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.

REFERENCES

1. M. A. Tumanyan and G. B. Kirillicheva, *Otcrytiya*, № 6 (1986).

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

V. V. Malaitsev and O. Ya. Azhipa

UDC 612.124.017.1:547.96].014.46.08

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 6, pp. 645–646, June, 1993
Original article submitted January 23, 1993

Key Words: *dopamine; immunogenicity; epitope*

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

Russian Research Center of Molecular Diagnostics and Treatment, Moscow; Cancer Research Center, Russian Academy of Medical Sciences, Moscow. (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0006-0726$12.50

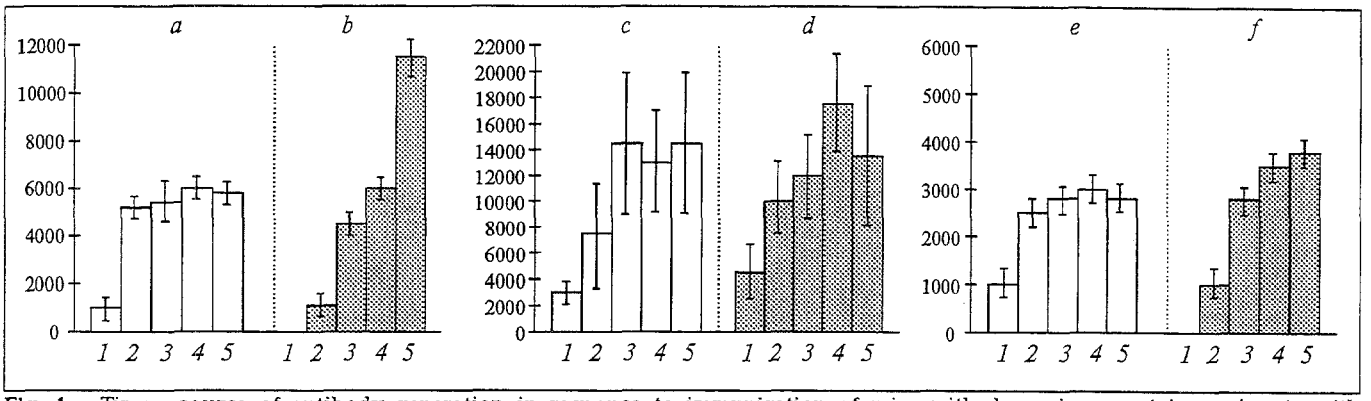

Fig. 1. Time–course of antibody generation in response to immunization of mice with dopamine–protein conjugate with different epitope density. Abscissa: number of immunizations; ordinate: reverse titer values ($M \pm m$). Hapten–protein molar ratio: *a*) 4:1; *b*) 5.8:1; *c*) 8.8:1; *d*) 14:1; *e* and *f*) 18–22:1. Dose per mouse: *a*–*e*) 100 μg; *f*) 10 μg.

of antigen [2]. The immunogenicity of hapten-protein conjugates decreases if parameters such as epitope density and molecular weight deviate from the optimal values [3].

Here, using dopamine-protein conjugates as a model, we studied the effect of their main characteristics on the ability to induce the primary and secondary immune response. The choice of hapten was dictated by the need to generate antibodies to dopamine. This publication presents the first stage of the investigation.

MATERIALS AND METHODS

Dopamine-bovine serum albumin (BSA) conjugates with a differing epitope density were prepared by the glutaraldehyde method as earlier described [1]. The different level of dopamine incorporation into the BSA molecule was achieved by varying the synthesis conditions (ratio of reactive agents and incubation time).

BALB/c mice were immunized with preparations of DA_4-BSA, $DA_{5.8}$-BSA, $DA_{8.6}$-BSA, $DA_{8.8}$-BSA, $DA_{10.6}$-BSA, DA_{14}-BSA, and $DA_{18\text{-}22}$-BSA in a dose of 100 μg/mouse three weeks apart. In the case of $DA_{18\text{-}22}$-BSA conjugates we also used a dose of 10 μg/mouse. The conjugates were dissolved in 0.05 M carbonate buffer containing 10^{-3} M $Na_2S_2O_5$ (pH 8.5) and administered intraperitoneally in Freund's adjuvant.

The titers of specific antibodies in mice sera were determined three weeks after each immunization from the final point of the titration curve using indirect ELISA modified for low-molecular-weight monoamines [4]. For the removal of antibodies to the protein carrier, prior to titer determination, the sera (500 μl, diluted 1:100) were exhausted on glutaraldehyde-modified BSA. The nonspecific binding of antibodies with modified BSA after exhaustion constituted 0.8% with respect to binding with DA-BSA conjugate. The data obtained were processed statistically using Fisher-Student's test.

RESULTS

The data on the immunogenicity of dopamine-protein conjugates with different epitope density are presented in Fig. 1. The preparations $DA_{8.8}$-BSA and DA_{14}-BSA induced the most strongly expressed primary immune response. The character of the response to $DA_{8.6}$-BSA and $DA_{10.6}$-BSA was analogous to that in the case of $DA_{8.8}$-BSA and DA_{14}-BSA (the data are not presented). Conjugate DA_{14}-BSA proved to be most effective. The intensity of the primary immune response to this preparation was comparable to that registered upon hyperimmunization with DA_4-BSA. Conjugates with a low hapten density (DA_4-BSA and $DA_{5.8}$-BSA) and high-valence conjugates ($DA_{18\text{-}22}$-BSA) did not differ in their ability to induce the primary immune response. The antigen titers in mice sera after single immunization with DA_4-BSA and $DA_{5.8}$-BSA were more than three times lower than those in mice immunized with DA_{14}-BSA. The difference in the primary immune response was even more pronounced for $DA_{18\text{-}22}$-BSA and DA_{14}-BSA, the antibody titer in the latter case being more than four times higher.

Synthesis of $DA_{18\text{-}22}$-BSA conjugates was accomplished with a 4-fold excess of glutaraldehyde with respect to hapten, which constituted a 670-fold excess of it vis-a-vis the protein and led to a significant polymerization of BSA. In their electrophoretic mobility, these conjugates were close to 140 kD proteins.

In view of this, we thought it advisable to test two immunization doses of these preparations: 100 and 10 μg/mouse. However, we failed to enhance the immunogenicity of $DA_{18\text{-}22}$-BSA by decreasing its immunization dose.

The same tendency was traced in the response kinetics, except that the immunogenicity of $DA_{5.8}$-BSA after hyperimmunization (4 injections) attained that of $DA_{8.8}$-BSA and DA_{14}-BSA.

Thus, the immunogenicity of dopamine-protein conjugates increased in the following order: DA_4-BSA - $DA_{5.8}$-BSA - DA_{14}-BSA and decreased with a further increase of hapten valence. The optimal immune response developed at a hapten valence of 5.8-14. It may be supposed that the partial tolerogenic properties of thymus-dependent antigens such as dopamine-protein conjugates are accounted for both by specific processes (that are more typical for low-substituted antigens) and by nonspecific processes that are connected with alterations in the physicomolecular characteristics of antigen. The latter is directly connected with the mode and conditions of hapten-protein coupling. High-substituted conjugates (DA_{18-22}-BSA) acquire tolerogenic properties due to nonspecific mechanisms rather than to specific blocking of receptors.

REFERENCES

1. O. Ya. Azhipa and O. V. Makarova, *Biotekhnologiya*, **5**, 46-50 (1991).
2. V. A. Lyashenko and A. A. Vorob'ev, *Molecular Bases of Antigen Immunogenicity* [in Russian], Moscow (1982).
3. R. Z. Dintzis, M. Okajima, *et al.*, *J. Immunol.*, **143**, 1239-1244 (1989).
4. N. Mons and M. Geffard, *J. Neurochem.*, **48**, 1826-1833 (1987).
5. M. Sela, *The Antigens*, New York-San Francisco-London (1974), pp. 154-158.

Effect of Defensins on the Blood Level of Corticosterone and the Immune Response During Stress

O. V. Shamova, M. P. Lesnikova, V. N. Kokryakov, E. K. Shkhinek, and E. A. Korneva

UDC 616.154:577.175.53] — 092:612.017.1] — 02:613.863 — 092.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 6, pp. 646—649, June, 1993
Original article submitted January 21, 1993

Key Words: *defensins; stress; immune response; corticostatic effect*

An important role of the interaction between the neuroendocrine and immune systems in the formation of the defense response of the organism during stress is now commonly acknowledged [5,9]. Nevertheless, the specific mechanisms of this process remain the object of recent studies. In this connection, the data reported by Canadian scientists [15] on the ability of defensins (cationic cyclic polypeptides possessing antimicrobial properties and located in the granular apparatus of neutrophils and some macrophages [4,10]) to suppress adrenocorticotropic hormone (ACTH)-induced production of steroids by adrenal cells in culture are of certain interest. This property of the polypeptides has been termed by the authors corticostatic. The ability of defensins to exert a corticostatic effect has been regarded by researchers as one of the probable molecular mechanisms of the immune effect on the activity of the hypothalamus-pituitary-adrenocortical system (HPACS), which is based on the principle of negative feedback [8]. However, it is not yet certain whether such an adrenal-altering effect of defensins is true for the whole organism. We were the first to study the effect of exogenous defensins on some indexes of the organism's

Laboratory of Immunopathophysiology, Research Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg

0007-4888/93/0006-0728$12.50