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Essential Role of Endogenous Heat Shock Protein 90 of Dendritic Cells in Antigen Cross-Presentation

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Extracellular HSP90 associated with Ag peptides have been demonstrated to efficiently cross-prime T cells, following internalization by dendritic cells (DCs). In addition, the nature of cell-associated Ags required for cross-priming is implicated as peptides and proteins chaperoned by heat shock protein (HSP). However, the role of endogenous HSP in DCs during cross-presentation remains elusive. In this paper, we show that endogenous HSP90 is essential for cross-presentation of both soluble and cell-associated Ags in DCs. Cross-presentation of soluble OVA and OVA-loaded transporter associated with Ag processing-1-deficient cells by bone marrow-derived DCs and DC-like cell line DC2.4 was profoundly blocked by HSP90 inhibitors, whereas presentation of endogenously expressed OVA was only partially suppressed. Assays using small interfering RNA and heat shock factor-1–deficient DCs (with defective expression of HSP90α) revealed the pivotal role of HSP90α in cross-presentation. The results suggest that in addition to HSP90 in Ag donor cells, endogenous HSP90 in DCs plays an essential role during Ag cross-presentation and, moreover, points to a link between heat shock factor-1–dependent induction of HSP90α within DC and cytotoxic T cell immunity. The Journal of Immunology, 2010, 185: 2693–2700.

The heat shock proteins (HSPs) are known as molecular chaperones and many members of this family are induced by heat shock, which is called “heat shock response” (1). HSP is also known as a stress protein whose expression is increased by conditions that cause protein denaturation or unfolding in the cells (2). Thus, HSP is engaged in: 1) promoting protein folding/renaturing and preventing aggregation of misfolded proteins; 2) targeting misfolded proteins for degradation by the proteasome; and 3) facilitating protein transport.

In addition to the fundamental roles in the life cycle of cells, HSPs were implicated as a player in adaptive immunity, especially cross-priming (3). HSP90–peptide complexes reconstituted in vitro were shown to be efficient in promoting cross-presentation (4). Moreover, HSP90 in Ag donor cells is essential for cross-priming of certain Ags, because cells treated with HSP90-specific inhibitor lose their immunogenicity (3, 5). The rationale behind this observation is based on the concept that antigenic peptides associated with HSP90 in Ag donor cells are released by the HSP90 inhibitor, becoming free forms that might become inefficient in terms of cross-priming. This idea is also supported by the genetic evidence that heat shock factor-1 (HSF-1)–deficient Ag donor cells (H-2d) where HSP90 is mainly downregulated is less capable of cross-priming (6). Zheng and Li (6) have proposed that chaperoning either peptides or proteins by HSP90 in Ag donor cells is necessary for efficient cross-priming. These observations suggest that HSP90 associates with and protects Ag peptides (and/or proteins) from being degraded to keep their immunogenicity, which renders effective cross-presentation (priming) even by extremely tiny amount of Ags.

Our question in this report is, apart from the role of HSP in Ag donor cells, does HSP inside Ag acceptor cells, such as dendritic cells (DCs), play a role in Ag cross-presentation? This question is not yet clarified, although a role of HSP90 in endogenous MHC class I (MHC I) Ag processing pathway was demonstrated (3, 7, 8). We found that pharmacological inhibition of DC HSP90 resulted in profound suppression of cross-presentation, although it showed only partial inhibition in presentation of endogenously expressed Ag, thus, direct presentation. Experiments using small interfering RNA (siRNA) demonstrated that inducible form of HSP90, HSP90α, plays a dominant role in cross-presentation and so does HSP90β but to a lesser extent. We also report that HSF-1–deficient bone marrow-derived DCs (BMDCs) (H-2b), whose expression of HSP90α is mainly defective, are less efficient in their cross-presentation ability compared with normal BMDCs, whereas they are nearly comparable in direct Ag presentation.

Our results indicate that endogenous HSP90 in DCs plays a pivotal role in cross-presentation pathway and that the mechanism is at least different from its role in endogenous MHC I processing pathway.

Materials and Methods

Mice

C57BL/6 mice, OT-1 (H-2Kb–restricted, anti-OVA TCR transgenic) mice (9) were provided by Dr. W.R. Heath (Walter and Eliza Hall Institute, Melbourne, Victoria, Australia). TAP1–/– mice (10) were purchased from The Jackson Laboratory (Bar Harbor, ME), MyD88–/– mice (11) were...
provided by Dr. S. Akira (Osaka University, Osaka, Japan), and HSF-1−/− mice (12) were maintained in pathogen-free conditions in the Research Center for Allergy and Immunology, RIKEN, Yokohama Institute Animal Facility.

**Cells**

BMDCs were generated from C57BL/6 mice. The bone marrow was flushed out from the femurs and tibiae and cultured in complete RPMI 1640 with 10% FCS and 20 ng/ml GM-CSF (R&D Systems, Minneapolis, MN). Immature BMDCs were used in experiments on days 5 or 6. OT-I CD8+ T cells were isolated from splenocytes by magnetic separation with IMag system (BD Biosciences, Franklin Lakes, NJ). CD11b+ cells were deleted by negative selection prior to the positive selection of CD8+ cells. DC cell line DC2.4 was a gift from Dr. K. L. Rock (University of Massachusetts Medical School, Boston, MA) and maintained in RPMI 1640 (Sigma-Aldrich, St. Louis, MO) supplemented with 2 mM l-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acid, penicillin-streptomycin, 2-ME, and 10% FCS at 37°C in 5% CO2.

**Abs and reagents**

Rat anti-mouse IFN-γ, biotinylated rat anti-mouse IFN-γ Ab, and streptavidin-alkaline phosphatase (AP) were from BD Pharmingen (Franklin Lakes, NJ). Phosphatase substrate (p-nitrophenyl phosphate; Kirkegaard & Perry Laboratories, Gaithersburg, MD) was used in AP detection. rIFN-γ was purchased from R&D Systems. Rabbit anti-HSP90α isoform-specific Ab and rat anti-HSC70 (1B5) were obtained from StressGen Biotechnologies (Victoria, British Columbia, Canada), and rabbit anti-HSP90β-specific Ab was from Lab Vision (Fremont, CA), and rabbit anti-actin polyclonal Ab was from Sigma-Aldrich. Mouse anti-HSP90 mAbs (2A6-E9 and 4B6-G12) and mouse anti-OVA serum were raised in our laboratory. OVA (Sigma-Aldrich) was used as a model Ag. Anti-CD8α magnetic particles and anti-CD11b magnetic particles were from BD Biosciences. Radicicol was purchased from Sigma-Aldrich, 17-allylamino-17-demethoxygeldanamycin (17AAG) and epoxomicin were from InvivoGen (San Diego, CA) or BIO-MOL (Plymouth Meeting, PA), ubiquitin E1 inhibitor UBE1-A1 was from Biogenova (Rockville, MD), and proteasome inhibitor MG115 was from Sigma-Aldrich. 25-D1.16 mAb specific for OVA257–264-Kb (13) was provided by Dr. R. N. Germain (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) and biotinylated in our laboratory. Abs against CD11c and H-2Kb were purchased from BD Pharmingen.

**FIGURE 1.** Cross-presentation of OVA was inhibited by HSP90 inhibitors. A, BMDCs were pulsed with the indicated doses of soluble OVA and cocultured with OT-I CD8+ T cells. The culture supernatant was collected at 38 h, and the amount of IFN-γ released in the medium was measured by ELISA. B, BMDCs from WT or TAP1−/− mice were pretreated with 25 μM radicicol or 10 μM MG115 or DMSO for 15 min and pulsed with 2 mg/ml soluble OVA or 10−7 M OVA peptide (OVA257–264) in the presence of the inhibitor or DMSO. The amount of IFN-γ released by OT-I CD8+ T cells was measured as in A. C and D, BMDCs were treated with graded concentrations of radicicol (C) or 17AAG (D) and pulsed with 0.5 mg/ml soluble OVA. E and F, Cross-presentation of OVA in MyD88−/− BMDCs was also inhibited by HSP90 inhibitor. E, BMDCs from MyD88−/− or MyD88−/− mice were pulsed with the indicated amount of OVA. DCs were then cultured with OT-I CD8+ T cells, and the amount of IFN-γ released in the culture was measured. F, BMDCs from MyD88+ or MyD88−/− mice were treated with the indicated doses of radicicol and pulsed with 2 mg/ml OVA and cultured with OT-I CD8+ T cells. The culture supernatants of 38 h were subjected to ELISA for IFN-γ. The results are confirmed by at least three independent experiments.
sucrose in 10% w/v polyethylene glycol 1000 in RPMI 1640) containing OVA protein (0.5–1 mg/ml). Then, 45 ml warm hypotonic buffer (RPMI 1640/dH2O: 60%) was immediately added, followed by 2-min incubation. After washing twice, the cells were irradiated with 302-nm UV rays for 5 min using a transilluminator (α Innoteck, San Leandro, CA) and further incubated for 3 h to induce apoptosis and used as “Ag donor cell” for cross-presentation assay. BMDCs or DC2.4 cells (1 × 10^6 cells), pretreated with epoxomycin, radicicol, or DMSO (control) for 1 h, were washed three times and incubated with Ag donor cells (5 × 10^6 cells) for 3 h, fixed, and used as described previously.

**ELISA**

To determine the level of IFN-γ in the culture supernatant, the capture Ab was coated onto ELISA plates (Nunc, Naperville, IL), and the sample and serial diluted standards were incubated after blocking with 1% BSA and 0.05% Tween 20 in PBS. Captured IFN-γ was detected by biotinylated anti–IFN-γ Ab and AP-labeled streptavidin. p-Nitrophenyl phosphate tablet was dissolved in p-nitrophenyl phosphate buffer (0.05 M Na_2CO_3 and 0.5 mM MgCl_2) and used as AP substrate. The developed color was read at 405 nm by microtiter plate reader. Recombinant mouse IFN-γ (R&D number 458-ML) was used as standard.

**Endogenous OVA presentation assay**

To analyze endogenous Ag presentation, a plasmid encoding OVA protein (pcDNA3-3myc-TEV-flag-OVA) was transfected into DC2.4 cells by Nucleofector device (Amaxa Biosystems, Gaithersburg, MD). Transfected cells were incubated with or without HSP90 inhibitors for 3 h and then fixed with 0.5% paraformaldehyde, and 10^4 cells/well were cultured with 10^5 cells/well OT-I CD8^+ T cells. The culture supernatants were collected at 18–40 h of culture, and the amount of IFN-γ released was measured by ELISA.

**siRNA**

For knockdown of HSP90, piGENE PUR hU6 vector (Toyobo, Osaka, Japan) was used. The target sequence specific for HSP90α was GACC-CAAGACCAACCAATG and for HSP90β was GGACAAGATTGCAATGTG. The plasmid DNA (2 μg) was transfected into DC2.4 cells (4 × 10^5) twice by Nucleofector device (Amaxa Biosystems) on days 0 and 2. The cells were collected on day 3 and subjected to cross-presentation assay and Western blotting analysis. Lentivirus vector carrying short hairpin RNA (shRNA) sequence to HSP90α separated by IRES sequence from GFP gene (pGIPZ lentiviral shRNAmir clone V2LMM-79462), and its control empty vector was purchased from OPEN Biosystems (Huntsville, AL).

**Uptake of FITC-OVA**

FITC-labeled OVA was used to detect the uptake of OVA by APCs. To confirm the uptake in cross-presentation, BMDCs were treated with 25 μM radicicol as in the cross-presentation assay and cultured with 0.5 mg/ml FITC-OVA for 60 min. FITC-OVA was washed out three times to exclude free FITC-OVA and then analyzed by FACS.

**Western blotting**

For HSP90 knockdown, DC2.4 cells were lysed with 4× sample buffer (4% SDS, 2-ME, 20% glycerol, and 125 mM Tris-HCl) and loaded onto 10% SDS-PAGE. Proteins were transferred to polyvinylidene difluoride...
membrane and blocked by 3% skim milk dissolved in TBS buffer with 0.05% Tween 20. The membrane was blotted with rabbit anti-Hsp90α polyclonal Ab or anti-Hsp90β polyclonal Ab. Anti-actin polyclonal Ab was used as a control. HRP-conjugated goat anti-rabbit IgG (MBL, Nagoya, Japan) was used as secondary Ab, and the detection was conducted using chemiluminescent reaction.

Detection of MHC I Kb–OVA257–264 peptide complexes

H-2Kb–SINFEKL complexes expressed on the surface of BMDCs were analyzed by flow cytometry with biotin-conjugated 25-D1.16 mAb and avidin-PE-Cy5. BMDCs were treated with radicicol (25 μM) or MG115 (10 μM) and pulsed with soluble OVA protein (7 mg/ml) as described in cross-presentation assay and cultured for 8 h before staining.

Results

Inhibitors of HSP90 profoundly block cross-presentation of OVA by BMDCs

We first examined the effects of an HSP90 inhibitor on cross-presentation by GM-CSF–induced BMDCs. BMDCs were pulsed with OVA, then fixed and incubated with OT-I CD8+ T cells (specific to OVA257–264 plus Kb) (9) for 24–48 h. The quantity of labeled OVA (Supplemental Fig. 2). The toxicities of the inhibitors radicicol treatment as seen in the result of internalization of FITC-processing. The uptake of OVA by BMDCs was not altered by MG115 (Supplemental Fig. 1), indicating downregulation of OVA suppressed the expression of the complexes to a similar extent as the cell surface by 25-D1.16 mAb (13) indicated that radicicol treatment (Supplemental Fig. 3).

MHC I, MHC II, CD80, CD86, and CD40 were not affected by MG115 (10 μM) and pulsed with soluble OVA protein (7 mg/ml) as described in cross-presentation assay for 8 h before staining.

FIGURE 3. HSP90 inhibitor abrogated cross-presentation of cell-associated Ag. A, DC uptake of UV-induced apoptotic cells. TAPI-deficient splenocytes after UV irradiation were stained with PKH26 (ordinate). The cells were cocultured with indicated doses of radicicol-treated DC2.4 cells (stained with PKH67, abscissa) for 3 h and analyzed by FACS. B, Soluble OVA was osmotically introduced into TAPI-deficient spleen cells and irradiated with UV rays as in A (used as “Ag donor cell”). DC2.4 cells pretreated with or without radicicol as indicated were cocultured with the Ag donor cells for 3 h and subjected to cross-presentation assay. The results are representative of at least two independent experiments.
in cross-presentation assay. There was no OT-I CD8+ T cell reaction in cocultures of BMDCs pulsed with the culture medium (data not shown). Almost identical sensitivities of exogenous versus endogenous Ag presentation to graded doses of proteasome inhibitors MG115 and Lactacystin (Supplemental Fig. 4) ruled out the possibility that effect of HSP90 inhibitors against proteasome is involved in degradation of OVA Ag used in this study.

These results suggest that HSP90 is particularly involved in cross-presentation and less in processing of newly synthesized proteins.

**E1 inhibitor profoundly blocks both endogenous Ag presentation and cross-presentation by DCs**

Polyubiquitinylation is considered an essential and common step in proteasome-dependent processing of proteins. Inhibition of E1 ubiquitin activator results in abrogation of E3-mediated polyubiquitinylation and arrest of degradation of proteins by proteasomes, and hence, inactivation of the Ag processing pathway. Indeed, treatment of BMDCs with E1 inhibitor blocked the production of IFN-γ from OT-I CD8+ T cells in a dose-dependent manner (Fig. 2D). Furthermore, such treatment also suppressed the processing of endogenously expressed OVA (Fig. 2E). These effects are different from those of radicicol but similar to those of the proteasome inhibitor MG115.

**Radicicol treatment of DCs blocks cross-presentation of cell-associated Ag**

To examine the effect of radicicol on cross-presentation of cell-associated Ag, TAP1-deficient splenocytes osmotically loaded with OVA were UV irradiated to induce apoptosis and then incubated with DC2.4 cells pretreated with or without graded doses of radicicol or epoxomicin (a highly specific inhibitor for the proteasome). Interestingly, such treatment also suppressed the processing of endogenously expressed OVA (Fig. 3B). Taken together, the results suggest that radicicol-induced functional inactivation of HSP90 in DC2.4 cells reduces the ability to cross-present the cell-associated as well as soluble Ag.

**HSP90α mainly contributes to the cross-presentation of OVA by DCs**

Next, we determined the HSP90 family subtype responsible for cross-presentation. We transfected siRNA twice into DC2.4 cells and used the transfectant cells in cross-presentation assays. The siRNA to HSP90α markedly suppressed IFN-γ production albeit 50% reduction of the protein level as detected by Western blotting, whereas siRNA to HSP90β resulted in a milder suppression (Fig. 4A, 4B). Thus, HSP90α is mainly responsible for cross-presentation of OVA in DC2.4 cells. Application of another strategy to downregulate the level of HSP90α by using lentivirus-carrying shRNA sequence to HSP90α separated by IRES sequence from GFP gene failed to infect DC2.4 cells. However, transfection of the large lentiviral vector plasmid (~10 kb) twice by electroporation into DC2.4 cells and sorting of GFP-expressing cells by FACSVantage allowed us to identify again HSP90α to be responsible for cross-presentation (Fig. 4C). Downregulation of Hsp90α was shown in Fig. 4D. HSF-1–deficient BMDCs are defective in HSP90α and less efficient in cross-presentation

**HSF-1 is a major transcription factor for many HSPs and mediates heat shock response to various stimuli. Zheng and Li (6) reported**

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**FIGURE 4.** Knockdown of HSP90α in DC2.4 cells profoundly reduced production of IFN-γ from OT-I CD8+ T cells. A, DC2.4 cells were transfected with plasmid vectors encoding HSP90α and/or HSP90β–specific siRNA twice (days 0 and 2) and on day 3 were subjected to cross-presentation assay as shown in Fig. 1. Data are representative of three independent experiments. B, Western blotting analysis of HSP90α and HSP90β isoforms in Hsp90 knocked down cells in the same experiment with A. C, DC2.4 cell line was transfected twice (day 0 and 2) with 8 μg lentivirus vector carrying shRNA sequence to HSP90α, and GFP-positive cells were sorted by FACSAria. As a control, empty vector was used. On day 3, the DC2.4 cells were incubated with 1, 0.5, and 0.25 mg/ml OVA for 1 h and washed. After 2 h of incubation, the DC2.4 cells were fixed and used in cross-presentation assay. D, The DC2.4 cells used in C were examined for their expression of HSP90α by Western blotting.
significantly lower HSP90 expression levels in BMDCs of HSF-1−/− mice than WT BMDCs. We further investigated this evidence and found that HSP90α, but not HSP90β, was specifically reduced in HSF-1−/− BMDCs, although HSC70, grp78, and gp96 (both KDEL positive) were not (Fig. 5D). We took this advantage to address the role of HSP90α in cross-presentation and found that HSF-1−/− BMDCs is less capable of cross-presenting both soluble and cell-associated OVA (Fig. 5A, 5B). In contrast, no difference was detected between HSF-1−/− and HSF1+/− BMDCs in direct Ag presentation examined by transfection with plasmid DNA encoding OVA (pOVA) (Fig. 5C). There was no significant difference in MHC I Kb expression and CD11c population between HSF-1−/− and HSF-1+/− BMDCs (Fig. 5E). Also, there was no difference in uptake of apoptotic Ag donor cells by HSF-1−/− and HSF-1+/− BMDCs (Supplemental Fig. 5). These results led us to conclude that the cross-presentation ability was lower in HSF-1−/− BMDCs and that low expression of HSP90α is possibly responsible for the defective cross-presentation.

**FIGURE 5.** Cross-presentation efficiency was low in HSF-1−/− BMDCs. A, BMDCs induced from HSF-1+/− or HSF-1−/− mice were pulsed with the indicated doses of soluble OVA in the presence or absence of radicicol (20 μM) and cocultured with OT-I CD8+ T cells. The amount of IFN-γ released in the medium was measured by ELISA. B, Apoptotic cells were prepared by UV irradiation of OVA-loaded TAP1−/− splenocytes. BMDCs were treated with radicicol (20 μM) for 1 h and washed three times. The apoptotic cells were cultured with the BMDCs induced from HSF-1+/− or HSF-1−/− mice for 3 h. The mixture of BMDCs and apoptotic cells was then fixed and subjected to cross-presentation assay as in A. C, BMDCs induced from HSF-1+/− or HSF-1−/− mice were transfected with graded doses of pOVA as indicated and cultured for 3 h and then fixed. The cells were used in the assay as in A, D. Western blotting analyses of BMDCs used in A, E, BMDCs induced from HSF-1+/− or HSF-1−/− mice were stained with anti-CD11c and anti–H-2Kb Ab, and the expression levels were analyzed by FACS. Data are confirmed by at least four independent experiments.
**Discussion**

This report provides the evidence for the importance of endogenous HSP90 in Ag cross-presentation within DCs. Inhibitors of HSP90 abolished the ability of DCs to cross-present the exogenous soluble and cell-associated Ags, and the indispensability of HSP90 was confirmed by knockdown of HSP90 isoforms with siRNA. The physiological relevance was supported by using HSP-1–deficient BMDCs whose expression of HSP90α but not HSP90β is specifically downregulated, although HSF-1–mediated gene regulation is not restricted to HSP expression (12). It is possible that HSF-1, through HSP90α, is linked with cross-presentation, and hence, cytotoxic T cell immunity. HSP90β was reported to mediate cytosolic refolding of exogenous luciferase taken up by endocytosis, but there was no clarification whether HSP90β contributes to the cross-presentation (18). Downregulation of HSP90β by siRNA showed reduced cross-presentation ability, although the effect is lesser extent compared with HSP90α. It is possible that HSP90β is also playing a role in cross-presentation.

Several cytosolic pathways for Ag processing of cross-presentation have been proposed. The requirement for TAP is one of the issues and then whether or not the Ag goes into endoplasmic reticulum (ER) after degraded by proteasome is another. In a TAP-independent model, the Ags are degraded by endosomal proteases and loaded onto MHC I molecules within endosomes. Most studies report the requirement of TAP in cross-presentation. In a TAP-dependent model, two routes are suggested. Both involve Ag translocation from endosomes to cytosol for proteasomal degradation, and in one model, the digested Ags are imported into the ER by TAP, then the peptide loading will occur in the same way as the endogenous Ag loading. In another model, the proteasome-degraded Ags re-enter the endosomes and load onto MHC I molecules there. Mannose receptor is also suggested as the receptor for soluble OVA uptake and is involved in the TAP-dependent pathway (19). Our experimental setting showed that the soluble OVA cross-presentation was TAP dependent and proteasome dependent (Fig. 1B). Considering the involvement of HSP90 in Ag processing after uptake of OVA, chaperoning the Ag protein/peptide in cytosol before or after proteasome degradation is most likely to be the role of HSP90. From the results of HSP90 inhibitor effect on endogenous Ag presentation (Fig. 2C) and the inhibition of polyubiquitinylation of endogenous and cross-presentation (Fig. 2D, 2E), the HSP90 inhibitor seems to influence the step before proteasomal degradation of substrates most likely is the translocation of Ag from endosome to the cytosol.

During cross-presentation, exogenous Ag emerging across the endosomal membrane to the cytosol is believed to be in a relatively unfolded state because the membrane pore (∼5–8 Å in diameter) (20) is not large enough for passage of Ag (30–60 kDa, >30 Å) (21) through its native structure (22). Therefore, it is conceivable that cytosolic HSP such as HSP90 and HSP70 bind to unfolded exogenous Ag translocating from the endosome to the cytosol. To consider the role of HSP in cross-presentation, there might be two distinct mechanisms. First, HSP directs the C terminus of Hsc70-interacting protein-mediated polyubiquitylation of translocated unfolded proteins for the proteasomal degradation (22), (23). Second, HSP directs translocation of proteins from the endosome to the cytosol, which might resemble the observations that binding Ig protein (Grp78) and cytosolic HSP70/HSP90 drive polypeptides into the ER and the mitochondria, respectively (25), (26).

Our results showed that HSP90 of APCs is essential for cross-presentation of soluble and cell-associated Ag by DCs, and it should be clearly discriminated from previous observations that in vivo association of certain peptides with HSP90 in Ag donor cells is required for cross-priming by cellular Ag (3, 27, 28).

The Ags associated with HSP90 are derived from either postproteasomal degradation products (3) or newly synthesized polypeptides to be degraded by the proteasome (8) in Ag donor cells. Immunization with cells containing the HSP90–peptide complex generates peptide-specific CTLs, whereas HSP90 inhibitor-treated cells where the peptide is dissociated from HSP90, cannot cross-prime T cells (3, 5).

The effect of hsp90 inhibitors on presentation of endogenously expressed Ag was only marginal in this study, which is in contrast to results of previous studies including ours (3, 7, 8). However, cells used in this study are DC-related cells, thus, BMDCs and DC2.4 cell line, whereas those in the previous studies were tumor cell lines. In this context, it is possible that professional APCs are more flexible to have an alternative (HSP90 independent) mechanism for endogenous Ag processing pathway.

Ags from HSF-1–deficient cells were poorly reported to be poorly cross-presented to MHC I pathway by Zheng and Li (3). They showed that Kd-restricted nuclear protein, Hsp90cb, Hsp90db1, and CB6F1 (H-2d/b) mice with irradiated mouse embryonic fibroblasts (H-2d) expressing SV40 large T Ag resulted in inefficient cross-priming of large Ag-specific CD8+ T cells. They also showed that low cross-presentation ability of HSF-1−/− Ag donor cells in vitro cross-presentation assay (6). These results indicate HSF-1–mediated genetic control(s) affects cross-presenting ability of Ag donor cells. Because HSP90 was specifically downregulated in skin and mouse embryonic fibroblasts of HSF-1−/− mice (H-2d), it is plausible that chaperoning cellular Ags by HSP90 in Ag donor cells is involved in efficient cross-presenting ability. Despite that, HSF-1−/− mice we used in this study were C57BL/6 origin (H-2b) and separately produced genetically ablated will be necessary to fully demonstrate the role of HSP90 in Ag cross-presentation.

However, it is also true that HSF-1 is linked with other immunity-related events such as IL-6 production (12). The production of cytokines and chemokines might influence cross-presentation ability of APCs. In this context, a study using cells or mice whose HSP90 is genetically ablated will be necessary to fully demonstrate the role of HSP90 in Ag cross-presentation.

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**Disclosures**

The authors have no financial conflicts of interest.

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