EXPERIMENTAL GENETICS

Comparative Study of Mechanisms of Antiproliferative Action of Mafosfamide and Cyclosporin A in Low Doses

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The existence of interstrain variations in sensitivity to the antiproliferative action of alkylating agents [2] has raised the question about the precise mechanisms of these differences. It is widely accepted that the cytostatic action of alkylating agents is the result of DNA-DNA linkage [11] and/or alkylation of amino acid residues, which affects the biological activity of protein molecules [4]. It seems that the aforesaid mechanisms are significant for the suppression of malignant cell proliferation when there is no alternative but irreversible damage of cell reproduction. However, it is necessary to look for more specific mechanisms if the alkylating drug is used not as a crucial cytostatic but as a regulator of immune reactions.

In the studies reported here we tested some mechanisms of the susceptibility of murine lymphocytes to the antiproliferative effect of alkylating agents in comparison with cyclosporin A (CsA) action. CsA affacts the early steps of T cell activation, one of the key events being the blockade of interleukin-2 (IL-2) production [7, 9] and IL-2 receptor (Tac) expression [5, 6, 10]

MATERIALS AND METHODS

BALB/cJlac, DBA/2J, CC57BR/Mv, and C57Bl/6J mice were obtained from the Russian Academy of Medical Sciences Care Units Stolbovaya and Rappolovo (CC57BR). Male mice weighing 22-24 g were used. All animals were kept on a standard diet and had free access to water.

The immunodepressants used were Mafosfamide (Asta Z 7654, Asta-Werke, Germany) and Cyclosporin A (Sandimmune, Sandoz, Switzerland).

Lymphoid cells were isolated from murine spleen with a glass homogenizer. Inhibition of Con A stimulated by Mf of CsA was evaluated at six different concentrations within the dose range of 0.1 to 30 μg/ml and 0.03 to 10 μg/ml, respectively. The cells were incubated for 1 h at 37°C in a humidified atmosphere containing 5% CO_2 and washed. Fresh culture medium with Con A in a final concentration of 20 μg/ml was added. The cells were incubated for 72 h under the same conditions. Four hours before the end of cultivation, each well was pulsed with 40 kBq of ^{3}H-thymidine. The cells were harvested and counted on a liquid scintillation counter.

The spleen cells (native of preincubated with different concentrations of antiproliferative agents) in a concentration of 5×10^6 cells per ml were resuspended in the culture medium with 20 μg/ml Con A to perform the inhibition of IL-2 production. The cells were incubated (see above) for 4 h in the wells (1 ml per well) of 24-well plates (Nunc, Denmark) The cells were then washed twice in the same plate with a serum-free medium, resuspended again in the culture

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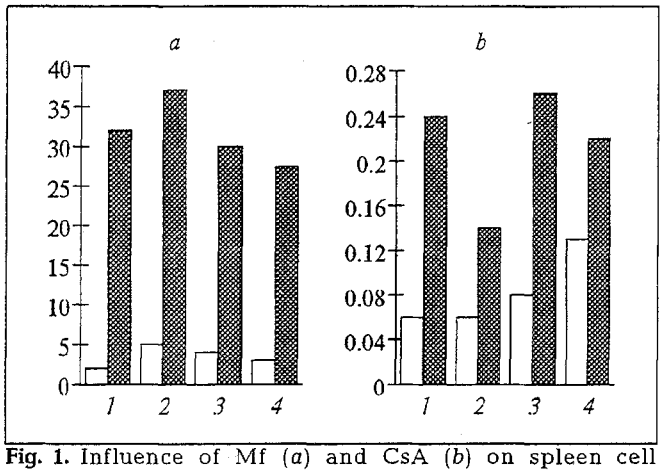

Fig. 1. Influence of Mf (*a*) and CsA (*b*) on spleen cell proliferation and IL−2 production in mice of various strains. Cell proliferation (left bar); IL−2 production (right bar). Here and in Fig. 2 the ordinate shows the ED_{50} values (mg/ml). Here and in Figs. 2 and 3: *1*) C57Bl/6, *2*) CC57BR; *3*) BALB/c; *4*) DBA/2.

medium, and incubated for 18-20 h. The supernatant were collected and stored at -20°C until use. The amount of IL-2 in separated samples was determined by their ability to maintain the growth of an IL-2-dependent cytotoxic T-cell line (CTLL) [8]. The titration curves of the tested samples and the controls (recombinant IL-2) were analyzed by probit analysis [1, 3].

The response to IL-2 by Mf- or CsA-treated murine thumocytes was tested with the comitogenic action of IL-2. The effectiveness of IL-2 utilization was evaluated by the area under the titration curve. For comparison of the result normalization was performed: X/min (X), where min(X) is the minimal value of the X series.

Statistical analysis was performed by Wilcoxson-Mann-Whitney's *U* test and probit analysis.

RESULTS

Significant differences between the various strains of mice in their sensitivity to the antiproliferative action of Mf were demonstrated. Thus, the cells of C57Bl/6 and DBA/2 mice were determined as highly sensitive and the cells of CC57BR and BALB/c mice as low-sensitive (Fig 1, *a*). The same investigations carried out with CsA demonstrated a different distribution of sensitivity types: the cells of C57Bl/6 and CC57BR were more sensitive than DBA/2 cells; the sensitivity of BALB/c cells was determined as intermediate (Fig. 1, *b*).

Our data show that Mf has a dose-dependent effect on IL-2 production by murine spleen cells. Despite the existence of some interstrain differences in suppression of IL-2 production by Mf, the absolute ED_{50} values were ten times higher than the doses which are able to produce 50 percent inhibitory effect on ConA-dependent spleen cell proliferation (Fig. 1, *a*). The same results were obtained with CsA (Fig. 1, *b*).

The study of the comitogeneic action of IL-2 showed that thymocytes of mice of different strains had different sensitivity to the antiproliferative action of Mf and CsA. For example, the DBA/2 mice were more sensitive to Mf, whereas the C57Bl/6 mice were more resistant (Fig. 2, *a*). In contrast, CsA suppressed the response to IL-2 in C57Bl/6 mice in the strongest manner, while DBA/2 proved to be a more resistant strain (Fig. 2, *b*).

These interstrain variations may have been related to differences in IL-2 receptor (IL-2R) expression. We checked this hypothesis, assuming that the level of thymocyte response to IL-2 without PHA reflects the presence of constitutive IL-2R, and the subtraction of this level from the level of thymocyte response to IL-2 with submitogeneic doses of PHA characterizes the induced (p55) IL-2R expression. The results show that the constitutive IL-2R were expressed in DBA/2 and BALB/c mice rather well. In contrast, their expression in C47Bl/6 and CC57BR mice was poor (Fig. 3, *a*). The best PHA-stimulated

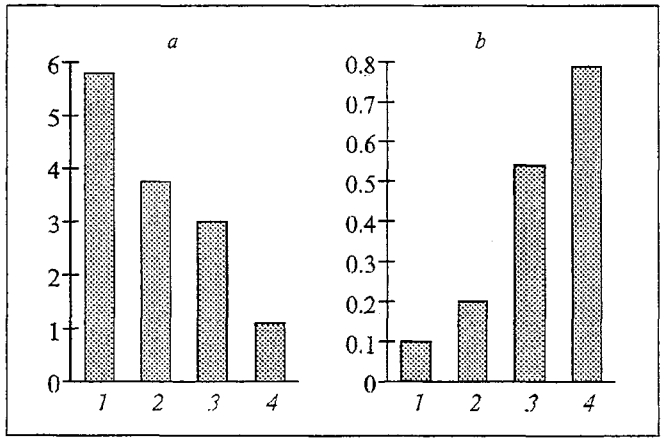

Fig. 2. Response to IL−2 of murine thymocytes treated with Mf (*a*) and CsA (*b*).

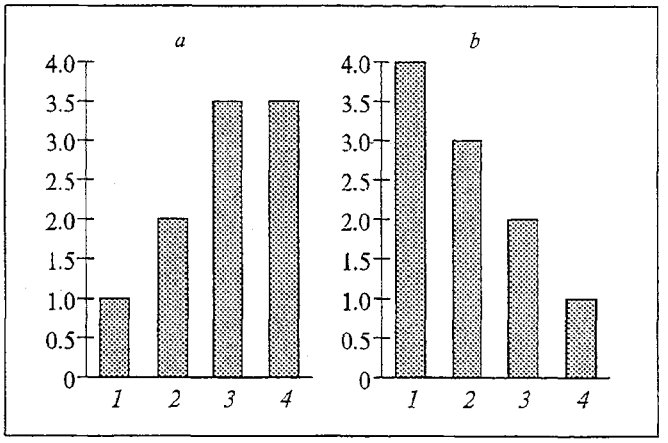

Fig. 3. Constitutive (*a*) and PNA−induced (*b*) IL−2R expression in mice of various strains (see text for the method of normalization).

IL-2R induction observed in C57BL/6 and the minimal induction in DBA/2 mice (Fig. 3, *b*).

The above data indicate that the inhibition of IL-2 production by Mf and CsA cannot play an important role in lymphoid cell sensitivity to the antiproliferative action of these drugs.

Our data obtained with murine thymocytes indicate the existence of a connection between IL-2R expression and cell sensitivity to the antiproliferative action of CsA. It is known that normal lymphocytes treated *in vitro* with CsA show significant inhibition of the light chain (p55, Tac) of IL-2R. It is likely that the response of CsA-treated thymocytes depends on the level of expression of constitutive (p75) IL-2R, as the cells of mice with high sensitivity to CsA reveal a low level of its expression, and on the other hand the cells of resistant mice show a high level of p75 IL-2R expression (Fig. 3, *b*).

A study of Mf efficacy on the thymocyte proliferative response showed that the DBA/2 mice were most sensitive and the C57Bl/6 mice most resistant. The cells of DBA/2 mice revealed a high level of constitutive and a low level of induced IL-2R, respectively. On the contrary we obtained a low level of constitutive IL-2R and a high level of induced IL-2R in C57Bl/6 mice (Fig. 3, *a, b*).

In conclusion this study indicates that the heavy (p75) chain of IL-2R may be a general target for Mf doses which are relatively low, but sufficient for cell proliferation suppression.

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