ABILITY OF LYMPHOCYTES TO PRODUCE FRACTION C_1' OF COMPLEMENT

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If sensitized sheep's erythrocytes are incubated with immune lymphocytes in the presence of components C_2', C_3', and C_4' of complement (reagent R_1) lysis of the erythrocytestakes place. The phenomenon of lysis is explained by liberation of complement C_1' by the lymphocytes.

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The study of the role of lymphocytes in production of the components of complement is essential for the understanding of some important problems in immunology. Fresh light would be shed on the problem of cytotoxicity of lymphocytes if it were proved that lymphocytes could produce complement or its components. Research to study this problem is only in its infancy [6, 7].

The object of the present investigation was to determine whether lymphocytes can produce fraction C_1' of complement.

EXPERIMENTAL METHOD

The problem was investigated by the classical scheme of titration of complement C_1' by the corresponding reagent R_1 containing components C_2', C_3', and C_4' [3]. The main modifications made to this system were as follows: instead of the protein fluid tested for C_1' activity, a suspension of lymphocytes mixed with sensitized sheep's erythrocytes was used. If lymphocytes can produce C_1', then the complex EAC_1' must be formed in such a system. This complex, in the presence of reagent R_1, reacts with fractions C_2', C_3', and C_4', resulting in lysis of the erythrocytes. Under these circumstances no titration of C_1' was carried out. Estimation of the ensuring hemolysis was qualitative in character.

Lymphocytes were obtained from Wistar rats 4 days after the animals received a single injection of 0.25 ml of a 50% suspension of sheep's erythrocytes. The cell suspension was prepared by carefully pressing minced lymph glands in Earle's solution through a Kapron mesh. The suspension was washed three times with large volumes of the same solution. Cells in a concentration of $5 \cdot 10^6$ lymphocytes/ml were added to an incubation medium consisting of 1.5 ml Eagle's medium with 5% bovine serum, inactivated by heating.

Heat-inactivated serum of the rats acting as donors of lymph glands was used to sensitize the sheep's erythrocytes [3]. When the titer of hemolysins in this serum could not be determined, sensitiziation was reinforced by dry rabbit's hemolytic serum with a titer of 1:100-1:200. Sensitized erythrocytes were added to the incubation medium in a concentration of 10^6 cells/ml.

Reagent R_1, obtained by dialysis against phosphate buffer, pH 5.4 ($\mu = 0.02$), was added to isotonicity and neutral pH, and treated with components C_3' and C_4' by the addition of 25% heat-inactivated guinea pig serum. The effectiveness of separation was tested by incubating fractions M and E separately and a mixture of equal volumes of them in the presence of the hemolytic system. Absence of hemolysis in the first two cases and its presence in the last case was used as the criterion of suitability of the R_1 reagent for use.

The scheme of the main experiment was as follows. After incubation of the suspension of sensitized erythrocytes and lymphocytes for 3 h at 37° the cells were sedimented by centrifugation and the supernatant was carefully drawn off and replaced by 1 ml of reagent R_1 diluted 1:5. After reincubation of the cells with R_1 for 1 h at 37°, the reagent R_1 was removed by careful aspiration, and it was replaced by 1.5 ml distilled

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TABLE 1. Hemolytic Action of Reagent R_1 on Sensitized Erythrocytes in Contact with Lymphocytes

Index	Experiment No.						
	1	2	3	4	5	6	7
Residual Hb (in % of control Hb)	46,7	60,6	88,2	91,1	68,2	105	94
Decrease in number of erythrocytes (in %)	53,3	39,4	11,8	8,9	31,8	—5	6
Values of criterion λ^2	2,73	2,67	0,93	0,49	3,60	0,33	0,75
Number of animals	4	4	4	4	3	3	3

water to produce cytolysis. Tests were carried out on 1 ml of carefully centrifuged supernatant. In this way the hemoglobin belonging to the intact erythrocytes was analyzed after interaction with R_1. The object of this procedure was to avoid distortion of the results possibly arising through pinocytic absorption of the liberated hemoglobin by the lymphocytes.

The control consisted of a suspension of sensitized erythrocytes in medium not containing lymphocytes. Specificity of the effect was tested by incubating sensitized erythrocytes and lymphocytes without subsequent treatment with reagent R_1.

The hemoglobin concentration was determined by the benzidine reaction [2, 4]. The mean statistical error in this investigation was ±15%.

Experiments were carried out on 25 animals. Each experiment included tests on 7-10 tubes containing the experimental cell suspension and the same number of controls. The experiments were repeated twice or three times. The Kolmogorov – Smirnov criterion was used in the statistical analysis [1].

EXPERIMENTAL RESULTS

The results are given in Table 1. In typical experiments (Nos. 1 and 2) the decrease in the number of erythrocytes because of hemolysis was 59.3 and 39.4% respectively. It is extremely important that the lymphocytes added to the incubation mixture should be freshly isolated. Experiment No. 3 demonstrates the results of a test in which sensitized erythrocytes were added to lymphocytes preliminarily incubated in standard medium for 24 h. The absence of a decrease in the erythrocyte count after treatment with reagent R_1 was explained by a morphological study of films made from the suspension, demonstrating death of most of the lymphocytes.

The factor liberated by the lymphocytes (C_1') was not found in the incubation medium in a free state. Treatment of the sensitized erythrocytes under standard experimental conditions with medium in which lymphocytes had been preliminarily incubated for 24 h did not cause loss of erythrocytes through hemolysis (experiment No. 4). There are two possible explanations of this: appearance of component C_1' on EA takes place only in the presence of viable lymphocytes, i.e., in contact with them [5], or on the other hand, component C_1' is liberated by lymphocytes into the medium but is inactivated during the period of incubation.

Finally, to explain the leading role of macrophages [7] in C_1' formation, an attempt was made to remove these cells from the lymphocyte suspension used. For this purpose a suspension of lymphocytes in Eagle's medium with 5% heat-inactivated bovine serum was slowly passed at 37° through a column packed tightly with glass wool. Elution of the cells was carried out with the same medium. Morphological examination of the suspension showed that its composition was comparatively homogeneous, consisting of small and large lymphocytes, lymphoblasts, with a few plasma cells. The results of an experiment with these cells are shown in Table 1 (experiment No. 5). A decrease in the number of erythrocytes (31.8%) was also found in this case. The somewhat smaller hemolytic effect compared with experiments Nos. 1 and

2 may be attributed to low activity of the reagent R_1, as was shown in the course of the experiments. This experiment casts doubt on the view that production of the first component (C_1') of complement is an exclusive property of macrophages.

Experiments Nos. 6 and 7 were carried out in accordance with the general scheme, but without subsequent treatment of the cells with reagent R_1. Freshly isolated lymphocytes were used in experiment No. 6, and lymphocytes preliminarily incubated for 24 h in experiment No. 7. As the results show, a hemolytic effect was absent in both experiments.

The cell population from the lymph glands of immune rats can thus exhibit activity of the first component of complement (C_1'). This cell population cannot produce at least one of the remaining components of complement (C_2', C_3', and C_4') essential for completion of hemolysis. Otherwise, hemolysis would have occurred without reagent R_1, for example, in experiments Nos. 6 and 7.

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