7. S. C. Grebe and J. W. Streilein, Adv. Immunol., 22, 120 (1976).
8. W. Savino, B. Wolf, S. Aratan, et al., Clin. Exp. Immunol., 55, 629 (1984).

IMMUNOGENICITY OF ARTIFICIAL ANTIGENS AS A FUNCTION OF NUMBER OF PROTEIN MOLECULES BOUND WITH POLYELECTROLYTES

R. M. Khaitov, M. I. Mustafaev,
A. Sh. Norimov, T. V. Abramenko,
and N. E. Sivuk

UDC 612.017.1.014.46:615.37

KEY WORDS: epitopic activity, artificial antigens; unnatural polyelectrolytes; covalent conjugates; polycomplexes.

Binding of protein and peptide antigens (of serum, bacterial, or viral origin) with artificial polyelectrolytes (PE; polybases and polycarboxylic acids), through complex formation or covalent bonding of the components, can yield highly immunogenic artificial antigens [3, 11, 12]. Correlation has been found between the immunostimulating properties of a series of polyelectrolyte polymer analogs and the immunogenicity of their protein mixtures, on the one hand, and the character of complex formation in these mixtures on the other hand [4]. It has been suggested that the physicochemical basis of manifestation of the immunologic properties of protein–PE complexes probably lies in the ability of free sites of the polymer chain in the composition of the complex (loops, free ends, and so on) to undergo cooperative sorption on the surface of immunocompetent cells [2, 5]. The critical character of the influence of the degree of polymerization of PE on their immunostimulating properties has recently been established [6].

Complex formation between proteins and PE is characterized by a significantly nonhomogenous distribution of protein globules among the polyions adsorbing them [7, 8]. The number of protein molecules per polymer chain depends on the degree of polymerization ($\bar{P}_\eta$) of PE and increases as a linear function of chain length. There is a certain critical value $\bar{P}_{\eta cr}$, above which nonstoichiometric polymer-protein complexes are formed, i.e., the "epitopic" density of the protein in the composition of the polyelectrolyte complex increases. Because of this, the study of the character of dependence of immunogenicity of artificial antigens on the length of the carrier polymer bound with the proteins, and on the immunostimulating activity of free PE, and the study of dependence of immunogenicity on the number of bound protein molecules in the composition of polymer homologs of soluble covalent conjugates are of great importance for the understanding of the mechanisms of immunogenicity of artificial antigens and of the "adjuvant" action of PE.

The aim of the present investigation was to study these problems.

EXPERIMENTAL METHOD

Fractions of polyacrylic acid (PAA) and poly-4-vinylpyridine (PVP) were obtained by known methods [9, 13]. PVP_R were obtained by quaternization of the PVP fractions with bromoacetic acid [7]. The average degree of quaternization was 50%. Narrow fractions of PAA with degrees of polymerization ($\bar{P}_\eta$) of 43 (PAA_1), 170 (PAA_2), 430 (PAA_3), 570 (PAA_4), and 1140 (PAA_5) were chosen as test objects; $\bar{P}_\eta$ for PVP_R was 10^3.

Covalent bonding of PAA and PVP_R was carried out through a stage of activation by carbodi-imide (CDI; from Serva, West Germany) [1, 11].

The immune properties of solutions of the conjugates were studied on C57BL/6 and (CBA × C57BL/6)F_1 hybrid mice weighing 22-24 g, obtained from the Stolbovaya Laboratory Animals Nursery, Academy of Medical Sciences of the USSR. The number of IgM- and IgG-antibody-forming

Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 597-600, November, 1985. Original article submitted July 8, 1984.

 0007-4888/85/0011-1566$09.50

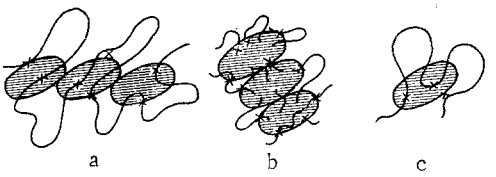

Fig. 1. Hypothetical scheme of structure of BSA-PAA conjugates with different degrees of polymerization ($\bar{P}_\eta$) of the polyacid carrier: a) $\bar{P}_\eta > 570$, b) $\bar{P}_\eta < 570$, c) $\bar{P}_\eta = 570$.

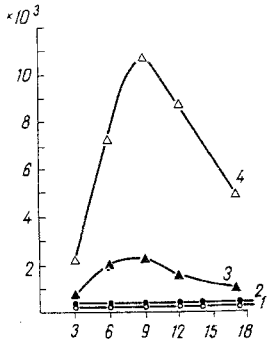

Fig. 2

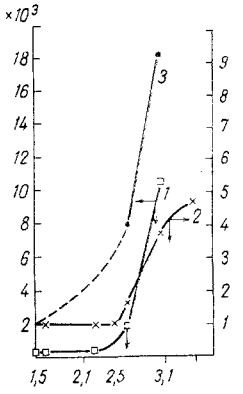

Fig. 3

Fig. 2. Kinetics of AFC accumulation during immunization of animals with a solution of pure BSA (1) and of its conjugates with PAA with different values of $\bar{P}_\eta$: 43 (2), 570 (3), 1140 (4). Abscissa, days after immunization; ordinate, number of AFC.

Fig. 3. Dependence of maximal number of AFC formed in response to injection of BSA-PAA conjugates on $\bar{P}_\eta$ of carrier polymer. Abscissa, log $\bar{P}_\eta$; ordinate: on left, number of AFC; on right, ratio of number of AFC in experiments to their number in control. 2 and 3) Dependence of relative number of AFC formed in response to injection of SRBC on degree of polymerization of PAA nd PAA*. Values of AFC for PAA and PAA* are equal.

cells (AFC) to bovine serum albumin (BSA) was determined 3, 6, 9, 12, and 17 days after intraperitoneal immunization in the spleen of the mice, by a modified method [14], using sheep's red blood cells (SRBC) loaded with BSA. Loading with BSA was carried out with the aid of $CrCl_3 \cdot 6H_2O$ (from Merck, West Germany).

The immunostimulating activity of PAA was studied on a model of the immune reaction of (CBA × C57BL/6)F_1 mice to a suboptimal immunizing dose of $5 \cdot 10^6$ SRBC. At the same time the mice were given an injection of PE in a dose of 50 mg/kg body weight. AFC were counted on the 4th-5th day by the local hemolysis in gel method [14].

The binding mechanism and structure of the soluble covalent conjugates formed were studied by methods of high-speed sedimentation, gel permeation chromatography, and viscosimetry [7, 8].

EXPERIMENTAL RESULTS

The results of the physicochemical measurements enabled the following hypothetical schemes of the particle structure of water-soluble conjugates to be suggested (Fig. 1). Binding of BSA with PAA in the case of covalent conjugation depends on the degree of polymerization of the polyion. A critical value $\bar{P}_\eta = \bar{P}_{\eta cr}$ exists, above which binding is effected by an irregular distribution of protein molecules among the PE adsorbing them. It is sug-

gested that the irregular distribution is due to positive interaction between protein globules in the composition of the complex particle (Fig. 1a). Under these circumstances the number of protein molecules bound with 1 PAA macromolecule increases as a linear function of the degree of polymerization of the polyion. At relatively low degrees of polymerization of PAA the character of binding of the components changes and the role of the carrier is assumed by protein globules. In that case macromolecules of conjugates are in all probability formed by BSA globules, in contact with one another, and "cross-linked" by many short chains of linear PE (Fig. 1b). There exists an intermediate degree of PAA polymerization when the soluble conjugate is equimolecular in composition (Fig. 1c).

The kinetics of AFC accumulation for pure BSA and for its conjugates with PAA with different values of $\bar{P}_\eta$, is shown in Fig. 2. A single immunization of mice with pure BSA and with BSA-PAA_1 conjugate did not lead to the production of a significant number of AFC against the protein. After injection of BSA-PAA_4 and BSA-PAA_5 conjugates, many more AFC accumulated in the spleen of the immunized mice than after immunization of the animals with pure BSA. The most marked immune response developed to injection of the BSA-PAA_5 conjugate.

Dependence of the number of AFC (IgM and IgG) at the peak of the immune response in mice immunized with covalent BSA-PAA conjugates on the degree of polymerization of the carrier polyacid is shown in Fig. 3 between semilogarithmic coordinates. The immune response to protein was exhibited only after certain critical values ($\bar{P}_{\eta cr}$) and increased sharply with an increase in P_η. Similar results also were obtained for covalent PVP_R-BSA conjugates [12]. In the latter case, five BSA molecules bound with one PE macromolecule.

It was shown previously that among the stable PE-protein complexes studied, the lowest degree of stimulation of AFC production was observed for the BSA-PVP (R_0, R_{16}) polycomplex. This complex was prepared at pH 4.2 in the absence of any specially added low-molecular-weight salt [10]. Under these conditions its characteristic composition ($\bar{N}i$) was from 3 to 4, i.e., 3 or 4 protein molecules were bound with 1 PE macromolecule. At physiological values of ionic strength and pH the soluble complex lost some protein molecules and was deposited as a residue in which only one protein globule was present per PE chain. It can be tentatively suggested that the same thing happens also when soluble BSA-PVP (R_0, R_{16}) complex is injected into the blood stream, i.e., under those conditions the number of protein molecules in the composition of the complex is reduced. That is evidently why the protein antigen in the composition of such a complex induces the weakest immune response.

An increase in the number of protein molecules in the composition of both complex and conjugate thus makes an important contribution to the immunogenicity of the artificial high-molecular-weight antigen.

The critical absence of dependence of the relative number of AFC on polyion length also was found during a study of the immunostimulating activity of PAA matrices not bound with proteins. The corresponding curves for fractions, and also the data for PAA fractions activated with CDI (PAA*), are given in Fig. 3. These last fractions can form stable conjugates *in vivo* with components of biosystems. The immunostimulating activity of the polyion was exhibited in both cases only after certain critical values of $\bar{P}_\eta$ had been reached, then rose sharply, and at high values of $\bar{P}_\eta$ virtually reached the limit.

When $\bar{P}_\eta = \bar{P}_{\eta cr}$, the composition of the conjugate corresponded to equimolar, but when $\bar{P}_\eta > \bar{P}_{\eta cr}$, as already mentioned, covalent cross-linking of protein and PE was accompanied by aggregation of protein molecules in the composition of the conjugate.

Thus when $\bar{P}_\eta > \bar{P}_{\eta cr}$ positive correlation is observed between the immunostimulating activity of the carrier polymer, the immunogenicity of the complex antigens, and the ability of the polymer molecule to aggregate protein globules, depending on the length of the PE chain. The critical character of dependence on $\bar{P}_\eta$, both in the case of manifest immunostimulating activity of PE and in the case of an immune response against the protein bound with PE, is evidence that common mechanisms operate in both cases and it is in agreement with the hypothesis of the role of cooperative interaction between complex antigens and components of the immune system. However, this last hypothesis is a necessary but insufficient condition. In our view, a further factor which plays an important role in immunogenesis is the "epitopic" density of the protein antigens in the composition of the carrier polymer. However, before a final solution to the problem of the role of the number of bound protein molecules in immunogenicity of protein–polymer conjugates can be finally solved, it is essential to obtain conjugates of such a kind that equal number of protein molecules is present on polymer chains with an equal degree of polymerization (equal length of molecule).

LITERATURE CITED

1. T. V. Abramenko, I. V. Vinogradov, V. A. Kabanov, et al., Zh. Mikrobiol., No. 11, 86 (1983).
2. I. V. Vinogradov, V. A. Kabanov, M. I. Mustafaev, et al., Dokl. Akad. Nauk SSSR, 263, No. 1, 228 (1982).
3. V. A. Kabanov, M. I. Mustafaev, A. Sh. Norimov, et al., Dokl. Akad. Nauk SSSR, 243, No. 5, 1330 (1978).
4. V. A. Kabanov, M. I. Mustafaev, V. V. Goncharov, et al., Dokl. Akad. Nauk SSSR, 250, No. 6, 1504 (1980).
5. V. A. Kabanov, R. V. Petrov, and R. M. Khaitov, Zh. Vses. Khim. Obshch. im. D. I. Mendeleeva, 27, No. 4, 417 (1982).
6. V. A. Kabanov, M. I. Mustafaev, A. V. Nekrasov, et al., Dokl. Akad. Nauk SSSR, 274, No. 4, 998 (1984).
7. V. A. Kabanov, M. I. Mustafaev, V. P. Evdakov, and A. D. Antipina, Mol. Biol., 11, 582 (1977).
8. V. A. Kabanov and M. I. Mustafaev, Vysokomol. Soed., 231, 225 (1981).
9. V. A. Kabanov, M. I. Mustafaev, V. V. Belova, and V. P. Evdakov, Biofizika, No. 5, 789 (1978).
10. V. A. Kabanov, M. I. Mustafaev, and V. V. Goncharov, Vysokomol. Soed., 231, 261 (1981).
11. R. V. Petrov, R. M. Khaitov, A. Sh. Norimov, et al., Dokl. Akad. Nauk SSSR, 248, No. 5, 1260 (1979).
12. R. V. Petrov, R. M. Khaitov, and R. I. Ataullakhanov, Immunogenetics and Artificial Antigens [in Russian], Moscow (1983).
13. A. G. Boyes and U. P. Strauss, J. Polymer Sci., 22, 463 (1956).
14. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963)

EXPERIMENTAL ANALYSIS OF THE IMMUNOSTIMULATING PROPERTIES OF VITAMIN A

K. D. Pletsityi

UDC 612.017.1.015.6:577.161.11+615.356:577.161.11/.015.46:612.017.1-063

KEY WORDS: vitamin A; immune response; T suppressors.

Administration of vitamin A to mice undergoing simultaneous immunization with γ-G-globulin, to which they were tolerant, abolishes this tolerance and induces antibody production, i.e., it has the action of an immunologic adjuvant [8]. Subsequently investigations were published in which administration of vitamin A to animals stimulated the immune response to certain T-dependent antigens [5, 6]. Meanwhile some investigators not only were unable to detect any stimulation of the immune response after administration of vitamin A, either *in vitro* or *in vivo*, but in some cases they even observed the development of immunosuppression in such cases [7, 13]. The effect of vitamin A on cellular immunologic reactions has been studied mainly on a model of skin transplantation and blast transformation of lymphocytes in response to the mitogenic stimulation with simultaneous addition of vitamin A to the culture. The results of these investigations were quite contradictory [3, 7, 14, 15].

This paper describes an experimental study of the effect of vitamin A on the T-dependent and T-independent humoral immune response with analysis of immunoregulatory activity of suppressor lymphocytes — cells responsible for regulation of the immune response. The effect of administration of vitamin A to the animals on the intensity of blast transformation of lymphocytes during mitogenic stimulation also was studied.

Laboratory for the Study of Nonspecific Resistance and Immunity, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 600-602, November, 1985. Original article submitted April 8, 1985.

0007-4888/85/0011-1569$09.50