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Heat shock proteins as vaccine adjuvants in infections and cancer

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In addition to maintaining cell homeostasis under physiological and stress conditions, some heat shock proteins (HSPs) are potent inducers of immunity and have been harnessed as vaccine adjuvants targeted to cancers and infections. HSPs are a group of ubiquitous intracellular molecules that function as molecular chaperones in numerous processes, such as protein folding and transport, and are induced under stress conditions, such as fever and radiation. Certain HSPs are potent inducers of innate and antigen-specific immunity. They activate dendritic cells partly through toll-like receptors, activate natural killer cells, increase presentation of antigens to effector cells and augment T-cell and humoral immune responses against their associated antigens. Their roles in priming multiple host defense pathways are being exploited in vaccine development for cancer and infectious diseases.

Challenges for vaccine development

Although vaccines have been highly successful in preventing many infectious diseases, there is an unmet need for novel vaccine strategies targeting infections of global importance, such as HIV, tuberculosis and malaria. An adjuvant is any substance that enhances immunity to an antigen with which it is mixed. The concept of vaccine adjuvants has mostly been restricted to those that stimulate antibody titers (e.g. measles, polio and pneumococcus) or, in the case of the Bacille Calmette–Guerin (BCG) vaccine, delayed type hypersensitivity responses. Antibody responses to pathogen antigens are easily measured and correlate with protection in many infectious diseases. Recently, the concept of adjuvants has been expanded to include soluble mediators and antigenic carriers that interact with surface molecules present on dendritic cells (DC) [e.g. lipopolysaccharide (LPS), Flt3 ligand and heat shock protein (HSP)] and viral vectors that infect antigen-presenting cells (APC; see Box 1) (e.g. vaccinia, lentivirus and adenovirus) [1]. By augmenting multiple host-defense pathways relevant to specific tumors and infectious pathogens, these novel approaches to adjuvant development could translate to enhanced efficacy.

Whereas vaccines have been successful for many infectious diseases, progress in immunotherapeutics for cancer has been modest. It is thought that cancer cells survive because they are not recognized by the immune system or fail to elicit an adequate immunologic response. Seen in this light, immunotherapeutic approaches for cancer involve attaching a 'danger signal' to previously unrecognized or poorly recognized 'tumor antigens and thereby enhancing their antigen-presentation capacity. HSPs could be used for this because they are known to augment innate and antigen-specific effector functions.

Heat shock protein families

Heat shock proteins (HSP) principally reside in the cytoplasm and nucleus of eukaryotic cells, with the exception of HSP60, which resides in mitochondria. A second set of stress proteins, glucose-regulated proteins (GRPs) resides in the endoplasmic reticulum. The GRPs are not readily responsive to heat shock or oxidative stress, but to reducing conditions (e.g. anoxia) and other conditions that interfere with the function of the endoplasmic reticulum.

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BOX 1

Glossary of immunologic terms

Antigen presentation: is the display of antigen as peptide fragments bound to MHC molecules on the cell surface.

Antigen presenting cells (APCs): are highly specialized cells that process antigens and display peptide fragments on MHC molecules on the cell surface. Dendritic cells are the main APCs for T-cells.

CD4 T cells: are T-cells that express the co-receptor protein CD4. CD4 T cells recognize peptides bound to MHC II molecules, and differentiate into T-helper (Th) 1 or Th2 effector cells that in-turn activate macrophages and B-cell responses to antigen. Th1 cells produce the cytokine interferon-γ and drive cellular immunity, whereas Th2 cells drive allergic responses.

CD8 T cells: are T-cells that express the co-receptor CD8. CD8 cells recognize antigens produced in the cytoplasm of a cell (e.g. viral antigens). Peptides derived from these antigens are transported by TAP (transporters associated with antigen presentation), assembled with MHC I molecules in the endoplasmic reticulum, and displayed on the cell surface. CD8 T cells can differentiate into cytotoxic T lymphocytes.

Cytotoxic T lymphocytes: are a subset of CD8 T cells that stimulate apoptosis of target cells. Targets include cells infected with virus and tumor cells.

Epitope: is a site on an antigen recognized by an antibody or T cell. A T-cell epitope is a short peptide derived from a protein antigen that binds to MHC molecules and is recognized by T cells.

Human leukocyte antigen (HLA): allelic designation of human MHC molecules.

Major histocompatibility complex (MHC): are cell-surface molecules crucial for antigen presentation. MHC I molecules present peptides generated in the cytosol to CD8 T cells, and MHC II molecules present peptides degraded in intracellular vesicles to CD4 T cells.

Natural killer (NK) cells: are large, granular non-T or -B lymphocytes important in innate immunity against a variety of intracellular pathogens. NK cells are also able to kill tumor cells.

Perforin: is a protein produced by CTLs and NK cells that lyse target cells.

Priming: is differentiation of antigen-naïve lymphocytes into effector or memory cells after antigen exposure.

Superantigen: in contrast to the usual antigen presentation by MHC II molecules that leads to activation of only those T cells recognizing specific antigen epitopes, a superantigen binds externally to a subset of T-cell receptors and to MHC II molecules, leading to activation of a much larger proportion of T cells than antigen-specific T-cell activation.

Toll-like receptors (TLR): are eceptors that recognize specific pathogen motifs (e.g. LPS, DNA sequences present on bacteria and viruses) and trigger a signaling pathway that activates the transcription factor, NF-κB. TLR activation leads to activation of dendritic cells and, in general, stimulate cellular immunity.

It is recognized today that HSP70, HSP110, and GRP170 comprise three distinguishable stress protein families that share a common evolutionary ancestor [2–4]. The existence of multiple HSP families in the cytoplasm and in the endoplasmic reticulum of (apparently) all eukaryotic cells argues for different functions of these protein families.

The different role of heat shock proteins

HSPs are present in all living cells. They can exist in an unbound state or bound to specific client proteins. HSPs function as molecular chaperones in numerous processes, such as protein folding, assembly and transport, peptide trafficking, and antigen processing under physiologic and stress conditions [5–10]. Expression of HSPs can be induced by several stressors, such as fever, oxidative stress, alcohol, inflammation and heavy metals, and by conditions causing injury and necrosis, such as infection, trauma and ischemic reperfusion. They serve to attenuate the damage and misfolding of proteins caused by these various stressors. HSPs bind to exposed hydrophobic sites on polypeptides and mediate conformational changes, prevent misfolding of peptides and facilitate transport across membranes, thus maintaining the function of crucial cellular pathways during stress.

HSPs are potent inducers of innate and antigen-specific immunity. Their role as 'danger signals' that prime multiple host defense pathways is being exploited in vaccine development for cancer and infections [11]. The activation of DCs is necessary for the initiation of primary and secondary immune responses and can be induced by motifs (motifs can be DNA, peptides, lipids or sugars etc.) present on pathogens via toll-like receptors (TLRs) or by endogenous danger signals released by tissues undergoing stress or necrosis. Examples of endogenous danger signals include HSPs, nucleotides, reactive oxygen intermediates, extracellular matrix breakdown products, neuromediators and cytokines (like the interferons) [12,13].

HSPs used to be considered exclusively intracellular proteins but it is now known that necrotic but not apoptotic cells release HSPs. This delivers a partial maturation signal to dendritic cells and activates the nuclear factor (NF)-κB pathway [14]. HSP release from cells might be a crucial signal that is able to activate the immune system to recognize 'dangerous' physiological situations [11]. This suggests that HSPs, which in living cells are intracellular proteins, might have developed an extracellular function related to the early evolution of the immune response.

Heat shock proteins activate dendritic cells and natural killer cells

HSP–peptide complexes purified from tumors or from cells infected with pathogens contain tumor- or pathogen-derived peptides, respectively. HSP-chaperoned peptides enter APCs through specific receptors, such as TLRs, scavenger receptors (LOX-1) and/or CD91, and prime T cells by increasing antigen display by major histocompatibility complex (MHC) class I and II molecules [10]. Certain HSPs also induce maturation of DCs and secretion of the proinflammatory cytokines interleukin (IL)-12 and tumor necrosis factor (TNF)-α, and chemokines [7,10,15–18]. The dual feature of certain HSPs – chaperoning antigens and serving as an adjuvant – has intrigued investigators to exploit HSPs for vaccine development.

Some HSPs are internalized by DCs through receptor-mediated endocytosis, activate DCs and enhance their antigen presentation capacity [19–22]. Other studies have shown internalization of HSP70 and HSP60 by surface receptors CD14, and TLR-2 or TLR-4 induce maturation of APCs [23]. The HSP gp96 binds to its receptor, CD91, and upregulates surface expression of MHC class II and the co-stimulatory molecule CD86, as well as secretion of

proinflammatory cytokines IL-6, IL-1, TNF-α, and IL-12 [23,24]. The combined effect of this interaction would be to activate DCs, increase antigen display and prime antigen-specific T-cell responses.

Some HSPs augment natural killer (NK) cell activity. NK cells play a crucial role in innate immunity and also modulate subsequent adaptive responses via cytokine production [e.g. interferon (IFN)-γ]. In addition to mediating host defense against infectious agents, NK cells have antitumor activity [25]. NK cells express activating and inhibitory receptors, which interact with MHC class I molecules on target cells. Human histocompatibility leukocyte antigen (HLA)-E is a non-classical MHC class I molecule that interacts with inhibitory CD94/NKG2A receptors expressed on the surface of NK cells and T cell subsets. A signal peptide derived from HSP60 binds to HLA-E during cellular stress, and interferes with recognition by CD94/NKG2A inhibitory receptors, leading to a reduced capacity to inhibit a major NK cell population [26]. Strbo *et al.* [27] demonstrated an essential role for perforin (a protein that disrupts membranes of target cells and induces apoptosis) produced by NK cells in the activation of innate and adaptive immunity by gp96 complexes. Surface expression of HSP70 on tumor membranes stimulated migratory and cytolytic activity of NK cells and induced apoptosis of tumor cells [28,29]. NK cells were required for HSP70-mediated induction of cytotoxic T-lymphocyte (CTL) responses that led to a therapeutic effect against lung metastases in animals [30]. In a therapeutic study, vaccination of patients with colorectal cancer with autologous tumor-derived HSP96 induced a significant boost of NK activity, as measured by cytokine secretion and cytotoxicity [31]. An autologous vaccine of leukocyte-derived HSP70 complexes administered to patients with chronic myelogenous leukemia (CML) increased the frequency of CML-specific IFN-γ-producing cells and IFN-γ-secreting NK cells in blood [32]. Taken together, these studies suggest that HSP-mediated NK cell activation could have a key role in eliciting antitumor responses (Figure 1).

One important concern about HSP-based vaccines relates to LPS contamination [33]. LPS is a potent activator of DCs via TLR-4 signaling and primes T-cell responses. Some reports argue that HSP preparations are contaminated by LPS, and it has been reported that depletion of LPS rendered HSP unable to activate APCs – indicating that LPS and not HSP might have activated APCs [34,35]. However, other investigators showed that LPS-free HSP preparations were potent activators of APCs [36,37]. In conclusion, HSP preparations need to be free of LPS for a proper analysis of their adjuvant properties in experimental systems and in clinical trials.

Heat shock proteins and antigen presentation

A central requirement of adaptive immunity is MHC I and II presentation of self and nonself peptides to CD4+ and CD8+ T cells. Endogenous antigens (produced within the cell) are gener-

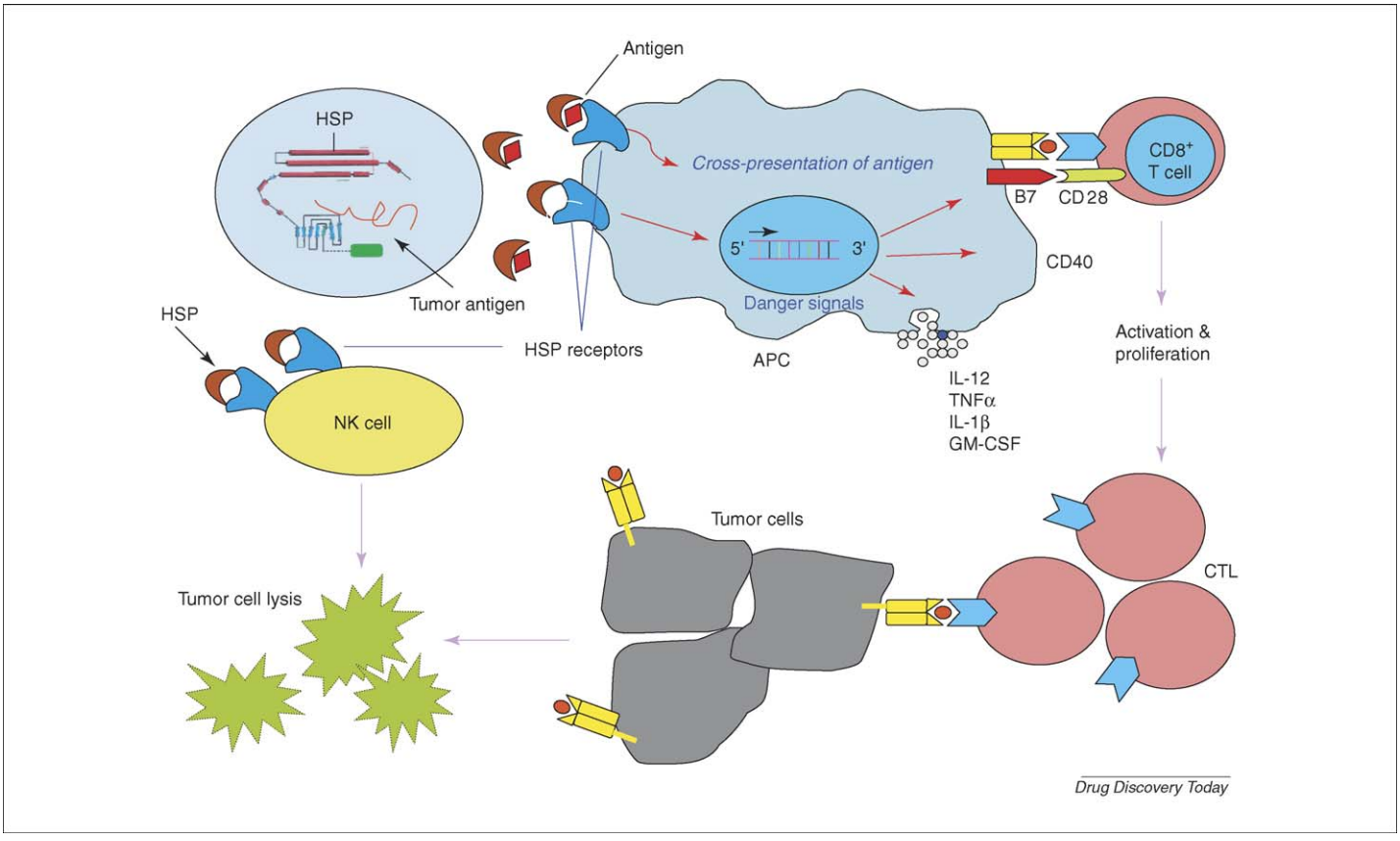

FIGURE 1

Heat shock proteins augment tumor antigen presentation and natural killer cell activity leading to tumor lysis. Heat shock proteins (HSPs) are induced under physiologic stress (e.g. fever and cell necrosis) and activate antigen-presenting cells (APCs) necessary for the initiation of primary and secondary immune responses. Antigen-specific cytotoxic T-lymphocyte (CTL) responses are initiated, leading to tumor cell apoptosis. Natural killer (NK) cells are capable of directly inducing tumor-cell lysis and enhancing CTL activity. Autologous patient-specific tumor vaccines have been generated by purifying HSP–antigen complexes from tumor specimens and are currently being evaluated in clinical trials.

ally presented by MHC I, and exogenous antigens by MHC II. Exogenous antigens can also be internalized and displayed by MHC I through a poorly understood process, known as cross-priming or cross-presentation. Cross-priming refers to re-presentation of exogenous cell-associated antigens by MHC molecules. An additional pathway with potential relevance for cross-priming was recognized when the induction of tumor immunity and CTL activation by administration of HSPs such as gp96, HSP70 and HSP90, was discovered [10]. MHC class I-restricted re-presentation of gp96-associated peptides and CTL activation is dependent on pathways that involve receptor-mediated endocytosis [38]. It is unclear whether HSPs directly interact with antigenic peptides and MHC molecules to broaden the repertoire of peptides available for presentation or whether HSP–peptide complexes act principally via alternative antigen-specific recognition pathways [39].

It is not known if a separate pathway evolved in addition to MHC that increases the antigenic repertoire of the cell and is exploited by HSP to augment antigen display, or whether this function of HSP is predominantly a laboratory-based phenomenon [39]. This question is of crucial importance because loss of tumor-antigen display through MHC molecules is a key mechanism by which some tumors and pathogens can evade immune surveillance.

Heat shock proteins in vaccine development against infectious diseases

HSPs could have a dual role in vaccine development against infectious diseases; pathogen-derived HSPs could be exploited as vaccine antigens and host- and pathogen-derived HSPs could be exploited as adjuvants. Because HSPs can be early targets in the immune response against pathogens, they are being exploited as antigens for vaccine development [40]. In addition, because HSPs potently stimulate innate and antigen-specific pathways, they are promising as vaccine adjuvants for a broad spectrum of pathogens. Pathogens with prolonged intracellular persistence (e.g. mycobacteria and some viruses) are logical targets for vaccines aimed at augmenting cellular immunity. However, the view that cellular immunity is restricted to intracellular pathogens and that humoral immunity is restricted to extracellular pathogens is overly simplistic. [41,42]. HSP-based vaccines target multiple innate and antigen-driven pathways, making this approach attractive for intracellular and extracellular pathogens.

Pathogen-derived HSPs can facilitate pathogen survival in the host and increase virulence, but some also act as immunostimulants in the host. HSP70 derived from *Toxoplasma gondii* induced DC maturation and stimulated IL-12 responses [43]. Long *et al.* [44] identified HSP60 as the ligand on *Histoplasma capsulatum*, a facultative intracellular fungal pathogen, that mediates binding to CD18 receptors on human macrophages. Immunization of mice with recombinant HSP60 from *H. capsulatum* conferred protection from a subsequent challenge [45].

Host defense against mycobacteria depends on cellular immunity. Tuberculosis is responsible for ~2 million deaths a year, with a substantial proportion occurring in HIV-positive people. The protection conferred by BCG vaccination has produced widely discrepant results in clinical trials, emphasizing the need for more-effective vaccines and more-reliable immunologic surrogates of protection [46]. Strategies to induce long-term immunity more effectively include the use of novel adjuvants and DNA vaccination.

HSP-based vaccines have been effective in several models of experimental tuberculosis. Vaccination with BCG peptides complexed to HSPs induced Th1 responses and was protective in murine pulmonary tuberculosis [47]. A DNA vaccine based on the HSP65 *Mycobacterium leprae* gene conferred protection both as prophylaxis and therapy in a mouse model of tuberculosis. Vaccination stimulated CD8+ lung cell activation, IFN-γ, TNF-α and reduction of lung injury [48]. In another study, DNA vectors containing *Mycobacterium tuberculosis* alanine–proline-rich antigen (Apa), and Hsp65 and Hsp70 mycobacterial antigens combined with BCG induced more robust immunity and conferred greater protection than BCG alone in tuberculosis in mice [49]. A DNA vaccine combination expressing mycobacterial Hsp65 and IL-12 was protective in a monkey model of tuberculosis [50]. Mycobacterial Hsp65 elicits different patterns of immune response depending on how it is administered [51].

Although HSPs isolated from tumors or infected tissues or loaded *in vitro* with peptides are, in some scenarios, able to elicit protective CD8 T-cell stimulation in the absence of CD4 help, this is not a universal feature. An HSP-based vaccine conferred protection in listeriosis in mice, but required CD4 help either through re-stimulation with peptide-loaded DCs or co-transfer of antigen-specific CD4 T cells [52].

Binding of HSP to viral complexes can enhance antiviral immunity, including NK activity, antibody-dependent cellular cytotoxicity, and CTL activities [53]. HSPs stimulated by stress can stimulate antiviral host defense [54]. Robust CD4+ and CD8+ T-cell responses are considered important immune components for controlling HIV infection; priming and expanding these responses are likely to be crucial for developing an effective HIV vaccine. HSP gp96 complexed to HIV-1 Gag-p24 peptide contained multiple MHC class I- and II-restricted epitopes suitable to induce effective CTL memory by simultaneously providing CD4 T-cell help [55].

Chemokine (C–C motif) receptor (CCR) 5 is a major co-receptor with the CD4 glycoprotein, mediating cellular entry of CCR5 strains of HIV-1 or simian immunodeficiency virus (SIV). Humans who express the homozygous δ32 CCR5 mutation are protected from acquisition of HIV infection. Bogers *et al.* [56] used a novel vaccine strategy in which HSP70 was covalently linked to the CCR5 peptides, SIV gpl20 and p27. Immunization of macaques with this complex led to induction of C–C chemokines and antibodies that block and downmodulate CCR5, as well as immune responses to the subunit SIV antigens that correlated with protection from SIV challenge. This vaccine was also effective in controlling mucosal SIV infection [57].

Two chaperone-based vaccines targeting chronic viral infections are currently in clinical development. These are chaperone E7 for treatment of anogenital warts and cervical dysplasia (Stress Biotechnologies) and AG-702 for treatment of genital herpes (Antigenics). Both use recombinant chaperone as carrier of defined pathogen-specific antigens, and have been shown to be safe and well-tolerated [58].

Heat shock protein and tumor vaccine development

Heat shock protein chaperone–peptide complexes from autologous tumors

It was hoped that increasing knowledge of tumor immunology and identification of immunodominant tumor antigens would

translate into therapeutic cancer vaccines. Despite some progress, we do not yet have a cancer vaccine that consistently induces tumor regression or improves patient survival. Experimental and clinical evidence suggests that immunotherapy can be combined with chemotherapy to enhance efficacy [59].

As an alternative to selecting a single antigen for tumor vaccine development, random mutations in cancer cells generate antigens unique to the individual, and could be important in generating custom-made autologous vaccines. Purification of chaperones from a cancer is believed to co-purify an antigenic peptide 'fingerprint' of the cell of origin [10]. Thus, a vaccine comprising chaperone–peptide complexes derived from a tumor would be expected to include a full repertoire of patient-specific tumor antigens and to obviate the need to identify CTL epitopes from individual cancers.

This advantage extends the use of chaperone-based immunotherapy to cancers where specific tumor antigens have not yet been characterized. Chaperone–peptide complexes from autologous tumors are a patient-specific vaccine, thus generating specific immunity only to the cancer from which the chaperones are isolated. This approach is expected to reduce the possibility of tumor escape from immunotherapy owing to reduced expression of a given tumor antigen by MHC molecules over time (antigen loss), because immunization with chaperones derived from tumor cells is directed against a diverse antigenic repertoire. No autoimmune reactions or severe side effects of the vaccine have so far been observed in immunized patients.

HSP–peptide complexes purified from tumor specimens (particularly HSP70 and gp96) serve as effective vaccines, producing antitumor immune responses in several animal models. HSP70 is frequently overexpressed in human tumors of different histological origins, but not in most normal tissue [60]. Within the cell, the HSP gp96 is directly associated with the antigen presentation machinery, specifically transporters associated with antigen presentation (TAP) and MHC class I molecules, and probably enhances antigen display. Thus, HSPs purified from cells are expected to bind a spectrum of cellular peptides [61]. The purification of these stress proteins from tumors would then be expected to co-purify a spectrum of peptides or a peptide fingerprint of the cell of origin. In the case of cancer cells, this presumably includes a subset of antigenic tumor-specific epitopes. The purified HSP, when used as a vaccine, would then present these tumor-specific peptides to the immune system (Figure 1). This approach represents a truly new idea in cancer therapy.

Heat shock proteins complexed to recombinant tumor antigens

HSPs can be used as autologous vaccines using patient-specific antigens derived from the tumor of an individual patient, and in 'global' vaccines using well-defined recombinant tumor antigens. In the personalized vaccine approach, the specific HSP-bound tumor peptides do not need to be identified. The rationale is that relevant patient-specific tumor antigens bound to HSPs present in the resected tumor can be extracted and purified and delivered back to the patient as a personalized vaccine containing the unique patient-specific repertoire of tumor antigens. The first autologous HSP vaccine – Oncophage® – used in clinical trials was heat-shock-protein–peptide complex (HSPPC)-96, a gp96 HSP peptide complex derived from resected tumor and then formulated for intradermal or subcutaneous administration.

Phase I and Phase II trials have been performed to define the safety and characterize immunologic responses of autologous HSPs in immunotherapy for cancer [62]. HSPPC-96 vaccines have been evaluated in early trials of pancreatic cancer, gastrointestinal tract cancers, lymphoma, chronic myelogenous leukemia, renal cell carcinoma and melanoma (www.antigenics.com/trials). The vaccines were well-tolerated, and measurable immune responses appeared to correlate with a positive clinical response in early-phase trials of melanoma [63] and colorectal cancer [64]. However, these trials enrolled small numbers of patients and were not powered to evaluate efficacy. Phase III studies evaluating HSPPC-96 in renal cell carcinoma [65] and melanoma are underway.

The global vaccine strategy involving recombinant HSPs and tumor antigens has important advantages and potential disadvantages over patient-derived tumor products. Advantages include: (i) HSPs efficiently bind substrate proteins; therefore, a highly concentrated vaccine would be presented to the immune system. (ii) A tumor antigen contains several peptides that can potentially be presented by MHC molecules – the specific peptides displayed on APCs will be determined by the individual's own MHC alleles. Use of a whole protein antigen that contains a large reservoir of potential peptides is expected to enable the individual's own MHC alleles to select the appropriate epitope for presentation. Such a chaperone complex vaccine increases the chance of poly-epitope directed T- and B-cell responses in a broad spectrum of MHC allelic backgrounds. (iii) Because a tumor specimen is not required for vaccine production, patients with no measurable disease or an inaccessible tumor can still be treated using this approach. This makes it an ideal adjuvant therapy for patients with completely resected disease. This approach is theoretically applicable as a preventive therapy for patients at high risk for cancer or for recurrence of cancer. (iv) Production of the recombinant chaperone complex is less time-consuming and far less expensive than patient-specific vaccines. In addition, vaccines can be generated in considerable quantities with uniformity between batches. (v) Immunodominant antigens recognized by autologous T cells have been characterized in several tumors (e.g. her-2/neu in breast cancer) and are targets for vaccine development. Using well-defined antigens, instead of whole tumor cells or lysate, allows better characterization of the resultant immune responses. (vi) This synthetic approach of combining antigen with chaperone can serve as a model to develop and evaluate many different antigen targets, either alone or in combination vaccines.

The main disadvantage is that this strategy will not capture the repertoire of antigens present in individual tumor specimens. The ability to specifically select peptides (dominant, subdominant and T-helper) allows the design of highly tailored chaperone vaccines (Figure 2). To minimize antigen escape, multiple peptides from different tumor antigens can be selected to create a polyvalent vaccine to generate a more vigorous and diverse immune response. Owing to HLA restrictions, peptides have to be carefully chosen to match the HLA phenotype of the patient.

We developed a vaccine, consisting of HSP110 complexed to the intracellular domain (ICD) of the breast cancer antigen her-2/neu, that elicited strong antigen-specific type I cellular immunity and

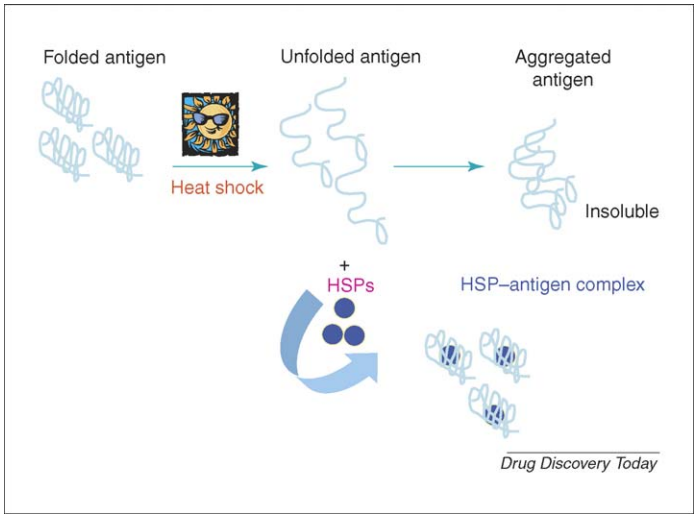

FIGURE 2

Generation of heat shock proteins tumor antigen complexes under heat shock conditions. Heat leads to unfolding of the purified antigens, exposing hydrophobic residues that bind to heat shock proteins (HSPs). Recombinant tumor antigens (e.g. her-2/neu associated with breast cancer) complexed to HSP110 led to induction of type I cellular immunity and CTL responses that were protective in animal models.

humoral responses [66]. This vaccine stimulated CD8+ and CD4+ T-cell responses against ICD in mice. *In vivo* depletion studies showed that the CD8+ T-cell response was independent of CD4+ T-cell help. Sensitization with the HSP110–ICD complex led to inhibition of tumor growth after challenge with mammary tumor cells [66], and also conferred protection against spontaneously occurring mammary tumors in susceptible mice [67].

Immunization with HSP110 complexed to gp100 (a human melanoma-associated antigen) protected mice against subsequent challenge with human gp100-transduced B16 melanoma [68]. Protection required CD4+ and CD8+ T cells. Vaccination also significantly suppressed the growth of established tumors in a therapeutic model. The antitumor response elicited by the HSP110–gp100 complex was more potent than that obtained using complete Freund's adjuvant.

Transducing tumor cells with heat shock proteins

A strategy of transducing tumor cells lines with HSP DNA has the potential advantage of increasing the repertoire of HSP-complexed antigens compared with using a single recombinant tumor antigen. This approach could be particularly promising in tumors in which dominant antigens are poorly characterized or a single HSP–antigen complex is inadequate to confer protection. We showed that transduced HSP110 overexpressing CT26 tumor had reduced tumor growth compared to the wild-type CT26 tumor in immunocompetent mice [69]. Immunization of mice with inactivated CT26–hsp110 cells significantly inhibited the growth of wild-type CT26 tumor, and was associated with an increased frequency of tumor-specific T cells after vaccination. Huang *et al.* [70] demonstrated that vaccination with an engineered melanoma cell line containing the transmembrane of the superantigen staphylococcal enterotoxin A and HSP 70 expressed on the cell surface led to augmentation of NK and CTL activities and protection against tumor challenge in mice.

Conclusions

Although vaccines have been highly successful in preventing several infections, there is an unmet need for novel vaccine strategies targeted to infections of global importance and to cancers. Certain HSPs are potent inducers of immunity and have been harnessed as vaccine adjuvants targeted to cancers and infections. HSP-based vaccine strategies – including vaccines derived from patient tumor material, from recombinant antigens and from transduced tumor cells lines – are promising for immunotherapy. Advanced clinical studies using autologous tumor-derived HSPs are underway. We expect several HSP-based immunotherapies will be translated into effective immunotherapy of cancer and infections in the future.

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