EFFECT OF SUBSTANCES OF POLYPEPTIDE NATURE ISOLATED FROM THE CEREBRAL CORTEX ON THE IMMUNE RESPONSE IN MICE

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UDC 612.825.015.2-06:612.017.1

The effect of low-molecular-weight polypeptides (mol. wt. < 10,000) isolated by acetic acid extraction from bovine cerebral cortex and white matter and red bone marrow on the primary immune response to sheep's red blood cells was investigated in experiments on 248 male CBA mice. Subcutaneous injection of the preparation from the cortex (where the θ-antigen cross-reacting with thymocytes is located) into the animals for the 5 days before and 3 days after immunization led to an increase of 2.5 times in the hemagglutinin titer and in the number of direct (IgM) and indirect (IgG) antibody-forming cells compared with the control. Preparations from the white matter of the brain and red bone marrow had no such effect.

KEY WORDS: cross-reacting antigens; thymus; brain; stimulation of immune response.

The θ-antigen, characteristic of the thymus-dependent population of lymphocytes in animals of various species, is known to be present in the brain also [1, 2, 6, 11]. This antigen is predominantly connected with the cerebral gray matter (cortex) and is virtually absent in the white matter [1, 2, 5, 12, 13]. It has also been shown that substances of polypeptide nature isolated from the thymus stimulate the immune response in animals, especially after thymectomy, when they take over the functions of receptors of T cells [3, 7, 9, 10]. The problem arises whether substances contained in the cerebral cortex possess a similar effect.

The object of this investigation was to compare the effect of substances isolated from the cerebral cortex and white matter and from the red bone marrow on the immune response in mice to thymus-dependent antigen.

EXPERIMENTAL METHOD

Preparations were obtained from the bovine cerebral cortex and white matter and red bone marrow by acetic acid extraction, followed by precipitation of the extracted substrate with acetone as described previously in [4]. These preparation were complexes of fractions of polypeptide nature with a molecular weight of under 10,000.

Two series of experiments were carried out on 248 male CBA mice weighing 16-18 g. The optimal dose and scheme of administration of the preparations were determined in the experiments of series I. For this purpose the preparations were injected subcutaneously into mice before immunization with the antigen, after immunization, and also at various times both before and after immunization in doses of 5 and 50 μg/g. The action of the preparations was assessed by reference to the hemagglutinin titer. In the experiments of series II (the main series), various indices reflecting the action of preparations injected subcutaneously in a dose of 5 μg/g daily for the 5 days before and the 3 days after immunization of the animals were determined. Control animals received physiological saline by a similar scheme. Sheep's red blood cells, thoroughly washed with physiological saline, were used as the antigen.

Laboratory of General Immunology, Department of Microbiology and Immunology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leingrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Ioffe.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 12, pp. 703-705, December, 1978. Original article submitted January 16, 1978.

0007-4888/78/8612-1631 $07.50

TABLE 1. Effect of Low-Molecular-Weight Polypeptides Isolated from Gray and White Matter of the Brain and Red Bone Marrow on Indices of Immune Response in Mice (M ± m)

Preparation	Reciprocal of serum hemagglutinin titer	Number of AFC/10^6 splenic karyocytes	
		direct (IgM)	indirect (IgG)
Physiological saline	202,7±32,6 (74)	15,2±0,4 (30)	16,5±0,8 (30)
Cerebral cortex (gray matter)	448,0±86,4* (30)	41,6±4,1* (30)	44,7±5,5* (30)
White matter of brain	276,1±41,6 (31)	16,4±1,4 (30)	20,2±1,7 (30)
Red bone marrow	176,0±30.3 (12)	11,3±2,0 (12)	15,0±1,1 (12)

Legend. 1. Asterisk denotes significance of difference from corresponding index in animals receiving physiological saline ($P < 0.01$). 2. Number of animals shown in parentheses.

The immune response was determined on the 4th day after a single intravenous immunization of mice with $1 \cdot 10^7$ red blood cells in the hemagglutination test and by determination of the number of direct (IgM) and indirect (IgG) antibody-forming cells (AFC) in the animals' spleens. Direct AFC were detected by the method of Jerne and Nordin [14] and indirect by the method of Dresser and Wortis [8]. Rabbit serum (1:400) against muscle IgG, isolated with the aid of caprylic acid [15], was used to detect indirect AFC. The number of direct and indirect AFC was expressed per 10^6 karyocytes. Antibodies were determined individually in each mouse and AFC in pools of spleens from three animals.

EXPERIMENTAL RESULTS

The results of the experiments of series I showed that injection of the preparation from the cerebral cortex (gray matter) into the animals stimulated the immune response. The most effective dose was 5 μg/g, if injected for the 5 days before and 3 days after immunization of the animals. Preparations of the white matter of the brain and red bone marrow did not affect antibody formation. In the experiments of the main series (II), all preparations were accordingly injected in a dose of 5 μg/g in accordance with the scheme mentioned above.

As Table 1 shows, the preparation from the gray matter of the brain had a marked stimulating effect on the immune response in the animals. Preparations of the white matter of the brain and red bone marrow had no such action. It will be noted that the number of direct and indirect AFC in animals receiving the cortical preparation was significantly higher than that in mice injected with the preparation from the white matter. After injection of the preparation from red bone marrow there was actually a tendency for the indices of the immune response in the mice to fall.

The results are evidence that the cerebral cortex, where the cross-reacting θ-antigen is located, also contains a factor of polypeptide nature, which can be isolated from it and which stimulates the immune response to thymus-dependent antigen. It must be emphasized that the biologically active factor was isolated from the cerebral cortex by the same method as the preparation isolated from the thymus (thymarin), which the writers described previously [4].

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TRANSFER OF BONE-MARROW MICROENVIRONMENT BY CLONES OF STROMAL MECHANOCYTES

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UDC 616.419-089.843-07: 616.419-003.917-092.4

Colonies consisting of clones of bone-marrow stromal fibroblasts, grown in monolayer cultures of mouse and guinea pig bone-marrow cells, transfer the hematopoietic microenvironment on retransplantation into the animal. Individual clones simultaneously form bone tissue and create a microenvironment for all three branches of medullary hematopoiesis: erythroid, myeloid, and megakaryocytic.

KEY WORDS: bone marrow, hematopoietic microenvironment.

The writers showed previously [6] that the formation of a new hematopoietic organ at the site of heterotopic transplantation of bone marrow takes place as a result of survival of stromal mechanocytes, which form a territory for colonization by hematopoietic cells, i.e., they create a hematopoietic microenvironment. Stromal mechanocytes are local, and not repopulating cells: In the hematopoietic tissue of radiochimeras they preserve their recipient origin, whereas in heterotopic grafts they remain of donor origin [8]. The hematopoietic organs contain clonogenic stromal precursors, which can be detected by the formation of colonies consisting of clones of fibroblasts in cultures [3]. During subculture of these cultures diploid strains of fibroblasts arise and, if retransplanted into the animal, they transfer the hematopoietic microenvironment [6]. The question arises whether individual clones of stromal mechanocytes can transfer the bone-marrow microenvironment or whether cooperation between several different clonogenic stromal precursors is required for this purpose. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Bone marrow cells from adult CBA mice and guinea pigs were used for cloning bone-marrow mechanocytes [5, 7]. Mouse bone marrow was extracted from the femora of the killed donors; guinea pig bone marrow cells were flushed out of the femur by means of a needle through the distal epiphysis of the anesthetized animal, after compression of the femoral artery. Cell suspensions were filtered through four layers of nylon and explanted into Roux flasks, the bottom of which was first covered with collagen gel. Guinea pig cells were cultured in medium No. 199 with 20% bovine serum, mouse cells in Fisher's medium with 15% embryonic serum. On the 16th-30th day colonies of fibroblasts together with the collagen gel were cut out and transplanted into animals. Colonies of mouse cells were transplanted into syngeneic recipients beneath the capsule of the kidney; colonies of guinea pig cells were autografted by introducing them in to the diaphysis of a homologous femur, freed from bone marrow and irradiated in a dose of 5000 R, which was then implanted into the muscle of the anterior abdominal wall. In control experiments collagen gel was implanted beneath the capsule of the kidney and irradiated cylinders of bone without colonies of fibroblasts were implanted into the abdominal muscle. After 30-90 days the kidneys and the bone cylinders were fixed in alcohol and formol, decalcified,

Laboratory of Immunomorphology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 12, pp. 705-707, December, 1978. Original article submitted May 26, 1978.

0007-4888/78/8612-1633$07.50