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Bacterial Heat Shock Proteins Enhance Class II MHC Antigen Processing and Presentation of Chaperoned Peptides to CD4⁺ T Cells

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

APCs process heat shock protein (HSP):peptide complexes to present HSP-chaperoned peptides on class I MHC molecules, but the ability of HSPs to contribute chaperoned peptides for class II MHC (MHC-II) Ag processing and presentation is unclear. Our studies revealed that exogenous bacterial HSPs (Escherichia coli DnaK and Mycobacterium tuberculosis HSP70) delivered an extended OVA peptide for processing and MHC-II presentation, as detected by T hybridoma cells. Bacterial HSPs enhanced MHC-II presentation only if peptide was complexed to the HSP, suggesting that the key HSP function was enhanced delivery or processing of chaperoned peptide Ag rather than generalized enhancement of APC function. HSP-enhanced processing was intact in MyD88 knockout cells, which lack most TLR signaling, further suggesting the effect was not due to TLR-induced induction of accessory molecules. Bacterial HSPs enhanced uptake of peptide, which may contribute to increased MHC-II presentation. In addition, HSPs enhanced binding of peptide to MHC-II molecules at pH 5.0 (the pH of vacuolar compartments), but not at pH 7.4, indicating another mechanism for enhancement of MHC-II Ag processing. Bacterial HSPs are a potential source of microbial peptide Ags during phagocytic processing of bacteria during infection and could potentially be incorporated in vaccines to enhance presentation of peptides to CD4⁺ T cells. The Journal of Immunology, 2004, 173: 5130–5137.
processing and presentation of chaperoned peptide. We also demonstrate that HSPs increase uptake of peptide and enhance binding of peptide to MHC-II molecules at acidic pH (as found in endosomes and phagosomes). We propose that bacterial HSPs may deliver HSP-bound bacterial peptides during phagocytic processing of bacteria, thereby promoting MHC-II presentation of bacterial Ags and CD4+ T cell responses during infection with bacterial pathogens. Furthermore, this mechanism could provide a basis for use of bacterial HSPs in vaccines to enhance priming of CD4+ T cell responses.

Materials and Methods

Cells and medium

Unless otherwise specified, incubations were at 37°C and 5% CO2 in standard medium containing DMEM (Invitrogen Life Technologies, Carlsbad, CA), 10% heat-inactivated FCS (HyClone, Logan, UT), 30 μM 2-ME (BioRad, Hercules, CA), 1 mM sodium pyruvate (Invitrogen Life Technologies), 10 mM HEPES buffer (Invitrogen Life Technologies), and antibiotics. B6D2F1/J and C57BL/6 female mice were from The Jackson Laboratory (Bar Harbor, ME). H2-DM+/– mice (24) were generously provided by L. Van Kaer (Vanderbilt University, Nashville, TN). MyD88–/– mice were generously provided by O. Takeuchi and S. Akira (Osaka University, Osaka, Japan) and bred onto C57BL/6 background for five to seven generations. B6D2F1/J mice were used for all experiments, except those involving knockout models, which used MyD88+/– or HLA-DMA mice with C57BL/6 mice for wild-type controls. Macrophages were derived from femur marrow cells cultured in bacterial grade dishes for 7–10 days in 25% LADMAC cell-conditioned medium (containing M-CSF (26)). To produce dendritic cells (27, 28), femur marrow cells were resuspended for 10 min in 0.83% NH4Cl to lyse erythrocytes; incubated for 1 h at 4°C with combined supernatants of B hybridomas GK1.5 (anti-CD4), 10 mM HEPES buffer (Invitrogen Life Technologies), and anionic dextran (Cytobeads, Biotechnik, Heidelberg, Germany), and isotype control IgG1 was from PROGEN Biotechnik (Heidelberg, Germany), and isotype control IgG1 was from Zymed Laboratories (San Francisco, CA). Cells were cultured in six-well plates (3 × 105 cells/well), and nonadherent cells were removed every 2 days by gentle swirling and replacement of half of the volume with fresh medium containing GM-CSF. Dendritic cells were harvested by pipetting on day 5, incubated with anti-murine CD11c microbeads (Miltenyi Biotec, Auburn, CA; 100 μl beads/4 × 105 cells) for 20 min at 4°C, resuspended in PBS with 0.5% BSA and 2 mM EDTA (4 × 105 cells/500 μl), isolated with a MACS MS column (Miltenyi Biotec), washed, and resuspended in standard medium.

HSPs, Abs, and reagents

E. coli Dnak (StressGen Biotechnologies, Victoria, Canada) was >90% pure by SDS-PAGE analysis, and MTB HSP70 (Lionex, Braunshweig, Germany) was >95% pure by SDS-PAGE analysis. We also prepared MTB HSP70 from E. coli BL-21 transformed with MTB HSP70 in pET-23 (Novagen, Madison, WI), obtained through the Tuberculosis Research Materials and Vaccine Testing Contract (Colorado State University, Fort Collins, CO), which drives expression of His-tagged MTB HSP70 (His tag on C terminus of MTB HSP70). E. coli were induced with isopropyl β-D-thiogalactoside for 4 h and lysed with BugBuster (Novagen). His-tagged MTB HSP70 was purified under native conditions with nickel columns (Qiagen, Valencia, CA). Similar results were obtained with MTB HSP70 (Lionex) and His-tagged MTB HSP70. Although LPS contamination was detected in HSP preparations with the E-TOXATE Limulus amebocyte lysate assay (Sigma-Aldrich, St. Louis, MO) with maximum experimental LPS concentrations of 0.22–1.1 μg/ml for E. coli Dnak and <0.14 μg/ml for MTB HSP70, control experiments without HSP, but with addition of LPS (from E. coli O127:B8; Difco, Detroit, MI) up to 1.5 μg/ml showed that LPS did not alter results or replicate HSP effects.

FITC-labeled extended OVA peptide (319–340) was added at 2.2 μg/ml to serum-free RPMI 1640 (BioWhittaker, Walkersville, MD), supplemented with 5% FCS, 50 mM HEPES buffer, MTB HSP70 and DnaK (StressGen Biotechnologies) or MTB HSP70 at 2 μg/ml in 40 mM Tris-HCl, pH 7.5, at 37°C with rotation for 1 h. Unbound peptide was removed using a MicroYm-10 or YM-30 centrifugal filter device (Millipore, Bedford, MA) three times for 20 min at 14,000 rpm with washes in 40 mM Tris-HCl. A negative control sample of 0.4 ml 40 mM Tris-HCl, pH 7.5, and 0.04 ml of uncomplexed peptide (1 μg/ml) using a microfilter device was processed to insure that unbound peptide was removed. The HSP-FTTC-peptide solution was analyzed with a Spectra Fluor Plus plate fluorometer (Tecan, Research Triangle Park, NC) to determine HSP-bound peptide concentration. DnaK and MTB HSP70 bound similar amounts of peptide with ~0.025 mol of peptide bound per mol of HSP (2.5% loading).

In comparison, studies with eukaryotic HSPs reported 1–5% loading with peptide sequences from model Ag (17, 29) and 20% loading with Ag peptide containing sequences known to promote HSP binding (6). To make latex bead-HSP:peptide, protein G-Fluoresbrite YG carboxylate microspheres (one micron diameter; Polysciences, Warrington, PA) were incubated overnight at 4°C with anti-MTB HSP71 Ab (Stressgen Biotechnologies), which recognizes MTB HSP70. The microspheres were then washed, incubated with MBT HSP70:peptide for 2 h at 4°C, and washed with PBS.

Flow cytometry

Macrophages were incubated in 24-well plates (6.7 × 104 cells/well) for 48 h at 37°C with 2 ng/ml IFN-γ. HSP-FTTC-labeled peptide complexes were added for 25 min. Cells were then washed in PBS, detached by scraping, fixed in 1% paraformaldehyde, and analyzed by flow cytometry.

Results

E. coli Dnak and MTB HSP70 promote processing and MHC-II presentation of chaperoned extended OVA peptide containing the OVA (323–339) epitope

Exogenous mammalian and bacterial HSPs have been shown to deliver antigenic peptides to APCs and promote MHC-I processing and presentation of HSP-chaperoned peptides, generating CD8+ T cell responses. We propose that exogenous HSPs may also deliver peptides for MHC-II processing and presentation to generate CD4+ T cell responses. Because bacterial HSPs are associated with peptides derived from bacterial proteins, including bacterial Ags, bacterial HSPs released during phagolysosomal processing of phagocytosed bacteria could deliver bacterial Ags for MHC-II processing and presentation, contributing to antibacterial CD4+ T cell responses. MHC-II Ag processing of HSP-chaperoned peptides has not been studied with either eukaryotic or bacterial HSPs. Our studies were designed to test the hypothesis that bacterial HSPs deliver exogenous peptides for MHC-II processing and presentation of constituent epitopes.

We tested whether an E. coli HSP, Dnak, could deliver a 22-mer extended OVA peptide (AESL KISQAVHAHAHAINEAGR (FITC conjugation at N terminus, 77% purity by HPLC)) from Biosynthesis (Lewisville, TX) was used for all experiments, except for those with HSP-peptide complexes bound to latex beads, which used FITC-labeled 17-mer extended OVA peptide GISASEL KISQAVHAHAHAINEARGVREVLKLLK (Biosynthesis; >86% purity by HPLC). FITC-labeled extended OVA peptide (0.04 ml at 1 mM in H2O) was incubated with 0.4 ml of E. coli Dnak (Stressgen Biotechnologies) or

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adapted this strategy for studies of MHC-II presentation of HSP-chaperoned peptide. APCs were incubated for 45 min with uncomplexed extended OVA peptide or E. coli DnaK:extended OVA peptide complexes. The cells were then fixed and incubated with DOBW T hybridoma cells to detect OVA (323–339):MHC-II complexes. Association of extended OVA peptide with E. coli DnaK significantly enhanced presentation of the OVA (323–339) epitope from the extended peptide by both macrophages (Fig. 1A) and dendritic cells (Fig. 1B). We conclude that E. coli DnaK can efficiently deliver chaperoned peptide for MHC-II presentation of constituent epitopes.

Additional studies were performed with MTB HSP70 to determine whether the above findings were idiosyncratic to E. coli DnaK or reflected properties shared broadly among bacterial HSPs. APCs were incubated with MTB HSP70:extended OVA peptide complexes for 60 min (Fig. 1C) or 45 min (Fig. 1D), fixed, and incubated with DOBW T hybridoma cells to detect OVA (323–339):MHC-II complexes. MTB HSP70 significantly enhanced MHC-II peptide presentation in both macrophages (Fig. 1C) and dendritic cells (Fig. 1D). The greatest relative enhancement of peptide presentation by MTB HSP with our readout assay was observed from 0.5–3 h of processing (Fig. 1E); plateau signal (maximum response of our T cell assay) was achieved within this time frame at typical concentrations of MTB HSP70:peptide, whereas presentation of uncomplexed extended OVA remained low from 0.5–3 h, but increased at longer times (beyond 3 h it is likely that MTB HSP70:peptide continued to produce peptide: MHC-II complexes that were not evident due to signal plateau).

Thus, bacterial HSPs from both E. coli and MTB can deliver extended peptide for enhanced MHC-II Ag presentation of a constituent epitope.

**Enhancement of MHC-II peptide presentation by bacterial HSP**

Although HSP contributions to MHC-II Ag processing and presentation have not been examined, some immune functions have been attributed to bacterial HSPs. MTB HSP70 and chlamydial HSP60 stimulate cytokine secretion through CD40 and TLR4, respectively (33, 34). These observations suggested that modulation of MHC-II Ag processing by bacterial HSPs could be explained by HSP signaling to produce generalized enhancement of macrophage MHC-II Ag processing and presentation, regardless of whether the presented peptides were directly chaperoned by the HSP.

To distinguish generalized effects of HSP signaling from enhanced processing specific to HSP-chaperoned peptide, we explored the requirement for binding of extended peptide to HSP. Macrophages were incubated for 45 min with HSP-complexed extended OVA peptide or equivalent concentrations of uncomplexed extended OVA peptide plus MTB HSP70. APCs were fixed and assessed for presentation of OVA (323–339):MHC-II complexes. The presence of MTB HSP70 did not enhance processing and presentation of uncomplexed extended OVA peptide (Fig. 2A). OVA (323–339) presentation was enhanced only if exogenous extended peptide was complexed to the HSP.

**FIGURE 1. E. coli DnaK and MTB HSP70 enhance MHC-II Ag processing in macrophages and dendritic cells.** Cells were incubated for 45 min (A, B, and D) or 60 min (C) with FITC-labeled extended OVA peptide complexed to E. coli DnaK or MTB HSP70. The cells were then fixed and incubated with OVA-specific MHC-II-restricted DOBW T hybridoma cells. Supernatants were assessed for IL-2 using a colorimetric CTLL-2 bioassay. A and B. E. coli DnaK enhances processing and presentation of extended OVA peptide by bone marrow-derived macrophages (A) and dendritic cells (B). C and D, MTB HSP70 enhances processing and presentation of extended OVA peptide by macrophages (C) and dendritic cells (D). E. MTB HSP70 produces rapid enhancement of extended OVA peptide processing by macrophages. Macrophages were incubated with 2.19 μM extended OVA peptide complexed to MTB HSP70 for the indicated periods. In this and other figures, the x-axes in panels showing dose-response experiments represent the concentration of extended OVA peptide, whether bound to HSP or uncomplexed. The results in each panel are representative of at least three independent experiments. Data points represent means of triplicate samples with SD. When error bars are not visible, they are smaller than the symbol width. Values of p resulting from a two-tailed t test comparing results with and without HSP are shown (+, p < 0.05; *, p < 0.01).
To determine whether enhanced MHC-II processing and presentation of chaperoned peptide were related to specific HSP properties, we compared MHC-II presentation of OVA (323–339) after exposure of macrophages to extended OVA peptide bound to HSP or BSA (BSA bound peptide, as reported previously (29)). APCs were incubated with MTB HSP70:extended OVA peptide complexes or BSA:extended OVA peptide complexes for 45 min, fixed, and incubated with DOBW T hybridoma cells to detect OVA (323–339):MHC-II complexes. MTB HSP70 significantly enhanced Ag presentation, but BSA did not enhance MHC-II peptide presentation in macrophages (Fig. 2B). Thus, HSP enhancement of Ag presentation was related to the ability of HSPs to promote processing or presentation of chaperoned peptide.

We performed experiments to determine whether TLR- and MyD88-dependent signaling by HSPs or potential microbial contaminants of bacterial HSP preparations (e.g., LPS) altered APC function to cause HSP enhancement of peptide presentation. LPS was excluded as a significant factor in our experiments, because LPS (up to 1.5 μg/ml, higher than maximal levels of LPS from HSP preparations) did not affect the processing of extended OVA peptide (data not shown). In addition, enhancement of MHC-II peptide presentation by MTB HSP70 was identical with wild-type and MyD88−/− macrophages (Fig. 2C). Finally, enhancement of MHC-II peptide presentation by MTB HSP70 was not affected by anti-CD40-blocking Ab (Fig. 2D). Although HSPs have been reported to signal through TLRs or CD40 to stimulate proinflammatory cytokines (33–37), our data indicate that enhancement of peptide presentation was not the result of signaling by HSPs or bacterial contaminants to alter overall processing functions of APCs. Furthermore, HSP-enhanced processing was specific to the HSP-chaperoned extended peptide that was delivered for processing and MHC-II presentation.

Active processing by APCs is necessary for enhanced presentation of HSP-chaperoned extended peptide

We tested whether bacterial HSP-chaperoned peptides required active processing, e.g., through endocytosis by viable APCs, or could be delivered directly to cell surface MHC-II molecules. To distinguish active processing from cell surface events, uptake and processing functions were inhibited by fixation or metabolic inhibition of APCs. Fixation of macrophages before addition of extended OVA peptide prevented enhancement of MHC-II peptide presentation by MTB HSP70 (Fig. 3A). Experiments with dendritic cells produced similar results; prefixation of dendritic cells prevented enhancement of MHC-II peptide presentation by MTB HSP70 (data not shown). Alternatively, macrophages were exposed to sodium azide and 2-deoxy-D-glucose for 90 min to deplete ATP and subsequently incubated with MTB HSP70:extended OVA peptide.
complexes for 45 min. This metabolic inhibition blocked enhancement of extended OVA peptide processing and presentation by MTB HSP70 (Fig. 3B). Macrophages were also exposed to chloroquine for 30 min to neutralize the pH of vacuolar compartments and subsequently incubated with MTB HSP70:extended OVA peptide complexes for 45 min. Chloroquine also blocked enhancement of extended OVA peptide processing and presentation by MTB HSP70 (Fig. 3C). Presentation of uncomplexed peptide was reduced by fixation or metabolic inhibition (Fig. 3, A–C), possibly reflecting loss of active peptide uptake, but sufficient signal remained to determine that HSP enhancement did not occur under these conditions. Thus, active APC function or processing was required for HSP enhancement of peptide presentation.

To confirm that bacterial HSPs enhance Ag processing through an active process involving cellular uptake, we attached HSP:peptide complexes to latex beads and used cytochalasin D to inhibit actin-dependent phagocytosis of these beads (Fig. 3D). Cytochalasin D inhibits actin-dependent phagocytosis of particles such as latex beads, but does not inhibit endocytosis of soluble proteins, which is not dependent on actin microfilament function. Thus, to make uptake of HSP70:peptide sensitive to cytochalasin D, we conjugated it to 1-μm diameter latex beads. Macrophages were incubated with or without cytochalasin D for 15 min before and during incubation with bead-conjugated MTB HSP70:extended OVA peptide. Cytochalasin D significantly inhibited processing of MTB HSP70:extended OVA peptide beads (Fig. 3D). Control samples showed that cytochalasin D completely blocked phagocytic alternate MHC-I processing of whole OVA protein conjugated to latex beads (data not shown), as previously observed (38, 39). Presentation of uncomplexed extended OVA peptide was unaltered by cytochalasin D, indicating that cytochalasin D did not cause general loss of macrophage peptide presentation function (Fig. 3E). This experiment confirms and extends conclusions from experiments shown in Fig. 3, A–C, using an independent approach. Because bacterial HSP enhancement of extended OVA peptide processing and presentation is abolished in macrophages that are unable to internalize or actively process HSP:peptide complexes, we conclude that MTB HSP70 peptide complexes must enter the acidic environment of intracellular vacuolar compartments to access MHC-II Ag processing mechanisms to which HSP-chaperoned Ag can contribute.

To examine the molecular requirements for efficient processing of bacterial HSP:peptide complexes, HLA-DM−/− macrophages were used. HLA-DM is expressed inside endosomal compartments and is required for removal of class II-associated invariant chain peptide and subsequent loading of MHC-II molecules with peptides. Enhancement of MHC-II peptide presentation by MTB HSP70 was abolished in macrophages that lacked HLA-DM (Fig. 3F).

**Bacterial HSPs enhance uptake of Ag**

Enhancement of MHC-II Ag processing by bacterial HSPs could be mediated by enhanced uptake of peptide Ag as well as enhanced intracellular processing mechanisms. To assess whether *E. coli* DnaK or MTB HSP70 enhance uptake of extended OVA peptide,
Bacterial HSPs facilitate delivery and binding of chaperoned peptide to MHC-II under acidic conditions that mimic the vacuolar environment.

In addition to enhancement of peptide uptake, bacterial HSPs may have active roles inside APCs to deliver peptides to MHC-II for binding and presentation. Although bacterial HSPs failed to deliver peptides by dendritic cells, similar to the results seen with macrophages (data not shown). In summary, MTB HSP70 and E. coli DnaK enhanced uptake of chaperoned extended OVA peptide through a mechanism that was reduced by metabolic inhibitors. Thus, bacterial HSPs mediate enhanced delivery of peptide Ags to APCs, and this mechanism may contribute to enhancement of MHC-II Ag processing and presentation.

**Figure 4.** MTB HSP70 enhances uptake of chaperoned peptides. Macrophages were incubated with or without 30 mM sodium azide and 5 mM deoxy-D-glucose for 90 min before and during the peptide incubation to inhibit endocytosis. MTB HSP70:peptide complexes produced a strong signal for peptide uptake with mean fluorescence value (MFV) of 293, and metabolic inhibitors reduced uptake to ~62% of this level (MFV = 182) (Fig. 4). In contrast, uptake of extended OVA peptide without HSP was much lower (MFV = 109) and was not substantially affected by addition of metabolic inhibitors (MFV = 99). Similarly, E. coli DnaK:extended OVA peptide promoted peptide uptake that was reduced by metabolic inhibitors to baseline uptake level (data not shown). In addition, MTB HSP70 enhanced uptake of chaperoned peptides by dendritic cells, similar to the results seen with macrophages (data not shown).

**Figure 5.** MTB HSP70 enhances MHC-II peptide presentation in acidic environments. Macrophages were fixed, incubated with MTB HSP70:extended OVA complexes in citrate buffered saline at pH 7.4 (A) or 5.0 (B) for 30 min, fixed again, and assessed for presentation of MHC-II:OVA complexes, as in Fig. 1. Data points represent means of triplicate samples with SD. 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. Values of p resulting from a two-tailed t test comparing results with and without HSP are shown (*, p < 0.01).

OVA peptide:MHC-II complexes beyond the background level observed in the absence of MTB HSP70 (Fig. 5A). At pH 5.0, however, MTB HSP70 enhanced formation of OVA peptide:MHC-II complexes (Fig. 5B). These results indicate that bacterial HSPs can assist in MHC-II peptide loading in acidic environments. The binding of uncomplexed peptide was higher at pH 5.0 than pH 7.4 (Fig. 5, A vs B), consistent with previous observations that MHC-II molecules optimally bind or exchange peptides under acidic conditions (40, 41), but HSP-complexed peptide produced even greater binding of peptide to MHC-II. Thus, acidic vacuolar pH may promote release of HSP-chaperoned peptides and their binding to MHC-II molecules.

**Discussion**

HSPs enhance alternate MHC-I Ag processing of chaperoned self, tumor, or viral peptides (reflecting the range of proteins synthesized by mammalian cells) to promote MHC-I cross presentation and CD8+ T cell responses (2, 4–6, 11, 13, 15, 16). It has also been demonstrated that overexpression of endogenous mammalian HSPs can enhance MHC-II presentation to CD4+ T cells (19–22), although specific Ag-processing functions to which HSPs may contribute have not been addressed. Furthermore, MHC-II processing of exogenous HSP:complexes, either mammalian or bacterial, has not been studied. In particular, there have been no studies to test whether bacterial HSPs, which are naturally associated with bacterial peptides, deliver chaperoned peptides for MHC-II Ag processing and presentation.

The microbial peptides that are chaperoned by bacterial HSPs are a potentially important source of Ag to promote antimicrobial immunity. We propose that microbicidal mechanisms cause release of bacterial HSPs from phagocytosed bacteria, potentially...
delivering antigenic HSP-chaperoned bacterial peptides into the phagolysosomal environment for processing and binding to MHC-II. In addition, bacterial HSPs released into the extracellular space (secondary to lysis of bacteria by complement or other mechanisms) may be internalized by APCs, allowing MHC-II processing of HSP-chaperoned bacterial Ags. In this study, we demonstrate that exogenous bacterial HSPs (E. coli DnaK and MTB HSP70) enhance uptake and MHC-II Ag processing of a chaperoned model peptide.

Some HSPs may stimulate signaling (e.g., chlamydial HSP60 signaling via TLR 4 (34) and MTB HSP70 signaling via CD40 (33, 37)) to increase expression of accessory factors (e.g., cytokines or costimulators), which may be important in vivo for generating responses by primary T cells. Our data establish a different mechanism whereby bacterial HSPs contribute directly to generation of peptide:MHC-II complexes from HSP-chaperoned peptide Ag (as detected by T hybridoma cells, which are less dependent on co-stimulatory molecules). This mechanism requires that peptides be complexed to the HSP and is independent of MyD88 and CD40. Thus, this enhancement by HSPs does not involve a generalized increase in MHC-II Ag presentation, but specifically involves the delivery of HSP-chaperoned peptides for processing and presentation by MHC-II.

Two potential mechanisms that could explain enhancement of MHC-II Ag processing by bacterial HSPs are increased uptake and enhanced intracellular processing. Both of these mechanisms are supported by our observations. Uptake of peptide complexed to either E. coli DnaK or MTB HSP70 was significantly higher than uptake of uncomplexed peptide as measured by flow cytometry. In some systems, CD91 has been reported to be involved in uptake of exogenous HSP:peptide complexes for alternate MHC-I processing and presentation (10, 18, 42), but MHC-II processing of MTB HSP70:peptide complexes was not altered by the addition of anti-CD91-blocking Ab (data not shown). The reason for this difference is unknown, but intracellular trafficking of CD91 and differences in compartmentalization of MHC-II and alternate MHC-I processing may contribute. These observations coupled with the finding that bacterial HSP enhanced uptake of peptide in the present studies suggest that receptors other than CD91 may also be involved in uptake of HSPs.

In addition to assisting in uptake, bacterial HSPs may enhance intracellular processing of chaperoned Ag. Although bacterial HSPs did not enhance binding of peptide to MHC-II at neutral pH, HSP-enhanced binding was evident at pH 5.0. Thus, HSPs may release chaperoned peptides at the acidic pH of vacuolar compartments, making these peptides available for binding to MHC-II. We speculate that this could involve direct interactions between HSPs and MHC-II, leading to release of chaperoned peptide, but this hypothesis requires future experimental testing. It is interesting to note that MHC-II molecules optimally bind or exchange peptides under acidic conditions (40, 41). Thus, acidic vacuolar pH may promote both release of HSP-chaperoned peptides and their binding to MHC-II molecules.

We propose that bacterial HSPs deliver antigenic HSP-chaperoned peptide Ags that contribute to the generation of CD4+ T cell responses during infection of mammalian hosts with bacterial pathogens. This mechanism could be particularly important during infection with bacterial pathogens for which CD4+ T cell responses contribute to host immunity. APCs may encounter bacterial HSPs following phagocytosis and phagolysosomal degradation of bacteria, which may release bacterial HSPs directly into the phagosomal Ag-processing environment (where MHC-II molecules are present). Alternatively, bacterial HSPs that have been released in the extracellular space by microbicidal mechanisms (e.g., complement) may be internalized for subsequent intracellular processing and MHC-II presentation. In addition to physiological roles in processing of bacterial Ags, bacterial HSPs have the potential to contribute to vaccine efficacy. HSPs, including bacterial HSPs, could be incorporated in vaccines to stimulate CD4+ T cell responses that are crucial to immune responses against bacteria.

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