NATURE OF A HUMORAL FACTOR ARISING AFTER HEPATECTOMY AND STRENGTHENING THE IMMUNE RESPONSE

A. Ya. Kul'berg and D. N. Evnin

UDC 612.112.94.017.1

The mechanism of potentiation of the immune response to sheep's red cells in rabbits produced by homologous serum from partially hepatectomized donors was studied. The active factor does not possess antibody specificity and its action can be completely suppressed by the polyvalent proteinase inhibitor Trasylol. The potentiating action of the proteinase is unconnected with the liberation of highly immunogenic complexes from the surface of the sheep's red cells,and it can accordingly be postulated that the enzyme directly activates cells participating in the immune response.

An important aspect of research into the mechanisms of immunogenesis is the study of the nature of intercellular cooperation essential to the production of the various forms of immune response [10, 11]. In this connection it is an interesting fact that 2-4 h after partial hepatectomy a factor capable of potentiating the immune response to sheep's red cells in intact recipients of the same species can be found in the blood serum [1-3]. This phenomenon cannot be regarded as the result of hormonal changes arising in stress, for the serum of animals undergoing a mock operation did not contain factors stimulating antibody production [1, 3].

An attempt to assess the nature of the humoral factor responsible for the immunostimulating properties of the serum of hepatectomized animals is described below.

EXPERIMENTAL METHOD

Rabbits weighing 2.3-2.5 kg were used. Partial hepatectomy was performed on the donor rabbits, when the left lobe of the liver (35-40% of the total mass of the organ) was removed, and the animals were exsanguinated 4 h after the operation. A mixture of sera from 6-10 donors was used for intravenous injection into normal rabbits simultaneously with the antigen. Rabbits of the experimental groups each received 5 ml of serum of hepatectomized donors and 5×10^8 sheep's red cells. The animals of the control groups received the antigen and either physiological saline or normal rabbit serum. The titers of antibodies against sheep's red cells in the recipients' serum were determined by the direct hemagglutination and immune hemolysis tests. The titers of 7S-antibodies were estimated after inactivation of the 19S (IgM)-hemagglutinins with 0.2 M solution of 2-mercaptoethanol at pH 7.3 [5]. Some animals were killed on the 7th day after immunization in order to determine the number of antibody-forming cells (AFCs) in the spleen by the local hemolysis in agar gel method [7].

The stroma was obtained from the sheep's red cells by the method of Kabat and Mayer [8]. The preparations of stroma used were completely freed from soluble products by repeated washing with cold isotonic NaCl solution. The "normal" antibodies were adsorbed on the stroma of the sheep's red cells by

Laboratory of Immunochemistry, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Department of Biochemistry, Kursk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 3, pp. 83-86, March, 1974. Original article submitted March 19, 1973.

TABLE 1. Changes in Immune Response to Sheep's Red Cells in Rabbits Produced by Trasylol

Group of animals	No. of animals	Titer of hemagglutinins ($\log_2$)	Titer of 7S-antibodies ($\log_2$)	Titer of hemolyzins ($\log_2$)	No. of AFCs per 10^6 spleen cells
1	6	9,8±0,3	4,8±0,7	13,3±0,2	1379±15
2	11	5,2±0,4	3,0	9,8±0,2	303±4,9
3	6	4,0±0,26	3,0	9,2±0,3	286±4,8
4	11	5,1±0,3	3,0	9,8±0,3	302±7,2
5	6	5,8±0,3	3,0	10,3±0,2	—

Note. Group 1) Injection of 5 ml serum obtained 4 h after hepatectomy; 2) injection of 5 ml serum obtained 4 h after hepatectomy and incubated before injection with 500 K.I.U. Trasylol for 1 h at 20°C; 3) injection of 5 ml serum obtained 4 h after hepatectomy, simultaneously with injection of 500 K.I.U. Trasylol; 4) injection of 5 ml normal rabbit serum; 5) injection of 5 ml normal rabbit serum incubated before injection with 500 K.I.U. Trasylol for 1 h at 20°C.

TABLE 2. Content of Hemagglutinins (HA) in Recipients' Blood after Injection of Serum of Hepatectomized Donors Incubated with Sheep's Red Cells

Group of animals	No. of animals	Before immunization		On 7th day after immunization	
		Titer of HA ($\log_2$)	Titer of 7S-HA ($\log_2$)	Titer of HA ($\log_2$)	Titer of 7S-HA ($\log_2$)
1	7	2,6±0,2	0	2,7±0,2	0
2	5	2,8±0,2	0	3,0±0,3	0

Note. Group 1) Injection of 5 ml normal rabbit serum adsorbed twice on stroma of sheep's red cells at 0°C and then incubated with sheep's red cells for 8 h at 37°C; 2) injection of 5 ml serum obtained 4 h after hepatectomy and treated before injection as in group 1.

incubating the serum twice with the stroma at 0°C for 20 min [8], followed by centrifuging twice in the cold at 6000 and 12,000 g for 40 min.

EXPERIMENTAL RESULTS

Two basic assumptions guided the choice of approach to the study of the nature of the factor appearing in the blood immediately after partial hepatectomy and capable of stimulating the immune response in intact recipients of the same species. First, transformed "normal" 19S-hemolyzins, whose migration from the depots into the blood stream could be induced by a nonspecific stimulus (partial hepatectomy), could act as the stimulating factor. The ability of 19S-hemolyzins to potentiate the immune response to sheep's red cells has been proved experimentally [6]. Second, the factor potentiating the immune response could be a nonspecific biostimulator liberated from destroyed or damaged liver cells.

Tests of the first hypothesis showed that the titer of "normal" hemagglutinins in the mixture of sera of the partially hepatectomized donors was identical to the titer of those antibodies in a mixture of normal rabbit sera, namely 1:4 g ($\log_2$); the titer of normal hemolyzins in both sera was 1:64 ($\log_2 = 6$). Removal of all "normal" antibodies by adsorption on the stroma of the sheep's red cells did not abolish the stimulant effect of the serum of the hepatectomized donors. Consequently, the inducer of immunogenesis arising after hepatectomy does not possess antibody specificity.

If the nonspecific stimulator appearing in the blood immediately after hepatectomy is a product liberated from liver cells destroyed or damaged during the operation, it could be the proteolytic enzymes of

the lysosomes of the hepatocytes themselves or of the Kupffer cells. This hypothesis was confirmed by experiments in which serum from the hepatectomized animals was injected into recipients simultaneously with the polyvalent proteinase inhibitor Trasylol [4, 13], given in a dose of 500 K.I.U. (kallikrein inhibitor units). As Table 1 shows, in the dose used Trasylol did not affect the immune response to sheep's red cells but it completely abolished the stimulant effect of the serum of the hepatectomized animals.

One possible mechanism by which the proteinase could affect the immune response could be by liberating highly immunogenic complexes from the surface of the red cells. To test this hypothesis the sera of hepatectomized and intact rabbits, first completely freed from "normal" antibodies, were incubated with sheep's red cells for 8 h at 37°C and, after separation of the cells by centrifuging twice in the cold at 6000 g, they were injected into intact rabbits. According to the results given in Table 2, incubation of the serum of the partially hepatectomized rabbits with sheep's red cells did not lead to the liberation of antigenic complexes from the surface of the erythrocytes, for the content of antibodies in the animals of the control and the experimental groups did not increase but remained at the same level as the titer of "normal" hemagglutinins.

In the light of these observations it can be assumed that the proteinase present in the serum of partially hepatectomized animals can stimulate cells participating in the immune response directly. This hypothesis is confirmed also by the results of recently published work showing that inhibitors of proteolytic enzymes inhibit the transformation of lymphocytes in vitro induced by antigen or phytohemagglutinin [12] and that proteolytic enzymes themselves (trypsin) stimulate DNA synthesis in cell cultures in vitro [9].

The writers showed earlier that the factor stimulating immunogenesis and present in the serum of hepatectomized animals possesses marked species-specificity of its action [3]. This observation does not conflict with the conclusion that the stimulator is enzymic in nature, and it can be explained on the basis of the pattern of distribution of heterologous enzymes in the body. If foreign enzymes, bound with macrophages as an antigen, can be inactivated by this process, the homologous enzyme will evidently be able to penetrate unhindered into the germinal centers of the peripheral lymphoid organs and to exert a direct influence on the immunocompetent cells.

LITERATURE CITED

1. D. N. Evnin and A. Ya. Kul'berg, Dokl. Akad. Nauk SSSR, 197, No. 3, 711 (1971).
2. D. N. Evnin, L. G. Prokopenko, and A. Ya. Kul'berg, Byull. Éksperim. Biol. i Med., No. 10, 65 (1972).
3. A. Ya. Kul'berg and D. N. Evnin, Zh. Obshch. Biol., No. 5, 626 (1972).
4. F. A. Anderer and S. Hörnle, Z. Naturforsch., 206, 457 (1965).
5. N. Costea, V. Yarulis, and P. Heller, Blood, 26, 323 (1965).
6. C. Henry and N. K. Jerne, J. Exp. Med., 128, 133 (1968).
7. N. K. Jerne, A. A. Nordin, and C. Henry, in: B. Amos and H. Koprowski (Editors). Cell-Bound Antibodies, Philadelphia (1963), p. 109.
8. E. Kabat and M. Mayer, Experimental Immunochemistry [Russian translation], Moscow (1968), p. 140.
9. L. Mallucci, V. Wells, and M. R. Young, Nature, 239, 53 (1972).
10. J. F. Miller, A. Basten, et al., Cell Immunol., 2, 469 (1971).
11. G. F. Mitchell and J. F. Miller, J. Exp. Med., 128, 821 (1968).
12. H. Tchorzewski and A. Denys, Experientia, 28, 462 (1972).
13. J. Trautschold, E. Werle, and G. Rüdel, Arzneimittel-Forsch., 11, 1507 (1966).