POLYMER-METALLIC COMPLEXES OF MYCOBACTERIAL PROTEIN ANTIGEN PREVENT DISSEMINATION OF POSTVACCINAL INFECTION IN T-DEFICIENCY STATES

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Thymectomy in adult animals is followed by gradual depopulation of the heterogeneous thymus-dependent zones of the spleen and of lymph nodes and lymphoid tissue located in the submucosal layer of the intestine. Against the background of atrophy of the T-cell component of the lymphoid formations a T-cell immunodeficiency state is observed: suppression of the immune response and lowering of resistance to infection [3, 4].

It was shown previously that the development of serious complications after BCG vaccination may be connected in some cases with inhibition of the T-cell component of immunity [1]. Injection of 2 mg of living BCG mycobacteria without the caseous component into thymectomized adult mice led to the development of a disseminated process, culminating in death of the mice. This type of course of infection is observed in children with immunodeficiencies, in whom a high incidence of tuberculosis is observed.

In some cases BCG vaccination of such children is complicated by the development of disseminated BCG-itis and, as a rule, by superimposition of staphylococcal infection. Hence the need for the urgent development of new and safe methods of vaccinating children and adults with T-deficiency states. In this case artificial polymer subunitary vaccines may be found to be necessary [5].

Previously the writers made a comparative study of several versions of artificial immunogenic complexes (AIC) with polyacrylic acid, containing tuberculous antigens isolated from two cellular components of BCG mycobacteria [2]. Of the nine different antigens in the composition of the AIC, two glycoproteins obtained from the BCG cell membrane, and the total alkaline protein obtained from an ultrasonic digest of BCG cells, possessed a protective action and prolonged the life of mice infected with a virulent culture of ECG statistically significantly.

The aim of this investigation was to study the protective action of a polyelectrolyte complex of total alkaline BCG protein (TBP) on a model of tuberculous infection in experiments on adult thymectomized mice.

EXPERIMENTAL METHOD

The alkaline protein was isolated from an ultrasonic digest of BCG mycobacteria, grown in Sutton's liquid synthetic medium. Under these circumstances the pH of the ultrasonic digest of the BCG culture was adjusted to 8.0. After removal of the residue by centrifugation, complete precipitation with ammonium sulfate was carried out. For the next 24 h the resulting residue was washed 3 times with a 50% solution of ammonium sulfate, after which it was dissolved in an alkaline medium with pH 8.0. After dialysis the preparation was dried. The TBP contained 53% protein and 27% of reducing sugars.

As the polymer-complex former we chose the nontoxic copolymer (CP) N-vinylpyrrolidone with acrylic acid, with a composition of 50:50 moles % and molecular mass of 50 kD, synthesized by the radical copolymerization method. Binding of CP with TBP was brought about with copper ions by the addition of a 1% solution of $CuCl_2 \cdot 6H_2O$ to the mixture of solutions, and

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TABLE 1. Protective Activity of Triple Complex CP—Cu^{2+}—TBP

Experimental group	Immunized B mice		Survival rate	
	TBP	CP-Cu^{2+}-TBP	number of animals surviving/number dying	%
1) B-mice	—	—	0/12	
2) Immunized B mice				
a	+	—	1/15	6,6
b	—	+	23/27	85
3) Mice undergoing mock operation	—	—	20/20	100

the resulting complexes were studied by sedimentation viscosimetry, and UV-spectroscopy. The mice were immunized with a water-soluble triple complex of CP—Cu^{2+}—TBP, in which, for 2 carboxyl groups of CP there was only 1 g-ion of copper, the weight ratio TBP/CP being 2:1.

To obtain experimental B mice, adult CBA mice were thymectomized, irradiated, and restored with syngeneic bone marrow cells.

The animals were immunized intravenously with pure protein fraction in a dose of 100 μg or the equivalent amount of this fraction in the composition of the triple complex. After 1 month the mice were given an intravenous injection of 2 mg of living BCG vaccine. The control groups consisted of unimmunized B mice (pure control) and a group of mice undergoing a mock operation. The protective properties of TBP also were compared in the presence of Freund's incomplete and complete adjuvants.

EXPERIMENTAL RESULTS

In neutral aqueous solutions obtained by mixing solutions of CP and TBP, relatively unstable polyelectrolyte complexes are formed. However, the addition of Cu^{2+} ($CuCl_2 \cdot 6H_2O$) to a solution of a mixture of SP and TBP leads to the formation of a stable triple complex, in which copper ions play the role of cross-linking agent between functional groups of CP and protein. The results of physicochemical investigations will be published later. Here it must be noted that, in the chosen proportions of the components, ions of the metal coordinate both the intramolecular and the intermolecular functional groups, with the result that aggregates of the triple complex CP—Cu^{2+}—TBP are formed. In this structure the protein molecules are aggregated due to cross-linkage through metallic ions, and the complex is characterized by the high epitopic density of antigen molecules. Subsequently, a mixture of solutions of CP, TBP, and copper ions in which this type of complex is formed was used for the immunological experiments. The results of experiments to study the survival rate of the animals are given in Table 1. Survival rate (in %) was calculated as the ratio of the number of animals surviving to the number dying.

Two months after injection of 2 mg of living BCG vaccine, both the control (0/12) unimmunized B mice and mice vaccinated only with TBP (1/15) had died from the disseminated disease; 85% of the animals (23/27), immunized with the triple complex CP—Cu^{2+}—TBP remained alive. All animals of the control group (mice undergoing the mock operation, 20/20) were alive.

Under these conditions immunization with TBP in aqueous emulsion mixture of Freund's adjuvants, both complete and incomplete, did not lead to the formation of a protective effect.

Thus a triple immunogenic complex consisting of tuberculous protein antigen, CP of acrylic acid and vinylpyrrolidone, and copper ions, giving reliable protection and inducing marked immunologic protection in thymectomized T-deficient mice, as well as of such mice restored by bone marrow, against lethal dissemination of living, attenuated bacilli (BCG), was obtained for the first time.

The results of these experiments show that complex compounds of antigen and polyelectrolytes, constructed on the basis of nonimmunogenic thymus-dependent proteins of agents of microbial infection, exhibit the property of a thymus-independent immunizing preparation, providing immune protection in the presence of a marked deficiency of the T-cell component of the immunogenic system. Vaccinating preparations created in accordance with this principle are one possible way of developing methods and trends preventing not only undesirable aftereffects of ordinary corpuscular vaccinating molecules, but also of creating an effective immune defense against infection.

LITERATURE CITED

1. B. B. Moroz, B. B. Averbakh, et al., Byull. Éksp. Biol. Med., No. 12, 367 (1976).
2. R. Yu. Romanova, M. M. Averbakh, A. M. Moroz, and A. Sh. Norimov, Probl. Tuberk, No. 5, 49 (1988).
3. F. M. Burnet, Transplant. Rev., 7, 3 (1971).
4. J. F. Miller, A. H. Marshall, and R. G. White, Adv. Immunol., 2, 111 (1962).
5. R. V. Petrov and R. M. Khaitov, Vaccine, 3, 392 (1985).

ROLE OF CHORIONIC GONADOTROPIN IN THE REGULATION OF ANTIGEN-INDEPENDENT DIFFERENTIATION OF IMMUNOCOMPETENT SPLEEN CELLS

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Recently chorionic gonadotropin (CG) has attracted the attention of research workers not only as an important reproductive placental hormone and tumor cell marker [11], but also as an active modulator of immune reactions. It is important to emphasize that processes of reproduction and oncogenesis are closely connected both with each other and with the immune system, for which CG is a mediator of intercellular interactions [5, 6]. The role of gonadotropin, secreted by lymphocytes under mixed culture conditions is not completely clear, but the fact itself indicates the need for its involvement in the immune response. Considering that in the gestation period the hormone persists for a long time in the body not only during pregnancy, but also in the developing fetus, it is important to know what effect it may have on maturation processes of immunocompetent cells, which as a rule take place at the antigen-independent stage of differentiation.

The aim of this investigation was to determine the effect of CG and its action, indirectly through ovarian hormone, on processes of antigen-independent differentiation of the T and B cells of the spleen and on its radioresistant stroma.

EXPERIMENTAL METHOD

Experiments were carried out on 400 male (CBA × C57BL/6)F_1 hybrid mice weighing 20-22 g. Some animals underwent ovariectomy under ether anesthesia. The ovariectomized mice were used in the experiments after 1 month. CG was injected subcutaneously on alternate days for 19 days in doses of 40 or 200 U, which is comparable with the average serum level of the hormone in pregnant women in the 1st and the 2nd and 3rd trimesters respectively.

The experiments were conducted in the following variants: 1) injection of CG into donors of T lymphocytes; 2) injection of CG into donors of B lymphocytes; 3) injection of CG into recipients which then underwent lethal irradiation (219.3 mCi/kg) and restoration by syngeneic splenocytes, together with thymus-dependent antigen, namely sheep's red blood cells (SRBC). T Lymphocytes were obtained by fractionation of splenocytes on a column with nylon fiber [9], and B cells from a suspension of splenocytes after treatment with anti-BaO serum and guinea pig complement. The purity of the fractionated cell populations was estimated in the cytotoxic test with anti-BaO-serum [7] and with EAC-rosette formation [1]. In the population enriched with B cells 0-2% of T lymphocytes was present, whereas in the population enriched with T cells, 1-6% of B lymphocytes was present.

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