Genetic Heterogeneity of Heat Shock Protein Synthesis and Sensitivity of Mouse Splenocytes to Antiproliferative Effect of Alkylating Agents

A. L. Pukhal'skii, V. K. Vikulova, A. P. Toptygina, and V. N. Lyashko

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The intensity of heat shock protein synthesis by splenocytes is assessed in mice of two strains with genetically different sensitivity to antiproliferative effect of alkylating effect of maphosphamide. The content and *de novo* production of heat shock proteins in resistant BALB/c mice are higher than in sensitive DBA/2 mice. Exposure to heat shock increases cell resistance to antiproliferative action of maphosphamide.

Key Words: *heat shock proteins; alkylating agents; maphosphamide; interstrain differences*

Strain-specific differences in the sensitivity of lymphoid target cells to antiproliferative action of alkylating agents determine the suppressive effect of these agents on immune response of mice of different genotypes [1,11,13]. Recent studies of molecular mechanisms of the effects of alkylating agents on immunocompetent cells showed that antiproliferative effect of low doses of alkylating drugs on the lymphoid cell is due to blocking of the receptor β-subunit for interleukin-2 [12]. This does not explain the mechanisms of genetic variability of the sensitivity of lymphocytes stimulated by mitogens to antiproliferative action of alkylating compounds. On the other hand, cells of all live organisms are known to react to stress by rapid acceleration of few specific genes coding for heat shock proteins (HSP) [8]. One of HSP functions is cell protection from untoward consequences of stress. Eukaryotes contain multiple copies of genes belonging to the HSP70 family. Two groups of HSP70 proteins were identified in mice: proteins with molecular weight of 68 kD induced under increased temperature (inducible HSP) and with molecular weight of 70-74 kD, or constitutive HSP [3,4]. The expression of HSP genes is increased in some vertebrates living in hot climate [15]. Similar data were obtained in study of human populations traditionally living in the arid zone [5].

Interstrain differences in sensitivity to some xenobiotics, including alkylating compounds, can be caused by differences in the production of HSP in animals of different genotypes. Mice of two strains were shown to be opposite by splenocyte sensitivity to antiproliferative effect of alkylating agents: DBA/2 mice were identified as sensitive and BALB/c as relatively resistant [1,13].

MATERIALS AND METHODS

DBA/2J and BALB/cJlac mice weighing 22-24 g from *Stolbovaya* Breeding Center, Russian Academy of Medical Sciences were used. Maphosphamide (MP, Asta Medica AG) was the alkylating agent.

Lymphoid cells were isolated from mouse spleen homogenate, washed twice, and resuspended in RPMI-1640 medium with 10% inactivated equine serum, 2×10^{-3} M HEPES, 2 mM L-glutamine, 2.8×10^{-6} M 2-mercaptoethanol, and 20 μg/ml gentamicin. Pools of cells from 4 animals of the same strain were used. After 3-min preliminary warming, the cells were incubated for 1 min in a water bath at 42.5°C and then incubated at 37°C for 1 h. Control cells were

Medical Genetics Research Center, Russian Academy of Medical Sciences, Moscow

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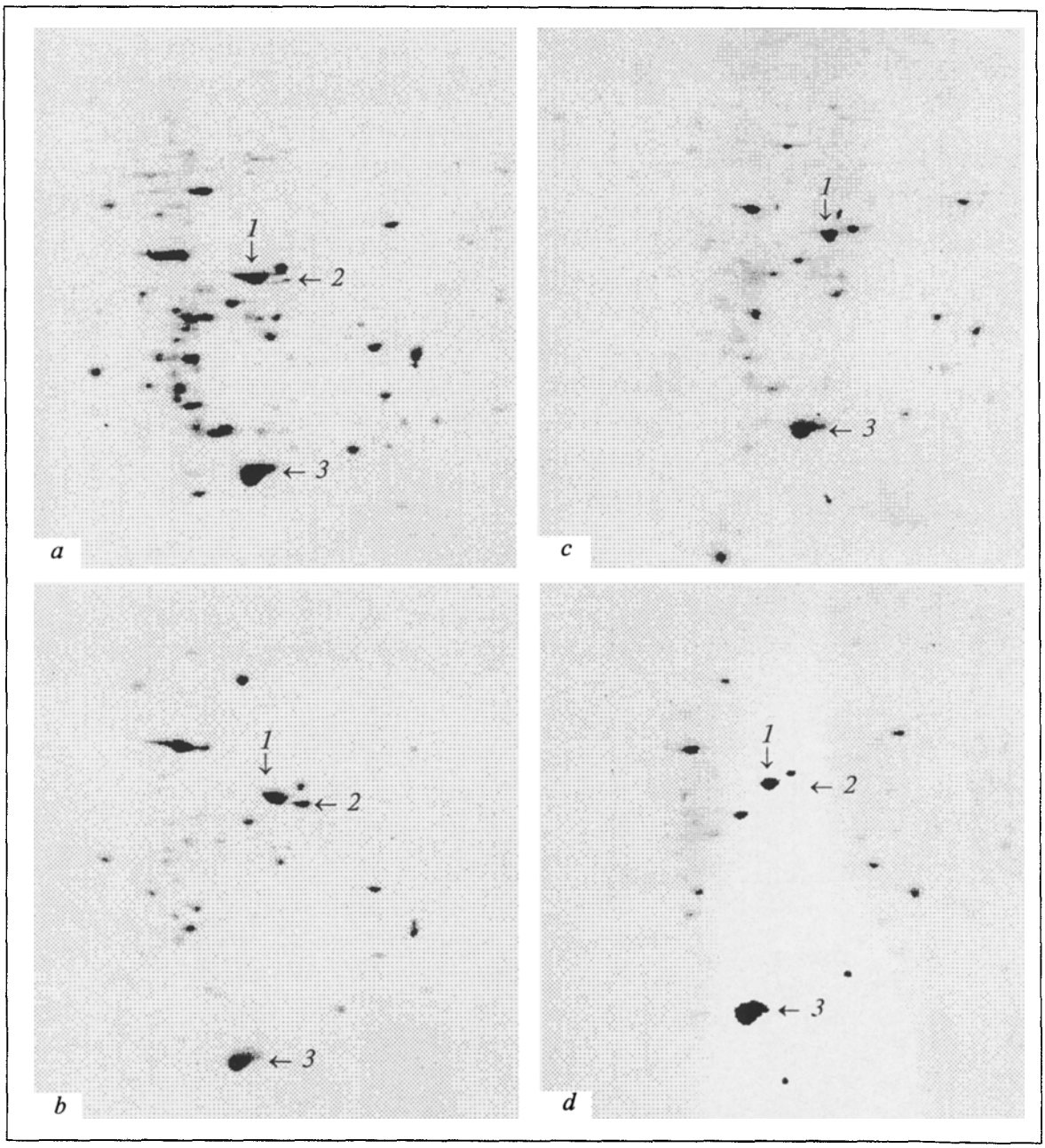

Fig. 1. Production of heat shock proteins-70 by intact splenocytes (*a*, *c*) and cells exposed to heat shock (*b*, *d*) of BALB/c (*a*, *b*) and DBA/2 (*c*, *d*) mice. Arrows show constitutive (*1*) and inducible (*2*) heat shock proteins and actin (*3*).

incubated for 4 min at ambient temperature and then for 1 h at 37°C. Culturing was carried out in flat-bottom 96-well Nunc plates, 2×10^5 cells per well.

Proliferative response to concanavalin A (ConA, Calbiochem) was inhibited by 1-h incubation of cells in the presence of 6 different concentrations of MP (0.01-30 μg/ml). Cells were incubated at 37°C in humid atmosphere with 5% CO_2 for 1 h, after which the plates were centrifuged at 400*g* for 10 min at 4°C, the supernatant was removed with Transtar-96 (Costar), and the cells were resuspended in fresh medium with ConA in a final concentration of 5 μg/ml. Control wells contained cells incubated without MP in medium with ConA or culture medium alone. Cells were incubated under the same conditions for 72 h. Four hours before the end of culturing, 40 kBq of ^{3}H-thymidine was added into each well. The cells were transferred onto filters, and their radioactivity was measured in a liquid scintillation spectrophotometer. The results were expressed in rpm.

For assessing the intensity of HSP70 production, the cells after 1-min incubation at 42.5°C and 1-h restoration at 37°C were incubated in the presence of ^{14}C-labeled protein hydrolysate for 1 h. Labeled

cell lysates were examined by two-dimensional electrophoresis [2,9]. Proteins were imaged by autoradiography, after which two-dimensional autoradiograms were processed by densitometric scanning. HSP in the cells were measured by Western immunoblotting [14] using rabbit polyclonal antibodies (H7) to calf HSP-73 [6] and goat antirabbit antibodies conjugated with horseradish peroxidase (Institute of Vaccines and Sera, St. Petersburg).

RESULTS

The differences in the intensity of synthesis of 70 kD proteins before and after heat shock are presented at autoradiogram (Fig. 1). Computer scanning helps more accurately assess the quantitative differences in the production of these proteins (Fig. 2, *a*). Obviously, heat shock suppresses the production of virtually all proteins in lymphoid cells, except actin. The production of HSP notably increases under such conditions. The production of HSP is more intensive in lymphoid cells of BALB/c mice than in DBA/2 mice. This is true for both constitutive and inducible HSP, and these differences do not depend on exposure of cells to heat shock.

The total count of HSP70 in mice of different strains was assessed using H7 antibodies. Western immunoblotting showed that the total content of HSP70 in BALB/c mice (intact and exposed to heat shock) is higher than in the cells of DBA/2 mice (Fig. 3).

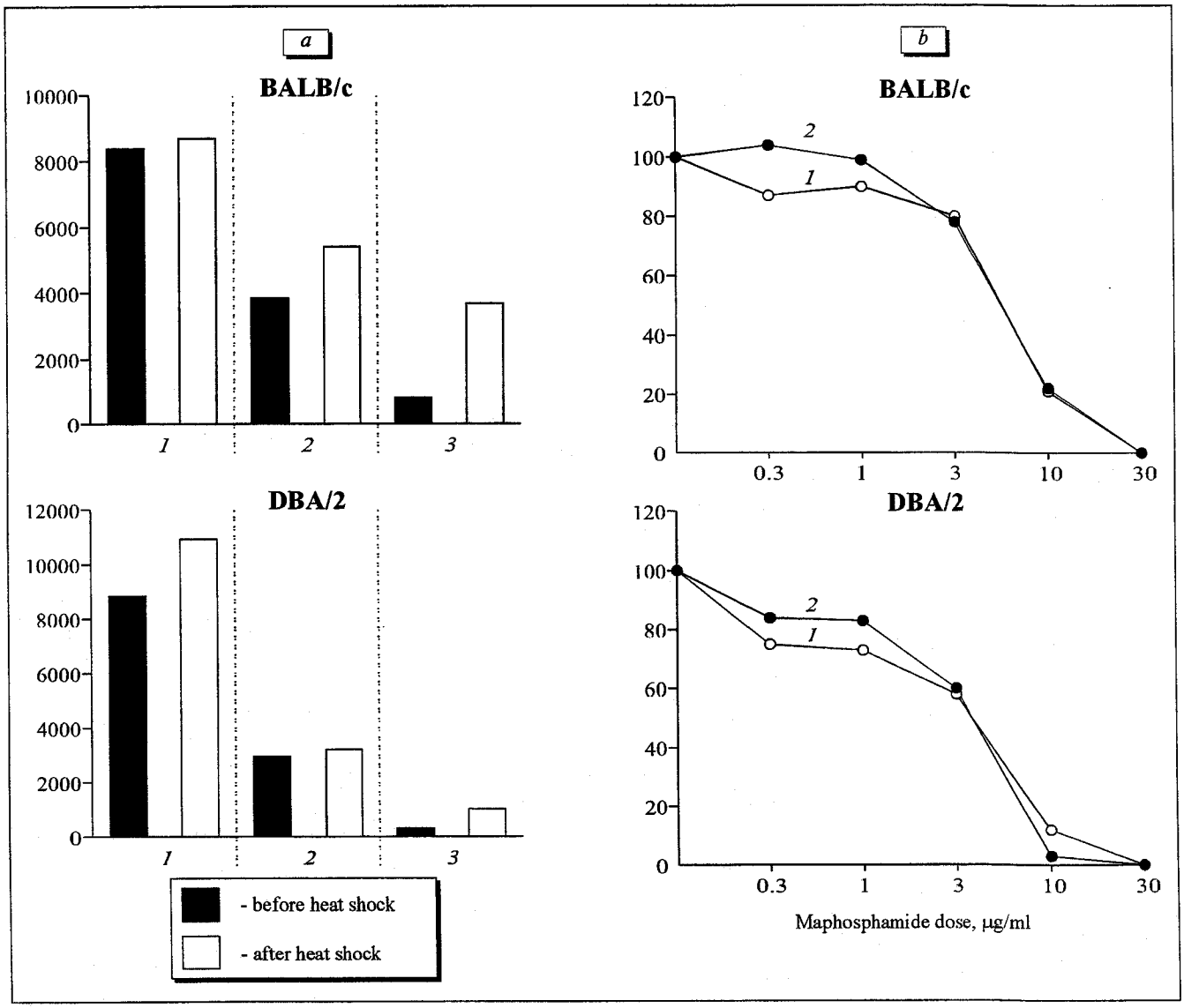

Fig. 2. Effect of heat shock on the production of heat shock proteins-70 and sensitivity of splenocytes to antiproliferative effect of maphosphamide. *a*) densitometric characteristics of autoradiograms (area×density) for BALB/c and DBA/2 mice. *1*) actin; *2*) constitutive and *3*) inducible heat shock proteins. *b*) effect of different maphosphamide doses on proliferative response of splenocytes to ConA. *1*) intact cells; *2*) cells exposed to heat shock. Ordinate: proliferative response, % of control.

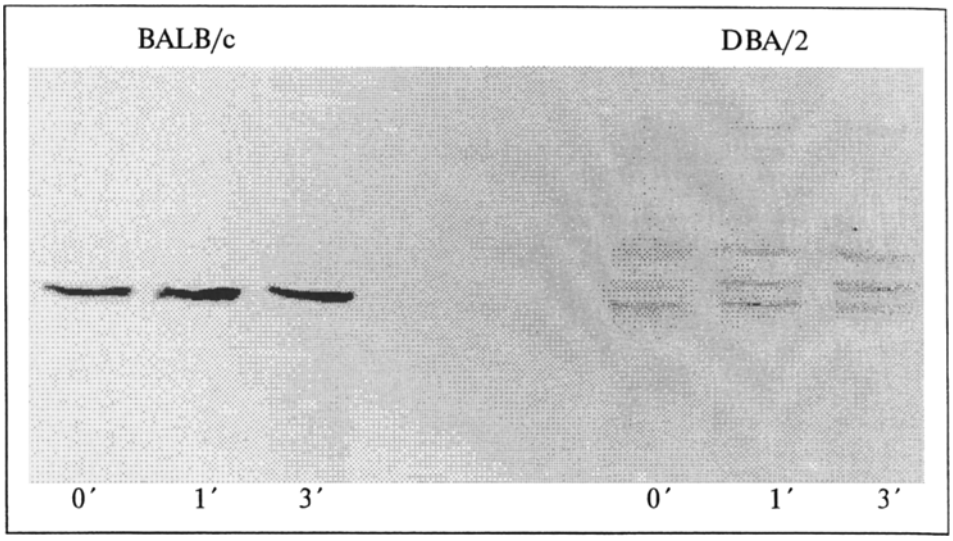

Fig. 3. Absolute quantities of heat shock proteins detected by Western immunoblotting with antibodies to heat shock proteins-68. Splenocytes of BALB/c and DBA/2 mice were examined before or after 1- and 3-min heat shock.

We selected the conditions of heat shock so that the proliferative response of splenocytes to ConA was not decreased. The cells of both strains exposed to heat shock were less sensitive to antiproliferative effect of MP than intact lymphocytes (Fig. 2, *b*). These results were reproduced in two independent experiments on splenocytes from individual DBA/2 mice (Table 1).

Hence, the intensity of HSP70 production is different in mice of different strains. The cells of BALB/c mice characterized by a higher content of HSP were more resistant to antiproliferative action of alkylating agents. The detected correlation might be not accidental, and the induction of HSP production by hyperthermia did increase cell resistance to MP, although the mechanism of such defense is not clear. On the other hand, we know that HSP play the role of chaperon proteins in the processes of assembly, spiralization, and transport of different cell proteins. In a stressed cell, HSP70 proteins can dissolve the denatured proteins and then remove them and promote respiralization of partially denatured molecules [7,16]. Previously we showed that MP inhibition of lymphocyte response to mitogenic stimulation is mediated by blocking the β-chain receptor for interleukin-2 [12], but it is not clear which, specifically, site of the chain is alkylated. Probably, alkylation is performed within the cytoplasmatic domain of the receptor and disturbs its three-dimensional structure. HSP molecules may protect the site of probable alkylation and/or repair the structures damaged by MP. In such a case a high level of HSP production should better protect the interleukin-2 receptors from denaturing agents. It is also probable that protective mechanisms of this kind are switched on in L-929 cells, whose resistance to cytotoxic effect of tumor necrosis factor is increased as a result of stress (e.g., heat shock) [10].

Our data indicate the contribution of the heat shock genes to cellular mechanisms determining the individual sensitivity of an organism to xenobiotics.

TABLE 1. Effect of Thermal Shock on Sensitivity of DBA/2 Mouse Splenocytes to Antiproliferative Effect of Maphosphamide

Mouse No.	Area under curve, % of control×mg/ml		Δ
	intact cells	cells exposed to thermal shock	
1	235.5	266.5	28
2	257.5	287.5	30
3	264	271.5	7.5
4	315	322	7
5	230	244	14
6	216.5	233	16.5
7	246.5	356	109.5
8	241	362.5	24.5

Note. $p<0.01$ (Wilcoxon test for bound totalities) *vs.* intact cells.

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