ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/5328995

The effect of vaccination with the lysate of heat-shocked tumor cells on nitric oxide production in BALB/c mice with...

Article *in* Cell Biology International · August 2008

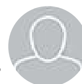
DOI: 10.1016/j.cellbi.2008.03.014 · Source: PubMed

CITATIONS
10

READS
53

4 authors:

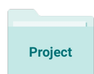
Seyed Mahmoud Hashemi
Shahid Beheshti University of Medical Sciences
57 PUBLICATIONS **1,001** CITATIONS
SEE PROFILE

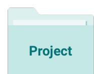

Zuhair Mohammad Hassan
Tarbiat Modares University
148 PUBLICATIONS **1,819** CITATIONS
SEE PROFILE

Sara Soudi
Tarbiat Modares University
28 PUBLICATIONS **355** CITATIONS
SEE PROFILE

Shahram Shahabi
Urmia University of Medical Sciences
32 PUBLICATIONS **341** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:


MSC for EAE View project


ElectroMagnetoChemotherapy: Simulation and Experiments View project

All content following this page was uploaded by Seyed Mahmoud Hashemi on 11 January 2015.

The user has requested enhancement of the downloaded file.

ELSEVIER

Cell Biology International 32 (2008) 835−840

Cell Biology International

www.elsevier.com/locate/cellbi

The effect of vaccination with the lysate of heat-shocked tumor cells on nitric oxide production in BALB/c mice with fibrosarcoma tumor

Seyed Mahmoud Hashemi [a], Zuhair M Hassan [a,*], Sara Soudi [a], Shahram Shahabi [b]

[a] *Department of Immunology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
[b] *Department of Microbiology, Immunology and Genetics, Urmia University of Medical Sciences, Urmia, West Azarbaijan, Iran*

Received 26 September 2007; revised 14 November 2007; accepted 26 March 2008

Abstract

The aim of this study was to investigate the effect of heat shock protein-70 (HSP-70) on splenocyte proliferation and nitric oxide (NO) production in the BALB/c mice fibrosarcoma tumor model. To do so, HSP-70 was induced in the lysate of heat-shocked tumor cells and WEHI-164 cells (mouse fibrosarcoma cell line) were injected subcutaneously into the right flank of inbred BALB/c mice to establish a tumor model. Three animal bearing tumor groups were applied: the test group; vaccinated with HSP-70 enriched tumor lysate; control group I, vaccinated with tumor lysate only; and control group II, which received PBS. Using immunoblot analysis, an increase of HSP-70 expression was detected in the lysate of heat-shocked cells in comparison with non-heat-shocked cells. The effect of the test lysate on NO production was measured both *in vitro* and *in vivo* in the peritoneal macrophages and splenocytes of tumor bearing mice, respectively. The result showed a significant increase in NO production both *in vitro* by peritoneal macrophages and *in vivo* after immunization with HSP-70 enriched tumor lysate. In addition, tumor growth was significantly postponed and the proliferation of splenocytes was increased in the test group. Our results indicate that the lysate of heat-shocked tumor cells was more potent than that of non-heat-shocked tumor cells in inducing anti-tumor immunity. Since production of NO by HSP-activated antigen presenting cells (APCs) is likely to affect innate immunity and tumor growth, the probable mechanism of postponing tumor growth would be NO production by innate immune cells. These findings provide a useful therapeutic model for developing novel approaches to cancer treatments.
© 2008 Published by Elsevier Ltd on behalf of International Federation for Cell Biology.

Keywords: Heat shock proteins; HSP-70; Nitric oxide; Cancer immunotherapy; Fibrosarcoma; Tumor cell lysate

1. Introduction

Although significant advances have been made for some subtypes, many cancers remain resistant to conventional therapies. Immunotherapy, which involves stimulating the immune system to attack tumor cells, has long been investigated as an alternative to conventional therapies. It is attractive, as the specificity of the immune system gives it the potential to target tumor cells while leaving normal cells unharmed (Parney et al., 2002). It has been known for some time that heat shock proteins (HSPs) bind and chaperone a large number of peptides derived from the cells (Gething and Sambrook, 1992; Srivastava and Udono, 1994). HSPs such as gp96, HSP-70 and HSP-90 elicit protective immune responses against challenge with the antigenic context from which they are isolated. This immunogenicity results from the receptor mediated uptake, processing and re-presentation of HSP-associated peptides by MHC I molecules of APCs to cognate T cells. HSP−APC interaction also results in the activation of a proinflammatory program of gene expression, culminating in the production of cytokines TNF-α, IL-12, IL-1 and GM-CSF, and the up-regulation of Ag-presenting and co-stimulatory molecules such as MHC II and CD86 (Panjwani et al., 2002; Srivastava, 2002; Srivastava and Udono, 1994). Early studies have shown that fractionated tumor cell lysate has the capacity to reduce tumor cell growth in mice (Srivastava et al., 1986). The ability of human melanoma-derived HSP-70 to stimulate autologous melanoma-specific T

* Corresponding author. Department of Immunology, School of Medical Sciences, Tarbiat Modares University, P.O. Box 14115 111, Tehran, IR Iran. Tel.: +98 21 88011001x3565; fax: +98 21 88006544.
E-mail address: hasan_zm@modares.ac.ir (Z.M. Hassan).

1065-6995/$ - see front matter © 2008 Published by Elsevier Ltd on behalf of International Federation for Cell Biology.
doi:10.1016/j.cellbi.2008.03.014

cells has been also shown *in vitro* (Castelli et al., 2001). Clinical trials of tumor-derived HSPs have been conducted in patients with a broad range of malignancies including lymphoma, renal cell carcinoma, melanoma, colorectal cancer, gastric cancer, pancreatic cancer and breast cancer (Srivastava, 2002). In a recent phase II clinical trial, vaccination of metastatic melanoma patients with autologous gp96 proteins induced clinical and tumor-specific T-cell responses in a substantial proportion of patients (Belli et al., 2002). It has previously been shown that HSP-70 up-regulation in tumor cells, through heat- or drug-induced stress or by gene transfection (Hashemi et al., 2007; Ito et al., 2001; Todryk et al., 1999, 2004), increased tumor immunogenicity and protected animals from challenge by a wild-type tumor. Additionally the interaction of gp96 and HSP-70 with a variety of murine and human APCs and APC cell lines resulted in the induction of iNOS and the release of NO, which was discovered to have antitumor effects when activated macrophages were cultured with tumor cells (Panjwani et al., 2002). NO is produced by activated macrophages through the enzymatic action of iNOS on L-arginine. The induction of iNOS can be detected in macrophages recovered from individuals undergoing active infection. *In vitro*, iNOS induction occurs in response to stimuli such as IFN-γ, endotoxin and TNF-α (Panjwani et al., 2002). The aim of the present study was to enhance whole cell lysate vaccine immunogenicity through increased HSP expression by heat stress without any purification of HSPs or using any adjutants. In this study, we immunized the mouse model for fibrosarcoma tumors with the lysate of heat-shocked WEHI-164 tumor cells. Tumor growth and several immunological parameters such as splenocytes proliferation and NO production by mouse splenocytes and normal macrophages were detected.

2. Materials and methods

2.1. Mice and tumor models

Female inbred BALB/c mice (6–7 weeks old) were purchased from the Pasteur Institute, Tehran, Iran. They were given sterilized water and autoclaved standard mouse chow *ad libitum* throughout the study. To establish subcutaneous tumors, BALB/c mouse fibrosarcoma cells (WEHI-164) were purchased from the Pasteur Institute, Tehran, Iran. Some 5×10^5 cell/100 µl were injected subcutaneously into the right flank of the inbred BALB/c mice to establish a tumor model (Panthel et al., 2006). Palpable tumors started to develop after 7 days. Tumor growth was monitored every 5 days with vernier calipers after tumor challenge until the experiment was completed. Tumor volume (mm^3) was calculated by the formula: length $\times$ width$^2 \times \pi/6$ (Zeng et al., 2003).

2.2. Cell culture and vaccine preparation

The WEHI-164 cells were cultured in Dulbecco's Modified Eagle's Minimal Essential Medium (GIBCO BRL, USA) supplemented with 10% fetal bovine serum (GIBCO BRL, USA) and incubated in an incubator with 5% CO2 and 95% air at 37 °C. The WEHI-164 cells in the logarithmic growth phase were heated by direct immersion of the cell culture dishes in a water bath, the temperature of which was controlled within ±0.1 °C. After heat treatment of the WEHI-164 cells at 42 °C for 60 min and incubation periods of 8 and 12 h, the cells were collected by detachment with trypsin/EDTA (GIBCO BRL, USA), washed 3 times in PBS and then resuspended in PBS (5×10^5 cell/100 µl). Tumor cell lysate was prepared following a previously published method (Kim et al., 2006; Schnurr et al., 2001). Briefly, the cell suspensions in the medium were disrupted by five cycles of freeze-thaw using liquid nitrogen and a 37 °C water bath. Large particles were removed by centrifugation (20 min, 3000 $\times$ rpm) and the supernatants were passed through a 0.2 µm filter.

2.3. Western blotting for HSP-70 expression

HSP-70 expression was investigated using Western blotting analysis. Equal amounts of the proteins (1.3 mg/ml), determined by Bradford assay from the lysate of 5×10^5 WEHI-164 cells, were fractionated on 10% SDS-PAGE gels and transferred into a polyvinylidene difluoride membrane. After washing, the membrane was blocked with 10% skim milk at room temperature for 1 h and incubated with mouse monoclonal antibody against HSP-70 (1:1000 R&D systems) at 4 °C overnight. After washing, the membrane was incubated with horseradish peroxidase-conjugated anti-mouse antibody (1:1000, Sigma) at room temperature for 1 h. After washing, the immunoreactive bands were detected by 0.5 mg/ml diaminobenzidine (DAB, Sigma) and 0.1% H_2O_2 in PBS.

2.4. Vaccination protocol

Ten days after tumor challenge, the mice of the three groups were treated with the lysate of 1×10^6 cell/ml of the heat-shocked tumor cells at 42 °C for 60 min and incubation periods of 12 h for the test group; the lysate of 1×10^6 cell/ml non-heat-shocked tumor cells for control group 1; and PBS for control group 2. All treatment groups contained six mice. Vaccination was performed subcutaneously into the flank of the mice at weekly intervals for three consecutive weeks.

2.5. Lymphocytes proliferation index

One week after the last vaccination, splenocytes were isolated from the three sacrificed mice. The splenocytes from the untreated mice were used as a negative control. The spleens were passed through 100-µm filters to obtain a single-cell suspension and erythrocytes were lysed at room temperature using ACK lysis buffer (NH_4Cl, $KHCO_3$, Na_2EDTA). The spleen cells were washed and resuspended in RPMI 1640 (GIBCO BRL, USA) supplemented with 10% FCS (GIBCO BRL, USA). The cells, at a concentration of 1×10^5 cell/well, were cultured with the lysate of heat-shocked and non-heat-shocked WEHI-164 cells (1×10^5 cell/well) and ConA (5 µg/ml) (Sigma) as a positive control in a total volume of

200 μl. The plates were incubated for 5 days at 37 °C in a 5% CO_2 humidified atmosphere. The lymphocytes proliferation was determined by an MTT [3, (4, 5-dimethylthiazal-2-yl)-2, 5-diphenyl tetrazolium bromide] assay. The cells, cultured in 96-well plate, were incubated for 4 h in the presence of MTT (5 mg/ml) (Sigma) followed by addition of 0.1 ml dimethyl sulfoxide (DMSO). The formazan crystals were dissolved and absorbance was read at 490 nM by an ELISA reader.

2.6. Nitrite measurement

2.6.1. Nitrite production by splenocytes

One week after the last vaccination, the splenocytes were collected as described above. A total of 1×10^6/ml spleen cells were washed and resuspended in RPMI 1640 (GIBCO BRL, USA) containing LPS (1 μg/ml) plus IFN-γ (50 U/ml) in 96-well plates. Then, 48 h after incubation, the supernatants were collected and tested for nitrite. Nitrite was measured by adding 100 μl of Griess reagent (1% sulphanilamide and 0.1% naphthylenediamine in 5% phosphoric acid) to 100 μl samples of the medium. The optical density at 550 nm (OD 550) was measured with a microplate reader. The concentrations were calculated by comparison with OD 550 of the standard solutions of sodium nitrite prepared in culture medium.

2.6.2. Nitrite production by peritoneal macrophages

Nitrite production was measured in the supernatants of peritoneal macrophages from the normal mice. Briefly, the cells were cultured in 96-well plates with 200 ml of culture medium until the cells reached confluence (approximately 200,000 cells per well). In order to induce iNOS, fresh culture medium containing LPS (1 μg/ml) plus IFN-γ (50 U/ml) was added. Nitrite accumulation in the medium was measured 24 h after the application of LPS/IFN-γ. To assay the effect of the lysate of the heat-shocked and non-heat-shocked cells on nitrite production, the lysate was added, and nitrite production was measured as previously described.

2.7. Statistical analysis

A Student's *t* test was used to calculate the significance of statistical comparisons. In all the analyses, statistical significance was claimed at the 5% level ($P < 0.05$) and for noticeable results ($P < 0.1$). All computations were performed with Statistical Package, version 11.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. HSP-70 expression by Western blotting analysis

To assess the level of HSP-70 in the supernatants of the lysate of the heat-shocked WEHI-164 cells, two groups of the cells were shocked with different temperatures and the lysate of the non-heat-shocked WEHI-164 cell was used as control. The results indicated a significant increase in the level of HSP-70 accumulation in the cells after heat-shock treatment compared with the non-heat-shocked control cells (Fig. 1).

3.2. Tumor volume following the vaccine therapy

The changes of the tumor volume in the three groups of mice were assessed as shown in Fig. 2. The results indicated that the tumors in the test group grew more slowly than those in the control groups. Immunization of the mice with the lysate of heat-shocked tumor cells significantly ($P < 0.01$) suppressed tumor growth compared to the control groups (on days 20, 25, 30 and 35). Similar results were obtained in two separate experiments.

3.3. Lymphocyte proliferation index following vaccine therapy

To estimate the proliferation of lymphocytes of the mice vaccinated with the lysate of the heat-shocked tumor cells, all the three groups of mice were used and the protocol in Fig. 3 was performed. The results indicated a significant increase in the proliferation index of the transformation of the lymphocytes in the test group compared with the control groups.

3.4. Nitrite production by normal mice peritoneal macrophages

In this study, we detected the effect of the lysate of heat-shocked tumor cells on nitrite production by normal mice peritoneal macrophages. NO production by peritoneal macrophages was significantly ($P < 0.01$) higher after *in vitro* treatment with the lysate of heat-shocked tumor cells in comparison with treatment with the lysate of non-heat-shocked tumor cells and media as negative control (Fig. 4).

3.5. Nitrite production by splenocytes

We assessed nitrite production by the splenocytes from the mice vaccinated with heat-shocked vs. non-heat-shocked necrotic tumor cells. Nitrite oxide production by the splenocytes from the mice treated with the lysate of heat-shocked and non-heat-shocked tumor cells was significantly ($P < 0.01$) higher than in the PBS treated mice after stimulation with LPS and IFN-γ (Fig. 5).

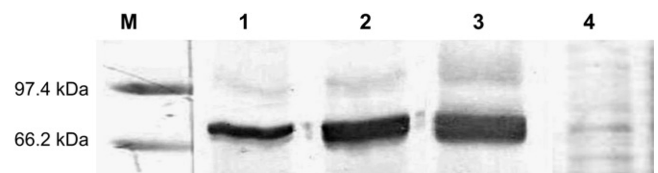


Fig. 1. HSP-70 expression of heat-shocked tumor cells. Western blot analysis of HSP-70 expression in WEHI-164 cells, maintained at 37 °C (1); heat-shocked up to 42 °C, 60 min, (8 h recovery in 37 °C) (2); (12 h recovery in 37 °C) (3); and molecular weight marker (M).

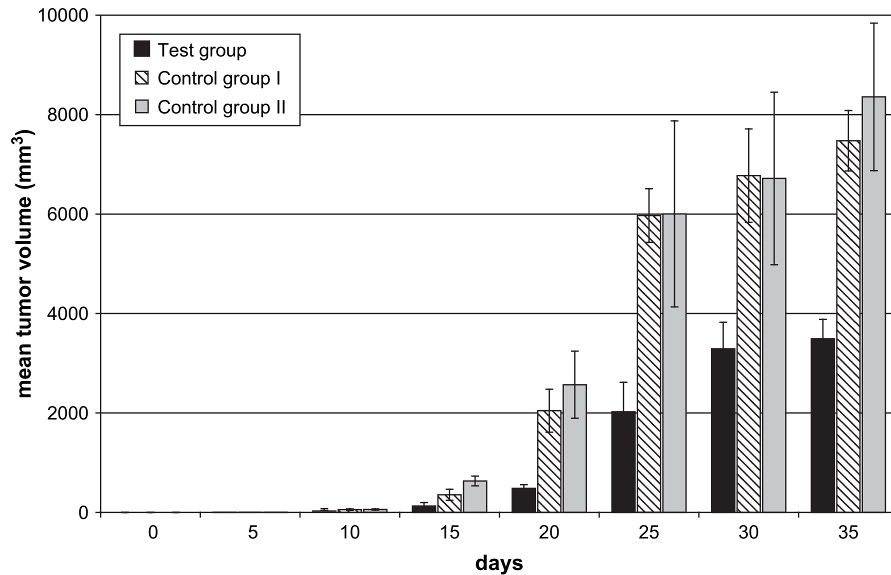


Fig. 2. Change of tumor volumes after immunization. Test group: treated with lysate of heat-shocked tumor cells. Control group I, lysate of non-heat-shocked tumor cells. Control group II, PBS. The values are mean ± SD of tumor volumes ($n = 6$ mice per group).

4. Discussion

HSPs have the ability to chaperone and present a broad antigenic repertoire of tumor cell peptides. There is now comprehensive experimental evidence that the antigenicity of tumor-derived HSP-70, HSP-90 and gp96 preparations results from diverse arrays of endogenous peptide antigens complexed with these stress proteins. Vaccination with tumor-derived stress protein/peptide complexes leads to their uptake and processing by professional APCs and presentation of associated tumor peptide antigens to cytotoxic T cells. This induces a tumor-specific cytotoxic T-cell response (Heike et al., 1999). In addition, HSP-70 itself activates polymorphonuclear cells to produce proinflammatory cytokines suggesting that HSPs are natural adjuvants involved in innate immunity (Ito et al., 2005). Many studies have shown that HSPs, purified from tumor cells, are potent immune modulators and can lead to stimulation of both the innate and adaptive immune responses to tumors (Srivastava, 2002; Srivastava and Udono, 1994). Vaccination with HSPs isolated from tumor cells circumvents the need to identify specific tumor antigens and, hence, extends application of HSP-based immunotherapy to the majority of cancers (Wang et al., 2000). Although irradiated heat-shocked tumor cells (Todryk et al., 2004) and purified HSPs from tumor cells (Srivastava, 2002) have been both reported to elicit anti-tumor immune response, there

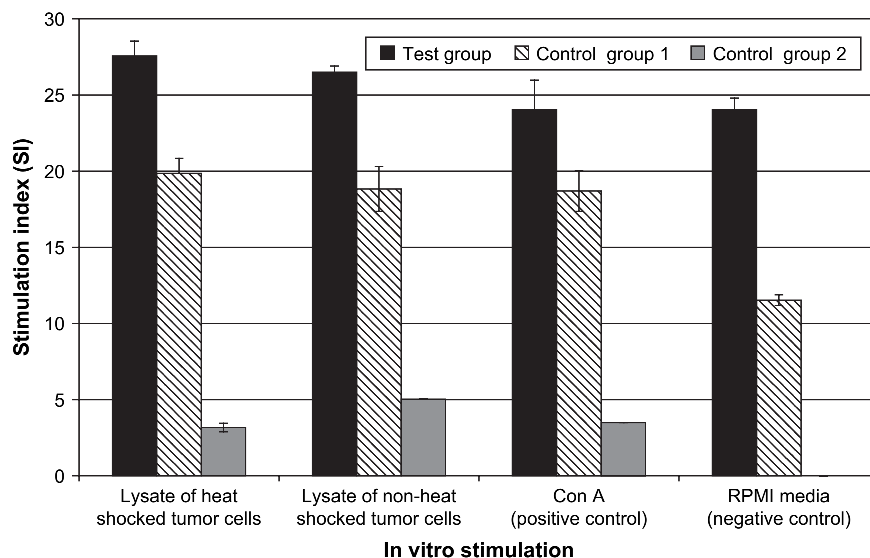


Fig. 3. Proliferation of splenocytes from mice treated with the lysate of heat-shocked tumor cells. The mean ± SD of triplicate determinations are shown ($n = 3$ mice per group).

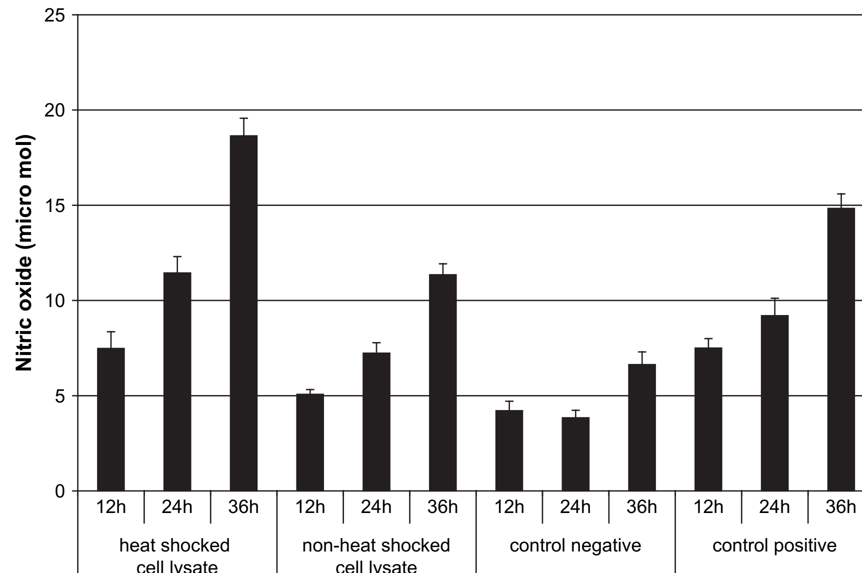


Fig. 4. NO production by peritoneal macrophages from normal mice in the presence of different stimulator. Stimulation with LPS and IFN-γ (control positive) and cell culture media (control negative), ($n = 3$ mice per group, the values are mean ± SD).

has been little information about the cellular immune response to the lysate of heat-shocked tumor cells. Our hypothesis was that after cellular hyperthermia, HSPs would be induced as peptide chaperones and immunostimulators. This could improve the anti-tumor effects of the whole tumor lysate. Our previous results with the same tumor model indicated a significant increase in the CD8+ lymphocytes as well as a significant increase of cytotoxic activity and IFN-γ production by splenocytes (Hashemi et al., 2007); this might be the cause of the decrease in the tumor size. Since there has been little information about the effect of lysate of heat-shocked tumor cells on NO production as an anti-tumor mediator of innate immune system, we assume that the effect of this vaccination procedure is due to NO production. To assess our hypothesis, the lysate of heat-shocked fibrosarcoma cells was administered to the BALB/c mice fibrosarcoma tumor model. Western blot analysis showed an increase of HSP-70 level in the heat-shocked cells compared with the non-heat-shocked controls, indicating that HSP-70 was induced during the cellular hyperthermia. Our findings showed that the immunization of mice with this heat-shocked tumor cell lysate was terminated to a significant reduction in tumor size. Accordingly, a significant increase in the index of splenocyte stimulation and proliferation were significant in the test group. Furthermore, an increase in NO production by peritoneal macrophages was observed after treatment with the lysate of the heat-shocked tumor cells. HSP–APC interaction has been shown previously to result in the secretion of an array of inflammatory cytokines by APCs (Basu et al., 2001; Binder et al., 2000). Additionally, the interaction of the gp96 and HSP-70 with a variety of murine and human APCs and APC cell lines results in the induction of iNOS and NO production (Panjwani et al., 2002). Furthermore, HSP-90 has been shown to be an important endogenous protein enhancer of iNOS. HSP-90 associates with iNOS in cells, and this association facilitates NO synthesis (Yoshida and Xia, 2003). In all recent studies, different HSPs have been purified from tumor cells and the effects of the purified HSPs on NO production have been detected. In the present study we have not purified any kind of HSPs; heat treatment of the tumor cells caused enrichment of the cells with HSP-70 and probably other HSPs that may induce NO production. We also analyzed one of the important heat inducible HSPs, HSP-70, and the result indicate a significant increase in its expression after heat treatment. We know that tumor cells in normal condition express different HSPs, as detected about HSP-70 in the lysate of non-heat-shocked tumor cells (Fig. 1). One of the functions of the macrophages is to provide a defense mechanism against tumor cells. In recent decades, the mechanism of macrophages killing tumor cells has been studied extensively. Macrophage-mediated tumor cytotoxicity is a slow, cell-to-cell contact-dependent process

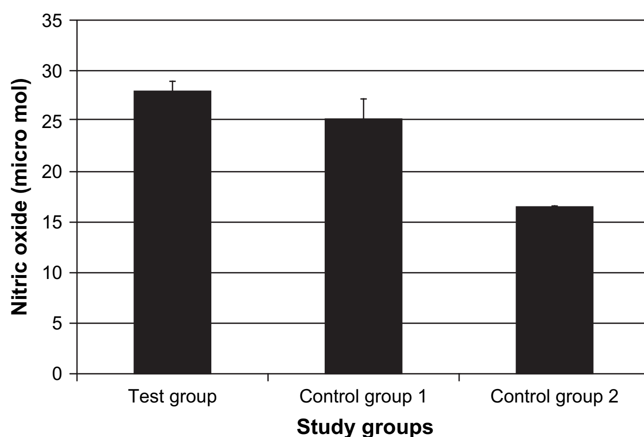


Fig. 5. NO production by splenocytes from immunized mice ($n = 3$ mice per group, the values are mean ± SD).

requiring 1–3 days. The susceptibility to macrophage-mediated tumor cytotoxicity varies greatly among different tumor cells (Klimp et al., 2002). The tumor cytotoxic function of macrophages requires stimulation with cytokines such as IFN-γ. Activated macrophages secrete several substances that are directly involved in the killing of tumor cells, i.e., TNF and NO (Klimp et al., 2002). Taken together, TNF and NO are considered as the most important mediators directly involved in killing tumor cells. These mediators are most effective when there is a close cell-to-cell contact. In addition to production of TNF-α, NO production has been shown to be a major tumoricidal mechanism of activated macrophages *in vitro* and *in vivo*. The toxic effects of NO and its derivatives on target cells are due to several mechanisms (Klimp et al., 2002). First, NO mediates loss of iron from cells thereby inactivating iron–sulfur cluster-containing enzymes in the citric acid cycle and the mitochondrial electron transport. Second, NO is capable of inducing zinc release from zinc-containing proteins, thereby inducing disulfide formation, which inhibits DNA-binding activity of zinc finger type transcription factors. Third, NO is capable of influencing the activity of ion channels, thereby destroying the mitochondrial membrane potential (Klimp et al., 2002). Overall, the present study suggests that increased expression of HSPs through heating, within whole tumor cell vaccines, is a means of enhancing immunogenicity without genetic modification or inclusion of potentially toxic adjuvants. Our findings also suggest that the lysate of the heat-shocked tumor cells can activate the monocytes and macrophages. The lysate of the heat-shocked tumor cells appears to exert its anti-tumor effects by stimulating the production of NO and through other mechanisms such as secretion of the cytokines including IL-1b and TNF-α which should be analyzed in detail in future studies.

Acknowledgments

We gratefully thank Dr. Nafiseh Pakravan for critical reading and helpful comments on the manuscript.

References

Basu S, Binder RJ, Ramalingam T, Srivastava PK. CD91 is a common receptor for heat shock proteins gp96, HSP-90, HSP-70, and calreticulin. Immunity 2001;14:303–13.

Belli F, Testori A, Rivoltini L, Maio M, Andreola G, Sertoli MR, et al. Vaccination of metastatic melanoma patients with autologous tumor-derived heat shock protein gp96-peptide complexes: clinical and immunologic findings. J Clin Oncol 2002;20:4169–80.

Binder RJ, Anderson KM, Basu S, Srivastava PK. Cutting edge: heat shock protein gp96 induces maturation and migration of CD11c+ cells *in vivo*. J Immunol 2000;165:6029–35.

Castelli C, Ciupitu AM, Rini F, Rivoltini L, Mazzocchi A, Kiessling R, et al. Human heat shock protein 70 peptide complexes specifically activate antimelanoma T cells. Cancer Res 2001;61:222–7.

Gething MJ, Sambrook J. Protein folding in the cell. Nature 1992;355:33–45.

Hashemi SM, Hassan ZM, Soudi S, Ghazanfari T, Kheirandish M, Shahabi S. Evaluation of anti-tumor effects of tumor cell lysate enriched by HSP-70 against fibrosarcoma tumor in BALB/c mice. Int Immunopharmacol 2007;7:920–7.

Heike M, Weinmann A, Bethke K, Galle PR. Stress protein/peptide complexes derived from autologous tumor tissue as tumor vaccines. Biochem Pharmacol 1999;58:1381–7.

Ito A, Fujioka M, Tanaka K, Kobayashi T, Honda H. Screening of cytokines to enhance vaccine effects of heat shock protein 70-rich tumor cell lysate. J Biosci Bioeng 2005;100:36–42.

Ito A, Shinkai M, Honda H, Kobayashi T. Heat-inducible TNF-alpha gene therapy combined with hyperthermia using magnetic nanoparticles as a novel tumor-targeted therapy. Cancer Gene Ther 2001;8:649–54.

Kim HS, Choo YS, Koo T, Bang S, Oh TY, Wen J, et al. Enhancement of antitumor immunity of dendritic cells pulsed with heat-treated tumor lysate in murine pancreatic cancer. Immunol Lett 2006;103:142–8.

Klimp AH, de Vries EG, Scherphof GL, Daemen T. A potential role of macrophage activation in the treatment of cancer. Crit Rev Oncol Hematol 2002;44:143–61.

Panjwani NN, Popova L, Srivastava PK. Heat shock proteins gp96 and HSP-70 activate the release of nitric oxide by APCs. J Immunol 2002;168:2997–3003.

Panthel K, Meinel KM, Sevil Domenech VE, Geginat G, Linkemann K, Busch DH, et al. Prophylactic anti-tumor immunity against a murine fibrosarcoma triggered by the Salmonella type III secretion system. Microbes Infect 2006.

Parney IF, Farr-Jones MA, Kane K, Chang LJ, Petruk KC. Human autologous *in vitro* models of glioma immunogene therapy using B7–2, GM-CSF, and IL12. Can J Neurol Sci 2002;29:267–75.

Schnurr M, Galambos P, Scholz C, Then F, Dauer M, Endres S, et al. Tumor cell lysate-pulsed human dendritic cells induce a T-cell response against pancreatic carcinoma cells: an *in vitro* model for the assessment of tumor vaccines. Cancer Res 2001;61:6445–50.

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

Srivastava PK, DeLeo AB, Old LJ. Tumor rejection antigens of chemically induced sarcomas of inbred mice. Proc Natl Acad Sci USA 1986;83:3407–11.

Srivastava PK, Udono H. Heat shock protein-peptide complexes in cancer immunotherapy. Curr Opin Immunol 1994;6:728–32.

Todryk S, Melcher AA, Hardwick N, Linardakis E, Bateman A, Colombo MP, et al. Heat shock protein 70 induced during tumor cell killing induces Th1 cytokines and targets immature dendritic cell precursors to enhance antigen uptake. J Immunol 1999;163:1398–408.

Todryk SM, Eaton J, Birchall L, Greenhalgh R, Soars D, Dalgleish AG, et al. Heated tumour cells of autologous and allogeneic origin elicit anti-tumour immunity. Cancer Immunol Immunother 2004;53:323–30.

Wang XY, Kaneko Y, Repasky E, Subjeck JR. Heat shock proteins and cancer immunotherapy. Immunol Invest 2000;29:131–7.

Yoshida M, Xia Y. Heat shock protein 90 as an endogenous protein enhancer of inducible nitric-oxide synthase. J Biol Chem 2003;278:36,953–8.

Zeng Y, Feng H, Graner MW, Katsanis E. Tumor-derived, chaperone-rich cell lysate activates dendritic cells and elicits potent antitumor immunity. Blood 2003;101:4485–91.

View publication stats