Detection of Complement-Producing Cells with Macrophage Antiserum

Using a modification of the hemolytic plaque technique with sensitized sheep erythrocytes and guinea-pig complement lacking C'4 (R4 reagent), evidence has been presented that bone marrow and spleen cells of guinea-pig produce hemolytically active C'4, the fourth component of complement[1]. Certain cells in these tissues have also been stained with specific C'4 rabbit antiserum[2] conjugated with fluorescein isothiocynate[1]. However, the techniques employed in these studies did not permit the identification of the types of cells involved. Recent investigations have shown that highly specific heterologous antisera can be prepared with cultured peritoneal exudate cells[3]. This offers a useful tool for the dissection of various cell types capable of producing components of complement. The present study describes the use of a highly specific rabbit macrophage antiserum for the identification of cells producing C'4 in guinea-pig peritoneal exudates.

Material and method. Peritoneal macrophages of adult guinea-pigs of the Hartley strain were obtained and purified by the technique of BLOOM and BENNETT[4]. The resulting cell suspension contained over 99% pure macrophages. Anti-macrophage sera were raised in albino rabbits by the method of LEVEY and MEDAWAR[5] and characterized as described elsewhere[3]. The pooled antiserum was absorbed with an insoluble antigen-antibody complex[6], heated at 56 °C for 30 min and sterilized by Seitz filtration before use. R4 reagent was prepared from guinea-pig serum as previously described[2]. For the test, cells were harvested by rinsing the peritoneal cavity with Hanks' BSS containing 10 units/ml heparin and washed 3 times with Hanks' BSS. A volume of 0.3 ml of the cell suspension (10^6 cells/ml) was added to tubes containing 0.5 ml of macrophage antiserum diluted 1:10 and 0.2 ml of either guinea-pig serum or R4 reagent, the latter at a 1:20 dilution. The tubes were incubated at 37 °C for 30 min in an atmosphere of 5% CO_2 and 95% O_2. Immune cytolysis resulting from the action of specific antibody and complement was assessed in a haemocytometer with 0.4% trypan-blue supravital stain in Hanks' BSS.

Results. The results are summarized in the Table. Neither macrophage antiserum (MAS) nor R4 alone had any demonstrable effect on peritoneal cells. The combination of macrophage antiserum and R4 resulted in the lysis of 72% of the macrophages in 10 min. Only 12% of the macrophages remained viable after 30 min observation. In contrast, 86% of the lymphocytes were unaffected by the same treatment. The use of fresh guinea-pig serum (GPS) in place of R4 reagent facilitated the immune lysis of macrophages but again had little effect on the lymphocytes. The combination of either macrophage antiserum and guinea-pig serum heated at 56 °C for 30 min (ΔGPS) or normal rabbit serum (NRS) and fresh guinea-pig serum (GPS) did not produce significant cell death. It thus appears that peritoneal macrophages release C'4 to complement the other components in the R4 reagent, giving rise to immune lysis by specific anti-macrophage serum. Further experiments were performed in order to determine whether C'4 was passively absorbed on cell surface or actively secreted by the cells. The cell suspension was incubated with the metabolic inhibitor potassium cyanide (0.01 *M* KCN) at 37 °C for 10 min, washed 3 times with Hanks' BSS and tested for immune lysis with macrophage antiserum and R4 reagent. The absence of significant immune cytolysis with R4 but not with whole guinea-pig serum clearly indicate that KCN at the concentration employed has no adverse effect on the activity of C'4, and that the complement component was actively secreted by the macrophage cells.

Discussion. Although the experiments do not distinguish between the release of preformed complement and de novo synthesis by the macrophages, the method provides a sensitive technique for the demonstration and identification of cells producing a biologically active component of complement. In a previous study[1], the application of the hemolytic plaque technique commonly employed for detection of antibody-producing cells proved inadequate for the study of C'4-producing cells, due apparently to the low concentration of component released. The problem was circumvented by devising a close-packed single-cell layer in free suspension for the demonstration of hemolytic plaques. However, the method still suffers from the lack of sensitivity on account of the fact that the manifestation of complement secretion by the cells under study is dependent upon lysis of a sufficient number of surrounding red cells to produce discernible plaques. The present approach, made feasible by the high specificity of the antiserum, offers a direct means for demonstration and identification of complement-secreting cells without the need for a secondary phenomenon, e.g. hemolysis of red cells. With the availability of highly specific tissue antisera and purified complement components, this method might prove useful for the direct identification of cell types responsible for producing other components of the complement system[7].

Immune cytolytic activity of macrophage antiserum and complement on peritoneal macrophages and lymphocytes[a]

Treatment	Time (min)	% Viable macrophages	% Viable lymphocytes
MAS + R4	0	100	100
MAS + R4	10	28	95
MAS + R4	30	12	86
MAS + GPS	10	3	93
MAS + ΔGPS	30	86	83
NRS + GPS	30	85	87
MAS	30	86	90
R4	30	87	85
MAS + R4[b]	30	82	86
MAS + GPS[b]	30	1	82

[a] See text for explanation of immune lysis. [b] Experiments were performed with cells pre-incubated with 0.01 *M* KCN.

Résumé. Exposé d'une méthode utilisant un antisérum macrophage hautement spécifique de lapin pour rendre apparente et identifier des cellules sécrétant du C'4 dans les exudations du péritoine du cobaye.

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