BLOCKING FACTOR OF NORMAL ANIMAL SERUM INHIBITING IMMUNOLOGICAL RESPONSES in vitro

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The sera of Wistar and noninbred rats of different ages and sexes were found to contain an extremely thermostable factor, resistant to various chemicals, which inhibits the action of normal and immune antibodies against the red blood cells and serum antigens of rats. The blocking factor is relatively species specific and is more active in respect of the action of normal than of immune antibodies. The activity of the blocking factor is connected with α_1-globulin.

KEY WORDS: serum blocking factor; heterophilic antibodies; hemagglutination.

The study of the role of factors inhibiting immunological reactions in vivo and in vitro under normal conditions and during the development of certain pathological processes is of great scientific and practical interest. Several workers have discovered factors inhibiting the cytotoxicity of antibodies and of sensitized lymphocytes during the growth of malignant tumors [5, 9, 10], after transplantation of organs and tissues in tolerant animals [14], during immunization with heterogeneous antigens [1, 13], and in allergic and other processes [3]. The nature and mechanism of action of the blocking factors (BF) are extremely varied. For instance, some workers consider that BF may be antibodies [8], others that they are an antigen–antibody complex [14], or an α-globulin with low molecular weight [6, 7, 11], a glycoprotein [1, 11], or urea or guanidine [4].

In the investigation described below some immunological and physicochemical properties of a component of the sera of normal animals which inhibits the action of natural (heterophilic) and immune antibodies in immunological reactions, were studied. This component of the sera, in line with the usage of other workers, was called BF.

EXPERIMENTAL METHOD

The sera of Wistar and noninbred rats of both sexes, taken from 12-14-day embryos, at birth, at the age of 6 days, and from adult animals were used. BF were studied in agglutination and gel-diffusion tests and also by immunoelectrophoresis in the usual manner. In the agglutination test rat erythrocytes and isolated testicular cells were used as the antigens, and in the gel-diffusion and immunoelectrophoresis tests, rat sera, either native or heated to 100°C for 30 min (supernatant 1), were used. The sera of normal mice, guinea pigs, rabbits, dogs, sheep, pigs, cattle, horses, and cocks and human group A(II) serum were used as the source of heterophilic antibodies and the immune antibodies were obtained from the sera of rabbits immunized with the erythrocytes, native sera, and supernatant 1 or rats. For the gel-diffusion and immunoelectrophoresis tests the sera of immune rabbits concentrated by MacErlean's method [12] were used. To study the immunological properties of the BF, single and double precipitation of the test antigens by the method described previously [2] was carried out. Antigens of supernatant 1 were precipitated by antibodies contained in fraction IV of the concentrated rabbit immune serum. Next, supernatant 2 obtained after single precipitation of the test antigens, and supernatant 3, obtained after double precipitation, were studied in the

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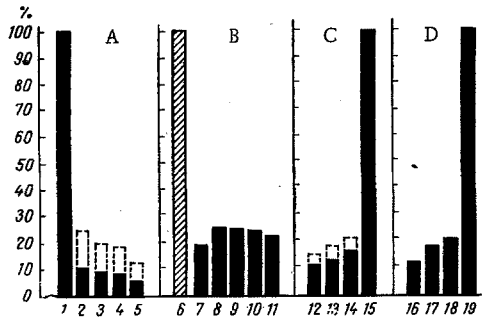

Fig. 1. Characteristics of BF of normal rat sera in a system of rat erythrocytes + standard cock serum. A) BF in ontogeny; B) resistance of BF to various substances; C) thermostability of BF; D) action of BF in the course of time. 1) Standard control; 2) embryos; 3) newborn rats; 4) rats aged 6 days; 5) adult rats; 6) treatment of BF supernatant 1 with 0.25% trypsin solution; 7) treatment of native serum with 0.25% trypsin solution; 8) with ether; 9) with ethyl alcohol; 10) with butyl alcohol; 11) with acetone; 12) 100°C for 10 min; 13) 100°C for 30 min; 14) 100°C for 60 min; 15) 100°C for 120 min; 16) simultaneously; 17) 30 sec; 18) 60 sec; 19) 90 sec. Maximal value of BF shown by broken lines.

immunoelectrophoresis test. Before the tests were carried out protein was determined by Lowry's method on a spectrophotometer at 750 μ. In addition, some of the physicochemical properties of BF were studied, such as thermostability and resistance to the action of ether, acetone, butyl and ethyl alcohols, and 0.25% trypsin solution (Difco). Native erythrocytes and testicular cells from rats and the same cells treated with trypsin for 10 or 30 min at 37°C were used in the tests.

EXPERIMENTAL RESULTS

Investigations by the agglutination test showed that the sera of the mice did not cause agglutination of the rat erythrocytes. Sera of rabbits, guinea pigs, dogs, sheep, and man reacted with rat erythrocytes in dilutions of 1 : 8-1 : 24. Heterophilic antibodies were present in higher titer in the serum of pigs and cattle (1 : 20-1 : 100). The highest titer of antibodies against rat erythrocytes was found in cock sera (1 : 60-1 : 240). Because of these finds, cock sera with the highest titer of heterophilic antibodies (1 : 200-1 : 240) were used in the main experiment.

The results of the study of immunological and physicochemical properties of BF from rat sera are summarized in Fig. 1. The results of the tests are given as percentages. A cock serum that agglutinated rat erythrocytes in the highest titer (taken as 100%) was used as the control.

The results showed that on addition of rat erythrocytes plus cock serum as diluting solution for the rat serum to the system the agglutination reaction was weakened by 10-20 times; i.e., it amounted to 5-10% of the standard control level. When this sytem was diluted with rat embryonic serum the agglutination reaction amounted to 10-25% of the standard control. A similar pattern was found when the system was diluted with the sera of rats of another age. The sera of male and female Wistar and noninbred rats were found to have a blocking action. The BF of rat sera also exhibited its action when the agglutination test was carried out with erythrocytes and heterophilic antibodies of all the above species of normal animals and man.

The BF of the rat sera was extremely thermostable. Its activity fell a little on heating the sera to 100°C for 60 min, but on heating to the same temperature for 120 min the BF was completely inactivated. In these experiments both native rat sera and supernatant 1 thus had a blocking action in the tests.

Treatment of native rat sera and supernatant 1 with ether, acetone, and ethyl and butyl alcohols weakened the BF activity only very slightly.

Treatment of the native rat sera with 0.25% trypsin solution solution did not inactivate the BF contained in them. The same treatment of supernatant 1 led to total inactivation of the BF contained in it.

It is interesting to note that on treatment of rat erythrocytes with 0.25% trypsin solution the BF activity of both native sera and supernatant 1 was completely abolished. A different picture was observed when agglutination of rat testicular cells by heterophilic antibodies was tested. Whereas in the control the reaction could take place in dilutions of sera between 1 : 20 and 1 : 30, after the addition of native rat serum or supernatant 1 the reaction took place only in dilutions of 1 : 2-1 : 4. However, by contrast with erythrocytes, treatment of the testicular cells with 0.25% trypsin solution did not weaken the BF. This fact suggests that surface antigens of erythrocytes and of rat testicular cells are different after trypsinization.

The relative species specificity of BF of the sera and supernatant 1 of the rats was established. On the one hand, sera of cattle, guinea pigs, and rabbits had no blocking action when added to a system of rat erythrocytes plus cock serum, and on the other hand, rat sera and supernatant 1 had a blocking action on the agglutination reaction between human group A(II) erythrocytes and heterophilic cock antibodies. Fetal calf serum and bovine serum had a blocking action in the same test.

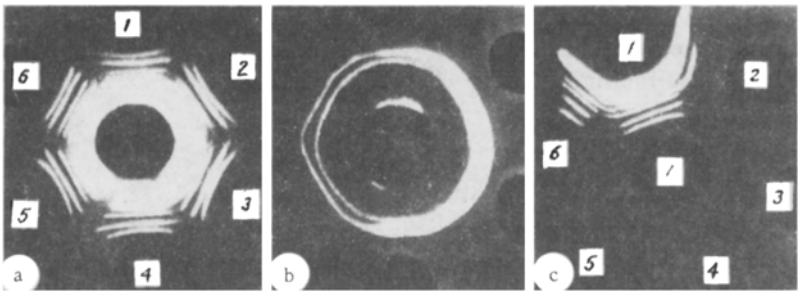

Fig. 2. Gel-diffusion test of rabbit immune sera with antigens from rat sera: a) Central well contains native rat serum; 1) undiluted fraction IV of concentrated serum of a rabbit immunized with native rat serum; 2, 3, 4, 5, and 6) the same rabbit serum diluted with physiological saline in ratios of 1 : 1, 1 : 2, 1 : 3, 1 : 4, and 1 : 5, respectively; b) central well contains supernatant 1, all peripheral wells contain fraction IV of concentrated serum of a rabbit immunized with supernatant; c) central well contains native rat serum; 1) undiluted fraction IV of concentrated serum of a rabbit immunized with native rat serum; 2, 3, 4, 5, and 6) the same rabbit serum diluted with native rat serum in ratio of 1 : 1, 1 : 2, 1 : 3, 1 : 4, and 1 : 5.

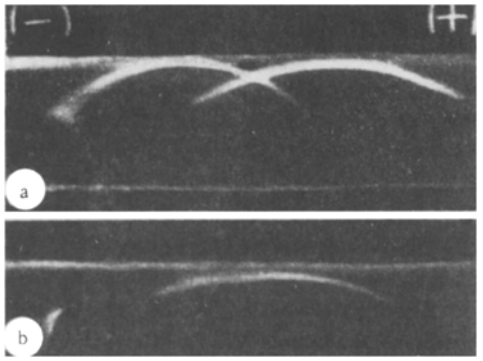

Fig. 3. Immunoelectrophoresis of fraction IV of concentrated serum of rabbit immunized with supernatant 1, with antigens from supernatants 1 and 2: a) Well contains supernatant 1, gutter contains fraction IV of concentrated serum of rabbit immunized with supernatant 1; b) well contains supernatant 1, gutter contains supernatant 2.

The standard titer of BF of native rat sera was established as the smallest quantity of serum which could completely suppress the agglutination reaction. This titer was conventionally taken as the unit. The minimal titer of BF of supernatant 1, determined by dilution of the supernatant with physiological saline, was much higher, namely 0.1-0.2 units of the standard. In other words, BF of supernatant 1 was more active than BF of native rat sera.

The fixation time of heterophilic antibodies on the surface of rat erythrocytes also could be determined with the aid of BF contained in supernatant 1 (Fig. 1).

To study the immunological properties of BF experiments were carried out with the sera of rabbits immunized with supernatant 1 and with native rat sera and also with rat erythrocytes. The sera of rabbits immunized with rat erythrocytes agglutinated rat erythrocytes in dilutions of 1 : 360-1 : 640. On the addition of BF of rat sera and supernatant 1, however, the intensity of the reaction was reduced by 2-4 times, i.e., the BF of rat sera and supernatant 1 was more active in the natural than in the immune system.

In the gel-diffusion test the sera of immune rabbits gave 5-6 bands with antigens from rat sera (fraction IV of concentrated serum) but only two bands with antigens from supernatant 1 (Fig. 2a, b). On dilution of the rabbit sera with rat sera or supernatant 1 the reaction was negative; i.e., the action of the immune antibodies in the test with soluble antigens was blocked (Fig. 2c). Immunoelectrophoresis of fraction IV of concentrated serum from a rabbit immunized with supernatant 1 with these same antigens gave two lines — one in the β_1-globulin zone, the other in the α_1-globulin zone (Fig. 3). After a single precipitation of the test antigens in supernatant 2 only one line could be detected, in the α_1-globulin zone (Fig. 3b). In the agglutination test, supernatant 2 continued to have an inhibitory action although this action was weaker than in the control (supernatant 1). After double precipitation of the test antigens during immunoelectrophoresis with supernatant 3 not a single line could be seen. Supernatant 3 lost its activity with respect to agglutination of erythrocytes by heterophilic antibodies.

The combined immunological investigation this revealed an extremely thermostable factor, resistant to the action of several chemical substances, in rat sera which inhibits the action of normal and immune antibodies. The activity of this factor is linked with α_1-globulin.

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