OPSONINS AND ERYTHROPHAGOCYTOSIS

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The ability of the granulocytes and reticulum cells of the rat bone marrow to phagocytose mouse erythrocytes under the influence of opsonins was investigated. Phagocytosis of mouse erythrocytes, both untreated and treated with formaldehyde, was compared. By the use of untreated erythrocytes and uninactivated rat immune serum, moderate phagocytosis was observed, and it was weaker if inactivated serum was used. No phagocytosis was observed with rat immune serum. Conversely, if formaldehyde-treated erythrocytes and these same sera were used, much stronger phagocytosis was observed.
KEY WORDS: erythophagocyosis; opsonins; bone marrow.

Phagocytosis of erythrocytes by granulocytes in vivo is observed in a number of immunological blood diseases. Erythrophagocytosis has been investigated in vitro by many workers [5-8].

Erythrophagocytosis takes place through the action of serum factors, known as opsonins, on erythrocytes [1, 2, 12]. Of the 30 plasma proteins tested, 13 possessed opsonic activity; proteins with low molecular weight did not stimulate phagocytosis [1, 10]. The first four fractions of complement [1, 12] and antibodies of Ig immunoglobulins of classes M and G are currently regarded as opsonins. IgA antibodies are indifferent [12]. Opsonic activity is also a feature of α- and β-globulins and of albumin [4].

Bonin and Schwartz consider that erythrophagocytosis is impossible without the presence of opsonizing antibodies requiring complement [5]. Other workers, however, have observed phagocytosis of group A_1 erythrocytes in vitro under the influence of α-agglutinins only [6, 8, 9]. Nikolov [3], after comparing phagocytosis by granulocytes using untreated and formaldehyde-treated human group A and B erythrocytes, found in the second case a fivefold increase in the intensity of phagocytosis when anti-A and anti-B sera were used.

The object of the present investigation was to study the opsonizing action of certain antibodies and the effect of formaldehyde treatment of erythrocytes on their ability to undergo phagocytosis. Simultaneously with this, the ability of mature bone marrow cells to undergo phagocytosis was studied.

EXPERIMENTAL METHOD

To obtain immune sera containing opsonins against mouse erythrocytes, Wistar rats were immunized with erythrocytes from noninbred albino mice. The opsonizing action of these sera against untreated and formaldehyde-treated mouse erythrocytes was tested in a system of mouse erythrocytes – rat bone marrow cells – rat or mouse serum. Control experiments were carried out to test the action of: uninactivated rat immune serum on untreated erythrocytes; inactivated rat immune serum on untreated erythrocytes; rat nonimmune serum on untreated erythrocytes; mouse nonimmune serum on untreated erythrocytes; physiological saline on untreated erythrocytes. For these five control series there were five corresponding experimental series in which, instead of untreated erythrocytes, erythrocytes treated with formaldehyde were used.

The scheme of immunication of the rats and preparation of the serum was as follows: Each of ten rats received an intraperitoneal injection of 1 ml of a 10% suspension of mouse erythrocytes, washed three times with physiological saline, on the first, third, fifth, tenth, and fifteenth days. Two days after the last immunization the rats were exsanguinated by cardiac puncture under ether anesthesia. The serum thus obtained was divided into two equal portions: One portion was poured into ampuls and immediately frozen at −20°C, the other was inactivated at 56°C for 30 min before being poured into ampuls and frozen, also at −20°C. Unimmunized rats were exsanguinated by the same method, and their serum also was poured into ampuls and kept at −20°C.

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TABLE 1. Number (in %) of Phagocytic Rat Bone-Marrow Cells

Erythrocytes	Serum tested				
	uninactivated immune	inactivated immune	nonimmune rat serum	nonimmune mouse serum	physiological saline
Untreated	18,48±0,10	7,87±0,062	0	0	0
Treated with formaldehyde	36,46±0,0947	27,28±0,114	19,3±0,022	5,55±0,22	0

TABLE 2. Phagocytic Number of Rat Bone-Marrow Cells

Erythrocytes	Serum tested				
	uninactivated immune	inactivated immune	nonimmune rat serum	nonimmune mouse serum	physiological saline
Untreated	0,61±0,0047	0,17±0,0017	0	0	0
Treated with formaldehyde	1,48±0,006	1,12±0,0061	0,40±0,0024	0,07±0,00036	0

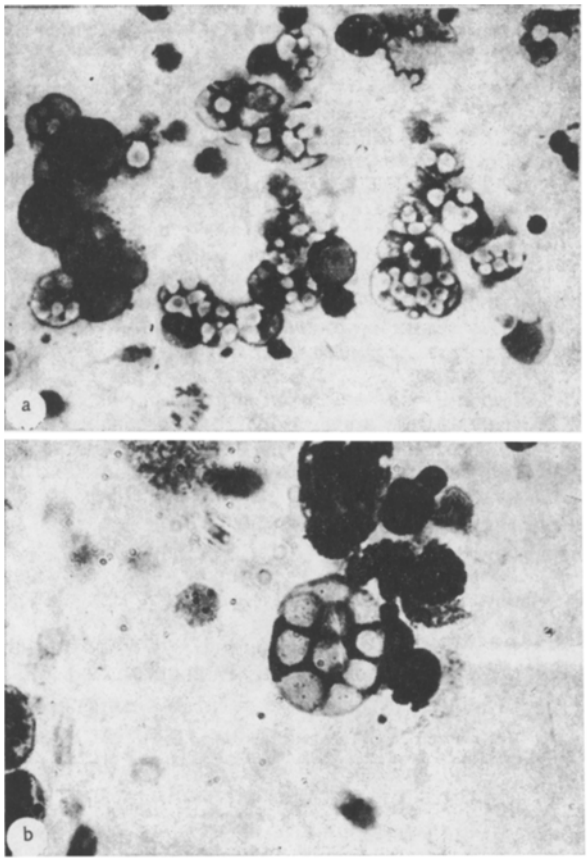

Fig. 1

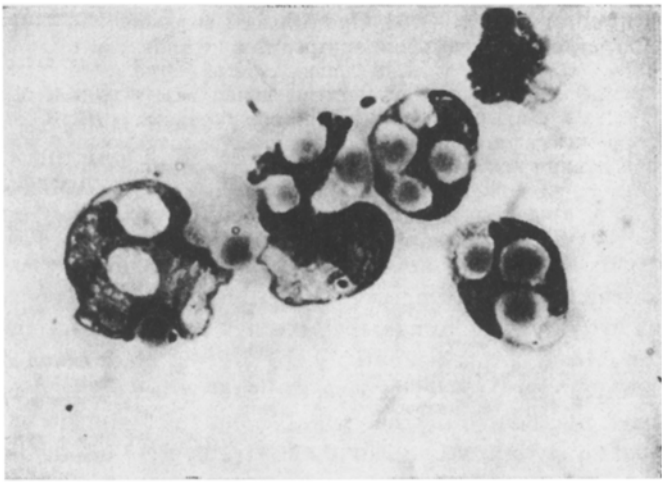

Fig. 2

Fig. 1. Erythrocyphagocytosis by rat bone-marrow cells: a) General view; b) cell with 10 ingested erythrocytes. Difco Quick Stain.

Fig. 2. Erythrocytes phagocytosed by young cells of myeloid series. Difco Quick Stain.

Mouse serum was obtained from blood taken from the caudal veins of several mice. Eagle's solution was used as the saline.

The titer of agglutinins and hemolysins was determined by the usual test tube method. The intensity of agglutination was designated ++++, +++, ++, and +. Bone marrow cells were obtained from healthy nonimmunized Wistar rats by flushing from the long bones with Eagle's medium. A suspension of bone-marrow cells (40,000-60,000 cells/ml) was then prepared in a Potter's homogenizer.

Mouse erythrocytes treated with formaldehyde by the method of Moskowitz and Carb [11] were used as the object for phagocytosis. Untreated mouse erythrocytes, washed three times with physiological saline, served as the control.

Phagocytosis was determined by the following method: 0.20 ml of the suspension of bone-marrow cells was centrifuged at 1000 rpm for 2 min. Three drops of the different types of sera or of physiological saline and 2 drops of a 30% suspension of untreated or formaldehyde-treated erythrocytes were added consecutively to the cell residue. After mixing, the contents of the tube were incubated at 37°C for 2 h, and films were then made on slides and stained by the rapid method with Difco Quick Stain. Under the microscope 100 bone-marrow cells were counted and the percentage of phagocytic cells and the phagocytic number were calculated.

From each sample three films were prepared as described above on slides, and 1000 cells were counted in them.

EXPERIMENTAL RESULTS

The rat immune sera used in the experiments had an agglutinin titer of 1:32 (the undiluted serum gave a reaction of +++ to ++). The hemolysin titer of the uninactivated immune serum was 1:2.

Altogether 10 experiments were carried out and their results are summarized in Table 1.

The strongest phagocytosis was observed with uninactivated rat immune serum. When erythrocytes treated with formaldehyde were used the percentage of phagocytic cells was doubled.

When inactivated rat immune serum and formaldehyde-treated erythrocytes were used the percentage of phagocytic cells was almost four times greater than in the experiments with untreated erythrocytes.

In experiments with nonimmune rat serum phagocytosis was observed only when formaldehyde-treated erythrocytes were used.

When nonimmune mouse serum was used, weaker phagocytosis than in the previous group also was found, only with formaldehyde-treated erythrocytes.

If, instead of the sera, Eagle's medium was used no phagocytosis was observed either with formaldehyde-treated or with untreated erythrocytes.

Similar results were obtained when the phagocytic number was determined (Table 2).

When phagocytosis was carried out with uninactivated rat immune serum, examination of the stained film revealed cells which had ingested different numbers of erythrocytes – from one to ten (Fig. 1). All stab cells and mature granulocytes had strong phagocytic activity. Younger cells of the myeloid series, which phagocytosed erythrocytes in precisely the same way, also were found, but infrequently (Fig. 2). Phagocytic activity also was manifested by reticulum cells. In the control experiment using untreated erythrocytes, the picture was very uneven and varied. Phagocytosed erythrocytes or their fragments were infrequently seen in the stained films; mainly ghost erythrocytes were found, or the erythrocytes were already broken up in the vacuoles.

When the other sera were used the intensity of erythrophagocytosis gradually diminished and completely disappeared in the samples with physiological saline.

The results show that the stronger the effect of the opsonising antibodies on the erythrocytes, the less intensively the latter were phagocytosed. Furthermore, phagocytic activity was strongest if formaldehyde-treated erythrocytes were the object of phagocytosis. Species differences also were found between the action of rat and mouse serum, but only when formaldehyde-treated erythrocytes were used.

The model of erythrophagocytosis used in this investigation shows that all mature and some young bone-marrow cells of the myeloid series are able to carry out phagocytosis. Since the largest number of ingested erythrocytes with this model is small, the phagocytic number can be determined accurately.

Some workers consider that the surface activity of erythrocytes is an obstacle to their phagocytosis [14, 16]. Formaldehyde-treated erythrocytes are not surface-active and, although their antigens remain unchanged, the cells cannot be agglutinated [11]. Formaldehyde thus acts as a stimulator of erythrophagocytosis; the antigen–antibody complex is formed on a surface-inactive cell, which is more easily phagocytosed.

Perkins and Leonard [13] showed that the degree of erythrophagocytosis depends on antigenic relations between the erythrocytes and the phagocytic cells: The more widely removed these cells are from the species point of view, the higher the percentage of phagocytosis [13, 16]. If the species difference is great, opsoniza-

tion of the erythrocytes is not essential [15]. In the present experiments features of the species difference between rat and mouse were clearly manifested when formaldehyde-treated erythrocytes were used. The success of the attempt to use bone marrow instead of peripheral blood granulocytes offers the prospects of working with small animals, from which it is difficult to obtain a leukocytic concentrate from the peripheral blood.

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MECHANISM OF ACTION OF HEPAIN ON LYMPHOCYTES in vitro

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In experiments on CBA mice in which a 2% suspe ·on of red blood cells was used as the antigen, heparin was shown to prevent the migration .. antibodies from antibody-producing cells substantially in vitro. On the addition of substances with the properties of detergents (Triton X-100, deoxycholate) in vitro to a suspension of plaque-forming spleen cells treated with heparin, the ability of the cells to form plaques was partially restored. It is concluded that heparin is able to interact with the outer membrane of immunocompetent cells and to inhibit migration of antibodies synthesized by them into the surrounding medium.

KEY WORDS: heparin; detergents; plaque-forming cells.

Considerable evidence of the ability of heparin to inhibit the development of diseases based on autoimmune conflict has accumulated recently in the literature [1, 2, 8, 11].

The inhibitory action of heparin on plaque formation in vitro has been demonstrated [3, 4]. During the analysis of the results of these investigations it was postulated that the mechanism of action of heparin could be connected with its adsorption of the cell membrane of the lymphocyte, as a result of which obstacles could arise to the migration of antibodies, which will be followed by inhibition of the reaction in progress.

The object of the investigation described below was to test this hypothesis.

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