6. S. Bhakdi, M. Roth, A. Sziegoleit, and J. Tranum-Jensen, *Infect. Immun.*, **46**, 394-400 (1984).
7. R. T. Dean, *Brit. J. Cancer*, **55**, Suppl. 8, 39-45 (1987).
8. M. K. Johnson and K. S. Aultman, *J. Gen. Microbiol.*, **101**, № 2, 237-241 (1977).
9. Y. Kanbayashi, M. Hotta, and J. Kogama, *J. Biochem.* (Tokyo), **71**, № 2, 227-237 (1972).
10. E. W. Kellogg and J. Fridovich, *J. Biol. Chem.*, **252**, № 19, 6721-6728 (1977).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *Ibid.*, **193**, № 1, 265-275 (1951).
12. T. D. Oberly and Y. L. Duncan, *Infect. Immun.*, № 4, 683-687 (1971).
13. G. M. Vercelotti, B. S. Van Asbeck, and H. S. Jacob, *J. Clin. Invest.*, **76**, 956-962 (1985).
14. K. Weber and M. Osborn, *J. Biol. Chem.*, **244**, № 16, 4406-4412 (1969).

Analysis of the Immunomodulating Effect of SK in Mice of Different Strains

E. I. Zlishcheva, G. B. Kirillicheva, A. Ya. Shurygin, I. G. Baturina, and G. L. Ratgauz

UDC 615.276.2.076.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 3, pp. 278–280, March, 1993
Original article submitted October 13, 1992

Key Words: *SK immunomodulator; 5'-nucleotidase; macrophages; resistance to staphylococcal infection; interstrain differences*

In recent years immunomodulators enhancing nonspecific resistance have been increasingly more widely used for a directed influence on immune reactions and for the correction and regulation of immune processes [3]. This poses the problem of the optimal choice of agents in the context of a given infection, and the elaboration of optimal dosages and regimens. At Kuban State University a new immunomodulator of microbial origin has been obtained. The preparation, named SK, represents a mixture of polypeptides with a molecular weight in the range of 500 to 4000 D [6].

Our aim was to study specific features of the immunomodulatory effect of SK in mice of different strains. Immunomodulatory activity was assessed according to the changes in: a) the 5'-nucleotidase (5'-N) activity in the peritoneal exudate macrophages (PEM) and b) the susceptibility of mice to infection with *Staphylococcus aureus* strain Wood-46. There is some information indicating significance of 5'-N for nucleotide assimilation, energy supply of the cells, and the implementation of genetic information [5,9,10]. The product of the enzymatic reaction, adenosine, is considered today to be one of the main regulators of vital physiological functions, including the work of the immune system [7,11].

MATERIALS AND METHODS

Male mice of the CBA and C57Bl/6 strains weighing 16-18 g were used. The animals received a single subcutaneous (sc) or intraperitoneal (ip) injection of SK, 0.2 μg or 5 μg per mouse. The following day ecto-5'-N activity in the PEM was determined according to a modified method [4]. At this time the mice were given sc 2.5×10^9, 5×10^9 or 10×10^9 cells from a one-day culture of *St. aureus* strain Wood-46. The mice were observed for 15 days. The effect

Kuban State University, Krasnodar; N.F. Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow. (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

0007–4888/93/0003–0300$12.50

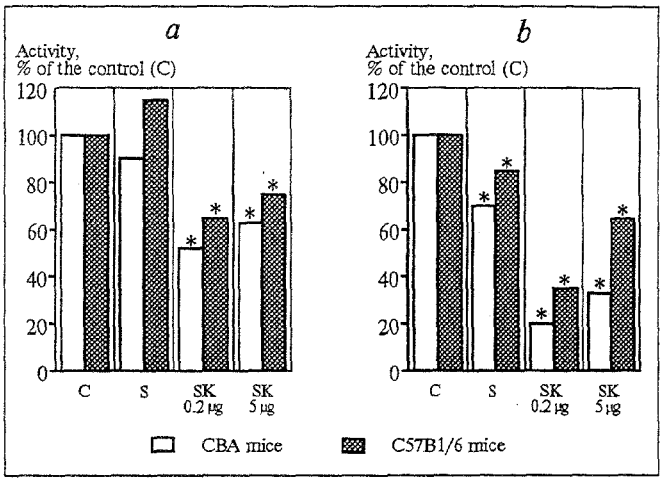

Fig. 1. 5'-nucleotidase activity in the peritonal exudate macrophages of CBA and C57Bl/6 mice. *a*) after sc injection; *b*) after ip injection. Asterisks: $p<0.05$ when compared to C.

of SK was evaluated by the difference in the percentage of survival in the SK-treated and untreated (control) groups.

RESULTS

Preliminary experiments on the effect of SK on 5'-N activity revealed that the preparation exhibits an immunomodulatory influence when given in doses of 0.2 μg and 5 μg. Figure 1 shows the levels of 5'-N activity in the PEM of CBA and C57Bl/6 mice 24 hours after sc and ip administration of SK. Mice of both strains exhibited decreased enzyme activity, the effect being more prominent in CBA mice. The reduction of enzyme activity was especially strong in mice which had received 0,2 μg of the preparation, regardless of the route of injection.

Earlier [1], a correlative dependence was shown in C57Bl/6 mice treated sc with different immunomodulators betweeen the PEM 5'-N levels and the subsequent resistance to staphylococcal infection. A similar relationship was also observed in the experiments described here.

Thus, in the C57Bl/6 mice sc inoculation with SK enhanced resistance to staphylococcal infection, the highest protective effect being obtained for a dose of 0.2 μg, the one which induced the maximal reduction of 5'-N activity (Fig. 2). Ip injection of SK was more effective than sc administration and again correlated with the reduction of PEM 5'-N activity. Yet, unlike the situation with sc injection, the relationship between the resistance and the level of 5'-N activity was less pronounced. For instance, the maximal decrease in enzyme activity was detected following SK injection in the dose of 0.2 μg, whereas the maximal level of resistance was attained with the use of 5 μg SK. Here it is worth pointing out that even an injection of saline was followed a day later by a certain enhancement of the resistance when compared with the intact animals. The rise of resistance here was accompanied, as in the case of SK injection, by a drop in the PEM 5'-N activity. This is apparently evidence of the predominant role of the nonspecific component of resistance following ip injection of SK. The noted differences in the nature of the effect of the preparation for different routes of inoculation might perhaps also be due to the predominant role of the direct effect of the preparation upon the PEM membrane in the course of ip challenge.

The CBA mice did not exhibit increased resistance to staphylococcal infection following ip SK injection, moreover, ip injection led to increased sus-

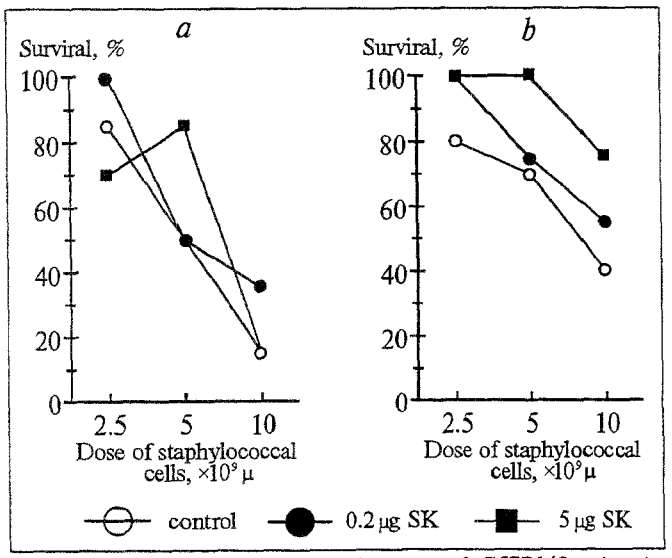

Fig. 2. Effect of SK on the resistance of C57Bl/6 mice to *Stasphylococcus aureus* strain Wood-46 infection. Other designations see Fig. 1.

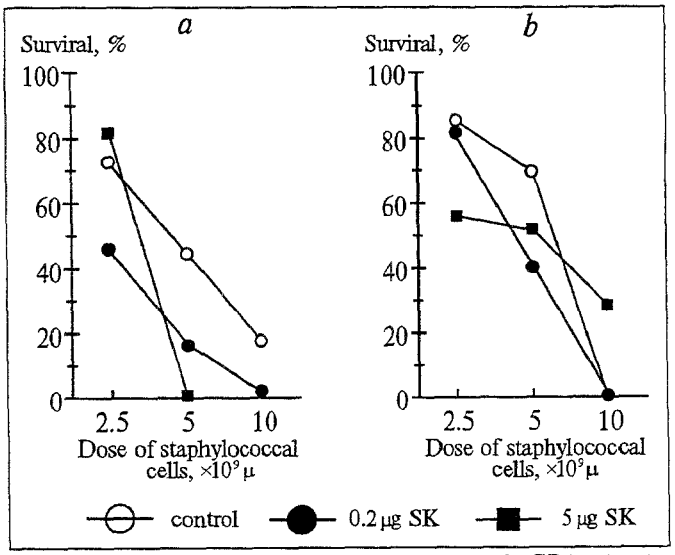

Fig. 3. Effect of SK on the resistance of CBA mice to *Staphylococcus aureus* strain Wood-46 infection. Other designations see Fig. 1.

ceptibility (Fig. 3). We would stress here that in mice of this strain, sc challenge with SK is characterized by an inverse correlation between the PEM 5'-N activity and susceptibility to staphylococcal infection. No correlation was revealed following ip inoculation, that is, a significant change in enzyme activity was not accompanied by any appreciable change in the susceptibility to infection.

Thus, the results of our study show that the type of SK action upon the PEM 5'-N activity and resistance depend on the dose and route of administration, as well as on the strain of animal.

The results do not provide an unequivocal explanation of the relationship between the PEM 5'-N activity and resistance to staphylococcal infection. The effect of SK on the activity of PEM 5'-N may result from a direct or indirect influence of the preparation on this ectoenzyme of the macrophages and/or from the redistribution of the macrophages in the organism and the accumulation of macrophages with low 5'-N activity in the peritoneal cavity [8].

In conclusion, we would stress the genotype-dependent relationship discovered between the PEM 5'-N activity and susceptibility to staphylococcal infection after sc injection of SK. This relationship was direct in the mice of the C57Bl/6 strain and inverse in the CBA mice.

It was shown that after sc administration of immunostimulators the changes in 5'-N activity are intimately related to the peculiarities of the action of the preparation upon the endocrine system indexes [2].

As changes in the neuroendocrine system are of crucial importance for the development of the organism's nonspecific resistance, studies of the effect of SK on the neuroendocrine system indexes in mice of different strains should make a significant contribution to our understanding of diverse effect of SK on the resistance to infection.

REFERENCES

1. G. B. Kirillicheva, V. A. Bekhalo, M. S. Solov'eva, *et al., Immunomodulators in Infection Pathology* [in Russian], Moscow (1988).
2. G. B.Kirillicheva, V. V. Mit'kin, M. S. Solov'eva, *et al., Byull. Eksp. Biol.*, **112**, № 9, 280-282 (1991).
3. D. N. Lazareva and E. K. Alekhin, *Stimulators of Immunity* [in Russian], Moscow (1985).
4. M. A. Tumanyan and G. B. Kirillicheva, *Immunologiya*, № 5, 27-31 (1984).
5. E. M. Khvatova, A. N. Sidorkina, and G. E. Mironova, *Brain Nucleotides (Metabolism and Assessment in Oxygen Deficiency)* [in Russian], Moscow (1987).
6. *Pat. № 1295557 SSSR. A Method of Obtaining the Peptide Fraction of Koumiss Possessing Immunostimulating Activity.* A. Ya. Shurygin, E. I. Zlishcheva, M. A. Senicheva, *et al.*
7. J. Barankiewier and A. Cohen, *Europ. J. Immunol.*, **15**, 627-631 (1985).
8. J. Bursuker and R. Goldman, *RES. J. Reticuloendoth. Soc.*, 33, 207-220 (1983).
9. H. Fleit, *et al., J. Biol. Chem.*, 250, № 23, 8889-8892 (1975).
10. R. Itoh, *Biochim. Biophys. Acta*, **659**, 31-37 (1981).
11. R. E. Riet and S. H. Polmar, *J. Immunopharmacol.*, **8**, № 3, 329-337 (1986).