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The HSC73 Molecular Chaperone: Involvement in MHC Class II Antigen Presentation

Naveed Panjwani, Omid Akbari, Sylvie Garcia, Melanie Brazil, and Brigitta Stockinger

Heat shock proteins (HSP) are conserved proteins, many of which share the ability for indiscriminate peptide binding and ATPase-coupled peptide release. In this paper, we show that heat shock cognate protein (HSC73), a constitutively expressed member of the HSP70 family, could be a candidate for chaperone activity within the MHC class II presentation pathway. HSC73 expression in macrophages was shown to overlap with expression of MHC class II; overexpression of HSC73 in stable transfectants of a macrophage line markedly enhanced their presentation of exogenous Ag without affecting presentation of processing independent peptide. Ag from an exogenous source was demonstrated to associate with HSC73 in macrophages, and this association was sensitive to ATP treatment and inhibited by deoxyspergualin, an immunosuppressive agent that has previously been shown to bind specifically to HSC73. Furthermore, deoxyspergualin reduced Ag presentation by macrophages in relation to the amount of HSC73 expressed in these cells. The data are consistent with a potential role for HSC73 in binding and protecting peptides from extensive degradation and/or facilitating the kinetics of peptide transfer to MHC class II molecules. The Journal of Immunology, 1999, 163: 1936–1942.

D4 T cell responses are initiated by APC displaying peptides bound to the groove of MHC class II (MHC-II) molecules. These are synthesized in the endoplasmic reticulum and form nonpeptide receptive complexes with invariant chain (Ii) (1). The MHC-II/Ii complex is routed to meet peptides generated by denaturation and proteolysis of internalized Ag in progressively acidified endocytic compartments. Progressive degradation of Ii by aspartic and cysteine proteases occurs to a final product, the class II-associated Ii peptide (CLIP), blocking the peptide binding groove of the MHC-II molecule (2, 3). The CLIP is removed with the help of H2-M molecules, and, following this, association with antigenic peptide occurs (4). The compartment in which these complexes are formed has been defined in various systems to have late endosomal to early lysosomal characteristics, termed MIIC (reviewed in Refs. 5 and 6). The peptide/MHC-II complex is then expressed on the cell surface for recognition by CD4 T cells.

Studies of the processing and loading of antigenic peptides onto MHC-II molecules to date leave unexplained how peptides evade complete proteolysis and survive to sufficient length (typically 9–16 aa) to associate with MHC-II molecules (7). Furthermore, although H2-M can accelerate the association of peptides with MHC-II molecules (8), it possesses no peptide binding properties and cannot therefore function directly as a peptide loading factor. Both of these functions would benefit from chaperone molecules, which are exemplified by heat shock proteins (HSPs). These are a conserved group of protein families, of which the HSP60 and -70 families share the properties of promiscuous binding of unfolded peptides and ATP hydrolysis-coupled peptide release (9–12). HSPs could conceivably behave as Ag scavengers in degradative compartments, protecting peptides from catabolic death and transferring them rapidly to vacant MHC-II molecules, using the energy of ATP hydrolysis. Experiments in which APCs have been subjected to heat shock showed evidence for modulation of MHC-II Ag presentation capacity (13, 14) and an increase in the generation of antigenic peptides in recovered endosomal fractions (14), as well as increased recovery of SDS-stable Ab MHC-II dimers (15). However, the presumed influence of up-regulation of HSPs in these experiments was difficult to distinguish from other possible effects of heat shock, such as up-regulation of lysosomal enzymes. Abs against hsp70 family members have also been shown to affect Ag processing (16, 17).

In the present study, we examined the role of a single defined HSP, the constitutively expressed 73-kDa heat shock cognate protein (HSC73), as a candidate for interaction with antigenic peptides and/or MHC-II loading in APCs. HSC73 is expressed in the cytosol (18), but is also present in lysosomes, as demonstrated by confocal (19) and electron microscopy (20), and by subcellular fractionation techniques (21). The subcellular localization of HSC73, therefore, could place it in proximity to MHC-II molecules in MIICs. Also known as HSC70, the physiological activities of this protein thus far characterized include: uncoating of clathrin-coated pits (22) and transport of cytoplasmic proteins to lysosomes for degradation (23, 24). Also, interaction of HSC73 with HLA-DR molecules has been demonstrated (25).

In this paper, we investigate the involvement of HSC73 in Ag presentation within the MHC-II presentation pathway. Overexpression of HSC73 in macrophages results in enhancement of Ag processing. We show that endocytosed Ag interacts with HSC73 and that this interaction is ATP-sensitive and inhibited by the immunosuppressive drug 15-desoxyspergualin (DSG), which was previously shown to specifically interact with HSC73.

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2 Address correspondence and reprint requests to Dr Brigitta Stockinger, Division of Molecular Immunology, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K. E-mail address: b-stocki@nimr.mrc.ac.uk
3 Abbreviations used in this paper: MHC-II, MHC class II; HSP, heat shock protein; HSC, heat shock cognate protein; DSG, desoxyspergualin; HEL, hen egg lysozyme.
Materials and Methods

Construction and transfection of HSC73 cDNA expression construct

The HSC73 cDNA was obtained from Dr. J. Fred Dice (Tufts University, Medford, MA) and subcloned into the β-actin expression vector (26) at the SalI site downstream of the IVS-1 intron to generate the β37/2.7 construct. 97.2 cells were transfected with the β37/2.7 construct using the Fugene-6 reagent (Boehringer Mannheim, Indianapolis, IN), as detailed in the manufacturer’s protocol. Transfectants were selected for G418 resistance and individual clones picked and amplified under selection.

Cell lines and culture

The cell line 97.2 (27) is a bone marrow-derived macrophage cell line of the haplotype H-2k, generated by R. Palacios in the Basel Institute for Immunology (Basel, Switzerland). Expression of MHC-II molecules was induced by culture with 400 U/ml IFN-γ for 48 h. The hen egg lysozyme (HEL)-specific T cell hybridoma 2G7.1 (28) recognizes epitope 1–18 of HEL presented in the context of H-2Kb, whereas 3A9 recognizes HEL epitope 46–61 in the context of H-2A	het (29). The A18 hybridoma recognizes epitope 106–121 of the fifth component of mouse complement (C5) (30). Cells were maintained in IMDM medium (Sigma, St. Louis, MO) containing 5% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 × 10−6 M ME (all Sigma). The IL-2-dependent cell line CTLL was maintained in medium as above supplemented with 5 μg/ml IL-2. Dendritic cells were generated ex vivo from bone marrow as previously described (31).

Western blotting

Untransfected 97.2 cells and HSC73 transfectants were lysed at 5 × 10⁶ cells/ml using 1% Brij-96 containing buffer (10 mM Tris-HCl, 1 mM EDTA, 0.15 M NaCl) supplemented with 200 μM PMSF, 5 mM iodoaceticamide, 5 μM leupeptin, and 1 μg/ml pepstatin A (all from Sigma). A total of 25 μl of each lysate was separated on an 8% SDS-PAGE gel, followed by transfer onto nitrocellulose membrane and immunoblotting with the anti-HSC73 Ab 13D3 (Maine Biotechnology, Portland, ME) and HRP-conjugated anti-mouse Ig (Southern Biotechnology Associates, Birmingham, AL), using enhanced chemiluminescence (Amersham Life Science, Buckinghamshire, U.K.) for detection, as detailed in the manufacturer’s protocol.

Ag presentation assays

Class II-restricted presentation to T cell hybrids was tested by coculture of 5 × 10⁴/well 97.2 cells (all macrophages were pretreated with 400 U/ml IFN-γ for 48 h) with 5 × 10⁶/well T hybridoma cells in flat-bottom 96-well plates (Costar, Cambridge, MA). Ag was given in serial dilution as synthetic peptide corresponding to HEL[1–18], HEL[46–61], or C5[106–121], or HEL protein (Sigma) or C5 protein. Alternatively, Ag was kept at a constant concentration and methyl-DSG was given in serial dilution from a maximal concentration of 20 μg/ml (DSG was obtained from Nippon Kayaku Co., Ltdo, Japan). All experiments involving DSG were performed in medium as described previously, with the substitution of 1 × Nutridoma (Boehringer Mannheim) for 5% FCS. Presentation was allowed to occur for 24 h in culture, following which, 50 μl of supernatant was harvested and transferred to a fresh plate for measurement of IL-2 by incubation with 5 × 10⁶/well CTLL cells. CTLL proliferation was measured by [3H]thymidine incorporation over 18 h. All values given are means of triplicate cultures, and all experiments were performed at least twice.

Immunofluorescence microscopy

Untransfected 97.2 and HSC73 transfectants, induced for MHC-II expression by IFN-γ (400 U/ml for 48 h) were grown on Lab-Tek chamber slides (Nalge Nunc, Naperville, IL) to semiconfluence and fixed with acetone at 4°C. Cells were permeabilized with 0.5% Triton X-100 (Sigma) and non-specific binding blocked with 0.5% aqueous gelatin (Sigma). Cells were then stained with the H2-E-specific mAb 14.4.4S (American Type Culture Collection, Manassas, VA; no. HB-32) conjugated with FITC and the HSC73-specific mAb 13D3 (Maine Biotechnology). 13D3 staining was visualized with anti-IgM Bio (Jackson Immunolab, West Grove, PA) and streptavidin-Texas Red (PharMingen, San Diego, CA). Cells were mounted in Citifluor mountant. Images of cells were acquired at the Confocal and Image Analysis Lab (CIAL) at the National Institute for Medical Research (London, U.K.) using an Olympus IX70 fluorescence microscope and a Photometrix cooled-CCD camera. Image deconvolution was performed using DeltaVision software to correct for out-of-focus light. Analytical flow cytometry of transfectants was performed using a FACScan (Becton Dickinson, Mountain View, CA), and the date were processed using Cellquest software (Becton Dickinson). Macrophages were stained with FITC-labeled anti-H2-Eβ Ab 14-4-4S, either untreated or after 48 h incubation with 400 U/ml IFN-γ to induce MHC-II synthesis.

Immunoprecipitation of HSC73 on latex beads

A total of 10 μg of HEL was added to 97.2 cells in culture, and uptake of the Ag was allowed to occur for 12 h. For DSG inhibition experiments, 5 μg/ml DSG was given in culture for 6 h before addition of Ag, and left in culture during Ag uptake. Cells were then washed extensively and subsequently lysed at 5 × 10⁶ cells/ml using 1% Brij-96 containing buffer supplemented with protease inhibitors, as detailed above. Lysates were incubated with mAb-coupled latex beads at 4°C for 8 h rotating. The anti-HSC73 mAb 13D3 and the isotype-matched control mAb 6-68 (specific for Th1.2) were coupled to LB8 0.8-μm diameter latex beads (Sigma), as described previously (32). Supernatant was removed and latex beads washed extensively with lysis buffer and resuspended in IMDM as a 1:1000 stock suspension. Beads were used in Ag presentation assays as above, using 2 × 10⁷/well bone marrow-derived dendritic cells as APC. Beads were given in serial dilution from a maximal starting concentration of 5% of stock suspension.

ATP treatment of HSC73 immunoprecipitates

Anti-HSC73 and control mAb immunoprecipitates, made as described above, were pelleted to remove IMDM and resuspended to 0.1% suspension in 100 mM Tris-HCl, 50 mM MgSO₄ buffer, with or without 50 mM ATP added. Treatment was performed for 2 h at room temperature, after which beads were extensively washed with IMDM and resuspended in IMDM at 1:1000 stock suspension. Beads were then used in bone marrow-derived dendritic cell Ag presentation assays, as described above.

Results

Overexpression of HSC73 in macrophages and immunofluorescence analysis of intracellular distribution

To study the involvement of HSC73 in the MHC-II Ag processing pathway, this molecule was transfected into the macrophage cell (Becton Dickinson, Mountain View, CA), and the date were processed using Cellquest software (Becton Dickinson). Macrophages were stained with FITC-labeled anti-H2-Eβ Ab 14-4-4S, either untreated or after 48 h incubation with 400 U/ml IFN-γ to induce MHC-II synthesis.

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line 97.2. 97.2, like fresh bone marrow macrophages, show relatively suboptimal Ag processing capacity for soluble exogenous Ag (33), so that changes in Ag presentation capacity are easily detected. HSC73 was placed under the control of the constitutive human b-actin promoter (26), and a panel of independent clones stably transfected with this construct was generated. 97.2 cells stably transfected with the empty b-actin vector only were used as a control. HSC73 transfectants were shown to overexpress the protein compared with vector-only transfected cells and untransfected cells (Fig. 1A).

Immunofluorescence and subcellular fractionation studies have previously shown colocalization of HSC73 with lysosomal markers (19, 21). For HSC73 to be a physiological participant in MHC-II peptide loading, however, it is required to be present particularly in MHC-II compartments. To determine whether this was the case, HSC73 transfectants and untransfected APC were stained with anti-HSC73 and anti-H2-Ek Abs and analyzed by confocal microscopy. Fig. 1B shows that HSC73 is localized in spherical organelles in both HSC73 transfectants and untransfected APC; the majority of these spherical organelles also stain positive for MHC-II. Thus, a subset of HSC73 in transfected and control 97.2 macrophages colocalizes with MHC-II compartments.

**Overexpression of HSC73 enhances Ag processing**

HSC73 transfectants and vector-only control transfectants were assayed for their ability to process and present Ag to three different

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**Table 1. Expression of H2-Ek with or without IFN-γ treatment**

<table>
<thead>
<tr>
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<th>MFI without IFN-γ</th>
<th>MFI + IFN-γ</th>
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<tbody>
<tr>
<td>Vector control</td>
<td>6.21</td>
<td>89.38</td>
</tr>
<tr>
<td>H73.5</td>
<td>7.96</td>
<td>92.29</td>
</tr>
<tr>
<td>H73.14</td>
<td>7.46</td>
<td>75.84</td>
</tr>
<tr>
<td>H73.18</td>
<td>9.64</td>
<td>92.08</td>
</tr>
<tr>
<td>H73.23</td>
<td>10.58</td>
<td>80.61</td>
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</table>

*Vector control transfectants and four HSC73 transfectants were analyzed for expression of H2-Ek either without IFN-γ treatment or after a 48-h treatment with 400 μg/ml IFN-γ. Mean fluorescence intensity (MFI) is shown for both conditions.*

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**FIGURE 2.** Functional assays of Ag presentation by a panel of HSC73 overexpressing transfectants to T cell hybridsomas 2G7.1 (A and B) and A18 (C and D) and 3A9 (E and F). IL-2 production by T cells is shown, as measured by incorporation of [3H]thymidine by the IL-2-dependent cell line CTLL. Results from four independent HSC73 transfectants (○, H73.5; □, H73.14; ◊, H73.18; △, H73.23) are shown together with vector-only transfected controls (■). A and E, HEL protein as Ag, given in 2-fold titration from a maximum concentration of 100 μg/ml HEL[1–18] (B) and HEL[46–61] (F) synthetic peptide, given in 5-fold titration from a maximum concentration of 5 μM. C, C5 protein as Ag, given in 2-fold titration from a maximum concentration of 10 μg/ml. D, C5[106–121] synthetic peptide, given in 5-fold titration from a maximum concentration of 5 μM.
MHC-II-restricted T cell hybridomas. Ag was given either as processing-dependent native protein, or as processing-independent peptide. MHC-II expression on all transfectants was similar to control transfectants, indicating that HSC73 overexpression does not influence the levels of class II (Table I). HSC73 transfectants showed greatly improved capacity to present native HEL protein to the HEL-specific H-2E\(^k\)-restricted T cell hybrid 2G7.1, compared with control transfectants (Fig. 2A). Presentation of HEL peptide was not significantly different between HSC73 transfectants and vector-transfected controls (Fig. 2B). Similarly, presentation of native C5 protein to the H-2E\(^k\)-restricted T cell hybridoma A18 was strongly enhanced in HSC73-transfected 97.2, compared with control transfectants, whereas presentation of peptide was equivalent in all APC (Fig. 2, C and D). The increase in presentation was not only evident for Ag presented with H-2E\(^k\), but also for HEL presented in the context of H-2A\(^k\) (Fig. 2, E and F; HEL-specific T cell hybrid 3A9). Since peptide given exogenously associates with cell surface MHC-II molecules, rather than intracellular class II (34), the enhancement of Ag presentation by HSC73-transfected macrophages most likely reflects a change in intracellular processing events.

Internalized Ag associates with HSC73 and is released in an ATP-dependent manner

If HSC73 fulfills a role in the MHC-II processing pathway, one would expect it to interact with Ag internalized by the APC. We used an immunoprecipitation strategy to examine whether antigenic activity could be coimmunoprecipitated with HSC73 from APCs fed with Ag. HSC73-transfected and untransfected 97.2 cells were fed HEL in culture and then lysed, followed by immunoprecipitation of HSC73 with anti-HSC73 mAb coupled to latex beads. As a control, parallel immunoprecipitations were conducted using an irrelevant isotype-matched mAb coupled to latex beads. Immunoprecipitates were then added to cultures of bone marrow-derived dendritic cells and the HEL-specific T cell hybridoma 2G7.1. HSC73 immunoprecipitates from both transfected and untransfected 97.2 cells contained antigenic activity, as seen in activation of the HEL-specific T cell hybrid by dendritic cells exposed to latex beads carrying HSC73 immunoprecipitate; control immunoprecipitates contained insignificant amounts of antigenic material (Fig. 3). To exclude the possibility that association of HSC73 and HEL occurred post lysis, a control experiment was performed with a mixture of lysate from HEL-fed untransfected macrophages and lysate from unfed HSC73 transfectant. This would provide the overexpressed HSC73 present in the lysate of transfecteds with a source of HEL in vitro. As shown in Fig. 3D, whereas HEL co-immunoprecipitated with HSC73 from Ag-fed transfecteds resulted in presentation by dendritic and T cell activation, no such interaction was detected in the lysate mixtures; in this experiment, no antigenic material was coimmunoprecipitated from lysate of Ag-fed untransfected macrophages. This emphasizes that HSC73 interacts with exogenously sourced Ag in vivo in a form that can be processed and presented by dendritic cells. Furthermore, HSC73-overexpressing transfecteds consistently yielded high amounts of antigenic material, whereas untransfected macrophages did not always do so, indicating that greater abundance of HSC73 in the transfecteds allows increased binding of internalized Ag.

Peptide release from chaperones of the HSP70 family is coupled to ATP hydrolysis (9, 10). To examine whether the Ag/HSC73 interaction demonstrated above was subject to dissociation upon ATP hydrolysis, HSC73-Ag complexes immunoprecipitated from Ag-fed APC lysates from HSC73-transfected and untransfected 97.2 cells were treated with ATP, or with buffer alone as a control. Treated immunoprecipitates were subsequently fed to bone marrow-derived dendritic cells to assay for presentation to the HEL-specific T cell hybridoma 2G7.1. HSC73 immunoprecipitates from HSC73-transfected, as well as untransfected 97.2, cells were shown to lose antigenic activity if treated with ATP before feeding to dendritic cells (Fig. 4), whereas control-treated control complexes retained their antigenic activity. Thus, the antigenicity associated with HSC73 was shown to be lost in an ATP-dependent manner.

Interaction of HSC73 with exogenous Ag is DSG-sensitive

DSG is an immunosuppressive agent that was shown to specifically bind to HSC73 (35, 36). DSG has a peptidomimetic structure and is thought to bind and preclude peptide binding to HSC73. It has previously been shown to affect MHC-II Ag processing without affecting MHC-II expression (37). We therefore used DSG as a reagent to probe the involvement of HSC73 in MHC-II processing.
To investigate directly whether binding of Ag to HSC73 could be prevented by DSG, HSC73-transfected and untransfected 97.2 cells were incubated with DSG before and during exposure to exogenous HEL protein. Cells were subsequently lysed and HSC73 immunoprecipitated, as described above. Immunoprecipitates were fed to dendritic cells for presentation to HEL-specific hybridoma 2G7.1. It was found that incubation with DSG during Ag uptake abolished antigenic activity from HSC73 immunoprecipitates of both HSC73-transfected and untransfected 97.2 cells, whereas immunoprecipitates from cells incubated with Ag in the absence of DSG were antigenic (Fig. 5A). To test whether the absence of a response was indeed due to a direct effect of DSG on binding of HEL peptides by HSC73, rather than an inhibitory effect on processing by DC, an experiment was set up mixing DSG containing HSC73.14 precipitates with precipitate from HSC73.14 fed with HEL in the absence of DSG. Stimulation by the mixture of precipitates did not markedly reduce T cell activation (Fig. 5C), confirming that the action of DSG is more likely on association of HEL peptides with HSC73, rather than on processing by the dendritic cells. To ensure that the action of DSG was HSC73-specific, 97.2 transfectants overexpressing HSP60 were also given Ag in presence or absence of DSG, followed by lysis and immunoprecipitation of HSP60. HSP60-overexpressing transfectants exhibit enhancement of Ag processing and presentation similar to HSC73 transfectants (our unpublished data). HSP60 immunoprecipitates, however, were antigenic in DC presentation assays, regardless of the presence of DSG before lysis and immunoprecipitation (Fig. 5C). This implies that DSG acted specifically on HSC73 and was capable of disrupting Ag/HSC73 interaction.

DSG inhibits presentation of native protein by HSC73 transfectants and abolishes presentation by nontransfected macrophages

Since DSG blocked the interaction of HSC73 with internalized antigenic material, we next investigated whether the presence of DSG in the Ag presentation assays would reduce or abolish presentation of exogenous HEL to T cells. Increasing amounts of DSG were given together with Ag, either native HEL protein or HEL[1–18] peptide in cocultures of HSC73 transfectants or untransfected controls and T cells. DSG inhibited presentation of HEL protein in HSC73 transfectants and virtually abolished presentation in control 97.2 cells (Fig. 6). Presentation of peptide remained unaffected up to a concentration of DSG of 10 μg/ml; higher amounts of DSG appear to be toxic. Notably, a 6-fold higher concentration of DSG was required to inhibit processing of native HEL.

![Figure 4](http://www.jimmunol.org/)  
**FIGURE 4.** Effect of ATP treatment on Ag/HSP complexes. 97.2 macrophages fed with 10 mg/ml HEL were lysed and HSC73 immunoprecipitated with 13D3 (anti-HSC73)-coupled latex beads. Ag/HSP complexes precipitated from HEL-fed cells were treated with ATP (□) or buffer alone (■) and added to dendritic cells (2 × 10⁴/well) and 2G7.1 cells (5 × 10⁴/well) to assay for coimmunoprecipitated antigenic activity. Figure shows IL-2 production by T cells as measured by incorporation of [³H]thymidine by the IL-2-dependent cell line CTLL. Treatment with ATP resulted in loss of antigenicity of immunoprecipitates from both HSC73.5 transfectant (A) and untransfected 97.2 cells (B).

![Figure 5](http://www.jimmunol.org/)  
**FIGURE 5.** Effect of DSG on antigenicity of HSP immunoprecipitates. Ag/HSP complexes were precipitated from 97.2 cells fed with 10 mg/ml HEL incubated with DSG during Ag uptake (■) or not treated with DSG (□) and added to dendritic cells (2 × 10⁴/well) and 2G7.1 cells (5 × 10⁴/well) to assay for coimmunoprecipitated antigenic activity. IL-2 production by T cells is shown, as measured by incorporation of [³H]thymidine by the IL-2-dependent cell line CTLL. DSG incubation resulted in loss of antigenicity of anti-HSC73 immunoprecipitates from untransfected 97.2 cells (Ai), HSC73.5 (Aii), and HSC73.14 transfectants (Aiii). B, LK1 (anti-HSP60) immunoprecipitates from HSP60 transfectants were also analyzed as a control for the specificity of the action of DSG on HSC73 only. DSG did not cause loss of antigenicity of anti-HSP60 immunoprecipitates. C, 2.5% bead suspension of precipitate from HSC73.5 transfectants fed with HEL (column A) or HSC73.5 fed with HEL in the presence of DSG (column B) or a 1:1 mixture of both (column C) were added to dendritic cells, as described above, and incubated with 2G7 hybridoma cells.
and presentation of HEL protein by HSC73-overexpressing APC than was required for untransfected controls. This indicates that availability of HSC73 is a critical factor in MHC-II Ag processing.

Discussion

The aim of this study was to investigate whether HSC73, a constitutively expressed member of the HSP70 family, could be a candidate for chaperone activity within the MHC-II presentation pathway. HSC73 has been shown to present in lysosomal compartments by a variety of techniques (19–21). In addition, both the HSC73-binding drug DSG as well as Abs to HSP70 family members have been shown previously to inhibit MHC-II Ag processing and presentation (16, 37). Mechanistically, there are a number of ways in which a chaperone such as HSC73 could facilitate Ag processing. It could bind and protect denatured Ag and protect epitopes from being lost by proteolytic degradation. The evidence of Pépin et al. (14), who have shown that up-regulation of HSP expression can increase the yield of antigenic activity in lysosomal fractions subsequently recovered, supports this idea. HSC73 itself has been shown to be unusually resistant to lysosomal degradation (38).

An alternative and contiguous mechanism to peptide stabilization could involve the rapid transfer of bound, unfolded antigenic peptides by HSC73 to class II molecules whose peptide binding groove has been rendered vacant by the catalytic action of H2-M. Such a rapid transfer would be assisted by the ability of HSC73 to eject bound peptides using the energy of ATP hydrolysis.

We have shown that overexpression of HSC73 in an APC line enhances processing and presentation of protein Ag. Presentation of peptide was unaffected by HSP73 overexpression, and the levels of surface MHC-II were unchanged. We have further shown that HSC73 immunoprecipitated from Ag-fed cells could act as a source of Ag in Ag presentations assays, indicating that HSC73 is able to interact with and bind internalized Ag in APCs. The strategy used to test the coprecipitation of antigenic material did not impose any constraints on the form of Ag bound to HSC73; since the complex was subjected to processing by dendritic cells, the Ag could have been just partially unfolded, rather than fully processed. ATP treatments of these immunoprecipitates caused loss of antigenic activity, suggesting that disruption of the HSC73-Ag interaction is ATP-coupled in the same way as interactions of HSP70 family chaperones with unfolded or misfolded proteins in the endoplasmic reticulum and mitochondria have been shown to be (39). We have extended the findings (19, 21) that HSC73 is present in lysosomal compartments to show that the chaperone also colocalizes with MHC-II molecules, and is therefore correctly positioned to participate in Ag processing within the MHC-II pathway. A three-way complex between MHC-II, HSC73, and peptide, such as that suggested by Auger et al. (25), could serve to create a localized concentration of HSC73-bound antigenic peptide, which in turn would accelerate the formation of peptide/MHC-II complexes. In this scenario, MHC-II molecules could act as partner proteins to HSC73 and serve to dictate a specific function for the ubiquitous chaperone, as other partner proteins have been shown to do for HSP70s in other cellular contexts (40).

Studies of Ag processing compartments have left unclear whether antigenic epitopes are invariably generated in compartments where association with class II can occur. Harding et al. (41) have shown that certain epitopes from the same Ag require processing in different parts of the endocytic chain. This suggests that transport of antigenic peptides from degradative compartments to compartments where association with MHC-II molecules can occur is necessary for these epitopes to be displayed. HSC73 has been shown to be able to carry proteins to endolysosomal compartments from other subcellular locations (23, 24), and could conceivably fulfill this function. Also, the nonclassical entry of cytosolic proteins (42) into class II Ag processing compartments could be achieved this way. It is interesting to note that the chaperone activities of HSP70 family members are already established in the loading of MHC class I molecules and in the transport and protection of their corresponding peptides (43–45).

Our studies also probed the function of HSC73 in MHC-II Ag processing using the HSC73-specific reagent methyl 15-DSG. We showed that DSG blocked the interaction of HSC73 with internalized Ag and that it had an inhibitory effect on Ag presentation that could be diminished by overexpressing HSC73. Taken together, these results confirm and extend the observations of Hoeger et al. (37), who showed that DSG was capable of arresting presentation of processing dependent Ags, but not peptide, by human monocytes to specific T cell clones. Our results suggest that interaction of HSC73 with Ag is a crucial component of the MHC-II Ag processing pathway. The disruption of Ag/HSC73 interaction by DSG also offers an explanation of the results of Wang et al. (46), who showed that positive selection of thymocytes is affected by in vivo administration of DSG, since a lack of MHC-II molecules loaded with the appropriate ligands on thymic APCs could suspend the presentation of positively selecting stimuli to thymocytes, resulting in a phenotype analogous to the absence of crucial proteolytic enzymes (47).
Further studies are required to clarify a number of issues raised by the experiments detailed in this paper. The possibility exists that certain epitopes would not survive complete proteolytic degradation in the absence of stabilization by HSC73. In addition, the postulated function of HSC73 would render the ATPase activity of this chaperone essential, and this could be addressed by studying the functional effects of ATPase disabled HSC73. The ability of HSC73 to accelerate the formation of SDS-stable (peptide-loaded) MHC-II dimers could be investigated biochemically using in vitro systems, as well as immunological reagents, recognizing peptide-loaded MHC-II molecules.

References