Arch. Immunol. Ther. Exp. (2016) 64:1–18
DOI 10.1007/s00005-015-0370-x

REVIEW

Antigen Cross-Presentation and Heat Shock Protein-Based Vaccines

Katerina Zachova[1] · Michal Krupka[1] · Milan Raska[1,2]

Received: 28 January 2015 / Accepted: 31 August 2015 / Published online: 30 October 2015

Abstract Vaccines currently in the clinical use contain adjuvants stimulating preferably Th2 type of immune response associated with the production of specific antibodies, mostly of neutralizing isotypes. This kind of immune response is effective only against some types of pathogens and has limited effect against tumors and many viruses where parallel activation of antigen-specific humoral and cell-mediated immunity is required. One of the main objectives of the current vaccine research is the development of approaches leading to the induction of antigen-specific $CD8^+$ T cell response including cytotoxic T lymphocyte (CTL). Induction of antigen-specific $CD8^+$ T cell response to exogenously delivered antigen requires their cross-presentation by antigen presenting cells, especially dendritic cells. The cross-presentation principles seem to be crucial for effective activation of CTL. In this paper, we discuss some approaches to employing heat shock proteins for induction of antigen-specific $CD8^+$ T cells in the context of cross-presentation and cross-priming principles.

Keywords Cross-presentation · Heat shock protein (hsp) · Dendritic cells

✉ Milan Raska
raskamil@uab.edu

[1] Department of Immunology, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, Olomouc 77900, Czech Republic

[2] Department of Immunology, University Hospital Olomouc, Olomouc, Czech Republic

Introduction

Antigen presentation on major histocompatibility complex (MHC) molecules is one of the most crucial steps during the recognition of various antigens by the antigen-specific cellular and humoral immunity. It is executed by functionally specialized cells called antigen presenting cells (APC) comprising dendritic cells (DCs), monocyte/macrophages, and B cells. Activation of naïve $CD4^+$ T cells is the consequence of the antigen processing by APC which is initiated by engulfment of the antigen by APC, followed by digestion and presentation of peptide fragments on MHC-II molecules; a situation typical for antigens of the exogenous origin such as bacterial ones. In contrast, endogenous antigens such as tumor or viral antigens are processed to peptide fragments and presented via MHC-I molecules; process ongoing inside nearly all nucleated somatic cells. This mechanism ensures a specific elimination of tumors and virus-infected cells by activated antigen-specific cytotoxic $CD8^+$ T cells (CTL: cytotoxic T lymphocyte). They are activated during prior interaction of their naïve precursors with the same peptide presented by the MHC-I on DCs (Jung et al. 2002). Nevertheless, such DCs should encounter the respective antigen from their surroundings because they approach cancer cells or virally infected cells as healthy ones and, thus, are not processing these antigens as endogenous. Therefore, the immune system developed several pathways to ensure MHC-I presentation of exogenous antigens by DC or macrophages, collectively called cross-presentation pathways. Here, we will describe basic principles of cross-presentation and will discuss utilization of heat shock proteins (hsp) as one of the known agents leading to effective cross-presentation of associated antigens serving as a promising system for development of novel vaccines for induction of both humoral and cell-mediated immunity.

Stimulation of Naïve CD4$^+$ T Cells Through MHC-II

The classical MHC-II presentation pathway is initiated once exogenous antigen is endocytosed by APC through formation of endocytic vesicles using various mostly receptor-mediated mechanisms including clathrin-dependent endocytosis or clathrin-independent endocytosis including actin-driven pathways such as phagocytosis or pinocytosis (Donaldson et al. 2009; Grant and Donaldson 2009). Endocytic vesicles are delivered to vacuolar domains of pleiomorphic early endosomes (EE) giving rise to multivesicular endosomes/bodies (MVBs) (Woodman and Futter 2008). In the case of phagocytosis, endocytic vesicles called early phagosomes exchange their content with EE (Desjardins 1995; Desjardins et al. 1994). Both MVBs or early phagosomes migrate deeper toward the cell center, decrease their intraluminal pH, and acquire hydrolytically degradative lysosomal hydrolases by either repeated fusion/fission or direct fusion with resting lysosomes (Desjardins et al. 1994) leading to the formation of phagolysosomes, a final degradative organelle where the antigens are cleaved in highly acidic pH into short peptides (15–35 amino acids long) and ultimately to dipeptides and amino acids (Coffey and De Duve 1968).

Inside APC, the EEs fuse with vesicles containing MHC-II molecules targeted to endosomes. There, peptides generated from engulfed antigens are finally associated with MHC-II molecules, which are then transported to the APC cell surface for interaction with CD4$^+$ T lymphocytes. Acidification of endosomes or phagosomes must be under control in APCs (especially in immature DCs, which are the most effective in the initiation of the MHC-II presentation) because rapid acidification associated with abundant activation of acidic pH optimum-exhibiting proteases (especially cathepsin L and legumain) leads to an extensive antigen cleavage, diminished MHC-II presentation, and reduced activation of the antigen-specific CD4$^+$ T cell response (Trombetta et al. 2003).

Stimulation of Naïve CD8$^+$ T cells Through the MHC-I Canonical Pathway

Proteins destined for MHC-I presentation are either synthesized in the cytosol (viral antigens) or delivered to the cytosol from endoplasmic reticulum (ER). Misfolded, damaged, or abnormal proteins (tumor antigens) are delivered by active ER-to-cytosol transport facilitated probably by the retrograde activity of the translocation pore complex formed with contribution of Sec61 and Derlin-1 proteins (Lilley and Ploegh 2004; Wiertz et al. 1996; Ye et al. 2004). Sec61 was initially identified in the ER membrane as the factor involved in the inverse process—cytosol-to-ER lumen translocation of proteins synthesized either on cytosolic or ER-associated ribosomes allowing their cotranslational or posttranslational transport into ER lumen for further ER processing, followed for example by relocation to Golgi apparatus, and secretion (Rapoport et al. 1996). Within the cytosol, proteins destined for MHC-I presentation are ubiquitinated, fragmented by the proteasome, and partially trimmed by not completely identified cytosolic aminopeptidases or hydrolases (Saveanu et al. 2002). Peptide fragments are thereafter translocated by the transporter associated with antigen processing (TAP1 and TAP2) to ER lumen where they are loaded onto membrane-anchored MHC-I (Cresswell et al. 1999). Before binding to MHC-I, peptides are trimmed by the ER aminopeptidase 1 or 2 (ERAP-1, ERAP-2) to 8–10 amino acid long fragments optimal to fit the MHC-I peptide-binding groove (Serwold et al. 2001, 2002). Afterwards, the MHC-I with a loaded peptide is transported by vesicular traffic through the Golgi complex to the cell surface where the MHC-I/peptide complex is recognized by T cell receptors (TCR) on CD8$^+$ T cells (Cresswell et al. 1999). This mechanism fits well to effector functions of antigen-specific CTLs, which kill virally infected or tumor cells.

Stimulation of Naïve CD8$^+$ T Cells by Exogenous Antigens Through MHC-I

It was discovered that some exogenously delivered antigens are recognized not only by CD4$^+$ T cells but also by CD8$^+$ T cells (Bevan 1976). As CD8$^+$ T cell activation requires presentation of antigen fragments through MHC-I, which, at the time, was thought to be solely an endogenous pathway, it was quite a surprising observation. DCs and probably also macrophages are the only cells endowed with the capability to activate naïve CD8$^+$ T cells to become antigen-specific CTLs, by providing the necessary costimulatory signals. In agreement with classical concept stating that MHC-I presentation takes place only for intracellularly synthesized antigens, it should be assumed that during initiation of CD8$^+$ T cell responses, only virally infected or malignantly transformed DCs could activate naïve CD8$^+$ T cells, which is not realistic. Therefore, in accordance with the above-described MHC-I-dependent recognition of exogenously delivered antigens by CD8$^+$ T cells, it was concluded that DCs should be able to process exogenous antigens for MHC-I presentation. This was designated as cross-priming (Bevan 1976) to specify the MHC-I-dependent stimulation of effector CTL response. In contrast, the more specific term cross-presentation describes only the part of the cross-priming mechanism responsible for the processing of the exogenous antigen for MHC-I presentation.

Uniqueness of DC During Exogenous Antigen Processing for Cross-Presentation

Proteins destined for cross-presentation are either phagocytosed or pinocytosed by DCs. This implies that similarly to MHC-II presentation, DCs digest the engulfed antigens only partially, leaving their proteolytic fragments of appropriate size fitting to the MHC-I peptide-binding groove. Immature DCs could control alkalization/acidification of their phagosomes and they exhibit attenuated lysosomal proteolysis (Delamarre et al. 2005; Savina et al. 2009; Trombetta et al. 2003). The propensity of immature DCs for limited degradation of antigens could be documented by the fact that commensal bacteria can be recovered even several hours after their phagocytosis (Macpherson and Uhr 2004). In contrast to DCs, rapid degradation of internalized antigens occurs in macrophages and especially neutrophils which are characterised by an extensive degradation of phagocytosed antigens.

Cross-Presenting DC Populations

Not all subsets of murine or human DCs could cross-present antigens. This led to many doubts about the existence of cross-presentation because the majority of the former DC experiments were performed using in vitro-derived DCs mostly from bone marrow precursors (BMDC) or from peripheral blood monocytes (MoDC) stimulated by mixture of IL-4 and granulocyte–macrophage colony-stimulating factor (GM-CSF). In mice, the dominant cross-presenting DC subset is referred to as CD8$\alpha\alpha^+$ CD11c^{high} DC population (den Haan et al. 2000; Dudziak et al. 2007; Pooley et al. 2001; Schnorrer et al. 2006). In humans, the dominant cross-presenting DC subpopulation is characterized by the surface expression of human blood DC antigen 3 (BDCA-3, also called thrombomodulin or CD141), C-type lectin domain family 9A (CLEC9A, also called DNGR1), and Receptor for XCL1 and XCL2 chemokines (XCR1, also called GPR5) (Schreibelt et al. 2012). The BDCA-3$^+$ CLEC9A$^+$ DCs were found in lymph nodes, bone marrow, and tonsils (Caminschi et al. 2008; Jongbloed et al. 2010). For clinical applications, exploitation of BDCA-3 DCs from peripheral blood as the easiest cell source is hampered by their very low count—they represent only $\sim$0.03 % of total human peripheral blood mononuclear cells (Jongbloed et al. 2010). Recently performed computational genome wide expression profiling clustered human BDCA-3$^+$ DC with the mouse CD8$\alpha\alpha^+$ (Robbins et al. 2008). Human BDCA-3$^+$ DC and mouse CD8$\alpha\alpha^+$ DC share several phenotypic similarities including the expression of Toll-like receptor (TLR)-3, Nectin-like protein 2, and CLEC9A (Caminschi et al. 2008; Edwards et al. 2003; Galibert et al. 2005; Huysamen et al. 2008; Sancho et al. 2009).

Basic Principles of Cross-Presentation

Since initial reports about the cross-priming phenomenon (Bevan 1976), several more or less overlapping pathways responsible for cross-presentation of exogenous antigens have been described. In principle, three crucial steps should take place: fragmentation of antigen to peptides of size corresponding to the MHC-I groove, binding to MHC-I, and transport of MHC-I/peptide complex to the cell surface. There are two ways in which antigens can be fragmented and associated with MHC-I: (a) the antigen is relocated from endosome into cytosol and targeted to immunoproteasome followed by processing in a way similar to canonical MHC-I presentation (cytosolic pathway), or (b) the antigen is fragmented inside the phagocytic vesicle (predominantly endosome), associated here with recycling MHC-I, and transported to the cell surface, without being exposed to the cytosolic milieu (vacuolar pathway; Table 1). Furthermore, overlap between both pathways was reported based on the observation that cytosolic antigen could be relocated back to endosome for MHC-I presentation (Gromme et al. 1999). The following sections describe individual cross-presentation pathways identified so far, using the cytosolic and vacuolar pathway classification. The identification of a particular pathway and its principal components such as transporter associated with antigen processing (TAP), proteasome, lysosome, and lysosomal proteases was facilitated by the use of various inhibitors and gene knock-out mice (Table 2).

Endosome-to-Cytosol Pathway

The endosome-to-cytosol pathway (Fig. 1) was described almost 20 years ago in macrophages as the pathway allowing induction of CD8$^+$ T cells response to corpuscular antigens as the lysosome-independent, but proteasome-, TAP-, and ER-to-Golgi transport-dependent pathway (Table 1) (Kovacsovics-Bankowski and Rock 1995). Later studies identified involvement of this pathway in cross-presentation of soluble antigens as well (Ichiyanagi et al. 2010; Imai et al. 2011). The mannose receptor (MR) has been studied concerning its involvement in the initiation of the endosome-to-cytosol cross-presentation of soluble antigens such as ovalbumin (OVA) from chicken egg white (Burgdorf et al. 2006). DC could internalize antigens bound to MR into stable EE compartments which do not undergo lysosomal transformation (Burgdorf and Kurts 2008; Segura et al. 2009). Tumor susceptibility gene

Table 1 Principal contributors in individual cross-presentation pathways

	Endosome-to-cytosol pathway	Endosome-to-cytosol-to-endosome pathway	Vacuolar pathway	Gap junction pathway	Note
Soluble antigens	Supports (Ikeuchi et al. 2010)	Supports (Ackerman et al. 2005)	Supports (Chen and Jondal 2004)	Supports (Saccheri et al. 2010)	
Corpuscular antigens	Supports (Kovacsovics-Bankowski and Rock 1995)		Supports (Song and Harding 1996)		
Ag targeting to mannose receptor	Supports (Zehner et al. 2011)	Supports (Saveanu et al. 2009)			
Ag targeting to TLR-2	Supports entering of exogenous hsp to the cell (Asea et al. 2002)	Supports entering of exogenous hsp to the cell (Asea et al. 2002)	Supports entering of exogenous hsp to the cell (Asea et al. 2002)		Pathway nonspecific
Ag targeting to CD40	Supports transport of Ag to EE (Chatterjee et al. 2012)	Supports transport of Ag to EE (Chatterjee et al. 2012)	Supports transport of Ag to EE (Chatterjee et al. 2012)		Pathway nonspecific
Ag targeting to DEC205	Supports transport of Ag to late endosome (Chatterjee et al. 2012)	Supports transport of Ag to late endosome (Chatterjee et al. 2012)	Supports transport of Ag to late endosome (Chatterjee et al. 2012)		Pathway nonspecific
Ag targeting to CD11b/CD18^{+} cells via/by adenylate cyclase toxin CyaA	Supports transport of Ag to cytosol (Holubova et al. 2012; Mackova et al. 2006)	Supports transport of Ag to cytosol (Holubova et al. 2012; Mackova et al. 2006)			
Proteasome	Supports (Delamarre et al. 2003; Rodriguez et al. 1999)	Supports (Delamarre et al. 2003; Rodriguez et al. 1999)		Supports (Neijssen et al. 2005)	
TAP	Supports (Cresswell et al. 1999)	Supports (Cresswell et al. 1999)		Supports (Cresswell et al. 1999)	
IRAP	Supports (Saveanu et al. 2009)	Supports (Segura et al. 2009)		Supports (Saveanu et al. 2009)	
ERAP	Supports (Serwold et al. 2002)			Supports (Serwold et al. 2002)	
Ubiquitination	Supports (Hershko et al. 1984)	Supports (Hershko et al. 1984)			
Sec61	Supports	Supports		(Imai et al. 2005; Mukai et al. 2011)	

Table 2 Approaches used to discriminate individual cross-presentation pathway involvement

Approach	Cross-presentation pathway	Note	References
In vitro			
Chloroquine	Inhibits vacuolar pathway Supports endosome-to-cytosol pathway	Lysosomotrophic agent, inhibits acidification of endosomal/lysosomal compartments Increases membrane permeability leading to Ag escape into the cytosol	(Accapezzato et al. 2005; Gromme et al. 1999)
NH_4Cl	Supports endosome-to-cytosol pathway Inhibits exchange of peptides on MHC-I	Lysosomotrophic agent Inhibits acidification of endolysosomal vesicles	(Accapezzato et al. 2005; Gromme et al. 1999)
Chlorpromazine	Inhibits clathrin-mediated endocytosis	Allows distinguishing between phagocytosis and clathrin-dependent endocytosis	(Moron et al. 2003)
Cytochalasin B	Inhibits phagocytosis	Allows distinguishing between phagocytosis and clathrin-dependent endocytosis Inhibits the non-coated-pit endocytosis by preventing polymerization of actin filaments	(Gurnani et al. 2004)
Cytochalasin D	Studied on endosome-to-cytosol pathway	Inhibitor of phagocytosis or macropinocytosis Causes cytoskeleton disruption and impairs cross-presentation of antigens derived from phagocytosed cellular structures	(Fonteneau et al. 2003; Sampath and Pollard 1991)
2-APB	Inhibits gap junction pathway	Inhibits gap junction transport	(Bai et al. 2006)
Amiloride	Inhibits macropinocytosis	Allows distinguishing between phagocytosis and clathrin-dependent endocytosis Inhibits acidification of endolysosomal vesicles	(Nakamura et al. 2008)
Dimethylamiloride	Inhibits pinocytosis	Allows distinguishing between phagocytosis and clathrin-dependent endocytosis	(Fonteneau et al. 2003; Tanaka et al. 2010)
Epoxomycin	Inhibits proteasome-dependent, cytosolic pathways	Inhibits proteasome	(Andrieu et al. 2003; Janda et al. 2004)
MG-132	Inhibits proteasome-dependent, cytosolic pathways	Inhibits proteasome	(Baker et al. 2011; Mant et al. 2012; Pham et al. 2012)
Leupeptin	Inhibits vacuolar pathway	Lysosomal cysteine-protease inhibitor	(Campbell et al. 2000; Kurotaki et al. 2007; Libby and Goldberg 1978)
18 β-glycyrrhetinic acid	Inhibits gap junction pathway	Inhibits gap junction formation by dephosphorylation of connexins	(Guan et al. 1996)
Brefeldin A	Inhibits endosome-to-cytosol and Gap junction pathways	Inhibits anterograde ER–Golgi transport Blocks MHC class I-peptide complex turnover on the cell surface	(Benke et al. 2006; Fujiwara et al. 1988; Shakushiro et al. 2004)
Exotoxin A from *P. aeruginosa*	Sec61 inhibitor	Inhibits protein translocation from the ER to the cytosol	(Ackerman et al. 2006)

Table 2 continued

Approach	Cross-presentation pathway	Note	References
Lactacystin	Inhibits proteasome-dependent, cytosolic pathways	Inhibits proteasome	(Craiu et al. 1997; Giodini et al. 2009)
B. abortus VirB		Directs fusion of phagosome with the ER	(Celli et al. 2003)

Approach In vivo	Note	References
TAP knock-out	TAP knock-out DCs show lower MHC-I expression and generally reduced cross-presentation	(Merzougui et al. 2011; Sigal and Rock 2000; Van Kaer et al. 1992)
CD11c-DTr	Mice carrying simian diphteria toxin receptor under control of the murine CD11c promoter synthesizing allow specific killing of $CD11c^{+}$ DC	(Jung et al. 2002)
	Model confirmed indispensable role of $CD11^{+}$ DC in cross-presentation and mounting of CTL to intracellular bacterium *Listeria monocytogenes* and malaria parasite *Plasmodium yoelii*	
OT-I	OT-I T cell receptor-transgenic mouse line produces T cells recognizing an $H\text{-}2^{b}$-restricted OVA epitope—SIINFEKL	(Garulli et al. 2008; Sathe et al. 2011)
	T cells allow specific tracing of SIINFEKL presented on MHC-I in vitro and in vivo	
TLR-2, -3, -4, -7, or -9	TLR-2, -3, -4, -7, or -9 are dispensable for generation of OVA-specific CTLs in naive mice immunized with mycobacterial hsp70-OVA	(Mizukami et al. 2012)
	TLR-2, -3, -4, -7, or -9 are dispensable in mycobacterial hsp70-OVA-dependent rejection of a tumor expressing endogenous OVA	
MyD88 knock-out	Myd88 is dispensable for generation of OVA-specific CTLs in naive mice immunized with mycobacterial hsp70-OVA	(Kawai and Akira 2010; Mizukami et al. 2012)
	Myd88 is indispensable in mycobacterial hsp70-OVA-dependent rejection of a tumor expressing endogenous OVA	
IRAK4 knock-out	IRAK-4-deficient animals are completely resistant to a lethal dose of LPS	(Mizukami et al. 2012; Suzuki et al. 2002)
	IRAK4 is indispensable in mycobacterial hsp70-OVA-dependent rejection of a tumor expressing endogenous OVA	
IL-18R knock-out	Lack of IL-18R signal reduces DCs maturation and production of TNF-α	(Ghose et al. 2011)
Cat S knock-out	DC lacking Cat S lack the TAP-independent pathway	(Shen et al. 2004)
	Mice whose APC lack Cat S have limited cross-priming of particulate and cell-associated antigens	

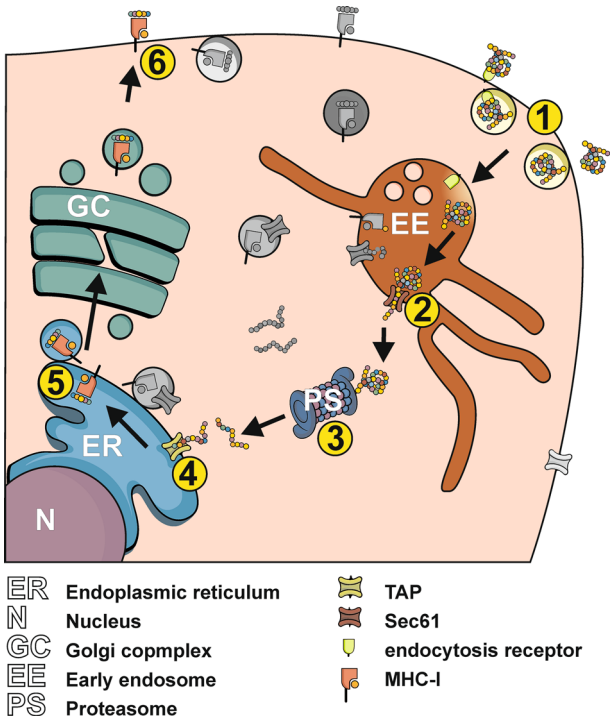

Fig. 1 Endosome-to-cytosol pathway of cross-presentation. Exogenous antigen is engulfed by a DC (*1*) leading to the formation of endocytic vesicles which fuse with EE. Antigen is transported out of the endosome vesicle with contribution of transporter complexes such as Sec61 (*2*), followed by cleavage of the antigen by proteasome and trimming to form short peptides (*3*) which are transported into the lumen or ER by TAP-assisted transport (*4*) according to canonical MHC-I presentation pathway. Inside the ER, peptides are loaded onto the MHC-I molecules (*5*) and subsequently transported as MHC-I/peptide complexes via ER and Golgi apparatus to the cell surface (*6*). *Gray color* is used for components not involved in this particular pathway

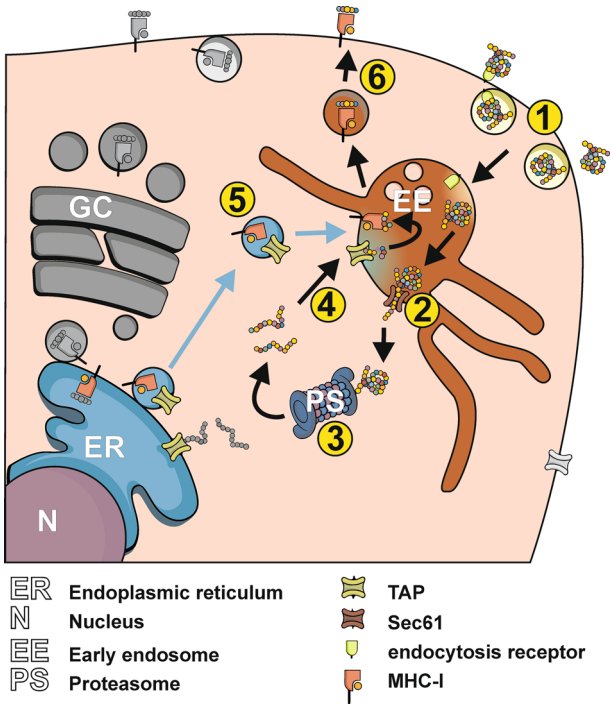

Fig. 2 Endosome-to-cytosol-to-endosome pathway. Exogenous antigen is engulfed by a DC (*1*) leading to the formation of endocytic vesicles (endosome, phagosome) which fuse with EE. Antigen is transported out of the endosome vesicle with contribution of transporter complexes such Sec61 (*2*), followed by cleavage of the antigen by proteasome and transport of peptide fragments back toward the endosome through TAP (*4*). The endosome/phagosome contains all proteins necessary for peptide transport across endosomal membrane and its subsequent association with MHC-I due to their supply from ER during fusion of ER-originating vesicles with EE (*5*). Once inside the EE, peptides are further processed and loaded onto the MHC-I molecules. MHC-I molecules with bound peptides are targeted to the cell surface by endosome sorting machinery (*6*)

101-regulated poly-ubiquitination of the cytosolic region of MR in the EE membrane recruits p97, a member of the formerly described ER-associated degradation machinery, responsible for antigen translocation across the endosomal membrane (Burgdorf et al. 2007; Zehner and Burgdorf 2013). On the EE membrane, p97 associates with Sec61 and Derlin-1. The mechanism by which Sec61 relocates from ER to EE is probably based on the transport of ER-originating vesicles toward EE or based on the existence of ER protrusions toward phagocytic vesicles leading to the formation of ER-phagosomes (Guermonprez et al. 2003; Mukai et al. 2011). Before translocation into the cytosol, endocytosed antigen must be unfolded and partially proteolytically degraded, depending on its physico-chemical nature (Singh and Cresswell 2010). Targeting the antigen to endosome-to-cytosol pathway could be facilitated also by complexing the antigens to various hsp (Basu et al. 2001).

Once the antigen locates within cytosol, it is targeted to proteasome by ubiquitination (Glickman and Ciechanover 2002) and further processed as for canonical MHC-I presentation pathway. Endosome-to-cytosol pathway was confirmed active both in vivo and in vitro (Moron et al. 2003; Norbury et al. 1997; Svensson and Wick 1999).

Endosome-to-Cytosol-to-Endosome Pathway

Here, the endocytosed antigen is also transported to cytosol and subsequently processed in proteasome but, contrary to previous pathway, fragments are transported back into endosome or phagosome (Fig. 2; Table 1) (Ackerman et al. 2003; Guermonprez et al. 2003), where all proteins necessary for efficacious binding of peptide to MHC-I (TAP, tapasin, lectin chaperones calnexin and calreticulin, Erp57, MHC-I) were identified (Ackerman et al. 2003; Houde et al. 2003). The presence of typical ER proteins within endosome/phagosome was explained by the fusion of EE with ER-derived vesicles (Guermonprez et al. 2003; Mukai et al. 2011), in the case of MHC-I molecule with the

contribution of invariant chain (CD74) associating with MHC-I in the ER and mediating trafficking of MHC-I to endolysosomes (Basha et al. 2012). However, the details of this mechanism remain controversial (Touret et al. 2005). Relocation of some MHC-I-loading machinery components from ER to EE could be induced in murine BMDC by lipopolysaccharide (LPS). Both TLR-4 and MyD88-signaling are involved (Burgdorf et al. 2008). Still, it remains to be determined whether and what ER chaperones can mediate efficient peptide exchange in an endosomal environment. Endosome-to-cytosol-to-endosome pathway relies on a peptide-trimming enzyme designated insulin-regulated aminopeptidase (IRAP or oxytocinase) localized within endosomes of murine inflammatory MoDC (Segura et al. 2009) and steady-state CD8αα$^+$ and CD8αα$^-$ DCs. Steady-state CD8αα$^+$ DC isolated from the spleen was the most abundant for IRAP and was identified to be the most effective in cross-presentation of both soluble and particulate antigens (Weimershaus et al. 2012). In accordance, mice deficient for IRAP exhibited reduced cross-presentation of phagocytosed antigens or of OVA endocytosed via TLR-2 and MR (Saveanu et al. 2009). Optimal IRAP pH ranging 5–8 assures its activity within EE and phagosomes. IRAP is associated with Rab14, a small GTP-ase that drives IRAP vesicle recruitment to the EE (Reed et al. 2013; Weimershaus et al. 2012).

Vacuolar Pathway

In addition to previous pathways, cross-presentation independent of antigen degradation in the cytosol was identified in vitro as the pathway insensitive to proteasome and TAP inhibitors. The vacuolar pathway was identified in vivo as the pathway responsible for induction of antigen-specific CD8$^+$ T cells using mice with inactivated genes involved in cross-presentation (Table 2). The name vacuolar cross-presentation pathway (Fig. 3; Table 1) was chosen to stress its independence on typically cytosol-localized proteasome. Endosomal or endolysosomal proteolysis has been attributed to the cysteine proteases (Campbell et al. 2000), particularly cathepsin S (Cat S) (Shen et al. 2004), as this cross-presentation is inhibitable by cysteine-protease inhibitors such as leupeptin, E64, and ZFA-FMK (Campbell et al. 2000) and almost abolished in Cat S-deficient DCs (Shen et al. 2004). In contrast, pepstatin A, an acid protease inhibitor, had little effect on the vacuolar cross-presentation at least for some antigens (Campbell et al. 2000). Cat S could cleave proteins even at neutral pH which is beneficial for cross-presentation as discussed above (Pillay et al. 2002). Generated peptides replace those on MHC-I molecules recycling to EE from the cell surface (Gromme et al. 1999; Schirmbeck et al. 1995) or MHC-I coming to

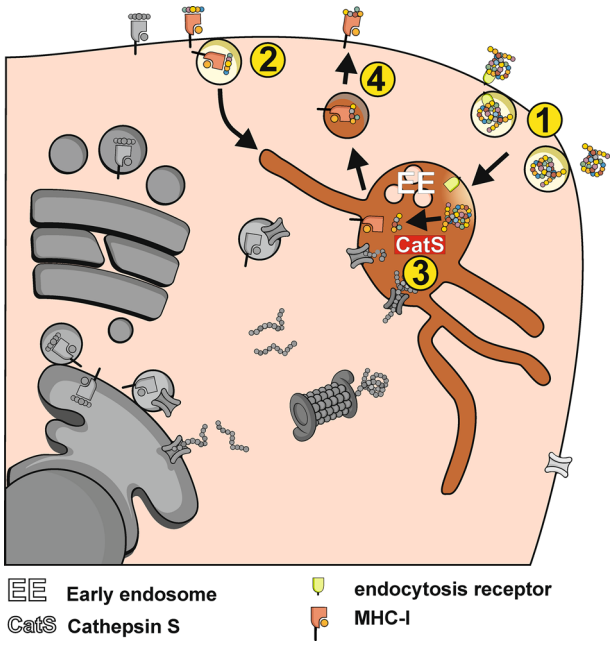

Fig. 3 Vacuolar pathway of cross-presentation. Exogenous protein is engulfed by a DC (*1*). Recycled molecules of MHC-I coming from the cell surface (*2*) fuse with the endosome. Protease cathepsin S present in the endosome cleaves the protein into short peptides that are loaded onto the MHC-I molecules (*3*). Peptides bound on the MHC-I molecules are transferred to the cell surface (*4*)

endosomes from ER by ER-originating vesicles. MHC-I/peptide complex is finally transported by EE-associated vesicular transport to the cell surface. The vacuolar pathway seems to be important for cross-presentation of particulate and cell-associated antigens such as those complexed with poly(lactic-coglycolic acid) beads, proteins associated with *E. coli*, viral proteins including influenza nucleoprotein and polymerase, virus-like particles, but it is active also for non-corpuscular antigen as exemplified by the OVA antigen or tumor antigen-originating peptide fragments associated with human hsp (Bachmann et al. 1995; Bendz et al. 2007; Campbell et al. 2000; Chen and Jondal 2004; Ruedl et al. 2002; Shen et al. 2004; Song and Harding 1996; Stober et al. 2002; Wick and Pfeifer 1996).

Gap Junction Pathway

Gap junctions serve for intercellular communication allowing various molecules exchange. The gap junction tunnels are highly ordered structures consisting of connexin (the most broadly expressed is Cx43) forming hexamers called connexons (Handel et al. 2007; Pang et al. 2009). The gap junction communication was observed in vitro both in murine DCs (Matsue et al. 2006) or human monocytes or DCs (Mendoza-Naranjo et al. 2007; Neijssen

et al. 2005). Neijssen et al. (2005) set up a human multicellular tissue culture that included a squamous cell carcinoma cell line as an antigen source, activated monocytes as presenting cells, and $CD8^+$ T cells as antigen sensors. There, the SCC line was transfected to express connexin 43 (Cx43), which was also expressed by the monocytes (activated by interferon-γ and tumor necrosis factor (TNF)-α). With the $CD8^+$ T cells serving as antigen-specific surface MHC-I/peptide sensors, the authors confirmed gap junction Cx43-mediated transport of peptides smaller than 1800 Da (corresponding to less than 10 amino acids) (Neijssen et al. 2005). Furthermore, they confirmed subsequent presentation of such peptides on MHC-I of APCs (Neijssen et al. 2005). Thus, the peptides obtained from adjacent cell could be cross-presented on MHC-I molecules of DCs or activated monocytes (Fig. 4; Table 1) (Handel et al. 2007; Pang et al. 2009). Although many tumor cells inactivate their gap junctions due to down-regulation of the Cx43 by ras, src, and neu oncogenes (Esinduy et al. 1995; Goldberg and Lau 1993), as well as many viruses (HSV-2, HPV-16), the gap junction-mediated peptide or small molecules transport still could take place, as demonstrated for melanoma cells with substantially reduced Cx43 expression (Benlalam et al. 2009). For future utilization of gap junctions in therapeutic anti-cancer vaccines, an important observation was made recently showing that infection with *Salmonella* can induce up-regulation of Cx43, both in human and murine melanoma cells (Saccheri et al. 2010).

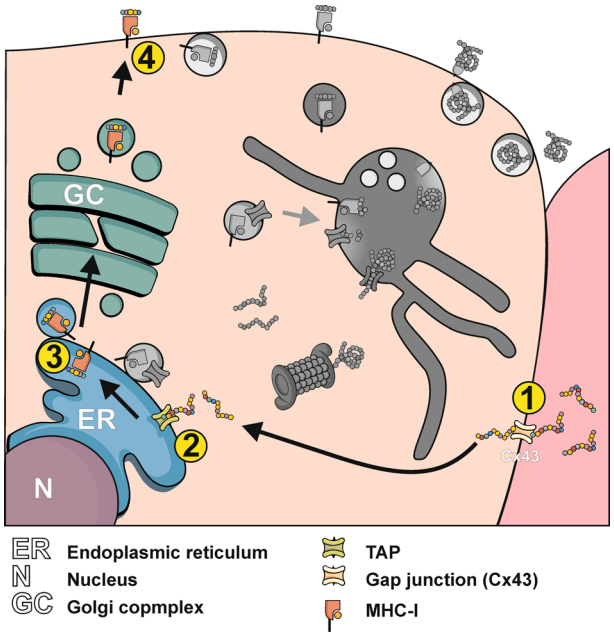

Fig. 4 Gap junction pathway of cross-presentation. Small molecule such as a peptide reaches the cytosol of an APC through the gap junction (*1*). The peptide is transferred inside the ER (*2*). Peptide loaded on MHC-I is targeted to the cell surface (*3*, *4*)

Cross-Presentation Applicability in Vaccine Design

Vaccines, currently in clinical use, contain adjuvants stimulating predominantly Th2 type of immunity and production of specific antibodies, mainly of neutralizing isotypes (Kaufmann 2004) This kind of immune response is effective only against some types of pathogens and has very limited effect against tumors and some viruses, where activation of cellular immunity is required. One of the main objectives of the current vaccine research is the development of approaches leading to elicitation of antigen-specific $CD8^+$ T cell responses including CTL activation. Knowledge about the cross-presentation principles seems to be crucial for effective CTL induction. Here, we will discuss some approaches employing hsp for induction of antigen-specific $CD8^+$ T cell response.

Heat Shock Proteins

The involvement of a class of cellular proteins in tumor-specific immunity was suggested in 1986 after successful protective immunization with a biochemically fractionated proteins isolated from murine methylcholanthrene-induced sarcoma, the predominant one being a 96 kDa protein (Srivastava and Das 1984; Srivastava et al. 1986). Subsequent research identified the 96 kDa protein in that fraction as the glucose-regulated protein Grp94, also called glycoprotein 96 (gp96) (Maki et al. 1990), complexed with tumor-specific peptides. Immunization experiments utilizing gp96 and later hsp70 preparations purified under native (non-denaturing) conditions from tumor cells induced cytotoxic immunity against the same type of tumor, from which the proteins were isolated (Tamura et al. 1997; Udono and Srivastava 1993). In contrast, hsp preparations obtained by the same method from normal, non-tumor tissue were not effective (Tamura et al. 1997). Similar effects were observed when preparations of another hsp superfamily member, hsp90, were tested. However, the immune response only reached about 10 % of values observed with hsp70 or gp96 preparations (Udono and Srivastava 1994). The hsp preparations could be separated into hsp and client peptides with molecular mass 1–5 kDa and these peptides, but not hsp per se, represent the tumor antigens recognized by immunization-elicited tumor-specific immunity. It was proposed that hsp-peptide complexes are internalized by APC followed by intracellular processing of client peptides and display of their fragments on MHC-I for activation of specific CTL response. In other words, the hsps were proposed as carriers enabling the cross-presentation of client peptides (Li and Srivastava 1993; Udono et al. 1994; Udono and Srivastava 1993, 1994).

Heat shock proteins belong to the super family of stress proteins a highly heterogeneous but evolutionary conserved group of proteins that can be divided into families according to molecular weight: small hsp (m.w. 12–43 kDa), hsp40, hsp60, hsp70, hsp90, and large hsps (m.w. 100–110 kDa) and include also glucose-regulated proteins, ubiquitin, and lectin chaperone—calnexin and calreticulin (David et al. 1993; Nauseef et al. 1995; Raska and Weigl 2005; Wada et al. 1995; Weigl et al. 1999). Heat shock proteins contribute to protection of the cell from external or internal stress factors, such as high temperature, osmotic stress, UV irradiation, inflammation, intense and cellular proliferation, which induce overexpression of several hsp members. Heat shock proteins are involved in the prevention of the nascent protein aggregations and in their proper folding (hsp60, hsp70, hsp40). Some hsps act as chaperones of intracellular signaling proteins, keeping them in an substrate-grabbable state (hsp90, hsp40, hsp70), others are involved in the protein degradation (ubiquitin, hsp104), and some hsps contribute to antigen presentation on MHC-I (calreticulin, calnexin, hsp70, hsp90, and gp96) (Berwin et al. 2002; Biswas et al. 2006; Doody et al. 2004; Ichiyanagi et al. 2010; Imai et al. 2011; Kato et al. 2012; Newman et al. 2014) and MHC-II molecules (Kropshofer et al. 1997; Matsutake et al. 2010).

In the vaccine research area, hsps of bacterial or parasite origin have been employed as protective antigens (Radwanska et al. 2000; Raska et al. 2004, 2005, 2008; Svanholm et al. 2000). In parallel, autologous hsp, as introduced above, has attracted attention as adjuvant molecules for the induction of antitumor immunity (Berwin et al. 2002; Biswas et al. 2006; Li and Srivastava 1993; Tamura et al. 1997; Udono et al. 1994; Udono and Srivastava 1993, 1994) and later also for induction of antiviral immunity (Hauser et al. 2004; Jazi et al. 2012; Krupka et al. 2015; Li et al. 2007; Meshkat et al. 2011; Rasoli et al. 2010). Furthermore, several studies demonstrated adjuvant activity also for a bacterial hsp such as hsp70 from *Mycobacterium tuberculosis* or DnaK from *E. coli* (Chen et al. 2004; Cheng et al. 2001; Tobian et al. 2004a, b). Adjuvant activity of hsp could be divided into two parts (Fig. 5). First, hsps facilitate receptor-mediated endocytosis of associated (complexed) antigens which are important for MHC-I cross-presentation of antigen-derived peptides, as was confirmed for example on peritoneal macrophages pulsed by native gp96 purified from cells overexpressing Vesicular stomatitis virus nucleocapsid N1 or β-galactosidase (Arnold et al. 1995; Suto and Srivastava 1995). Second, hsps activate APCs leading to the migration of APC to draining lymph nodes, expression of activation markers, and production of cytokines due to the activation of various intracellular pathways. For example, detail studies performed by Nicchitta's team focused on gp96 and murine BMDC confirmed that gp96 stimulates BMDC to enhanced expression of CD40 and CD86 receptors and proinflammatory cytokines IL-6, IL-12 p40/70 (Warger et al. 2006) in association with the activation of intracellular ERK signaling pathway (Reed et al. 2003). Other pathways identified to be involved in the response to gp96 include NF-κB, p38, and JNK (Basu et al. 2000; Bethke et al. 2002; Kuppner et al. 2001; Vabulas et al. 2002). Intensity and quality of DC activation by hsps, nevertheless, are dependent at least in some cases on the presence of LPS (endotoxin) which is actively copurified with various hsp proteins and which act upon DC synergistically with hsp (Warger et al. 2006), as will be discussed in detail further.

Hsp Contributes to Cross-Presentation of Hsp-Complexed Peptides and Facilitates Activation of DC

The process of internalization of hsp-complexed peptides, as the first step during representation of peptides on MHC, was explained by incubating monocytic and DC lines with hsp70 and gp96 labeled with gold particles. Electron microscopy revealed internalization of particles through receptor-mediated endocytosis (clathrin-coated vesicles) (Arnold-Schild et al. 1999) similarly to results obtained using fluorescently labeled gp96 (Grp94) (Wassenberg et al. 1999). Several cell surface receptors for hsp, mediating endocytosis and contributing to cross-presentation of hsp-associated peptides on MHC molecules, have been identified since first report in 2000 describing interaction of gp96 with CD91, a member of scavenger receptors family (Basu et al. 2001; Binder et al. 2000, 2002; Srivastava 2002). They include intercellular signaling receptor CD40 (Becker et al. 2002) and another scavenger receptor LOX-1 (Delneste 2004), scavenger receptor-A (SR-A), and SREC-1 (Berwin et al. 2003).

In parallel to facilitating endocytosis, hsp60, hsp70, hsp90, gp96 and, to a lesser extent, calreticulin activate DCs through additional set of cell surface receptors leading to the secretion of inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-12, and GM-CSF), nitric oxide, up-regulation of cell surface maturation markers (MHC-II, CD83, CD86, and CD40), and migration of DCs to draining lymph nodes; for details, see (Basu et al. 2000; Binder et al. 2004; Millar et al. 2003; Singh-Jasuja et al. 2000b; Todryk et al. 1999; Wallin et al. 2002; Zheng et al. 2001). Several DC-activating hsp receptors were identified including CD36, CD11b, SREC-1, SR-A, stabilin-1, and dectin-1 (Calderwood et al. 2007a, b; Kol et al. 2000; Murshid et al. 2011; Panjwani et al. 2000; Singh-Jasuja et al. 2000a). Scavenger receptor CD36, the second identified hsp receptor, is thought to be involved in endocytosis too, but direct evidence has not been provided yet.

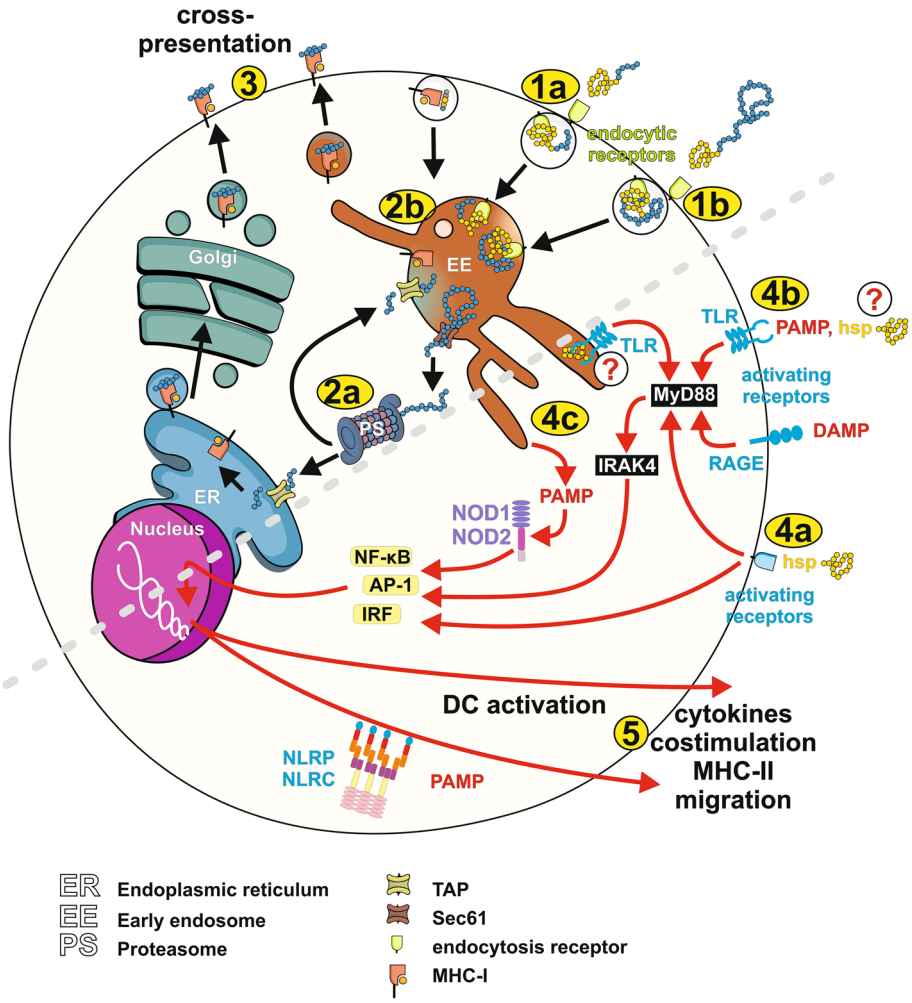

Fig. 5 Involvement of hsp proteins in cross-priming. Peptide (*1a*) or antigen (*1b*) (*blue chain*) complexed to hsp (*yellow chain*) is engulfed by CD91, LOX-1, CD36, or CD40 receptor-mediated endocytosis and antigen is processed either by cytosol (proteasome- and TAP-dependent) (*2a*) or vacuolar (*2b*) pathways leading to the representation of peptide on MHC-I on DC surface (*3*). Cross-presentation pathway connections are shown using *black arrows*. In parallel, hsp could activate DC through binding to cell surface-localized activating receptors (CD36, CD11b, SREC-1, SR-A, stabilin-1, dectin-1) (*4a*) or other surface PAMP or DAMP receptors (TLR, RAGE, etc.) (*4b*), although TLR involvement is still controversial. Endocytosed hsp could stimulate DC through intracellular TLR or other PAMP or DAMP receptors (*4c*), although this activation is also disputable. Although individual TLR involvement seems to be dispensable for cross-priming, subsequent signaling molecules MyD88 and IRAK4 are necessary. In addition to hsp, natural contaminants such as LPS or flagellin act as PAMPs which could activate DC through surface of intracellular receptors (*4b, c*). Selected DC activation interactions are represented by *red arrows*. *AP 1* activator protein 1, *DAMP* damage-associated molecular patterns, *IRAK4* interleukin-1 receptor associated kinase 4, *IRF* interferon regulatory factor, *MyD88* myeloid differentiation primary response gene 88, *NLRP* NALP: NACHT-LRR- PYD-containing protein, *NOD* nucleotide-binding oligomerization domain-containing protein, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *PAMP* pathogen-associated molecular pattern, *TLR* toll-like receptor

Additionally, three receptors involved in pathogen-associated molecular patterns (PAMP) signaling, CD14, TLR-2, and TLR-4, were reported to be involved in the activation of APCs by hsp (Ohashi et al. 2000; Zanin-Zhorov et al. 2003). Nevertheless, there is still an open discussion about hsp interaction with above receptors raised by frequently detected contaminations of hsp preparations with PAMPs, especially with LPS or flagellin both originating from *E. coli* cells, used for recombinant antigens production, or acquired during protein purification procedures. LPS seems to be the most serious one, due to its binding to the hydrophobic moieties of hsp (Gao and Tsan 2003a, b; Ye and Gan 2007). Using highly purified hsp70 and hsp60 preparations with LPS concentration below 1 EU/mg (measured by limulus amoebocyte lysate assay) of protein when proposing 10–30 μg of hsp preparation per dose, two groups demonstrated that hsps per se are not involved in CD14- or TLR-4-mediated signaling on monocytes and DCs (Bausinger et al. 2002; Gao and Tsan 2003a, b). On the other hand, the LPS at concentration

below 1 EU/mg has been accepted to be no longer inducing cytokine secretion in human monocytes and monocyte-derived DCs as well. Detailed analyses of gp96-mediated stimulation of murine monocyte-derived DCs (MDDC) in vitro identified substantial synergy between gp96 and LPS. Although LPS-free gp96 preparation at final concentration 20 μg of protein per ml or LPS at 0.25 ng/ml [corresponding approximately to 1.25 EU/ml (Zachova et al. 2009)] does not substantially activate MDDC, mixture of both gp96 and LPS (at the same final concentrations) induced substantial activation of MDDC (Warger et al. 2006). It was confirmed that, although cross-presentation and antigen-specific T cell activation develop in the absence of innate signaling through TLR-2, -3, -4, -7, and -9, mice lacking MyD88 or IRAK4 are unable to reject the tumor cells (Mizukami et al. 2012). Therefore, although innate signaling intracellularly transmitted by MyD88 or IRAK4 is important for activation of DCs and effective cross-priming, the initiation of MyD88, IRAK4 pathway could be achieved even without direct contribution of the above-specified TLRs, suggesting involvement of other extracellular or intracellular receptors from broader family of danger-associated molecular patterns signaling through MyD88 or IRAK4. Nevertheless, to accurately evaluate the immunological effects of hsp-antigens, controlling the parallel activation of DCs by concurrent LPS or other contaminations is necessary. For example, murine cross-presenting DC population ($CD8\alpha\alpha^+$) or human $BDCA\text{-}3^+$ cells after licensing by TLR ligands poly I:C could acquire full cross-priming capacity (Jongbloed et al. 2010; Poulin et al. 2010). Therefore, the high purity of the hsp preparations with maximal reduction of the immunologically active moieties in final preparation is needed for further experiments.

The cross-presentation mechanism involved in the processing of hsp-complexed antigens depends on the factors including size of complexed antigen, or N' versus C' orientation of antigen to hsp when recombinant fusogens are prepared, as an example of reverse vaccinology strategy. Tumor antigen Melan-A (MART1)- or tyrosinase-derived peptides complexed to recombinant human hsp70 were reported to be cross-presented in TAP-independent and *P. aeruginosa* exotoxin A- or endosomal acidification-insensitive manner indicating that both peptides are processed by vacuolar cross-presentation pathway without the need for extensive peptide processing before binding to MHC-I (Bendz et al. 2007). In contrast, the cross-presentation of OVA peptides from full length OVA fused to hsp70 is TAP dependent (Ichiyanagi et al. 2010; Imai et al. 2011). The TAP dependence is probably associated with requirement for OVA processing inside DC before presentation on MHC-I (Mizukami et al. 2012). In the case of recombinant fusion hsp-antigens, the cross-presentation of the antigen-derived peptides depends on the orientation of hsp and antigen subunits. If C'-terminal processing of the peptide for MHC-I presentation is needed, the antigen should be targeted to the proteasome as a part of cytosolic cross-presentation pathways whereas if C'-terminal processing is not required, the transfer of peptide from hsp to MHC-I can occur through vacuolar pathway (Bolhassani and Rafati 2008; Castellino et al. 2000; More et al. 1999).

Although autologous hsps are mostly reported as the MHC-I presentation-facilitating partners, they could assist also to MHC-II presentation of coupled peptides. Likewise, bacterial hsps such as *E. coli* DnaK and *M. tuberculosis* hsp70 have been identified to contribute in both MHC-I and MHC-II presentation of the associated peptides (Tobian et al. 2004b). Peptides presented by MHC-I are processed by cross-presentation pathways, whereas peptides presented by MHC-II are processed by canonical MHC-II pathway. Nevertheless, although gp96, for example, could bind in vivo both MHC-I and MHC-II restricted epitopes, it selectively primes $CD8^+$ T cell effector functions (Doody et al. 2004).

Clinical Applications of Hsps in Immunizations

So far a number of phase I to III clinical trials tested immunization of cancer patients with autologous tumor-derived hsp-peptide preparations or tumor-derived peptide preparations complexed with recombinant hsp70 or gp96. Studies focused on safety and effectiveness of particular formulation in therapeutic settings in patients with breast cancer, melanoma, glioma, cervical cancer, kidney cancer, pancreatic cancer, soft tissue sarcoma, leukemia, and lymphoma (Bolhassani et al. 2008; Kim et al. 2005; Li et al. 2005; Maki et al. 2007; Oki et al. 2007; Pilla et al. 2006; See et al. 2011; Testori et al. 2008; Wood et al. 2008). In the April 2008, patient autologous vaccine oncophage (vitespen; formerly HSPPC-96), based on affinity concanavalin A chromatography-purified gp96-peptide fraction from patient tumor tissue subsequently repurified on diethyl-aminoethyl ion-exchange chromatography (Jonasch et al. 2008), was approved in Russia for adjuvant therapy of renal cell carcinoma at intermediate-risk for disease recurrence. Neither food and drug administration (FDA) USA nor European medicines Agency approved Oncophage although since 2009 FDA granted orphan drug designation to Oncophage for the treatment of glioma, metastatic melanoma, and renal cell carcinoma.

Deeper understanding of the immunomodulatory effects of hsps, improved vaccine formulations, and combinations with other potentiating agents, such as cytokines or PAMPs, will be necessary to improve the efficacy of hsp-

based therapeutics before they can enter human medical practice.

Acknowledgments This paper was supported by grants LF_2014_020 and LF_2015_009 (Palacky University, Olomouc, Czech Republic) and grant CZ.1.07/2.3.00/20.0164 European Social Fund (Ministry of Education, Youth, and Sport, Czech Republic).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Accapezzato D, Visco V, Francavilla V et al (2005) Chloroquine enhances human CD8(+) T cell responses against soluble antigens in vivo. J Exp Med 202:817–828

Ackerman AL, Kyritsis C, Tampe R et al (2003) Early phagosomes in dendritic cells form a cellular compartment sufficient for cross presentation of exogenous antigens. Proc Natl Acad Sci USA 100:12889–12894

Ackerman AL, Kyritsis C, Tampe R et al (2005) Access of soluble antigens to the endoplasmic reticulum can explain cross-presentation by dendritic cells. Nat Immunol 6:107–113

Ackerman AL, Giodini A, Cresswell P (2006) A role for the endoplasmic reticulum protein retrotranslocation machinery during crosspresentation by dendritic cells. Immunity 25:607–617

Andrieu M, Desoutter JF, Loing E et al (2003) Two human immunodeficiency virus vaccinal lipopeptides follow different cross-presentation pathways in human dendritic cells. J Virol 77:1564–1570

Arnold D, Faath S, Rammensee HG et al (1995) Cross-priming of minor histocompatibility antigen-specific cytotoxic T-cells upon immunization with the heat-shock protein gp96. J Exp Med 182:885–889

Arnold-Schild D, Hanau D, Spehner D et al (1999) Cutting edge: receptor-mediated endocytosis of heat shock proteins by professional antigen-presenting cells. J Immunol 162:3757–3760

Asea A, Rehli M, Kabingu E et al (2002) Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. J Biol Chem 277:15028–15034

Bachmann MF, Oxenius A, Pircher H et al (1995) TAP1-independent loading of class-I molecules by exogenous viral-proteins. Eur J Immunol 25:1739–1743

Bai D, del Corsso C, Srinivas M et al (2006) Block of specific gap junction channel subtypes by 2-aminoethoxydiphenyl borate (2-APB). J Pharmacol Exp Ther 319:1452–1458

Baker K, Qiao SW, Kuo TT et al (2011) Neonatal Fc receptor for IgG (FcRn) regulates cross-presentation of IgG immune complexes by CD8-CD11b + dendritic cells. Proc Natl Acad Sci USA 108:9927–9932

Basha G, Omilusik K, Chavez-Steenbock A et al (2012) A CD74-dependent MHC class I endolysosomal cross-presentation pathway. Nat Immunol 13:237–245

Basu S, Binder RJ, Suto R et al (2000) Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. Int Immunol 12:1539–1546

Basu S, Binder RJ, Ramalingam T et al (2001) CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. Immunity 14:303–313

Bausinger H, Lipsker D, Ziylan U et al (2002) Endotoxin-free heat-shock protein 70 fails to induce APC activation. Eur J Immunol 32:3708–3713

Becker T, Hartl FU, Wieland F (2002) CD40, an extracellular receptor for binding and uptake of Hsp70-peptide complexes. J Cell Biol 158:1277–1285

Bendz H, Ruhland SC, Pandya MJ et al (2007) Human heat shock protein 70 enhances tumor antigen presentation through complex formation and intracellular antigen delivery without innate immune signaling. J Biol Chem 282:31688–31702

Benke D, Kruger T, Lang A et al (2006) Inclusion of Brefeldin A during dendritic cell isolation allows in vitro detection of cross-presented self-antigens. J Immunol Methods 310:12–19

Benlalam H, Jalil A, Hasmim M et al (2009) Gap junction communication between autologous endothelial and tumor cells induce cross-recognition and elimination by specific CTL. J Immunol 182:2654–2664

Berwin B, Rosser MFN, Brinker KG et al (2002) Transfer of GRP94(Gp96)-associated peptides onto endosomal MHC class I molecules. Traffic 3:358–366

Berwin B, Hart JP, Rice S et al (2003) Scavenger receptor-A mediates gp96/GRP94 and calreticulin internalization by antigen-presenting cells. EMBO J 22:6127–6136

Bethke K, Staib F, Distler M et al (2002) Different efficiency of heat shock proteins (HSP) to activate human monocytes and dendritic cells: superiority of HSP60. J Immunol 169:6141–6148

Bevan MJ (1976) Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. J Exp Med 143:1283–1288

Binder RJ, Han DK, Srivastava PK (2000) CD91: a receptor for heat shock protein gp96. Nat Immunol 1:151–155

Binder RJ, Kumar SK, Srivastava PK (2002) Naturally formed or artificially reconstituted non-covalent alpha2-macroglobulin-peptide complexes elicit CD91-dependent cellular immunity. Cancer Immun 2:16

Binder RJ, Vatner R, Srivastava P (2004) The heat-shock protein receptors: some answers and more questions. Tissue Antigens 64:442–451

Biswas C, Sriram U, Ciric B et al (2006) The N-terminal fragment of GRP94 is sufficient for peptide presentation via professional antigen-presenting cells. Int Immunol 18:1147–1157

Bolhassani A, Rafati S (2008) Heat-shock proteins as powerful weapons in vaccine development. Expert Rev Vaccines 7:1185–1199

Bolhassani A, Zahedifard F, Taghikhani M et al (2008) Enhanced immunogenicity of HPV16E7 accompanied by Gp96 as an adjuvant in two vaccination strategies. Vaccine 26:3362–3370

Burgdorf S, Kurts C (2008) Endocytosis mechanisms and the cell biology of antigen presentation. Curr Opin Immunol 20:89–95

Burgdorf S, Lukacs-Kornek V, Kurts C (2006) The mannose receptor mediates uptake of soluble but not of cell-associated antigen for cross-presentation. J Immunol 176:6770–6776

Burgdorf S, Kautz A, Bohnert V et al (2007) Distinct pathways of antigen uptake and intracellular routing in CD4 and CD8 T cell activation. Science 316:612–616

Burgdorf S, Scholz C, Kautz A et al (2008) Spatial and mechanistic separation of cross-presentation and endogenous antigen presentation. Nat Immunol 9:558–566

Calderwood SK, Mambula SS, Gray PJ Jr (2007a) Extracellular heat shock proteins in cell signaling and immunity. Ann NY Acad Sci 1113:28–39

Calderwood SK, Theriault J, Gray PJ et al (2007b) Cell surface receptors for molecular chaperones. Methods 43:199–206

Caminschi I, Proietto AI, Ahmet F et al (2008) The dendritic cell subtype-restricted C-type lectin Clec9A is a target for vaccine enhancement. Blood 112:3264–3273

Campbell DJ, Serwold T, Shastri N (2000) Bacterial proteins can be processed by macrophages in a transporter associated with antigen processing-independent, cysteine protease-dependent manner for presentation by MHC class I molecules. J Immunol 164:168–175

Castellino F, Boucher PE, Eichelberg K et al (2000) Receptor-mediated uptake of antigen/heat shock protein complexes results in major histocompatibility complex class I antigen presentation via two distinct processing pathways. J Exp Med 191:1957–1964

Celli J, de Chastellier C, Franchini DM et al (2003) Brucella evades macrophage killing via VirB-dependent sustained interactions with the endoplasmic reticulum. J Exp Med 198:545–556

Chatterjee B, Smed-Sorensen A, Cohn L et al (2012) Internalization and endosomal degradation of receptor-bound antigens regulate the efficiency of cross presentation by human dendritic cells. Blood 120:2011–2020

Chen LY, Jondal M (2004) Alternative processing for MHC class I presentation by immature and CpG-activated dendritic cells. Eur J Immunol 34:952–960

Chen K, Lu J, Wang L et al (2004) Mycobacterial heat shock protein 65 enhances antigen cross-presentation in dendritic cells independent of Toll-like receptor 4 signaling. J Leukoc Biol 75:260–266

Cheng WF, Hung CF, Chai CY et al (2001) Enhancement of sindbis virus self-replicating RNA vaccine potency by linkage of Mycobacterium tuberculosis heat shock protein 70 gene to an antigen gene. J Immunol 166:6218–6226

Coffey JW, De Duve C (1968) Digestive activity of lysosomes. I. The digestion of proteins by extracts of rat liver lysosomes. J Biol Chem 243:3255–3263

Craiu A, Gaczynska M, Akopian T et al (1997) Lactacystin and clasto-lactacystin beta-lactone modify multiple proteasome beta-subunits and inhibit intracellular protein degradation and major histocompatibility complex class I antigen presentation. J Biol Chem 272:13437–13445

Cresswell P, Bangia N, Dick T et al (1999) The nature of the MHC class I peptide loading complex. Immunol Rev 172:21–28

David V, Hochstenbach F, Rajagopalan S et al (1993) Interaction with newly synthesized and retained proteins in the endoplasmic reticulum suggests a chaperone function for human integral membrane protein IP90 (calnexin). J Biol Chem 268:9585–9592

Delamarre L, Holcombe H, Mellman I (2003) Presentation of exogenous antigens on major histocompatibility complex (MHC) class I and MHC class II molecules is differentially regulated during dendritic cell maturation. J Exp Med 198:111–122

Delamarre L, Pack M, Chang H et al (2005) Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. Science 307:1630–1634

Delneste Y (2004) Scavenger receptors and heat-shock protein-mediated antigen cross-presentation. Biochem Soc Trans 32:633–635

den Haan JM, Lehar SM, Bevan MJ (2000) CD8(+) but not CD8(−) dendritic cells cross-prime cytotoxic T cells in vivo. J Exp Med 192:1685–1696

Desjardins M (1995) Biogenesis of phagolysosomes—the kiss and run hypothesis. Trends Cell Biol 5:183–186

Desjardins M, Huber LA, Parton RG et al (1994) Biogenesis of phagolysosomes proceeds through a sequential series of interactions with the endocytic apparatus. J Cell Biol 124:677–688

Donaldson JG, Porat-Shliom N, Cohen LA (2009) Clathrin-independent endocytosis: a unique platform for cell signaling and PM remodeling. Cell Signal 21:1–6

Doody AD, Kovalchin JT, Mihalyo MA et al (2004) Glycoprotein 96 can chaperone both MHC class I- and class II-restricted epitopes for in vivo presentation, but selectively primes CD8 + T cell effector function. J Immunol 172:6087–6092

Dudziak D, Kamphorst AO, Heidkamp GF et al (2007) Differential antigen processing by dendritic cell subsets in vivo. Science 315:107–111

Edwards AD, Diebold SS, Slack EMC et al (2003) Toll-like receptor expression in murine DC subsets: lack of TLR7 expression by CD8 alpha(+) DC correlates with unresponsiveness to imidazoquinolines. Eur J Immunol 33:827–833

Esinduy CB, Chang CC, Trosko JE et al (1995) In vitro growth inhibition of neoplastically transformed cells by non-transformed cells: requirement for gap junctional intercellular communication. Carcinogenesis 16:915–921

Fonteneau JF, Kavanagh DG, Lirvall M et al (2003) Characterization of the MHC class I cross-presentation pathway for cell-associated antigens by human dendritic cells. Blood 102:4448–4455

Fujiwara T, Oda K, Yokota S et al (1988) Brefeldin A causes disassembly of the Golgi complex and accumulation of secretory proteins in the endoplasmic reticulum. J Biol Chem 263:18545–18552

Galibert L, Diemer GS, Liu Z et al (2005) Nectin-like protein 2 defines a subset of T-cell zone dendritic cells and is a ligand for class-I-restricted T-cell-associated molecule. J Biol Chem 280:21955–21964

Gao BC, Tsan MF (2003a) Endotoxin contamination in recombinant human heat shock protein 70 (Hsp70) preparation is responsible for the induction of tumor necrosis factor a release by murine macrophages. J Biol Chem 278:174–179

Gao BC, Tsan MF (2003b) Recombinant human heat shock protein 60 does not induce the release of tumor necrosis factor alpha from murine macrophages. J Biol Chem 278:22523–22529

Garulli B, Stillitano MG, Barnaba V et al (2008) Primary CD8 + T-cell response to soluble ovalbumin is improved by chloroquine treatment in vivo. Clin Vaccine Immunol 15:1497–1504

Ghose P, Ali AQ, Fang R et al (2011) The interaction between IL-18 and IL-18 receptor limits the magnitude of protective immunity and enhances pathogenic responses following infection with intracellular bacteria. J Immunol 187:1333–1346

Giodini A, Rahner C, Cresswell P (2009) Receptor-mediated phagocytosis elicits cross-presentation in nonprofessional antigen-presenting cells. Proc Natl Acad Sci USA 106:3324–3329

Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 82:373–428

Goldberg GS, Lau AF (1993) Dynamics of connexin43 phosphorylation in pp60v-src-transformed cells. Biochem J 295(Pt 3):735–742

Grant BD, Donaldson JG (2009) Pathways and mechanisms of endocytic recycling. Nat Rev Mol Cell Biol 10:597–608

Gromme M, Uytdehaag FG, Janssen H et al (1999) Recycling MHC class I molecules and endosomal peptide loading. Proc Natl Acad Sci USA 96:10326–10331

Guan X, Wilson S, Schlender KK et al (1996) Gap-junction disassembly and connexin 43 dephosphorylation induced by 18 beta-glycyrrhetinic acid. Mol Carcinog 16:157–164

Guermonprez P, Saveanu L, Kleijmeer M et al (2003) ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. Nature 425:397–402

Gurnani K, Kennedy J, Sad S et al (2004) Phosphatidylserine receptor-mediated recognition of archaeosome adjuvant promotes endocytosis and MHC class I cross-presentation of the entrapped antigen by phagosome-to-cytosol transport and classical processing. J Immunol 173:566–578

Handel A, Yates A, Pilyugin SS et al (2007) Gap junction-mediated antigen transport in immune responses. Trends Immunol 28:463–466

Hauser H, Shen L, Gu QL et al (2004) Secretory heat-shock protein as a dendritic cell-targeting molecule: a new strategy to enhance the potency of genetic vaccines. Gene Ther 11:924–932

Hershko A, Leshinsky E, Ganoth D et al (1984) ATP-dependent degradation of ubiquitin-protein conjugates. Proc Natl Acad Sci USA 81:1619–1623

Holubova J, Kamanova J, Jelinek J et al (2012) Delivery of large heterologous polypeptides across the cytoplasmic membrane of antigen-presenting cells by the Bordetella RTX hemolysin moiety lacking the adenylyl cyclase domain. Infect Immun 80:1181–1192

Houde M, Bertholet S, Gagnon E et al (2003) Phagosomes are competent organelles for antigen cross-presentation. Nature 425:402–406

Huysamen C, Willment JA, Dennehy KM et al (2008) CLEC9A is a novel activation C-type lectin-like receptor expressed on BDCA3(+) dendritic cells and a subset of monocytes. J Biol Chem 283:16693–16701

Ichiyanagi T, Imai T, Kajiwara C et al (2010) Essential Role of endogenous heat shock protein 90 of dendritic cells in antigen cross-presentation. J Immunol 185:2693–2700

Ikeuchi N, Futami J, Hosoi A et al (2010) Efficient cross-presentation of soluble exogenous antigens introduced into dendritic cells using a weak-based amphiphilic peptide. Biochem Biophys Res Commun 392:217–222

Imai J, Hasegawa H, Maruya M et al (2005) Exogenous antigens are processed through the endoplasmic reticulum-associated degradation (ERAD) in cross-presentation by dendritic cells. Int Immunol 17:45–53

Imai T, Kato Y, Kajiwara C et al (2011) Heat shock protein 90 (HSP90) contributes to cytosolic translocation of extracellular antigen for cross-presentation by dendritic cells. Proc Natl Acad Sci USA 108:16363–16368

Janda J, Schoneberger P, Skoberne M et al (2004) Cross-presentation of Listeria-derived CD8 T cell epitopes requires unstable bacterial translation products. J Immunol 173:5644–5651

Jazi MH, Dabaghian M, Tebianian M et al (2012) In vivo electroporation enhances immunogenicity and protection against influenza A virus challenge of an M2e-HSP70c DNA vaccine. Virus Res 167:219–225

Jonasch E, Wood C, Tamboli P et al (2008) Vaccination of metastatic renal cell carcinoma patients with autologous tumour-derived vitespen vaccine: clinical findings. Br J Cancer 98:1336–1341

Jongbloed SL, Kassianos AJ, McDonald KJ et al (2010) Human CD141 + (BDCA-3) + dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. J Exp Med 207:1247–1260

Jung S, Unutmaz D, Wong P et al (2002) In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. Immunity 17:211–220

Kato Y, Kajiwara C, Ishige I et al (2012) HSP70 and HSP90 differentially regulate translocation of extracellular antigen to the cytosol for cross-presentation. Autoimmune Dis 2012:745962

Kaufmann SH (2004) Novel vaccination strategies. Wiley-VCH, Weinheim

Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 11:373–384

Kim JH, Majumder N, Lin H et al (2005) Enhanced immunity by NeuEDhsp70 DNA vaccine Is needed to combat an aggressive spontaneous metastatic breast cancer. Mol Ther 11:941–949

Kol A, Lichtman AH, Finberg RW et al (2000) Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. J Immunol 164:13–17

Kovacsovics-Bankowski M, Rock KL (1995) A phagosome-to-cytosol pathway for exogenous antigens presented on MHC class I molecules. Science 267:243–246

Kropshofer H, Arndt SO, Moldenhauer G et al (1997) HLA-DM acts as a molecular chaperone and rescues empty HLA-DR molecules at lysosomal pH. Immunity 6:293–302

Krupka M, Zachova K, Cahlikova R et al (2015) Endotoxin-minimized HIV-1 p24 fused to murine hsp70 activates dendritic cells, facilitates endocytosis and p24-specific Th1 response in mice. Immunol Lett 166:36–44

Kuppner MC, Gastpar R, Gelwer S et al (2001) The role of heat shock protein (hsp70) in dendritic cell maturation: Hsp70 induces the maturation of immature dendritic cells but reduces DC differentiation from monocyte precursors. Eur J Immunol 31:1602–1609

Kurotaki T, Tamura Y, Ueda G et al (2007) Efficient cross-presentation by heat shock protein 90-peptide complex-loaded dendritic cells via an endosomal pathway. J Immunol 179:1803–1813

Li ZH, Srivastava PK (1993) Tumor rejection antigen GP96/GRP94 is an atpase– implications for protein-folding and antigen presentation. EMBO J 12:3143–3151

Li Z, Qiao Y, Liu B et al (2005) Combination of imatinib mesylate with autologous leukocyte-derived heat shock protein and chronic myelogenous leukemia. Clin Cancer Res 11:4460–4468

Li J, Ye ZX, Li KN et al (2007) HSP70 gene fused with Hantavirus S segment DNA significantly enhances the DNA vaccine potency against hantaviral nucleocapsid protein in vivo. Vaccine 25:239–252

Libby P, Goldberg AL (1978) Leupeptin, a protease inhibitor, decreases protein degradation in normal and diseased muscles. Science 199:534–536

Lilley BN, Ploegh HL (2004) A membrane protein required for dislocation of misfolded proteins from the ER. Nature 429:834–840

Mackova J, Stasikova J, Kutinova L et al (2006) Prime/boost immunotherapy of HPV16-induced tumors with E7 protein delivered by Bordetella adenylate cyclase and modified vaccinia virus Ankara. Cancer Immunol Immunother 55:39–46

Macpherson AJ, Uhr T (2004) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 303:1662–1665

Maki RG, Old LJ, Srivastava PK (1990) Human homologue of murine tumor rejection antigen gp96: 5′-regulatory and coding regions and relationship to stress-induced proteins. Proc Natl Acad Sci USA 87:5658–5662

Maki RG, Livingston PO, Lewis JJ et al (2007) A phase I pilot study of autologous heat shock protein vaccine HSPPC-96 in patients with resected pancreatic adenocarcinoma. Dig Dis Sci 52:1964–1972

Mant A, Chinnery F, Elliott T et al (2012) The pathway of cross-presentation is influenced by the particle size of phagocytosed antigen. Immunology 136:163–175

Matsue H, Yao J, Matsue K et al (2006) Gap junction-mediated intercellular communication between dendritic cells (DCs) is required for effective activation of DCs. J Immunol 176:181–190

Matsutake T, Sawamura T, Srivastava PK (2010) High efficiency CD91- and LOX-1-mediated re-presentation of gp96-chaperoned peptides by MHC II molecules. Cancer Immun 10:7

Mendoza-Naranjo A, Saez PJ, Johansson CC et al (2007) Functional gap junctions facilitate melanoma antigen transfer and cross-

presentation between human dendritic cells. J Immunol 178:6949–6957
Merzougui N, Kratzer R, Saveanu L et al (2011) A proteasome-dependent, TAP-independent pathway for cross-presentation of phagocytosed antigen. EMBO Rep 12:1257–1264
Meshkat Z, Soleimanjahi H, Mirshahabi H et al (2011) Strong immune responses induced by a DNA vaccine containing HPV16 truncated E7 C-terminal linked to HSP70 gene. Iran J Immunol 8:65–75
Millar DG, Garza KM, Odermatt B et al (2003) Hsp70 promotes antigen-presenting cell function and converts T-cell tolerance to autoimmunity in vivo. Nat Med 9:1469–1476
Mizukami S, Kajiwara C, Tanaka M et al (2012) Differential MyD88/IRAK4 requirements for cross-priming and tumor rejection induced by heat shock protein 70-model antigen fusion protein. Cancer Sci 103:851–859
More S, Breloer M, Fleischer B et al (1999) Activation of cytotoxic T cells in vitro by recombinant gp96 fusion proteins irrespective of the 'fused' antigenic peptide sequence. Immunol Lett 69:275–282
Moron VG, Rueda P, Sedlik C et al (2003) In vivo, dendritic cells can cross-present virus-like particles using an endosome-to-cytosol pathway. J Immunol 171:2242–2250
Mukai Y, Yoshinaga T, Yoshikawa M et al (2011) Induction of endoplasmic reticulum-endosome fusion for antigen cross-presentation induced by poly (gamma-glutamic acid) nanoparticles. J Immunol 187:6249–6255
Murshid A, Theriault J, Gong J et al (2011) Investigating receptors for extracellular heat shock proteins. Methods Mol Biol 787:289–302
Nakamura T, Moriguchi R, Kogure K et al (2008) Efficient MHC class I presentation by controlled intracellular trafficking of antigens in octaarginine-modified liposomes. Mol Ther 16:1507–1514
Nauseef WM, McCormick SJ, Clark RA (1995) Calreticulin functions as a molecular chaperone in the biosynthesis of myeloperoxidase. J Biol Chem 270:4741–4747
Neijssen J, Herberts C, Drijfhout JW et al (2005) Cross-presentation by intercellular peptide transfer through gap junctions. Nature 434:83–88
Newman RG, Dee MJ, Malek TR et al (2014) Heat shock protein vaccination and directed IL-2 therapy amplify tumor immunity rapidly following bone marrow transplantation in mice. Blood 123:3045–3055
Norbury CC, Chambers BJ, Prescott AR et al (1997) Constitutive macropinocytosis allows TAP-dependent major histocompatibility complex class I presentation of exogenous soluble antigen by bone marrow-derived dendritic cells. Eur J Immunol 27:280–288
Ohashi K, Burkart V, Flohe S et al (2000) Heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. J Immunol 164:558–561
Oki Y, McLaughlin P, Fayad LE et al (2007) Experience with heat shock protein-peptide complex 96 vaccine therapy in patients with indolent non-Hodgkin lymphoma. Cancer 109:77–83
Pang B, Neijssen J, Qiao X et al (2009) Direct antigen presentation and gap junction mediated cross-presentation during apoptosis. J Immunol 183:1083–1090
Panjwani NN, Popova L, Srivastava PK (2000) Activation of nitric oxide production in gp96-stimulated antigen presenting cells. Cell Stress Chaperones 5:391–392
Pham CD, Woo MY, Kim YS et al (2012) An anti-nucleic acid antibody delivers antigen to the cross-presentation pathway in dendritic cells and potentiates therapeutic antitumor effects. J Immunol 189:5755–5763
Pilla L, Patuzzo R, Rivoltini L et al (2006) A phase II trial of vaccination with autologous, tumor-derived heat-shock protein peptide complexes Gp96, in combination with GM-CSF and interferon-alpha in metastatic melanoma patients. Cancer Immunol Immunother 55:958–968
Pillay CS, Elliott E, Dennison C (2002) Endolysosomal proteolysis and its regulation. Biochem J 363:417–429
Pooley JL, Heath WR, Shortman K (2001) Cutting edge: intravenous soluble antigen is presented to CD4 T cells by CD8- dendritic cells, but cross-presented to CD8 T cells by CD8 + dendritic cells. J Immunol 166:5327–5330
Poulin LF, Salio M, Griessinger E et al (2010) Characterization of human DNGR-1 + BDCA3 + leukocytes as putative equivalents of mouse CD8alpha + dendritic cells. J Exp Med 207:1261–1271
Radwanska M, Magez S, Dumont N et al (2000) Antibodies raised against the flagellar pocket fraction of Trypanosoma brucei preferentially recognize HSP60 in cDNA expression library. Parasite Immunol 22:639–650
Rapoport TA, Jungnickel B, Kutay U (1996) Protein transport across the eukaryotic endoplasmic reticulum and bacterial inner membranes. Annu Rev Biochem 65:271–303
Raska M, Weigl E (2005) Heat shock proteins in autoimmune diseases. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 149:243–249
Raska M, Rybnikar A, Chumela J et al (2004) Recombinant protein and DNA vaccines derived from hsp60 Trichophyton mentagrophytes control the clinical course of trichophytosis in bovine species and guinea-pigs. Mycoses 47:407–417
Raska M, Belakova J, Wudattu NK et al (2005) Comparison of protective effect of protein and DNA vaccines hsp90 in murine model of systemic candidiasis. Folia Microbiol 50:77–82
Raska M, Belakova J, Horynova M et al (2008) Systemic and mucosal immunization with Candida albicans hsp90 elicits hsp90-specific humoral response in vaginal mucosa which is further enhanced during experimental vaginal candidiasis. Med Mycol 46:411–420
Rasoli M, Omar AR, Aini I et al (2010) Fusion of HSP70 gene of Mycobacterium tuberculosis to hemagglutinin (H5) gene of avian influenza virus in DNA vaccine enhances its potency. Acta Virol 54:33–39
Reed RC, Berwin B, Baker JP et al (2003) GRP94/gp96 elicits ERK activation in murine macrophages. A role for endotoxin contamination in NF-kappa B activation and nitric oxide production. J Biol Chem 278:31853–31860
Reed SE, Hodgson LR, Song S et al (2013) A role for Rab14 in the endocytic trafficking of GLUT4 in 3T3-L1 adipocytes. J Cell Sci 126:1931–1941
Robbins SH, Walzer T, Dembele D et al (2008) Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. Genome Biol 9:R17
Rodriguez A, Regnault A, Kleijmeer M et al (1999) Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. Nat Cell Biol 1:362–368
Ruedl C, Storni T, Lechner F et al (2002) Cross-presentation of virus-like particles by skin-derived CD8(−) dendritic cells: a dispensable role for TAP. Eur J Immunol 32:818–825
Saccheri F, Pozzi C, Avogadri F et al (2010) Bacteria-induced gap junctions in tumors favor antigen cross-presentation and antitumor immunity. Sci Transl Med 2:44ra57
Sampath P, Pollard TD (1991) Effects of cytochalasin, phalloidin, and pH on the elongation of actin filaments. Biochemistry 30:1973–1980
Sancho D, Joffre OP, Keller AM et al (2009) Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature 458:899–903
Sathe P, Pooley J, Vremec D et al (2011) The acquisition of antigen cross-presentation function by newly formed dendritic cells. J Immunol 186:5184–5192

Saveanu L, Fruci D, van Endert P (2002) Beyond the proteasome: trimming, degradation and generation of MHC class I ligands by auxiliary proteases. Molecular Immunol 39:203–215

Saveanu L, Carroll O, Weimershaus M et al (2009) IRAP identifies an endosomal compartment required for MHC class I cross-presentation. Science 325:213–217

Savina A, Peres A, Cebrian I et al (2009) The small GTPase Rac2 controls phagosomal alkalinization and antigen crosspresentation selectively in CD8(+) dendritic cells. Immunity 30:544–555

Schirmbeck R, Melber K, Reimann J (1995) Hepatitis B virus small surface antigen particles are processed in a novel endosomal pathway for major histocompatibility complex class I-restricted epitope presentation. Eur J Immunol 25:1063–1070

Schnorrer P, Behrens GM, Wilson NS et al (2006) The dominant role of CD8 + dendritic cells in cross-presentation is not dictated by antigen capture. Proc Natl Acad Sci USA 103:10729–10734

Schreibelt G, Klinkenberg LJ, Cruz LJ et al (2012) The C-type lectin receptor CLEC9A mediates antigen uptake and (cross-)presentation by human blood BDCA3(+) myeloid dendritic cells. Blood 119:2284–2292

See AP, Pradilla G, Yang I et al (2011) Heat shock protein-peptide complex in the treatment of glioblastoma. Expert Rev Vaccines 10:721–731

Segura E, Albiston AL, Wicks IP et al (2009) Different cross-presentation pathways in steady-state and inflammatory dendritic cells. Proc Natl Acad Sci USA 106:20377–20381

Serwold T, Gaw S, Shastri N (2001) ER aminopeptidases generate a unique pool of peptides for MHC class I molecules. Nat Immunol 2:644–651

Serwold T, Gonzalez F, Kim J et al (2002) ERAAP customizes peptides for MHC class I molecules in the endoplasmic reticulum. Nature 419:480–483

Shakushiro K, Yamasaki Y, Nishikawa M et al (2004) Efficient scavenger receptor-mediated uptake and cross-presentation of negatively charged soluble antigens by dendritic cells. Immunology 112:211–218

Shen L, Sigal LJ, Boes M et al (2004) Important role of cathepsin S in generating peptides for TAP-independent MHC class I crosspresentation in vivo. Immunity 21:155–165

Sigal LJ, Rock KL (2000) Bone marrow-derived antigen-presenting cells are required for the generation of cytotoxic T lymphocyte responses to viruses and use transporter associated with antigen presentation (TAP)-dependent and -independent pathways of antigen presentation. J Exp Med 192:1143–1150

Singh R, Cresswell P (2010) Defective cross-presentation of viral antigens in GILT-free mice. Science 328:1394–1398

Singh-Jasuja H, Hilf N, Scherer HU et al (2000a) The heat shock protein gp96: a receptor-targeted cross-priming carrier and activator of dendritic cells. Cell Stress Chaperones 5:462–470

Singh-Jasuja H, Scherer HU, Hilf N et al (2000b) The heat shock protein gp96 induces maturation of dendritic cells and down-regulation of its receptor. Eur J Immunol 30:2211–2215

Song R, Harding CV (1996) Roles of proteasomes, transporter for antigen presentation (TAP), and beta 2-microglobulin in the processing of bacterial or particulate antigens via an alternate class I MHC processing pathway. J Immunol 156:4182–4190

Srivastava P (2002) Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. Annu Rev Immunol 20:395–425

Srivastava PK, Das MR (1984) The serologically unique cell-surface antigen of zajdela ascitic hepatoma is also its tumor-associated transplantation antigen. Int J Cancer 33:417–422

Srivastava PK, Deleo AB, Old LJ (1986) Tumor rejection antigens of chemically-induced sarcomas of inbred mice. Proc Natl Acad Sci USA 83:3407–3411

Stober D, Trobonjaca Z, Reimann J et al (2002) Dendritic cells pulsed with exogenous hepatitis B surface antigen particles efficiently present epitopes to MHC class I-restricted cytotoxic T cells. Eur J Immunol 32:1099–1108

Suto R, Srivastava PK (1995) A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. Science 269:1585–1588

Suzuki N, Suzuki S, Duncan GS et al (2002) Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. Nature 416:750–756

Svanholm C, Bandholtz L, Castanos-Velez E et al (2000) Protective DNA immunization against Chlamydia pneumoniae. Scand J Immunol 51:345–353

Svensson M, Wick MJ (1999) Classical MHC class I peptide presentation of a bacterial fusion protein by bone marrow-derived dendritic cells. Eur J Immunol 29:180–188

Tamura Y, Peng P, Liu K et al (1997) Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science 278:117–120

Tanaka Y, Taneichi M, Kasai M et al (2010) Liposome-coupled antigens are internalized by antigen-presenting cells via pinocytosis and cross-presented to CD8 T cells. PLoS One 5:e15225

Testori A, Richards J, Whitman E et al (2008) Phase III comparison of vitespen, an autologous tumor-derived heat shock protein gp96 peptide complex vaccine, with physician's choice of treatment for stage IV melanoma: the C-100-21 Study Group. J Clin Oncol 26:955–962

Tobian AA, Canaday DH, Boom WH et al (2004a) Bacterial heat shock proteins promote CD91-dependent class I MHC cross-presentation of chaperoned peptide to CD8+ T cells by cytosolic mechanisms in dendritic cells versus vacuolar mechanisms in macrophages. J Immunol 172:5277–5286

Tobian AA, Canaday DH, Harding CV (2004b) Bacterial heat shock proteins enhance class II MHC antigen processing and presentation of chaperoned peptides to CD4+ T cells. J Immunol 173:5130–5137

Todryk S, Melcher AA, Hardwick N et al (1999) Heat shock protein 70 induced during tumor cell killing induces Th1 cytokines and targets immature dendritic cell precursors to enhance antigen uptake. J Immunol 163:1398–1408

Touret N, Paroutis P, Terebiznik M et al (2005) Quantitative and dynamic assessment of the contribution of the ER to phagosome formation. Cell 123:157–170

Trombetta ES, Ebersold M, Garrett W et al (2003) Activation of lysosomal function during dendritic cell maturation. Science 299:1400–1403

Udono H, Srivastava PK (1993) Heat shock protein 70-associated peptides elicit specific cancer immunity. J Exp Med 178:1391–1396

Udono H, Srivastava PK (1994) Comparison of tumor-specific immunogenicities of stress-induced proteins gp96, hsp90, and hsp70. J Immunol 152:5398–5403

Udono H, Levey DL, Srivastava PK (1994) Cellular requirements for tumor-specific immunity elicited by heat shock proteins: tumor rejection antigen gp96 primes CD8 + T cells in vivo. Proc Natl Acad Sci USA 91:3077–3081

Vabulas RM, Braedel S, Hilf N et al (2002) The endoplasmic reticulum-resident heat shock protein Gp96 activates dendritic cells via the Toll-like receptor 2/4 pathway. J Biol Chem 277:20847–20853

Van Kaer L, Ashton-Rickardt PG, Ploegh HL et al (1992) TAP1 mutant mice are deficient in antigen presentation, surface class I molecules, and CD4-8 + T cells. Cell 71:1205–1214

Wada I, Imai S, Kai M et al (1995) Chaperone function of calreticulin when expressed in the endoplasmic reticulum as the membrane-anchored and soluble forms. J Biol Chem 270:20298–20304

Wallin RPA, Lundqvist A, More SH et al (2002) Heat-shock proteins as activators of the innate immune system. Trends Immunol 23:130–135

Warger T, Hilf N, Rechtsteiner G et al (2006) Interaction of TLR2 and TLR4 ligands with the N-terminal domain of Gp96 amplifies innate and adaptive immune responses. J Biol Chem 281:22545–22553

Wassenberg JJ, Dezfulian C, Nicchitta CV (1999) Receptor mediated and fluid phase pathways for internalization of the ER Hsp90 chaperone GRP94 in murine macrophages. J Cell Sci 112:2167–2175

Weigl E, Kopecek P, Raska M et al (1999) Heat shock proteins in immune reactions. Folia Microbiol 44:561–566

Weimershaus M, Maschalidi S, Sepulveda F et al (2012) Conventional dendritic cells require IRAP-Rab14 endosomes for efficient cross-presentation. J Immunol 188:1840–1846

Wick MJ, Pfeifer JD (1996) Major histocompatibility complex class I presentation of ovalbumin peptide 257–264 from exogenous sources: protein context influences the degree of TAP-independent presentation. Eur J Immunol 26:2790–2799

Wiertz EJ, Tortorella D, Bogyo M et al (1996) Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. Nature 384:432–438

Wood C, Srivastava P, Bukowski R et al (2008) An adjuvant autologous therapeutic vaccine (HSPPC-96; vitespen) versus observation alone for patients at high risk of recurrence after nephrectomy for renal cell carcinoma: a multicentre, open-label, randomised phase III trial. Lancet 372:145–154

Woodman PG, Futter CE (2008) Multivesicular bodies: co-ordinated progression to maturity. Curr Opin Cell Biol 20:408–414

Ye Z, Gan YH (2007) Flagellin contamination of recombinant heat shock protein 70 is responsible for its activity on T cells. J Biol Chem 282:4479–4484

Ye Y, Shibata Y, Yun C et al (2004) A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. Nature 429:841–847

Zachova K, Krupka M, Chamrad I et al (2009) Novel modification of growth medium enables efficient E. coli expression and simple purification of an endotoxin-free recombinant murine hsp70 protein. J Microbiol Biotechnol 19:727–733

Zanin-Zhorov A, Nussbaum G, Franitza S et al (2003) T cells respond to heat shock protein 60 via TLR2: activation of adhesion and inhibition of chemokine receptors. FASEB J 17:1567–1569

Zehner M, Burgdorf S (2013) Regulation of antigen transport into the cytosol for cross-presentation by ubiquitination of the mannose receptor. Mol Immunol 55:146–148

Zehner M, Chasan AI, Schuette V et al (2011) Mannose receptor polyubiquitination regulates endosomal recruitment of p97 and cytosolic antigen translocation for cross-presentation. Proc Natl Acad Sci USA 108:9933–9938

Zheng H, Dai J, Stoilova D et al (2001) Cell surface targeting of heat shock protein gp96 induces dendritic cell maturation and antitumor immunity. J Immunol 167:6731–6735