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In Vivo Suppression of Heat Shock Protein (HSP)27 and HSP70 Accelerates DMBA-Induced Skin Carcinogenesis by Inducing Antigenic Unresponsiveness to the Initiating Carcinogenic Chemical

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Heat shock proteins (HSPs) are constitutively expressed in murine skin. HSP27 is present in the epidermis, and HSP70 can be found in both the epidermis and dermis. The purpose of this study was to investigate the role of these proteins in cutaneous chemical carcinogenesis and to determine whether their effects on cell-mediated immune function were a contributing factor. In vivo inhibition of HSP27 and HSP70 produced a reduction in the T cell–mediated immune response to 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene in C3H/HeN mice and resulted in a state of Ag-specific tolerance. When mice were pretreated with anti-HSP27 and anti-HSP70 Abs in vivo prior to subjecting them to a standard two-stage DMBA/12-O-tetradecanoylphorbol-13-acetate cutaneous carcinogenesis protocol, the percentage of mice with tumors was much greater (p < 0.05) in anti-HSP27– and HSP70–pretreated animals compared with mice pretreated with control Ab. Similar results were obtained when the data were evaluated as the cumulative number of tumors per group. Mice pretreated with HSP27 and HSP70 Abs developed more H-ras mutations and fewer DMBA-specific cytotoxic T lymphocytes. These findings indicate that in mice HSP27 and HSP70 play a key role in the induction of cell-mediated immunity to carcinogenic polyaromatic hydrocarbons. Bolstering the immune response to carcinogenic polyaromatic hydrocarbons may be an effective method for prevention of the tumors that they produce.

Heat shock proteins (HSPs) are highly conserved intracellular proteins that are present in large quantities in all cells (1). Their expression is increased following exposure to heat, oxidative stress, toxins, and glucose deprivation. These agents play an important role in the folding and unfolding of other proteins, serve as peptide chaperones, and help to transport proteins across membranes and within cells. Certain HSPs, including HSP27 and HSP70, are cytoprotective, impeding apoptotic pathways, thereby permitting cells to survive during conditions of stress, when it would be expected that there are increased amounts of unfolded proteins.

HSPs also play important roles in eliciting innate and adaptive immunity (2). HSPs, including HSP70, exert cytokine-like effects on maturation of APCs, enhancing TNF-α, IL-1β, IL-6, and IL-12 secretion from monocytes, macrophages (3–5), and dendritic cells (DCs) (6). They induce surface expression of B7 and MHC class II molecules on DCs (3, 7, 8) and stimulate the production of chemokines by macrophages and DCs (9). Such findings support the concept that HSPs are endogenous signals for APC maturation and may have a role in the regulation of immunity versus tolerance.

The effects of HSPs on the immune system have relevance for host defenses against tumors. For example, tumor cell lysates containing HSP70 as well as other HSPs, including gp96 and HSP90, have been used to protect animals against subsequent tumor challenge with autologous tumors. As a result, HSPs are being investigated as immunotherapeutic agents (10, 11). The role of HSPs in carcinogenesis (i.e., at earlier stages in cancer development prior to the time that tumors have formed) has not been investigated. This would have relevance for immunoprevention of tumors, in addition to their immunotherapeutic potential. In previous studies, we have shown that a cell-mediated immune response occurs following topical application of carcinogenic polyaromatic hydrocarbons (PAHs) in selected strains of mice (12, 13) and have postulated that immunization against the chemical that causes the tumors or to proteins mutated by the chemical, rather than to the tumor itself, may be an effective method of preventing, as opposed to treating, chemically induced tumors and may have more global activities.

Using proteomic mapping, we have shown that six molecular chaperones—HSP27, HSP60, HSP70, HSP84, ER60, and GRP78—are constitutively expressed in the skin of C3H/HeN and BALB/c mice (12). Higher levels of HSP27 are found in the epidermis. HSP70 family members are expressed constitutively within keratinocytes and are elevated in both epidermis and dermis after skin samples are heat shocked in vivo and in vitro (14–16).

We have previously found that HSP27 and HSP70 are important in the development of allergic contact hypersensitivity to
Materials and Methods

Animals and reagents

Adult female, 6- to 8-wk-old C3H/HeN mice were obtained from Charles River Laboratories (Wilmington, MA). Animals were maintained in accordance with institutional guidelines. Normal goat IgG and goat polyclonal anti-HSP27 and anti-HSP70 IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Sheep anti-rat IgG dynabeads were from Life Technologies (Carlsbad, CA). Hybridoma lines GK1.5 (anti-CD4), Lyt-2 (anti-CD8), and HB-32 (anti-IL-4) were acquired from the National Institutes of Health (Bethesda, MD). CD45R/B220 and rGM-CSF were obtained from BD PharMingen (San Diego, CA). DMBA, TPA, hexone(a)pyrene (B[a]P), and rIL-4 and were purchased from Sigma-Aldrich (St. Louis, MO).

In vivo treatment with anti-HSP Abs

To assess the contribution of HSP27 and HSP70 in contact hypersensitivity, the abdominal skin of mice was prepared by removing hair with an electric trimmer in conjunction with gentle brushing of the skin as described earlier (18). This was followed by administration of 2 μg control Ab or anti-HSP27, or a combination of anti-HSP27 and anti-HSP70 Abs in PBS for 2 h under occlusion with a bio-occlusive dressing (Tegaderm, 3M, Maplewood, MN) using previously established techniques (17). The site was examined for erythema and edema using a modified Draize scoring system of 0–3 (17, 19). All animals used for experiments had a Draize score of <1. Studies have shown that application of Ab to the shaved abdominal skin allowed the Ab to penetrate into the epidermis and dermis (17) and were confirmed for these experiments.

Contact hypersensitivity to DMBA and B(a)P

Contact hypersensitivity to DMBA was assessed as described previously (20). Briefly, the shaved abdominal skin of C3H/HeN mice was sensitized on day 0 with 100 μl of a 0.1% solution of DMBA (w/v in acetone). Five days later, a challenge dose of 20 μl 0.1% DMBA was painted on the ear after measuring baseline ear thickness. The increase in ear swelling was measured at 24-h intervals to quantitate the magnitude of the contact hypersensitivity response. Contact hypersensitivity to B(a)P was conducted in the same manner with the exception that on day 0 100 μl of a 0.1% solution of B(a)P was applied to the skin for sensitization and 20 μl 0.1% B(a)P was applied to the ear for elicitation of the response.

Generation of bone marrow–derived DCs

Bone marrow–derived DCs (BMDCs) were prepared from mice as described earlier with some modifications (21). Briefly, bone marrow cells were prepared from femurs and tibias of mice and were incubated in RPMI 1640 medium with a mixture of Abs against Il-α, CD45R/B220, Lyt-2, and GK1.5 (2 μg/106 cells) on ice for 1 h. The cells were subsequently washed once with HBSS by lysis of RBCs. Sheep anti-rat IgG dynabeads were used to remove different cellular populations from the cell suspension, according to the manufacturer’s instructions. Cells were washed once with HBSS and cultured in 10% FCS RPMI 1640 medium supplemented with recombinant mouse GM-CSF (10 ng/ml) and IL-4 (10 ng/ml) in 6-well plates (5 × 105 cells/well). On day 5, half of the medium was replaced with fresh medium, and cells were stimulated on the following day for the experiments.

Stimulation of DMBA primed lymph node cells with hapten-conjugated BMDCs for cytokine production

To assess Ag-specific cytokine production, BMDCs were used for in vitro stimulation of primed lymph node cells as described previously (18, 22). Mice were pre-treated with anti-HSP27 and anti-HSP70 or isotype control Ab as described earlier in this section and were then sensitized with 100 μl of a 0.1% solution of DMBA (w/v in acetone) on day 0. On day +5, mice were sacrificed, and lymph node cell suspensions were prepared by gentle pressure through a wire mesh screen. Ten million BMDCs were suspended in 1 ml DMBA solution (10 μM in DMSO) for 15 min. The cells were then washed three times with RPMI 1640 medium containing 5% FCS and resuspended in the culture medium. DMBA-primed lymph node cells (2 × 106/ml) were stimulated with DMBA-labeled BMDCs (2 × 105/ml). Cytokine concentrations in culture supernatants were measured 48 h after culture using cytokine-specific (IFN-γ, IL-17, IL-4, and IL-10) ELISA kits from Life Technologies, according to the manufacturer’s instructions.

Assessment of immunological tolerance to DMBA

Tolerance to DMBA was assessed by a modification of the protocol of Schwarz et al. (23). On day −14, the abdominal skin of mice was treated with 2 μg isotype control Ab or a combination of anti-HSP27 and anti-HSP70 Abs under occlusion with a bio-occlusive dressing. Immediately thereafter, 100 μl 0.1% DMBA was applied topically to the Ab-treated site. On day 0, mice were resensitized on the dorsal skin, a site that had not been treated with Ab, with 100 μl 0.1% DMBA. Five days later, mice were challenged with 20 μl 0.1% DMBA on the ear. The increase in ear swelling was measured at 24-h intervals as described above.

In vivo CTL activity

CTL activity was assessed using an in vivo Ag-specific cytokotoxicity assay using methods by Ingulli (24) and Hermans et al. (25). Mice were topically treated with HSP27 and HSP70 Abs as described above and were then transferred to the same site with DMBA. Eight days later, mice received an i.v. injection of 5 × 105 CFSE (Life Technologies)-labeled target spleen cells composed of two populations: CFSE low (6 μM–labeled cells) that were pulsed with 50 μM DMBA for 25 min, washed, and mixed 1:1 with unlabeled CFSE high (12 μM–labeled cells. After 16 h, spleens were harvested and processed into single-cell suspensions for flow cytometric analysis to quantify CFSE high and low cells. Flow cytometric acquisition of 2000 CFSE high cells were detected per sample. The percent DMBA-specific cytotoxicity was calculated as follows: 100 × (1 – [% CFSE low/naive]). Percent inhibition, which was calculated as follows: 100 × (1 – [% cytotoxicity]) average percentage of cytotoxicity IgG.

Skin tumorigenesis

A two-stage skin carcinogenesis protocol was used to study the effect of HSP27 and HSP70 on DMBA tumorigenesis in which DMBA was the initiating agent and TPA was the promoting agent, using methods that have been described previously (18). Anti-HSP (HSP27 and HSP70) Abs were applied topically on one time on the shaved dorsal skin of mice (10 mice/panel). Two hours later, the Ab-treated site was painted with 100 μl DMBA (0.1% w/v in acetone). One week after that, TPA (40 nmol in acetone) was applied twice weekly to the site that had been treated previously with DMBA. Mice were evaluated two times per week for tumors. Only tumors that had attained a size of ≥1 mm were present for 2 wk or longer were counted.

Detection of H-ras mutation at codon 61

The H-ras mutation in DMBA-induced skin tumors was performed as described with some modifications (26). Total RNA was isolated from the tumors using Trizol reagent (Life Technologies), according to the manufacturer’s instructions. One microgram of RNA from each sample was used as the starting material for RT-PCR (Promega, Madison, WI). For PCR amplification of CNA, the following primers were used: forward primer for both wild-type and mutant H-ras, 5′-CTAACGCCTGTGTTTFCGACAGC-3′; reverse primer for wild-type H-ras, 5′-CATGCGACCTACTCTCTCT-3′; and reverse primer for mutant H-ras, 5′-CATGCGACCTACTCTCTTA-3′. PCR was performed at 94˚C for 5 min, followed by 30 cycles of 94˚C 60 s, 55˚C 60 s, and 72˚C 60 s. Codon 61 C→A point mutation creates an XbaI restriction enzyme site, which is used to distinguish mutant from wild-type H-ras. The PCR product was digested with XbaI, and the products were separated by PAGE and visualized by ethidium bromide staining.

Statistical analysis

Data were analyzed by one-tailed Student t test, and the p values are indicated in the text and figure legends. The group difference was compared using ANOVA test, followed by Tukey’s post hoc test for multiple comparisons.
parison adjustment in one experiment. Differences were considered significant at $p < 0.05$.

**Results**

**HSPs 27 and 70 play a role in the development of contact hypersensitivity to DMBA**

To evaluate the role of HSP27 and HSP70 in the cell-mediated response to DMBA, C3H/HeN mice were contact sensitized to DMBA after local treatment of the skin with anti-HSP27 or anti-HSP70 Abs. Inhibition of the induction of DMBA contact hypersensitivity could be achieved by pretreatment with neutralizing Abs to either HSP27 or HSP70 Abs (Fig. 1A). Administration of both Abs together gave an additive effect ($p < 0.05$). Treatment with isotype control Abs did not diminish the contact hypersensitivity response to DMBA ($p < 0.05$). Results are expressed as the change in auricular thickness ± SEM.

**Studies on the murine contact hypersensitivity response to 2,4-dinitro-1-fluorobenzene have demonstrated that this response is**

\[ \text{Abs to either HSP27 or HSP70 Abs (Fig. 1A). Administration of both Abs together gave an additive effect. There was no inhibition of the contact hypersensitivity response with isotype control Abs (Fig. 1B). The effect of HSP27 and HSP70 neutralization on contact hypersensitivity to the PAHs B(a)P was also assessed. There was inhibition of the induction of B(a)P contact hypersensitivity also (Fig. 1C).} \]
mediated by IFN-γ and IL-17 (17, 22). Experiments were therefore also performed in which cells were isolated from the draining lymph nodes of mice sensitized to DMBA. The cells were then placed in culture with DMBA-labeled BMDCs. This served as an alternative method of assessing the immune response to the topically applied carcinogen. DMBA-labeled BMDCs stimulated the production of IL-17 and IFN-γ by primed lymph node cells, whereas they were not able to stimulate naive lymph node cells (Fig. 2). Pretreatment of mice in vivo with anti-HSP27 and anti-HSP70 Abs before DMBA application, significantly inhibited production of IL-17 and IFN-γ compared with untreated BMDCs (p < 0.05; Fig. 2). Moreover, pretreatment with anti-HSP Abs resulted in a corresponding increase in IL-4 and IL-10.

**Induction of DMBA-specific tolerance by HSP27 and HSP70 Abs**

Experiments were then conducted to determine whether treatment with anti-HSP Abs (HSP27 and HSP70) followed by DMBA application had an effect on subsequent attempts to sensitize mice to that carcinogen. Animals that had been sensitized with DMBA on anti–HSP Ab-treated skin were resensitized with 0.1% DMBA at a non–Ab-treated site after a resting period of 14 d; they were then ear challenged with DMBA 5 d after that. The ear swelling response of mice pretreated with anti-HSP Abs was suppressed compared with positive controls even though the second attempt to sensitize mice was through normal skin (Fig. 3A). This indicated that mice treated with anti-HSP Ab, followed by carcinogen application had become tolerant to DMBA.

To determine whether suppression of the induction of contact hypersensitivity was hapten specific and to exclude the possibility that the mice treated with a combination of anti-HSP27 and anti-HSP70 Abs and DMBA were also nonresponsive to other PAHs given subsequently, the following experiment was performed. Panels of mice were treated with a combination of anti-HSP27 and anti-HSP70 Abs, followed immediately thereafter by application of DMBA to the Ab-treated skin site. Two weeks later, animals were treated with either a sensitizing dose of B(a)P. Animals were ear challenged 5 d later. Although mice treated with DMBA plus anti-HSP Abs and then resensitized with DMBA had a significantly suppressed response to DMBA, mice treated with anti-HSP Abs plus DMBA, and then, with B(a)P behaved like normal mice in their ear swelling response to B(a)P (Fig. 3B). In a reciprocal manner, mice that were treated with B(a)P plus anti-HSP Abs and then resensitized with B(a)P had a significantly suppressed response to B(a)P (Fig. 3C), but mice that were treated with anti-HSP Abs plus B(a)P and then resensitized with DMBA had a normal ear swelling response to DMBA (Fig. 3D). The results indicate that animals rendered unresponsive to DMBA are capable of developing a normal cell–mediated immune response to other PAHs and that the combination of anti-HSP Abs followed by hapten sensitization induces a state of specific immunologic un-

**FIGURE 3.** Tolerance to DMBA follows HSP Ab treatment and is Ag specific. C3H/HeN mice (n = 5/group) were treated with anti-HSP Abs on the abdomen after which 0.1% DMBA was applied to the same site. After 14 d, the mice were resensitized on the shaved back with either 0.1% DMBA (A) or 0.1% B(a)P (B). Mice were ear challenged 5 d later with the same hapten that had been applied to the back. Ear swelling responses indicate that inhibition of CHS produced by anti-HSP Abs was present in the DMBA-sensitized mice (*p < 0.05) but not in the B(a)P-sensitized mice (*p > 0.05). In another set of experiments, C3H/HeN mice (n = 5/group) were treated with anti-HSP Abs on the abdomen after which 0.1% B(a)P was applied to the same site. After 14 d, the mice were resensitized on the shaved back with either 0.1% B(a)P (C) or 0.1% DMBA (D). Mice were ear challenged 5 d later with the same hapten that had been applied to the back. Ear swelling responses indicate that inhibition of contact hypersensitivity produced by anti-HSP Abs was present in the B(a)P-sensitized mice (*p < 0.05) but not in the DMBA-sensitized mice (*p > 0.05). Results are expressed as change in auricular thickness ± SEM.
responsiveness only to the carcinogen that was applied at the HSP-treated skin site.

**DMBA tolerant mice are more susceptible to cutaneous DMBA carcinogenesis**

When mice were treated with anti-HSP (HSP27 and HSP70) Abs and were then subjected to a DMBA initiation, TPA promotion skin carcinogenesis protocol, the number of tumors was significantly greater \( (p < 0.05) \) in anti–HSP Ab-treated mice compared with control Ab-treated animals (Fig. 4A). Similar results were obtained when the data were evaluated as the cumulative number of tumors and the number of tumors per tumor-bearing mouse (data not shown). Although animals in both treatment groups eventually developed tumors with this protocol, they arose more rapidly in anti–HSP Ab-treated mice. For example, by week 16, 100% of anti–HSP Ab-treated mice had developed tumors, whereas only 40% of mice treated with control Ab had tumors, and these differences were significant \( (p < 0.05) \) (Fig. 4B). The tumors from the immunologically unresponsive mice grew progressively and showed a significant \( (p < 0.05) \) increase in tumor volume compared with tumors from control Ab–pretreated mice (Fig. 4C, 4D).

**HSP27 and HSP70 contribute to reduced H-ras mutations in DMBA-induced tumors**

DMBA forms adducts with DNA, the most important of which are activating mutations in the \( H\)-ras oncogene. One frequently occurring mutation is an A→T point mutation in codon 61, resulting in a change from glutamine (Q) to leucine (L) \( (26–30) \). The frequency of the \( H\)-ras mutation was significantly greater \( (p < 0.05) \) in tumors from the anti–HSP Ab-treated mice than in mice treated with the control Ab (Fig. 5).

**Discussion**

Cancer is a multistep process in which biochemical and molecular abnormalities accumulate in target cells in a sequential manner over long periods of time. Many of these steps have been identified using experimental animal models in which carcinogenic PAHs are applied to the skin of animals \( (31, 32) \). Evaluation of the immune system in skin cancer development has focused primarily on immune responses to premalignant papillomas or invasive carcinomas. A vigorous host T lymphocyte–mediated immune response exists to PAH-induced tumors that have already progressed through the carcinogenesis pathway. These malignancies express tumor Ags on their cell surfaces that are recognized by immunocompetent lymphocytes \( (33) \). Immunization techniques against tumor Ags have been successful in protecting animals against the subsequent growth of tumors that express the same Ags, findings which support the immunosurveillance

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**FIGURE 4.** Effect of HSP27 and HSP70 Abs on DMBA/TPA induced cutaneous carcinogenesis in C3H/HeN mice \( (n = 10/\text{group}) \). There was a significantly higher \( (*p < 0.05) \) number \( (A) \), percentage of tumors \( (B) \), and tumor volume \( (C) \) in the group of mice, which was treated with anti-HSP Abs. \( (D) \) Groups of mice with tumors after 25 wk of DMBA/TPA treatment.
theory that was originally proposed by Thomas (34) and Burnet (35). However, in relation to the total time that it takes for tumors to develop, this is a late stage in the process. Previously, we have shown that administration of carcinogenic PAHs, such as DMBA, B(a)P, and 3-methylcholanthrene, to the skin of mice results in an Ag-specific response to the topically applied carcinogen, which is mediated by CD8⁺ T cells; CD4⁺ T cells have a regulatory role (12, 13, 18, 20). This raises the possibility that the development of a T cell response to the chemical that causes the tumor, rather than to the tumor itself, may be an effective method of eradicating mutant cells and preventing these tumors from developing in the first place. Previous studies have supported that concept (13, 18). Specifically, the immune response to PAHs is genetically determined in part by polymorphisms in the class I genes within the MHC (13). Mice that develop an immune response to PAHs developed significantly fewer tumors when subjected to a DMBA cutaneous carcinogenesis protocol compared with MHC congenic mice that did not develop immunity to DMBA (13). Strains of mice that did develop an immune response to PAHs also had a reduced number of DMBA–DNA adducts compared with those that did not (13). In addition, in mice subjected to a DMBA/TPA skin tumorigenesis protocol, greater numbers of tumors developed in CD8-deficient mice than in wild-type mice, and fewer tumors occurred in CD4-deficient mice, indicating that CD8⁺ T cells are effector cells and CD4⁺ T cells have a regulatory role (18).

Although suggestive that strains of mice that develop a T cell response to PAHs are resistant to the carcinogenic effects of these agents, a more direct method of evaluating whether Ag specific T cell–mediated immunity to PAHs confers resistance to the tumors that they produce would be to demonstrate that mice rendered immunologically tolerant to a carcinogenic PAH are more susceptible to PAH-induced skin tumors compared with mice that develop a cell-mediated immune response that agent. In this study, we observed that inhibition of the cell-mediated immune response to the carcinogen DMBA could be achieved by pretreating the skin with neutralizing Abs to HSP27 and HSP70. Loss of the immune response to DMBA was Ag specific and led to long-term immunological unresponsiveness to that molecule. This enabled us to test, in a very direct manner, the hypothesis that the presence of a cell-mediated immune response to PAHs confers resistance to their carcinogenic effects. We observed that this, in fact, was the case. Mice unresponsive to DMBA developed substantially greater numbers of tumors than mice in which a cell-mediated immune response was present. This was associated with fewer DMBA cytotoxic T cells and more H-ras mutations, which are known to initiate DMBA-induced skin tumors.

FIGURE 5. Increased expression of mutant H-ras in DMBA/TPA induced tumors from anti–HSP Ab-treated mice after 25 wk of DMBA/TPA treatment by RT-PCR. (A) Mutant H-ras is indicated by cleaved products (lanes 5 and 6) after digestion with XbaI. Lanes 1 and 2, Naive skin; lane 3, control Ab; lane 4, anti-HSP Ab; lane 5, control Ab; and lane 6, anti-HSP Ab (after digestion with XbaI). Densitometric analysis of mutant H-ras expression from gel (B) and quantitative PCR for mutant H-ras expression in tumors (C). The frequency of the H-ras mutation was significantly greater (*p < 0.05) in tumors from the anti–HSP Ab-treated mice than in mice treated with the control Ab.

FIGURE 6. HSP Ab pretreatment inhibits development of DMBA-specific CTLs. (A) In vivo CTL assay of DMBA-pulsed CFSE low target spleen cells. Mice were pretreated with Abs then sensitized, as indicated. After 8 d following treatment, mice (n = 3/group) received a 1:1 mixture of DMBA-pulsed CFSE low– and unpulsed CFSE high–stained splenocytes by i.v. After 16 h, splenocytes were harvested and analyzed by flow cytometry. The percent CFSE low population of total CFSE–labeled cells is shown in each histogram. The percent CFSE low population in naive mice provided an input baseline control of 53% (confirming the 50:50 input mix). (B) Bar graph of the mean ± SEM percent DMBA-specific cytotoxicity is shown for each group. Arrow indicates percent inhibition. *p < 0.05 by Student t test.
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The method we used for the induction of immunological tolerance was to first pretreat the area of skin used for immunization with Abs to HSP27 and HSP70. There are other methods of producing immunological tolerance to topically applied chemicals. These include oral or i.v. administration of hapten prior to sensitization, topical application of hapten to UV radiation–exposed skin, or i.v. injection of UV-irradiated hapten modified epidermal cells. However, most of those procedures require coadministration of the same or different carcinogenic agents (i.e., DMBA and UV radiation), which would obfuscate efforts to determine whether it was actually alterations in the immune response or differences in the amount of carcinogen that was administered to the animal. In vivo treatment with anti-HSP27 and anti-HSP70 Abs does not have those complicating effects and thus had a number of advantages for these studies.

The generation of immune tolerance by HSPs has been seen in other model systems. In an autoimmune disease model, it has been shown that HSP70 can promote immunogenic APC function and prevent the induction of tolerance. HSP70 increased inflammatory responses and elevated CTL function after enhanced Ag presentation by DCs, similar to what is observed after CD40 ligation. Thus, HSP70 promotes DC function and, together with Ag, triggered autoimmune disease in vivo. Initiation of T cell activation and immune function are crucially dependent on immunostimulatory Ag presentation by professional APCs, most notably DCs (36). Immunological tolerance to self-Ags also depends on the interaction of T cells with APCs, and can result in T cell deletion or induction of unresponsiveness (37, 38). In our model, pretreatment of mice with anti-HSP27 and anti-HSP70 Abs significantly inhibited the production of IL-17 and IFN-γ compared with untreated carcinogen-labeled BMDCs, and there was a corresponding increase in IL-4 and IL-10 production under the same conditions.

HSPs are known to bind TLR4 (5). In other studies, we have found that TLR4 results in the production of IL-12 by DCs, which in turn facilitates production of Tc1, IFN-γ–producing cells that mediate a vigorous cell mediated immune response (15). We have further shown that IFN-γ plays an essential role in preventing the development of carcinogenic PAH-induced tumors (16). Thus, it seems reasonable to postulate that following treatment with a carcinogenic PAH, such as DMBA, HSPs are released in the extracellular space. In that location, they bind to TLR4 resulting in IL-12 production, and biasing DCs toward activation of IFN-γ–producing T cells and cytotoxic T cells. Those T cells then proceed during the early stages of the chemical carcinogenesis pathway to eradicate mutant epidermal cells with the potential to eventually become DMBA-induced skin tumors. Thus, in the absence of HSPs, PAH-induced tumors are more likely to arise.

HSPs, including HSP27 and HSP70, have been associated with the development of cancer (39, 40). In some situations, their expression has been linked to a more aggressive behavior. HSP70 transgenic mice have an increased rate of T cell lymphoma (41), and when HSP70 was transfected into fibrosarcoma cell lines, they became more tumorigenic and resistant to the cytotoxic effects of TNF-α (42). In humans, increased HSP70 has been found in early liver carcinoma, and its expression has been associated with increased tumor grade and poorer prognosis (43). Similarly, significantly higher levels of HSP70 have been found in leukemia, breast, ovarian, and endometrial cancer cell lines compared with nontransformed cells, and there is a direct correlation between HSP27 expression and the grade of ovarian carcinoma (44). HSP27 overexpression has been observed in glial tumors as well (45). In contrast, high levels of HSP70 have been associated with improved outcomes. This is the case for osteosarcoma (46) and renal cell carcinoma (47) in which high levels of HSP70 are found. Moreover, reduced tumor levels of HSP27 have been associated with more aggressive tumors in oral cancers (48). In addition, head and neck cancers in which there is diminished expression of HSP27 have the poorest survival rates (49). Thus, differences in HSP effects may depend on the type of tumor being investigated.

PAHs are a major causative agent for various types of epithelial cancers. They present in tobacco smoke, charcoal-broiled food, and automobile emissions and are causative agents for cancers of the lung, head and neck, bladder, and breast. Although the majority of nonmelanoma skin cancers are caused by overexposure to UV light, there is clear evidence that carcinogenic PAHs are an etiologic factor or cofactor in a significant proportion of squamous cell carcinomas of the skin as well (50). Vaccination against PAHs is analogous to prevention against cervical cancer by administration of HPV vaccines (51) or against liver cancer by hepatitis B vaccines. This strategy has not been used before against chemical carcinogens. Efforts to bolster the immune response to carcinogenic PAHs or to proteins expressed by tumor-initiated cells could be an effective means of preventing their development.

Disclosures

The authors have no financial conflicts of interest.

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