NATURE AND MECHANISM OF PLAQUE FORMATION (VESICULOCYTOSIS)

V. S. Kislyakov

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N. N. Klemparskaya has concluded that plaque formation in peripheral blood preparations is the result of hemolysis of erythocytes by autohemolysins secreted by immunocompetent mononuclear cells – lymphocytes and plasma cells. However, this interpretation is not confirmed by an investigation of this process. Staining the plaques, microfilming, and other techniques have shown that plaque formation takes place as a result of separation of the cells (and not their hemolysis) by a substance secreted from the cell as a result of destructure processes with vesicle formation (vesiculocytosis). These cells in blood preparations carry out no vital activity and do not divide. Plaque-forming cells are polynuclear cells – mainly neutrophils – and not mononuclear.

KEY WORDS: autohemolysins; plaque formation.

The search for and development of methods of objective testing of immunoreactivity, including its initial changes, are of great practical importance [9]. The writer's attention has been drawn to Klemparskaya's method of detecting autohemolysin-producing cells [3]. According to Klemparskaya, plaque formation is the result of destruction of erythrocytes by autohemolysins liberated from mononuclear cells; consequently, this method can be used to estimate the number of cells producing autoantibodies, which is 1% in the blood of normal healthy persons (0-3%). This is equivalent to 104 autohemolysin-producing cells per 10^6 nucleated cells. According to data in the literature, this level of antibody-producing cells is possible only in the case of immunization with heterologous antigens. The question accordingly arises: Are the cells detectable by this method [3] in fact true producers of autohemolysins.

The mechanism of formation and nature of the plaque and also the type of cells possessing plaque-forming ability were studied.

EXPERIMENTAL METHOD

Klemparskaya's method [3] with minor modification [2] was used. The technique is as follows. Blood is taken from the finger in the usual way, filling a mixing chamber up to mark I, and fresh 10% sodium citrate solution is then added up to mark II. The contents are mixed in the chamber, two drops of suspension are expelled, a Goryaev's counting chamber is then filled, and it is at once placed in a Petri dish containing a circle of gauze 5-7 cm in diameter and 5-7 ml water. The dish is covered and incubated at 37°C. The number of plaques in 20 fields of vision is counted 4 h later by means of a microscope (ocular 5 ×, objective 20 ×, attachment magnification 1.5 ×). The number of plaques in 1 μl blood is then obtained by multiplying the total number of plaques in 20 fields of vision by ten.

The plaques also were photographed, microfilmed, and stained and the dynamics of their formation was observed. The effect of temperature and incubation time on the process of plaque formation and the intensity when material from bone marrow, gastric juice, and duodenal juice was used also were studied.

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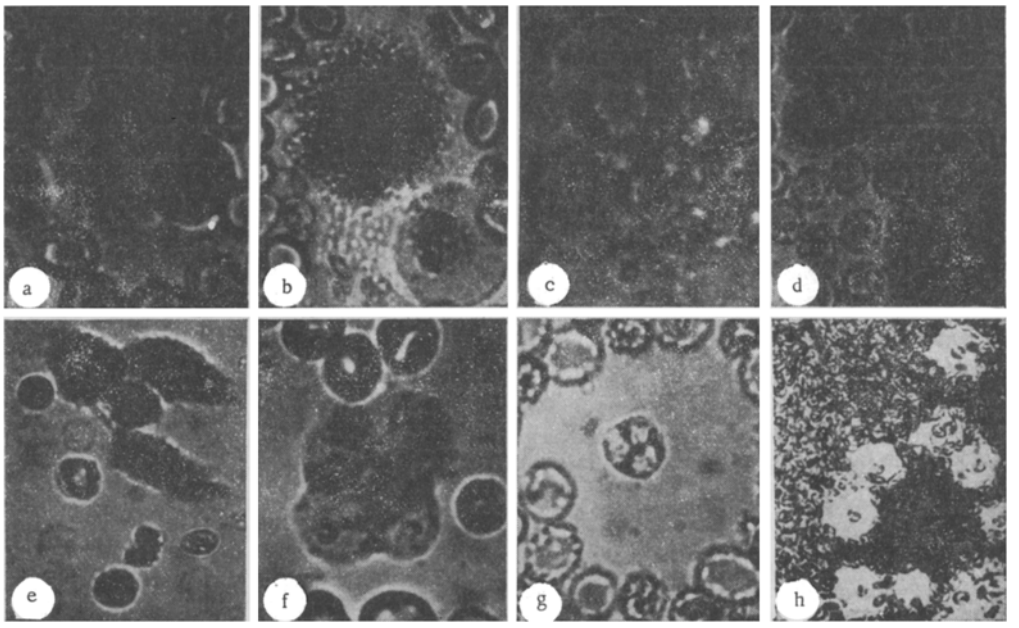

Fig. 1. Plaques in preparations of blood, gastric juice, and bone marrow: a) plaque in initial stage; ejection of substance from autohemolysin plaque-forming cells (APFC); b) various stages of vesiculocytes: above) membrane ruptured, below) membrane intact; c) APFC in gastric juice; d) variants of expulsion of substance from APFC: above) multijet, below) uniform; e) jets of substance escaping from cell; hemolyzed and nonhemolyzed erythrocytes; f) quadrivesicular vesiculocyte in formalinized preparation; g) APFC – cell with nucleus in free segments; h) condensation of layer of cells in middle of bone marrow preparation because of separation of their plaques. Staining: g and h) unstained, elsewhere stained by Pytel's method. Magnification: a-g) objective 40×, ocular 20×; h) objective 10×, ocular 20×.

Fig. 2. Plaque formation by leukocytes in suspension of ink. Magnification: objective 40×, ocular 20×.

EXPERIMENTAL RESULTS

The process of plaque formation was found to consist essentially of separation of the erythrocytes and not hemolysis. On supravital staining [6] erythrocyte ghosts could be seen in the preparation (Fig. 1e), but as a rule none were found in the zone of the "plaque" (Fig. 1a, b, d, f).

Formalinized erythrocytes [1] are resistant to hemolysis (immune, hypotonic, and so on). However, plaques are still formed in such preparations (Fig. 1f). Some workers [3] have found vital activity in progress in cells forming plaques on account of autohemolysins (APFC) in vitro, and division of these cells in as many as 26% of cases. However, on prolonged observation and photography of the same plaques it was found that the phenomenon interpreted by those workers as division is in fact the result of the original arrangement of APFC in the preparation in pairs. According to published data [3], plaques are formed by mononuclear cells. The present observations show that APFC are mainly polynuclear cells (Fig. 1g). Examination of bone marrow for APFC showed that they were two to four times more numerous than in blood preparations, a result which does not correlate with the number of lymphocytes and plasma cells. Preparations from duodenal secretion and gastric juice also contained APFC (Fig. 1c). There, just as in blood preparations, destructive changes in the cells with vesicle formation were found.

TABLE 1. Number of APFC in Subjects Examined Depending on Temperature, Incubation Time, and Method of Study (M ± m, n = 97)

Incubation time	37°		12—25°		Data of N. N. Klemparskaya (%)
	absolute	%	absolute	%	
4 h	920±56	16,1±0,7	488±50	83±0,7	—
24 h	1710±80	28,7±1,3	1020±81	16,6±1,0	1,8±0,3

The number of APFC in the blood correlates with the number of neutrophils. For instance, in a patient with lymphatic leukemia (leukocytes 32,000, neutrophils 0.5%, lymphocytes 98.5%) the number of APFC was 40/μl blood (0.12%), whereas in a patient with neutrophilia (leukocytes 7,900, neutrophils 98%, monocytes 1%, lymphocytes 1%) there were 2730 APFC/μl blood (36%). Supravital staining showed the structure of the plaques (Fig. 1). Destructive changes were found in the cells, a feature more characteristic of granulocytes and in harmony with existing data [5]. The essential character of the changes in APFC was similar to degenerative changes in "active" leukocytes [7]. Since plaque formation is based on the formation of vesicles (Fig. 1b, c, f), the swollen cells can be called vesiculocytes [2] and the phenomenon itself vesiculocytosis.

All factors depressing respiration and cell metabolism, according to Klemparskaya's observations [4], also interfere with plaque formation (heating at 56°C for 30 min, etc.). However, inactivation of complement at 56°C even for 4 h facilitated plaque formation. This indicates that plaque formation takes place in the absence of autoimmune hemolysis. The results in Table 1 show that with a rise in temperature and an increase in incubation time, the number of plaques rises.

Finally, in a model experiment, in which particles of ink were introduced instead of erythrocytes into a preparation containing isolated blood leukocytes, plaques also were formed (Fig. 2). In this case, it is clear that the ink particles simply separated, as is also the case with erythrocytes.

Since the number of plaques is connected with the aging process [3] and also with the state of the organism as a whole [3, 4], the process of plaque formation reflects changes in the granulocytes (age, functional, morphological, etc.). Probably during sensitization a certain proportion of the granulocytes, loaded with antibodies, because of changes in their properties, become subjected to the action of autocytolysins, which leads to plaque formation. Considering the available data [8] it can be postulated that interaction between the electric charges of the plaque and erythrocytes leads to their separation. The clinical importance of vesiculocytosis is a matter for further study.

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