD8OVA1.3 T hybridoma cells. B cells were activated with CpG ODN 1826 overnight before the addition of inhibitors and Ag, providing increased MHC-I expression and greater signal for inhibitor experiments that used short Ag pulses before fixation of B cells. Neither brefeldin A nor lactacystin substantially inhibited HSP70-OVA processing and presentation by B cells (Fig. 3*A*). A simultaneous positive control demonstrated that cytosolic MHC-I Ag processing of influenza virus M1 Ag by infected B cells was completely inhibited by brefeldin A and lactacystin (Fig. 3*B*). These data suggest that cross-processing of

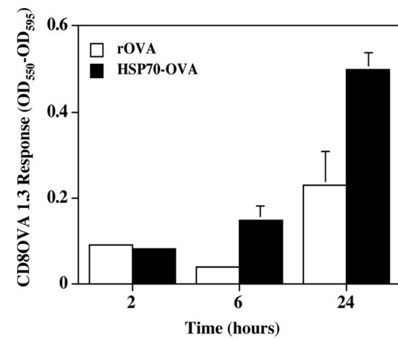

FIGURE 2. Kinetics of HSP70-OVA processing by B cells. B cells were incubated with 2400 nM HSP70-OVA or rOVA for 2, 6, or 24 h. Cells were then washed and fixed. Ag presentation was assessed with CD8OVA1.3 T hybridoma cells as described in Fig. 1. Results are representative of three independent experiments. Data points represent the mean of triplicate samples with SD.

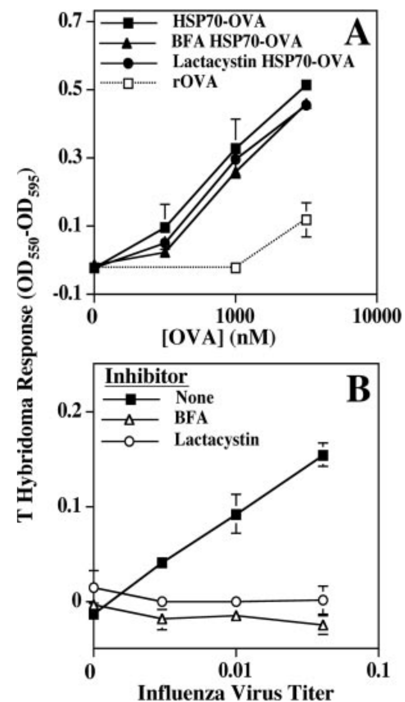

FIGURE 3. HSP70-OVA enhances alternate MHC-I Ag processing by vacuolar mechanisms. B cells were preactivated by overnight incubation with CpG ODN 1826, then exposed to brefeldin A (1 μg/ml) or lactacysin (20 μM) for 10 min before and during a 10-h incubation with Ag. The cells were fixed, and Ag presentation was assessed with T hybridoma cells as described in Fig. 1. *A*, B cells from B6D2F1/J mice were incubated with HSP70-OVA or rOVA with or without inhibitor. CD8OVA1.3 T hybridoma cells were used to detect SIINFEKL:K^b complexes. *B*, B cells from HLA-A2/K^b mice were incubated with influenza virus with or without inhibitor. Influenza virus titer refers to the dilution of the allantoic fluid containing live influenza virus. 4VA1 T hybridoma cells were used to detect HLA-A2 presentation of the influenza M1 peptide GILGFVFTL. *A* is representative of three independent experiments, and *B* is representative of two independent experiments. Data points represent the mean of triplicate samples with SD.

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HSP70-OVA occurs by vacuolar alternate MHC-I-processing mechanisms in B cells.

MTB HSP70-OVA promotes processing and MHC-I presentation of linked OVA sequence independent of MyD88, TLR2, TLR4, and CD40

HSPs have been proposed to impact T cell responses by two distinct mechanisms: enhancement of Ag delivery and HSP-mediated signaling. We explored the possible contribution of HSP signaling to enhancement of B cell cross-processing of HSP70-OVA. The potential roles of MyD88, TLR2, TLR4, and CD40 were tested, because these molecules have been reported to be involved in other HSP-mediated signaling effects (40–48) (although TLR signaling in response to HSPs is controversial (49)). We assessed cross-processing of HSP70-OVA by $CD40^{-/-}$, $TLR2^{-/-}$, $MyD88^{-/-}$, TLR4-deficient (C57BL/10ScN), and wild-type C57BL/6 or C57BL/10ScSn B cells. $CD40^{-/-}$, $TLR2^{-/-}$, and TLR4-deficient B cells were as efficient as wild-type B cells in processing of HSP70-OVA (Fig. 4, *A–C*). $MyD88^{-/-}$ B cells were slightly less efficient than wild-type cells, but they still showed substantial enhancement of HSP70-linked Ag (Fig. 4*D*). Thus, HSP70-OVA enhancement of OVA processing and MHC-I presentation did not involve TLR2, TLR4, or CD40 and was largely independent of MyD88.

Enhanced presentation of OVA epitope from HSP70-OVA is dependent on CD91

Mammalian HSPs gp96, HSP90, HSP70, and calreticulin all use a common receptor, CD91, also known as α_2-macroglobulin receptor (7, 9, 22), which is present on APCs. Our recent studies established that CD91 also facilitates uptake of bacterial HSPs (e.g., *E. coli* DnaK and MTB HSP70) and enhances presentation of peptides chaperoned by these HSPs (15). Previous studies in this area have been conducted with macrophages and dendritic cells, and receptors involved in HSP-mediated cross-processing by B cells have not been evaluated. We observed that addition of anti-CD91-blocking Ab inhibited processing and presentation of MTB HSP70-OVA by B cells (Fig. 5). Anti-CD91 did not affect the presentation of exogenous SIINFEKL peptide (data not shown). This finding reveals for the first time that CD91 can serve as a receptor for HSPs in B cells.

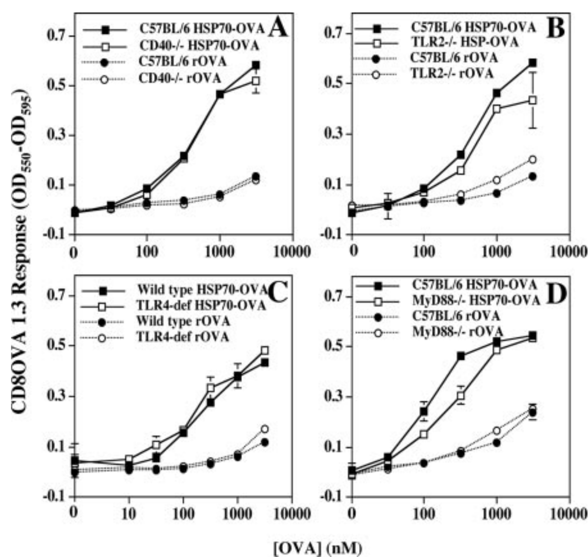

FIGURE 4. MTB HSP70-OVA enhances B cell alternate MHC-I Ag processing of OVA independent of CD40, TLR2, TLR4, and MyD88. B cells from $CD40^{-/-}$, $TLR2^{-/-}$, $MyD88^{-/-}$, TLR4-deficient (C57BL/10ScN), and wild-type (C57BL/6 or C57BL/10ScSN) mice were incubated for 24 h with HSP70-OVA, washed, and assessed for presentation of $OVA_{257–264}$:K^b complexes as described in Fig. 1. *A*, HSP70-OVA enhances processing independently of CD40. *B*, HSP70-OVA enhances processing independently of TLR2. *C*, HSP70-OVA enhances processing independently of TLR4. *D*, HSP70-OVA enhances processing independently of MyD88. The results in each panel are representative of at least three independent experiments. When error bars are not visible, they are smaller than the symbol width. Data points represent the mean of triplicate samples with SD.

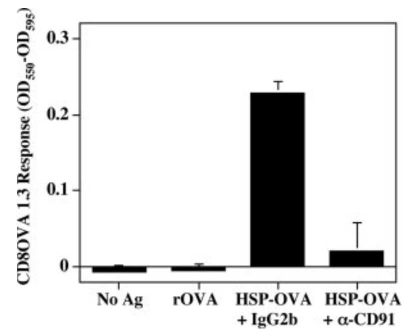

FIGURE 5. Enhanced MHC-I cross-processing of HSP70-OVA is dependent on CD91. B cells were incubated with or without 50 μg/ml blocking anti-CD91mAb (αCD91) or isotype control mouse IgG2b (IgG2b) for 30 min before and during a 16-h incubation with 300 nM HSP70-OVA or rOVA. Cells were washed, fixed, and assessed for Ag presentation as described in Fig. 2. The results are representative of three independent experiments. Data points represent the mean of triplicate samples with SD.

Discussion

HSPs enhance cross-processing and promote $CD8^+$ T cell responses to chaperoned peptides, and recombinant MTB HSP70 fusion proteins similarly have been shown to elicit $CD8^+$ T cell responses to antigenic epitopes included in the fusion protein (2–6, 10, 11, 14–16, 26, 27, 50, 51). Thus, HSPs and HSP fusion proteins can be used to elicit antitumor or antimicrobial $CD8^+$ T cell responses.

Almost all studies of immunogenicity of exogenous HSPs or HSP fusion proteins have assessed MHC-I Ag presentation in either macrophages or dendritic cells. The ability of B cells to process HSP-associated Ag has not been addressed. B cell APC function is well established for MHC-II presentation of Ags internalized via surface Ig for stimulation of $CD4^+$ T cells, but the overall role of B cells in MHC-I Ag presentation has been controversial. Naive or activated B cells can present cell-associated H-Y Ag to naive $CD8^+$ T cells (52). Heit et al. (32) recently demonstrated that covalent linkage of Ag to CpG DNA enabled B cell MHC-I cross-processing and -presentation to generate $CD8^+$ T cell responses, establishing the ability of B cells to cross-present Ags that are delivered in the appropriate molecular context or linkage. Similarly, we hypothesized that MTB HSP70-OVA fusion proteins enhance MHC-I cross-processing and -presentation of OVA by B cells.

In this study we established that exogenous bacterial HSP fusion protein (MTB HSP70-OVA) enhanced MHC-I cross-processing of the fused Ag sequence by B cells. HSP-enhanced cross-processing required linkage of Ag to the HSP, i.e., HSP70 did not substantially enhance processing of unlinked rOVA. Enhancement of cross-processing by HSP70 was generally independent of signaling receptors or signaling molecules that have been implicated in HSP-mediated danger signaling (TLR2, TLR4, CD40, and MyD88), but it was dependent on CD91, consistent with previous

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studies with HSP-chaperoned peptides (5, 9, 15, 22). Thus, the mechanism for HSP enhancement of cross-processing was not dependent on HSP signaling for general enhancement of MHC-I peptide presentation, and was specific to the HSP-linked Ag delivered for MHC-I cross-processing and -presentation. In other words, the mechanism that we have studied is related to HSP chaperone function (in this case the Ag is a covalently linked peptide sequence, not a noncovalently chaperoned peptide) and not to an HSP danger signal function (although such function could promote other B cell responses).

Our experiments were performed with HSP70-OVA fusion protein containing antigenic sequence covalently linked to the HSP, not a noncovalently associated HSP:peptide complex. It may be possible for HSPs to promote processing of Ag that is associated with HSP either by covalent linkage or noncovalent binding. Hypothetically, microbial polypeptide Ags that are noncovalently chaperoned by mammalian or microbial HSPs may also be cross-processed by B cells in pathophysiological situations in vivo. During bacterial infection, extracellular lysis of bacteria (e.g., by complement) could release bacterial HSPs containing chaperoned bacterial Ags to generate antibacterial $CD8^+$ T cell responses. Alternatively, lysis of virally infected host cells could release host HSPs containing chaperoned viral Ags to generate potentially protective antiviral $CD8^+$ T cell responses. These potential roles for HSPs in antimicrobial immunity remain to be tested.

The current studies focused on HSP70 fused to the rOVA antigenic sequence ($OVA_{230-359}$). With a length of 129 aa, this fused sequence is considerably larger than peptides used in studies of HSPs with antigenic peptides bound (chaperoned) by noncovalent interactions. Inclusion of long antigenic sequences in HSP fusion proteins could allow inclusion of multiple antigenic epitopes to generate protective responses to more than one agent or to achieve responses in patients with varying MHC haplotypes. Moreover, HSP fusion proteins are more stable than noncovalent HSP:peptide complexes and thereby may provide a more practical approach for vaccine development. HSP fusion proteins could be incorporated in vaccines to stimulate $CD8^+$ T cell responses that are crucial to immune responses against tumors, viruses, and certain intracellular bacteria. Our studies demonstrate for the first time that B cells can contribute to cross-processing of HSP fusion proteins for the generation of $CD8^+$ T cell responses.

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Disclosures

The authors have no financial conflict of interest.

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