Whereas in a culture of mouse lymph node cells isolated at the peak of the secondary immune response MP give a stimulating effect through modulation of helper–suppressor interactions of T lymphocytes [4], in the present case a direct influence of MP in cells secreting antibodies is observed; moreover, the increase in the intensity of antibody secretion under the influence of MP was accompanied by inhibition of cell proliferation. Thus, under the influence of MP the hybridoma cells switched from the proliferative into the secretory cycle. On the basis of the data given above, it can be postulated that different mechanisms controlling antibody secretion exist in the case of a pure homogeneous population of hybridoma B cells and a heterogeneous population of immune lymph node cells, for in the latter case MP probably have no direct action on B lymphocytes [4]. At the present time clone 2G6B3 of hybridoma B cells is used to evaluate the functional activity of MP and of the immunoregulatory preparation myelopide that has been created on their basis.

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PREPARATION OF SPECIFIC ANTISERA TO BRADYKININ AND THEIR INVESTIGATION BY ELISA

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UDC 615.373.3:577.175.853].07

KEY WORDS: immunization; bradykinin conjugates; specific antisera; ELISA

Bradykinin (BK) is involved in many physiological processes and also in the pathogenesis of acute and chronic diseases [1]. Specific antibodies to BK and its analogs are known to be antagonists of BK, blocking some of its biological functions [5, 6]. For that reason, immunoneutralization of kinins by active or passive immunization can be regarded as an important technical approach to the correction of BK-dependent pathological states. For this purpose, it is essential to have available a set of specific antisera and a reliable method of their identification.

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(Presented by Academician of the Academy of Medical Sciences of the USSR, I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 6, pp. 727-729, June, 1989. Original article submitted May 3, 1988.

0007-4888/89/0006-0849$12.50

TABLE 1. Titers of Rabbit Antisera to BK Obtained with the Aid of Protein and Erythrocytic Conjugates (data of ELISA)

Protein conjugate of BK	Erythrocytic conjugate of BK
1:32 000	1:1600
1:32 000	1:2400
1:16 000	1:400
1:16 000	1:800
1:25 000	1:400

The aim of this investigation was to obtain specific antisera to BK and to develop a method of enzyme-linked immunosorbent assay (ELISA) with high sensitivity for recording activity and specificity of the kinins.

EXPERIMENTAL METHOD

Immune sera against BK were obtained by immunizing rabbits with conjugates of a synthetic BK preparation (Serva) with protein or autologous erythrocytes. Conjugation with protein (bovine serum albumin – BSA) was carried out with the aid of p-toluene-di-isocyanate or carbodi-imide [6, 8]. In the conjugate thus obtained, for every molecule of albumin there were 9-12 BK molecules (data obtained by amino acid analysis). Conjugation with autologous rabbit erythrocytes was carried out with the aid of glutaraldehyde [4]. The effectiveness of conjugation was determined by the test with fluorescamine [3]. The resulting conjugate contained 1.1 micromole of BK to 1 ml of 20% erythrocyte suspension. Immunization with each conjugate was carried out on five male chinchilla rabbits (weight 2-2.5 kg) in accordance with the scheme: four immunization cycles separated by intervals of 14 days. With each injection 500 μg of the BK-BSA conjugate/kg body weight or per 50% suspension of erythrocytic conjugate (in a volume of 0.25 ml with an equal volume of Freund's complete adjuvant per rabbit) was injected at 5 points in the dorsal region, in a dose of 0.1 ml intradermally at each point. Blood was taken at the peak of the immune response 18 days after the beginning of immunization. The resulting antisera were lyophilized and kept at 4°C. The titer of specific antibodies to BK was determined by ELISA, using a method [2] with minor modifications on polystyrene panels (from "Soyuzmedpolimer," Leningrad). The technique of analysis was as follows: 0.1 ml of BK solution in 0.01 M bicarbonate buffer, pH 9.6, in a concentration of 10 μg/ml was introduced into each well of the panel and kept at 4°C for 18 h. The wells were then washed 4 times with buffered physiological saline, pH 7.0-7.2, with 0.05% Tween-20 and dried in air. The antigen-covered panels were incubated for 2 h at 37°C with antiserum and normal rabbit serum, titrated against physiological saline, in a volume of 0.1 ml per well. To reduce nonspecific sorption of the serum on the surface of the well, each well was coated again with BK solution of the same concentration and kept for 2 h at the same temperature. After washing 4 times with 0.02 M Tris-HCl buffer, pH 8.0, containing 0.05% Tween-20, 0.1 ml of a 1:500 dilution of conjugate of antibodies to rabbit immunoglobulins with peroxidase (produced by the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) was added as the label to each well, and the panels were incubated for 1 h at 37°C. The conjugate was then removed, the panels were thoroughly washed with the same buffer, and 0.1 ml of substrate containing 0.2% o-phenylenediamine (Serva) and 0.015% perhydrol in 0.05 M citrate buffer, pH 4.8, was added. After incubation for 30 min at 37°C the reaction was stopped by addition of 50 μl of 4 M H_2SO_4 to each well. The results were read on a "Titertek Multiscan" instrument at 495 nm. The titer was taken to be the highest dilution of the test serum in which the difference between optical density of the test serum and normal serum became not less than 0.1.

EXPERIMENTAL RESULTS

As a result of immunization of rabbits with conjugates of BK with protein and with autologous erythrocytes, specific antibradykinin sera were obtained. Specific antibodies were found in the immune sera by ELISA in all immunized animals (Table 1). In rabbits immunized with the protein conjugate, the antibody titer to BK reached 1:32,000, or 10-15 times higher than the titer after immunization with erythrocytic conjugate. Titration curves of both types of sera, plotted from mean values for each group of animals, are given in Fig. 1.

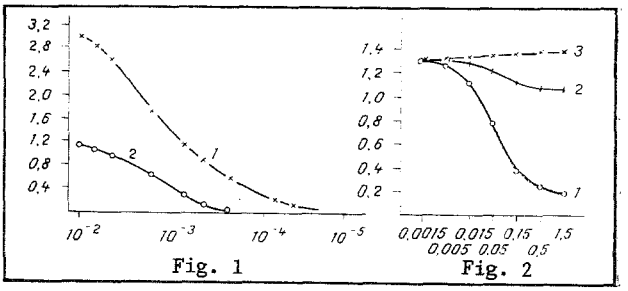

Fig. 1. Titers of antibodies to BK in antisera according to ELISA data. Abscissa, dilution of antiserum; ordinate, specific extinction at 495 nm (optical density units). 1) Antiserum obtained with the aid of protein conjugate of BK, 2) with erythrocytic conjugate of BK.

Fig. 2. Competitive inhibition by peptides of activity of antibodies to BK: BK (1) – standard curve, M-enkephalin (2), and angiotensin II (3). Abscissa, quantity of bradykinin in well (in μg); ordinate, specific extinction at 495 nm (optical density units).

The specificity of antisera to BK was analyzed by ELISA for comparison with angiotensin II (Serva) and M-enkephalin (Serva). These peptides were used as antigens for adsorption on the panels in the same concentrations as BK. Antisera obtained with the aid of the protein conjugate exhibited low crossed binding (1.25%) with angiotensin II and M-enkephalin. The erythrocytic sera did not exhibit crossed binding with peptides nonhomologous with BK.

Antigenic specificity of the antisera obtained also was tested by competitive inhibition with free peptides – BK, angiotensin II, and M-enkephalin. To determine the optimal ratio of antibodies and antigen, a two-stage determination was used. The bradykinin covering in a concentration of 1 μg/ml ensured the optimal response for antiserum of 1-1.5 optical density units at 495 nm: for antiserum obtained with the protein conjugate in a dilution of 1:1000, for erythrocytic antiserum in a dilution of 1:100. During performance of the inhibition test, simultaneously with antiserum an equal volume of titrated solution of the peptide was added simultaneously to each well of the panel within the range of 1.5-0.0015 μg (final concentrations 10^{-9}-10^{-12} mole/ml per well), and the panels also were incubated for 2 h at 37°C. The determination was subsequently carried out as described in the section "Experimental Method."

Specific inhibition of BK activity of the immune sera enabled a standard curve to be plotted for BK by the method in [7]. The minimal detectable dose of BK, calculated from the curve, was 10^{-12} mole/ml (Fig. 2). Analysis of the results of competitive inhibition of activity of the antibodies in the test sera with other peptides used in an adequate concentration compared with BK (the specific activity toward BK was taken as 100%), revealed 20% inhibition by M-enkephalin of the activity of serum obtained with the aid of the erythrocytic conjugate, and complete absence of inhibition by angiotensin II of activity of both types of sera. Immune serum obtained with the aid of the protein conjugate, according to ELISA data, had a high titer of antibodies specific for BK, whereas it contained antibodies to peptides nonhomologous to BK as impurities: to M-enkephalin and to angiotensin II. We postulated that the high immune response to BK obtained in rabbits was a combination of responses to BK and to BSA. For this reason, specificity of the immune serum was verified by carrying out the inhibition test with a purified BK preparation without the carrier (BSA). Additionally, covering of the solid phase with a double layer of antigen was carried out similarly for BK without BSA, thus preventing adsorption of nonspecific antibodies. The immune serum obtained by immunization with the erythrocytic conjugates did not cross-react with peptides nonhomologous with BK, although it had a lower titer of antibodies specific for BK.

The main results of this investigation were thus the obtaining of specific antisera to BK with the aid of erythrocytic conjugates (antisera of this kind were obtained for the first time) and investigation of the antisera, by the ELISA technique with the demonstration that this method can be used in future research with highly purified antibodies to BK.

Laboratory assistant M. V. Petrova assisted with the work.

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ICO-45 MONOCLONAL ANTIBODIES TO A NEW EPITOPE OF CD38 ANTIGEN

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UDC 616.155.02-007.12-07:
616.155.96-097-078.73

KEY WORDS: monoclonal antibodies; CD38 antigen; breast carcinoma

Monoclonal antibodies (McAb) against differential antigens of hematopoietic cells are widely used in the immunodiagnosis of hemoblastoses and in the study of fundamental problems in hematopoiesis [5]. New differential surface antigens of blood cells are continually being described and are broadening our ideas about the cell surface [3, 4, 6]. An important marker of leukemia cells is the T10 antigen, which was classed at the International Working Conference on Differential Antigens of Human Leukocytes with the CD38 cluster. In its structural organization, CD38 antigen very closely resembles antigens of the human class I major histocompatibility complex, but differs from it in the character of its distribution on hematopoietic cells. The T10 antigen is expressed on all thymocytes, null cells, NK cells, and also on activated T lymphocytes and plasma cells [7].

The aim of this investigation was to characterize ICO-45 McAb, directed against a new epitope of the CD38 antigen.

EXPERIMENTAL METHOD

Blood cells were fractionated by standard methods. Thymocytes were isolated from the thymus of children aged from 1 to 14 years, undergoing open heart operations. Expression of the antigen was investigated by the indirect surface immunofluorescence test (IFT) on living cells [2]. The reaction was read by means of a "light" fluorescence microscope and a "Spectrum 111" flow cytofluorometer. To study the effect of McAb on NK-cell activity, mononuclear cells from healthy blood donors were preincubated with McAb and rabbit complement, washed off, and used in the NK test against ^{51}Cr-labeled K-562 target cells. To determine the effect of the McAb on the blast transformation reaction, peripheral blood mononuclear cells from healthy donors were preincubated for 30 min with ICO-45 McAb, PHA was added, and the sample was then incubated for 48 h at 37°C in an atmosphere of 5% CO_2. ^{3}H-thymidine was then added. After 24 h the cells were transferred to filters and incorporation of ^{3}H-thymidine into the cell DNA was determined [1]. Competitive blockade was carried out with the aid of ^{125}I-labeled McAb and with human thymocytes, fixed with glutaraldehyde. The effect of McAb on secretion of active forms of oxygen by human neutrophils and

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(Presented by Academician of the Academy of Medical Sciences of the USSR, N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 6, pp. 729-731, June, 1989. Original article submitted August 27, 1988.

0007-4888/89/0006-0852$12.50