plasma membrane receptors which split off from the cells during desorption. The receptor "shedding" phenomenon after complementary structure fixation [2] underlies this hypothesis (which remains, however, to be verified).

Our conclusions based on model experiments may be of far-ranging importance. The endothelium is the most significant object for PMNL adhesive reactions in the body. Overcoming this barrier, the cells interact in succession with enditheliocytes and basal membrane components, adhering to them and disengaging themselves from the adhesive contacts. It is possible that here, too, the neutrophil-mediated modification of ligand structures determines the pattern of events.

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A Method for Primary Selection of Immunomodulators by Peritoneal Exudate Macrophage 5'-Nucleotidase Activity

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The problem of immunity modulation as acquired great significance of late because of the ever-increasing prevalence of immunodeficiency states of various origin. Quite a number of both natural and synthetic immunity modulators have been proposed to stimulate the organism's natural nonspecific resistance. The number of such modulators in increasing, complicating the investigation of the biological activity of these agents and the selection of the most effective of them.

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We have developed a method for primary selection of immunomodulato based on measuring the level of ecto-5'-nucleotidase (5'-n) activity of peritoneal exudate macrophages (PEM) [1]. The possibility of using this metabolic characteristic to assess the immunomodulating efficacy of drugs is based on the detected relationship between the type of immunomodulating effect and the trend of enzymological parameter changes. The activity of 5'-n has been found to change in different directions when exposed to immunosuppressives and immunostimulants.

Drugs characterized by immunostimulating activity are found to reduce PEM 5'-n activity, whereas immunosupressives, on the contrary, increase it. Drugs

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TABLE 1. Changes in Resistance to *S. typhi*−2 Infection and 5'−Nucleotidase Activity of Outbred Mice Peritoneal Macrophage Exudate after Subcutaneous Injection of Immunomodulators of Different Chemical Structure

Drugs injected	Class of chemical compounds	LD in mln. bacterial bodies (Reed & Mench)	Resistance index	m	% enzymatic activity vs. control
Salmosan	Bacterial polysaccharide	383.7	18.6	2.408	39.15
Proteus vulgaris o−somatic antigen polysaccharide	Bacterial polysaccharide	300.8	14.6	3.717	60.4
Taftsin	Tetrapeptide synthetic analog	76.7	3.7	4.965	81
Talikmedin	Phytoalkaloid	144.0	7.0	4.232	68.8
Pseudoephedrine	Phytoalkaloid	18.0	0.87	5.883	
Levamisole	Tetramisole synthetic isomer	40.0	1.9	4.461	72.5
Irradiation in dose 7 Gy		0.004	0.0002	7.837	127.4
Control		20.6	1	6.15	100

having no immunomodulating activity do not change PEM 5'-n activity.

MATERIALS AND METHODS

Animal infection with agents of infectious diseases is one of the most informative methods used for the selection of immunomodulators and assessment of the immunomodulating activity of drugs *in vivo*.

Multiannual use of the 5'-nucleotidase test for primary selection of immunomodulators and assessment of their immunomodulating effect has shown the efficacy and informative value of this method. Assessment of drugs immunomodulating properties in this test has yielded results coinciding with immunomodulating activity characterization based on the results of inoculation with infection agents.

RESULTS

Let us consider several examples of immunomodulating activity studies by the two methods mentioned above.

Table 1 presents the results of studies of immunomodulating activities of drug of various origin characterized by different chemical structure. Both methods used, the traditional one, based on changes in sensitivity to *S. typhi* infection, and the one suggested, based on changes in PEM ecto-5'-n activity, yield similar data about the presence of immunomodulating properties. The table shows that agents Nos. 1-4 and 6 are characterized by immunostimulating properties. This is indicated by a reaction to a varying degree of PEM 5'-n activity and an increased resistance index. Salmosan is characterized by the highest immunomodulating activity, levamisole by the least activity. Pseudoephedrine had no effect on the organism's non specific resistance 24 h after a subcutaneous injection to outbred mice. No changes in the metabolic parameter were observed either.

Figure 1 presents a planimetric image of a two-factor regression model of 5'-n activity effected by different salmosan doses at various periods after its injection. It can be seen that subcutaneous injection of the drug to C57B1/6 mice was associated with a prolonged monotonic decline of the enzymatic activity, augmenting with increase of the drug dose.

The results demonstrate a marked immunostimulating activity of salmosan in a wide range of doses, from 0.005 to 100 μg and higher per mouse. The immunomodulating effect manifests itself one day after injection and persists at the same level for up to 14 days, increasing with the dose increase. The immunostimulating effect of the drug is highest for a dose of 100 μg/mouse.

Table 2 presents data on the effects of salmosan in doses of 10 and 100 μg on the outcome of staphylococcal infection. Both tested doses of the drug were quite effective. The local focus of infection was reliably decreased if the animals were administered

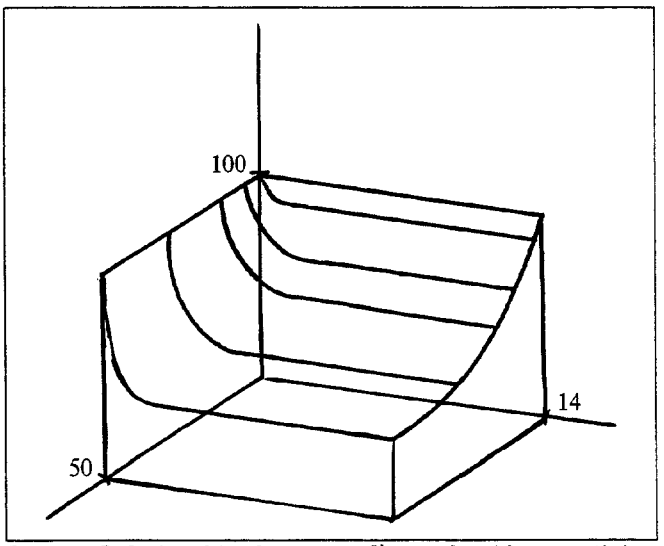

Fig. 1. Relationship between 5'−nucleotidase activity, salmosan dose, and time elapsed since subcutaneous injection to C57B1/6 mice (% of control).

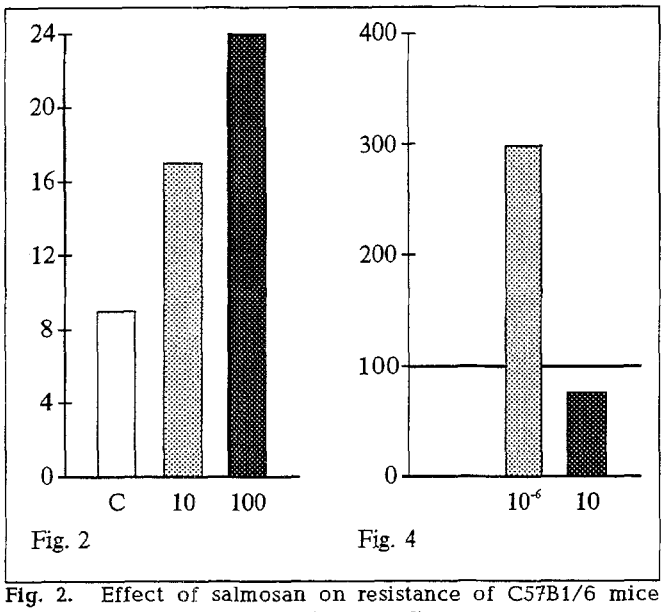

Fig. 2. Effect of salmosan on resistance of C57B1/6 mice to *Klebs. pneumoniae* K−2 (c: control).

Fig. 4. Effect of MOP−35 on sensitivity of C57B1/6 mice to staphylococcal infection (% mortality vs. control).

salmosan a day before infection. Salmosan in a dose of 100 μg, causing the maximal reduction of PEM 5'-n activity, had the highest protective effect in the staphylococcal infection test. Similar results were obtained in experiments with *Klebs. pneumoniae* K-2 infection of mice (Fig. 2).

Hence, our results on the immunomodulating activity of salmosan also indicate a sufficiently high informative value and efficacy of this method for the assessment of drug immunomodulating activity. The test with 5'-n may be used in experiments with other routes of immunomodulator administration, i. e., intravenous, intraperitoneal, oral, and intramuscular.

For instance, Fig. 3 presents the results of experiments on changes PEM 5'-n activity 5 min after i.v. injection of MOP-35, an immunomodulator of a metalloorganic origin. The dose-effect curve was irregular in this case. The activity of 5'-n increased in the dose range 10^{-8} to 10^{-2} μg per mouse and decreased in the dose range from 1 to 100 μg per animal. This means that the immunomodulating effect pattern was a function of the dose. The immunosuppressive effects of MOP-35 manifested itself in the dose range from 10^{-8} to 10^{-2} μg. The immunostimulating effect was much less marked, manifested best of all at a dose of 10 μg.

The results of assessment of the immunomodulating activity of MOP-35 by changed PEM 5'-n activity correlate with the data describing the effect of this agent on resistance to staphylococcal infection (Fig. 4).

Hence, the results of our studies on the immunomodulating activity of preparations of different origin and various chemical structure, such as salmosan, NOP-35, etc., are in line with current notions on the complex time and spatial structure of the immunomodulating effects of these preparations.

A characteristic feature of any immunomodulator is its dose range within which its immunomodulating properties manifest themselves. Immunomodulators differ significantly from each other in terms of this parameter; an optimal dose may vary from a single molecule to 100 μg and higher for various agents. This circumstance hampers a correct assessment of immunomodulating activity when drugs are used in doses beyond the range of the optimal values. The danger of underrating efficacy is particularly high for drugs showing immunomodulating activity within a narrow dose range. All this underscores the need for a judicious choice of test agent dose range. Moreover, in studies of immunomodulating effects one should remember that administration of the majority of immunomodulators is associated with phasic changes of the nature of immunomodulation over time: a peroid of immunostimulation may be followed by immunosuppression and vice versa. Assessment of 5'-n activity over time will help *in vivo* control the directional changes in immunomodulation. Specific features of the time course of immunomodulating effect are governed by the chemical nature of the immunomodulator, by the drug dose, route of administration, and the animal's genotype.

The authors of the 5'-nucleotidase test have developed the optimal schemes of its application to drugs with different chemical structure, permitting a maximal increase of its efficacy and informative value. The specificities of the immunomodulating effects of drugs are taken into account in these schemes with due consideration for their chemical structure, route of

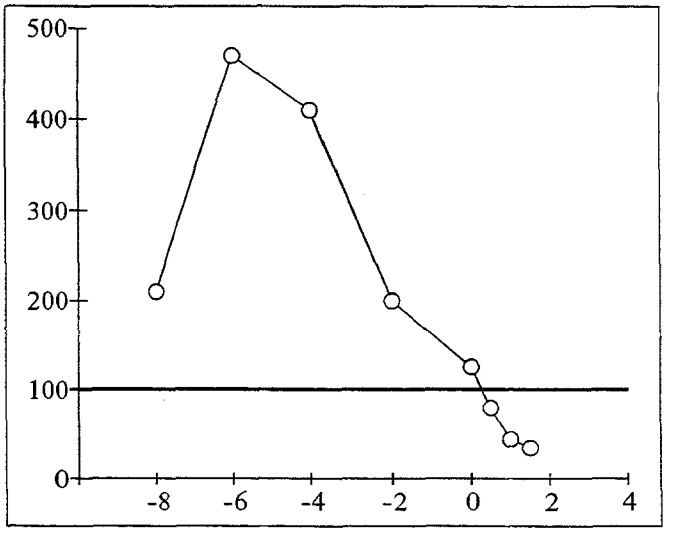

Fig. 3. Changes in activity of PEM 5'−nucleotidase 5 min after i.v. injection of MOP−35 (% of control).

TABLE 2. Local Skin Injury Index (in Points) on Day 14 after *Staphylococcus* Infection

Salmosan dose, μg	Number of mice	Local skin injury, points			
		0	+1	+2	+3
Control	5	—	2	2	1
10	6	4	2	—	—
100	6	6	—	—	—

Note. The drug was injected subcutaneously 24 h before infection with live *Staphylococcus* culture.

administration, etc.; the use of various mouse strains, choice of drug doses, and analysis of the time course of immunomodulating effect are specified in these schemes.

The suggested methods may be recommended both for the primary selection of newly synthesized agents and study of their immunomodulating effects at research institutions engaged in the development of immunomodulating agents and for plants manufacturing immunomodulating drugs for their quality control.

And, finally, we should like to mention one more aspect of the possible application of the 5'-nucleotidase test. Currently *in vitro* methods have been widely used in studies of the immunomodulating effects of drugs. Without in any way deprecating the significance of these methods in disclosing the mechanisms of immunomodulator action, we should like to emphasize the great significance of *in vivo* analysis of drug action. We insist on *in vivo* investigations because the problem of immunomodulation is one of the knotiest in biology and medicine. Analysis of published data and our own finding attests that any immunomodulator may show both immunostimulating and immunosuppressive activities depending on the specific conditions. Our knowledge of these conditions are quite insufficient. To understand the mechanisms of immunomodulator action, learn to perdict an immunomodulating effect, and to control it, we should concentrate out efforts on studies of these specific conditions. The use of the 5'-nucleotidase test, permitting *in vivo* monitoring of immunomodulator effect, can make a valuable contribution to the solution of this problem.

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Influence of Epitope Density on Immunogenic Properties of Hapten-Protein Conjugates

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One of the important considerations when generating antibodies to a hapten is selection of the optimal density of hapten epitopes on the carrier protein molecule, since antigens of this type with different hapten valence induce immune reactions of different intensity and character. The immunogenic properties of hapten-protein conjugates are determined by both the nature of the carrier molecule and the number of homogeneous hapten determinants [5]. It is known that a very high hapten density may lead to the inability of immunocompetent cells to respond to repeated administra-tions

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