FALSE PLAQUE FORMATION IN IMMUNOLOGIC REACTIONS

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The dynamics of the cellular autoimmune response can be determined by Jerne's method in one of its modifications. This method is based on the detection of cells producing autoantibodies, present in the peripheral blood and immunocompetent organs and tissues [5]. In the literature on the mechanism of plaque formation there are data which question the correctness of information obtained by the use of this modification [3, 4].

Those components of the modification which lead to an incorrect understanding of information received are analyzed below.

EXPERIMENTAL METHOD AND RESULTS

When the cell suspension is prepared for investigations by Jerne's method in Klemparskaya's modification [5] the spleen (kidneys, liver), in a sample weighing 150-200 mg, is cut into small pieces with scissors and the resulting mass is placed on Kapron gauze. Medium No. 199 or Hank's solution, or sterile physiological saline is applied above drop by drop, and mixed with the tissue by means of a rod. The filtrate contains isolated cells. The background for detection of hemolysis is citrated blood from the same animal. A mixture of cells and background is then made and a "pressed drop" preparation is obtained, and is protected against drying by means of petrolatum. After the preparation has remained for 20-30 min at room temperature it is placed strictly in the horizontal position in a refrigerator at 4°C for 18-20 h, after which the results are read.

It will be clear that during preparation of a cell suspension from the various organs, some of the cells will be destroyed when the tissue is cut into pieces with scissors and the suspension obtained, and this will lead to an increased concentration of salts and proteins in the suspension. In our experiments splenic tissue (and also other tissues of mice and rats) was taken in an amount of 200 mg and cut into pieces with scissors; the mass was placed on Kapron gauze, after which 1 ml of 0.9% sodium chloride solution was added. The resulting suspension was centrifuged for 10 min at 3000 rpm to separate whole cells and the stroma of the destroyed cells. The supernatant was collected and examined on a flame photometer [2] to determine the concentration of sodium, potassium, and calcium ions quantitatively (Table 1). The mean results of three series of experiments, with five experiments in each series, are given in Table 1.

If less physiological saline (i.e., 0.5 ml, as indicated in the modification) is taken to obtain the cell suspension the total quantity of ions is increased and this leads to greater hypertonicity of the solution. It was thus shown that the osmotic pressure of the solution in cell suspensions prepared from different tissues may

TABLE 1. Concentration of Ions in Supernatant (in %)

Test material	Sodium	Potassium	Calcium
Spleen	4,05	0,050	0,002
Liver	3,80	0,065	0,002
Kidneys	3,65	0,040	0,002
Physiological saline	0,86	0,002	0,0004

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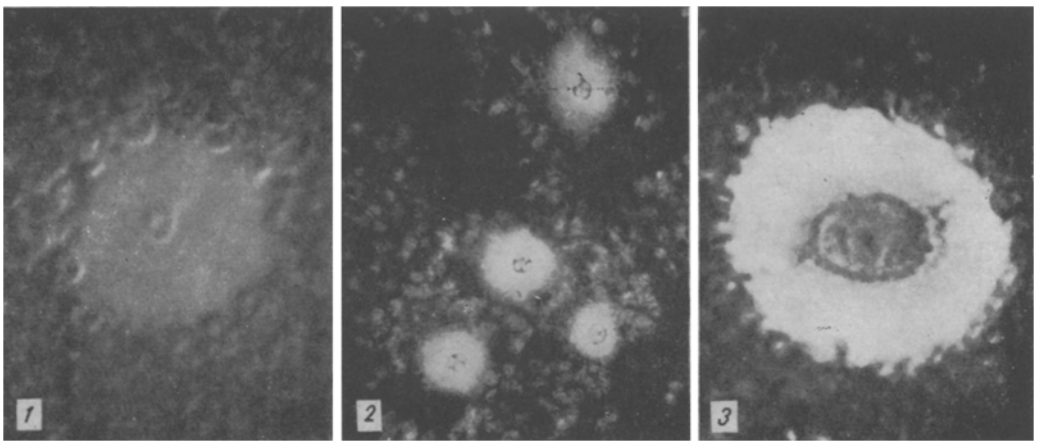

Fig. 1. "Immune" plaque obtained by Klemparskaya's modification. Phase-contrast microscopy, 400×.

Fig. 2. Halos around some blood leukocytes in the film. 200×.

Fig. 3. Halo around a tumor cell in blood. 900×.

be increased by 4-5 times or more.

The hypertonicity of the solution in the cell suspension also was determined from swelling of the tissues in hypo- and hypertonic solutions [7]. It was found that the weight of rat liver tissue in distilled water was increased by 146% compared with its weight in isotonic (physiological) solution, in 10% (hypertonic) sodium chloride solution the weight of the tissue was reduced by 87%, and in the supernatant obtained by the use of Klemparskaya's modification, it was reduced by 60%.

Some investigators have shown [1, 4] that living cells kept for a long time in hypertonic solutions die because of destructive processes. In Klemparskaya's modification plaques are formed in the course of 18-20 h, during which they are in a hypertonic solution, which may cause death of the cells rather than the production and secretion of autoantibodies. The total protein content in the course of preparation of the cell suspension, including enzymes, may reach 3-5 g/liter under these circumstances, and this produces additional oncotic (colloid-osmotic) pressure. Furthermore, variations in the weight of the tissue (150-200 mg) and in the volume of isotonic solution taken (0.5-1 ml) may lead to considerable variability in the composition of the medium in which the plaque-forming cells are kept.

Some workers consider that hemolysin antibodies which accumulated previously [5], and which destroy erythrocytes, can be rapidly separated from the cells. However, during an investigation of the structure of plaques obtained by Klemparskaya's modification in the phase-contrast microscope, no hemolyzed erythrocytes could be found but, by contrast, there was a destroyed plaque-forming cell. In our opinion these facts are evidence of absence of autoantibodies of the hemolysin type (Fig. 1).

To confirm that plaques are in fact formed in hypertonic solution, we carried out the following experiment. To one drop of blood (0.03 ml) we added 4 drops (0.12 ml) of 15% sodium chloride solution and made a "pressed drop" preparation. Plaques began to form in the course of 3-5 min. Moreover, similar plaques (we called them halos) formed in the course of 2-4 sec in a system of: ink, 15% sodium chloride solution and cells of immunocompetent tissues (Fig. 2). Ink as background particles for the detection of halo-forming cells was used on Professor Klemparskaya's advice. These methods reveal halo-forming blood, tumor, and lymphoid tissue cells (Fig. 3).

In our experiments, in order to study the effect of oxygen on plaque formation, sheep's blood was saturated with oxygen. However, in "pressed drop" preparations no plaques formed in the course of 2 h or more, although Klemparskaya [5] describes the rapid (after 15-20 min) appearance of plaques in preparations with both foreign and the animal's own erythrocytes (without isolation of the preparation with petrolatum), thus indicating the importance of access of oxygen to the plaque-forming cells during the period of their formation. In my opinion the author of the modification did not take into account drying of the liquid around the periphery of the preparation, leading to increased hypertonicity of the solution in which the plaque-forming cells were located.

It can be concluded from these results that in the modification [5] of Jerne's method arbitrary quantities of tissue sample and of isotonic fluid were used, and this led to the appearance of an unverifiable quantity of inorganic ions; "immune" plaques are formed when this modification is used under conditions of high osmotic pressure; investigation of such plaques in the phase-contrast microscope showed the absence of hemolysed erythrocytes, possible evidence of the absence of autoantibodies; the author of the modification in [6] showed that when hypertonic solutions are used false plaques are obtained, i.e., plaques of nonimmune nature, In my own opinion, the description is evidence that false plaques are plaques formed under conditions of increased osmotic pressure and they differ from "immune" plaques in their mechanism of formation.

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EXPRESSION OF VIRUS-SPECIFIC RNA IN CELLS OF MICE INFECTED WITH MAZURENKO AND RAUSCHER VIRUSES

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In 1957 a virus which subsequently proved to be highly leukemogenic for mice not only of the CC57Br line, but also for CC57W, C57BL, and C3H mice and also for noninbred rats, was isolated by N. P. Mazurenko from organs of CC57Br mice developing leukemia after receiving an injection of vaccinia virus.

Mazurenko virus has the morphology of particles of type C oncornaviruses [6]. The viriens are composed of 60-70S RNA and RNA-dependent DNA-polymerase, and the density of the virus particles in a sucrose gradient is 1.16 g/ml [3]. In its immunologic properties the virus belongs to the Friend, Moloney, Rauscher (FMR) group of viruses [2].

Despite the fact that Mazurenko virus was discovered more than 20 years ago, its molecular-biological properties have so far received little study. Yet some of the properties of this virus, distinguishing it from the mouse leukemia viruses known today, namely its extremely high oncogenicity, and the method of activation of the virus – make the study of its molecular-biological properties and their comparison with those of known mouse leukemia viruses most interesting.

The object of this investigation was to study expression of RNA of Mazurenko and Rauscher (RLV) viruses in tissues of various organs of CC57Br and BALB/c mice infected with these viruses.

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