REACTION OF SERA FROM RABBITS IMMUNIZED WITH SYNTHETIC POLY-L-ALANINE WITH MYOCARDIAL INTERSTITIAL CONNECTIVE TISSUE CELLS

I. M. Lyampert, E. V. Kochetkova, V. Yu. Kolesnikova, and G. A. Ugryumova

UDC 612.172.017.1-06:612.118.017.1.014.46:547.466.23

KEY WORDS: synthetic polypeptides; fibroblasts of myocardial interstitial connective tissue; cross reactions.

In some immunologic investigations synthetic polypeptides are used as analogs of mammalian tissue substances and also in order to study their antigenic structure. A polypeptide capable of inducing autoimmune encephalomyelitis in experimental animals, like the basic protein of myelin, has been synthesized [9, 12]. Investigation of the antigenic structure of collagen has shown that synthetic acetylated hydroxyproline inhibits the reaction of the sera of animals immunized with collagen with connective tissue. In this case the sera were tested by the indirect immunofluorescence (IF) method on tissue slices [11]. No reactions could be found with connective tissue when sera of animals immunized with synthetic acetylated hydroxyproline homopolymer were tested on tissues [11]. The passive cutaneous anaphylaxis method revealed cross reactions between collagen and a synthetic copolymer containing L-proline and L-glutamic acid [7]. Synthetic polymers containing one of the amino acids are usually nonimmunogenic when used for immunization without a carrier protein. At the same time it has been shown that certain homopolymers, such as poly-L-hydroxyproline, are immunogenic for guinea pigs but not for rabbits [11]. It should be pointed out that the obtaining of an immune response during immunization with synthetic polypeptides largely depends on the method of immunization [14].

This paper describes an attempt to obtain an immune response during immunization of rabbits with poly-L-alanine (PL-ala) as a result of its injection into lymph nodes with Freund's complete adjuvant [10].

L-alanine is known to be one of the amino acids present in collagen [3]. Accordingly the sera of animals immunized with PL-ala were tested by the indirect IF method on sections of myocardial interstitial connective tissue (ICT). By the use of the IF method it is possible to obtain nonspecific reactions because of the presence of Fc-receptors, reacting with Fc-regions of immunoglobulins [8], in the tissues. Despite the fact that Fc-receptors have not hitherto been found in myocardial ICT, additional control experiments were undertaken to exclude nonspecific reactions.

EXPERIMENTAL METHOD

Synthetic PL-ala and poly-L-lysine (PL-lys) (Serva, West Germany) were used for immunization. The polypeptide in a dose of 300 μg in 0.1 ml of 0.85% NaCl and 0.1 ml of Freund's complete adjuvant were injected into the lymph nodes of rabbits (1st cycle). The same quantity of polypeptide in 1 ml of 0.85% NaCl was injected 1 month later intravenously, intramuscularly, and subcutaneously into the region of the lymph nodes [10]. The 3rd and 4th cycles were given like the 2nd, at monthly intervals. Six rabbits were immunized with PL-lys and 22 rabbits (two batches, consisting of 12 and 10 animals respectively) with PL-ala. Sera obtained before immunization and after the 2nd, 3rd, and 4th cycles were tested by the indirect IF method. Antibodies against rabbit IgG, isolated by means of immunosorbent and labeled with fluorescein [2], were used in the IF experiments.

Preparation of IgG and of $F(ab')_2$-fragments of IgG were obtained from normal and immune sera [1]. These preparations were tested in the gel diffusion test with antiglobulin serum and compared with control preparations.

Laboratory of Streptococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 1, pp. 54-56, January, 1981. Original article submitted April 18, 1980.

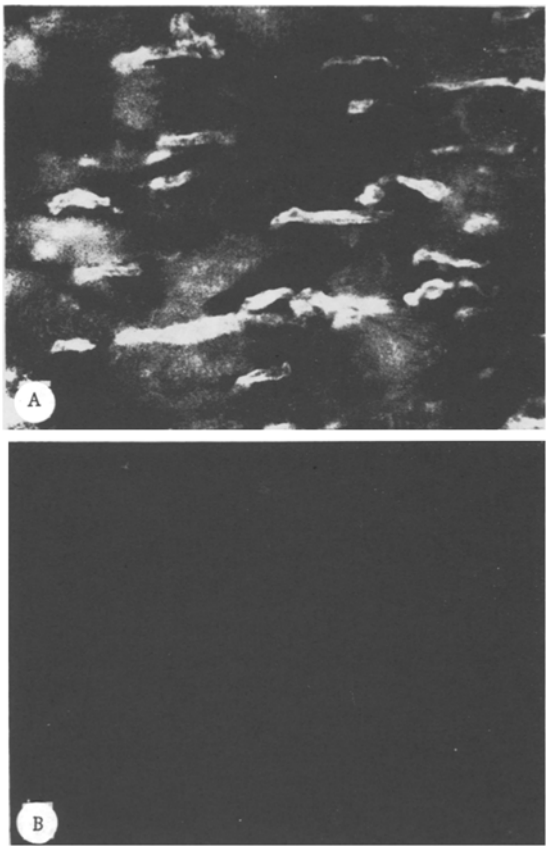

Fig. 1. Testing of sera from rabbits immunized with synthetic polypeptides. A) Serum of rabbit immunized with poly-L-alanine. Reaction with myocardial ICT cells; B) serum of rabbit immunized with poly-L-lysine. No reaction present. Indirect immunofluorescence method. Magnification: objective × 40, ocular homal × 3.

Sera against PL-ala in a dilution of 1:5 were absorbed with an equal volume of erythrocytes obtained from persons with different blood groups or from guinea pigs. Cultures of fibroblasts isolated from the guinea pig thymus or bone marrow also were used for absorption [6]. The cultures of fibroblasts ($1 \cdot 10^6$ cells), washed three times with phosphate buffer (pH 7.2), were added to 0.2 ml of serum diluted 1:8. The mixture of serum with erythrocytes or fibroblasts was incubated for 2 h at 37°C or for 18 h at 4°C.*

In control experiments serum against streptococcal antigens (antigens of the F-fraction), reacting with the sarcolemma of the myocardial muscle fiber [1], was used. Slices of myocardium from 22 healthy subjects aged from 6 to 25 years, dying from trauma, and also slices of bovine, pig, guinea pig, rat, and rabbit myocardium, were used. The technique of preparing and processing the slices was described previously [2]. The tissue slices were incubated with sera or with preparations of IgG or $F(ab')_2$-fragments of IgG for 2 h at 18-20°C. After washing with phosphate buffer (pH 7.2) the slices were incubated for 35 min with fluorescein-labeled antibodies. The reaction was estimated in the ML-2 luminescence microscope with a × 40 objective. A homal × 3 ocular was used for photography.

EXPERIMENTAL RESULTS

None of the sera obtained from the 28 rabbits before immunization or the sera of six rabbits taken after different cycles of immunization with PL-lys reacted with myocardial ICT from man or other species of

*The fibroblast culture was generously provided by A. A. Ivanov-Smolenskii, working in the Laboratory of Immunomorphology (Director, Professor A. Ya. Fridenshtein), N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

Fig. 2. Reaction of $F(ab')_2$-fragments of IgG isolated from serum against poly-L-alanine with myocardial ICT cells. Magnification: objective × 40, ocular homal × 3.

mammals. When the sera of animals immunized with PL-ala were tested, sera of 11 of the 22 rabbits reacted with myocardial ICT cells (Fig. 1). In some cases the intensity of the reactions increased after the 3rd or 4th cycles of immunization. Sera of four rabbits reacted with myocardial ICT in dilutions of 1:64-1:256, the rest in dilutions of 1:8-1:32. Positive reactions were found to all samples of human myocardium irrespective of blood group, and also to all slices of the myocardium of other species of mammals tested, except rabbits.

Weak reactions in low (1:8) dilutions of sera, which reacted intensively on myocardium of other species in higher dilutions, were discovered on slices of rabbit myocardium only in certain cases. Preparations of IgG and also of $F(ab')_2$-fragments of IgG (300 μg/ml), obtained from immune sera, reacted with human myocardial ICT cells. The same preparations obtained from normal sera did not react with these structures (Fig. 2). After absorption of the sera of rabbits immunized with PL-ala by a BCG culture (2 mg to 0.2 ml of serum diluted 1:8), and also by human and guinea pig erythrocytes, the reactions with the myocardial ICT cells were completely preserved. Absorption by cultures of fibroblasts completely abolished the reaction of these sera with ICT cells. Meanwhile the reaction of the control serum with sarcolemma was completely preserved after absorption with the same cultures of fibroblasts.

After immunization of rabbits with the synthetic homopolymer PL-ala, in one half of all cases sera reacting with myocardial ICT cells of man and other mammals, except rabbits, were thus obtained. Accordingly, the reactions found with myocardial ICT cannot be classed as autoimmune. This is a matter for further study, for the possibility cannot be ruled out that an analogous antigenic determinant is a "latent" determinant in the animals of this species or is localized in other tissues.

There is some evidence to suggest that the sera of animals immunized with PL-ala evidently contain antibodies against a true homopolymer, that react specifically with myocardial ICT cells. This view is based on the reaction with the tissues when sera with pure labeled antibodies against IgG are tested, positive reactions with ICT when sera obtained after immunization with PL-ala were tested, and the absence of such reactions with sera taken before immunization or after immunization with PL-lys, and also the positive reactions with preparations of IgG and $F(ab')_2$-fragments of IgG isolated from sera against PL-ala, but not from normal sera.

The production of antibodies reacting with ICT did not depend on immunization with the BCG present in the Freund's adjuvant. First, the use of BCG to absorb sera does not abolish these reactions, and second, the sera of animals immunized with PL-lys with Freund's complete adjuvant did not contain any such antibodies. Moreover, it was shown previously that sera of animals immunized with complete adjuvant only likewise do not react with myocardial ICT [5].

Cells reacting with antibodies against PL-ala were evidently fibroblasts, for absorption of the sera with a culture of fibroblasts abolished these reactions whereas absorption with erythrocytes had no effect. Absorption of antibodies by fibroblasts was not connected with Fc-receptors, for these cells did not react with normal sera and did not absorb antibodies reacting with sarcolemma from the control serum.

The immunogenicity of the homopolymer PL-ala was most likely determined by its insolubility and by the use of the method of Goudie et al. [10], which evidently ensures prolonged preservation of PL-ala in the lymphoid tissue. The study of cross reactions of other synthetic amino-acid homopolymers, and also of copolymers containing several amino acids, with tissues is interesting. Copolymers, as we know, are more immunogenic [14].

Subsequent testing of sera and antibodies isolated from them with the aid of immunosorbents by the IF method can be used to study the antigenic structure of tissues, including antigens of lymphocyte membranes and antigens of tumor tissues. Another important task is the isolation of antibodies with the aid of immunosorbents containing synthetic polypeptides from the sera of animals immunized with microbial antigens, and also from the sera of patients with autoimmune diseases, and the subsequent testing of these antibodies on tissues by the IF method.

This is a promising approach to the study of the structure of cross-reacting antigens of microorganisms and tissues, and also of autoantibodies in the sera of man and experimental animals.

Some recent investigations have been devoted to the creation of synthetic antigens – analogs of microbial antigens – with the aim of developing new methods of vaccination [4]. In this connection a detailed study of cross reactions between synthetic antigens and tissue antigens is essential in order to exclude the possibility of development of autoimmune reactions.

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