Simple Method for Preparing the Cellular Intermediate EACT₄, and Its Use for Estimation of the Second Component of Complement

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Sensitized erythrocytes carrying the first and fourth components of complement (EACT₄) were prepared by incubating optimally sensitized sheep erythrocytes with normal serum appropriately diluted in Mg²⁺-free diethybarbiturate buffer containing Ca²⁺. EACT₄ cells so prepared were found to be suitable for use in estimating the second component of complement (C2) in human serum, and the method is described here.

Subnormal concentrations of the second component of complement (C₂) are present in serum of patients with a genetic deficiency of this component or with certain other immune-complex and auto-immune diseases.

Two approaches are usually adopted to the estimation of C₂. One (I) is to use C₂-deficient serum, the restoration of the hemolytic activity of which depends on C₂ present in added test serum. This method requires the availability of serum from a C₂-deficient patient. The other approach is to incubate EACT₄ cells with test serum and then with EDTA-containing guinea pig or rat serum as a source of C₃ to C₉, and monitor the lysis of the EACT₄ cells, the extent of which depends on the concentration of C₂ in the test sample. For preparation of EACT₄ cells either purified components (2) or normal sera rendered deficient in C₂ or C₃ is used (3, 4). Purified components are costly, their activities do not remain uniformly constant, and sera rendered deficient in a component of complement are not always reliable.

Here we describe the preparation of EACT₄ cells suitable for the estimation of C₂ without the use of purified components or C₂- or C₃-deficient sera. We exploited the fact that, at higher dilution in Mg²⁺-free buffer, the Mg²⁺ concentration in serum is decreased so much that it no longer effectively allows adsorption of C₂ onto C₁₄ sites. Results of our C₂ estimation are compared with those obtained by the method of Ngan et al. (I).

Materials and Methods

Buffers

GVB: Isotonic buffer (pH 7.4) containing, per liter, 142 mmol of NaCl, 4.94 mmol of sodium diethybarbiturate, and 1.0 g of gelatin.

GVB⁺⁺: GVB containing, per liter, 0.15 mmol of CaCl₂ and 0.5 mmol of MgCl₂.

GI-GVB⁺⁺: GVB containing, per liter, 138 mmol of glucose, 0.15 mmol of CaCl₂, and 0.5 mmol of MgCl₂.

Ca⁺⁺-GVB⁺⁺: GVB containing 0.15 mmol of CaCl₂ per liter.

EDTA-GVB: GVB containing 40 mmol of EDTA per liter.

Sera

Sera were prepared by allowing blood samples to clot at 22 °C for 60 min at room temperature, then centrifuging (800 × g, 10 min). Sera thus obtained were kept on ice. A pool of normal serum was prepared from sera of 18 healthy volunteers. Individual normal serum samples (from 21 men and seven women) were obtained from healthy hospital personnel. C₂-deficient serum was obtained from a patient with frontoparietal scleroderma "en coup de sabre" associated with hereditary deficiency of C₂ (5). The pooled normal serum and all the other serum samples were stored in aliquots at −70 °C until use.

Erythrocytes, Antisera, and Complement

Sheep erythrocytes, amboceptor, and guinea-pig complement were from the National Institute of Health, Biltbewen, The Netherlands. Purified human C₂ was from Cordis Laboratories, Miami, FL. C-EDTA was prepared by diluting 1 mL of guinea pig serum to 12.5 mL with EDTA-GVB. Erythrocytes and amboceptor were stored at 4 °C. The other material was stored in small aliquots at −70 °C until use.

Procedures

Preparation of EACT₄ cells. EA cells (20 × 10⁶) were suspended in a final volume of 100 mL of Ca⁺⁺-GVB⁺⁺ containing 229 µL of the normal serum pool and incubated at 32 °C for 30 min, with shaking, followed by three washings with Ca⁺⁺-GVB⁺⁺ and two with GI-GVB⁺⁺.

Measurement of EACT₄ formation and decay. EACT₄ cells (2 × 10⁹) in 0.2 mL of GI-GVB⁺⁺ were incubated with 108 ng of C₂ in 0.2 mL of GI-GVB⁺⁺ and the reaction was stopped, in aliquots removed at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 10, and 15 min, by addition of 0.4 mL of EDTA-GVB. We then added 0.2 mL of C-EDTA and incubated the tubes at 37 °C for 1 h, with shaking. Controls for measuring spontaneous and 100% lysis were included. The tubes were then cooled, 0.2 mL of cold isotonic saline was added, and, after centrifugation, the absorbances of the supernates were measured at 412 nm and the percent lysis was calculated.

C₂-estimation with use of C₂-deficient serum. Various amounts of test serum in 0.6 mL of GVB⁺⁺, 0.2 mL of C₂-deficient serum (diluted 1 to 125 mL in GVB⁺⁺), and 0.2 mL of EA cells (2 × 10⁸) were incubated for 60 min at 37 °C, with shaking. Controls for spontaneous and 100% lysis were included. The percentage lysis was measured as described above. The C₂ CH50 units were calculated as described by Mayer (6). The results were expressed in percent of the normal serum pool.

C₂-estimation with use of EACT₄ cells. Various amounts of test serum in 0.2 mL of GI-GVB⁺⁺ were incubated with

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²Nonstandard abbreviations: C₁–C₉; complement components; C₁ to C₉; E, sheep erythrocytes; EA, sheep erythrocytes optimally sensitized with antibodies against E; EACT₄, sensitized erythrocytes carrying complement components C₁ and C₄ in the activated state; EACT₄, sensitized erythrocytes carrying complement components C₁ and C₄ in the activated state; and C-EDTA, reagent-guinea pig serum mixture (defined in text).

Received September 12, 1985; accepted December 20, 1985.
Results and Discussion

The EACT\textsubscript{4} cells gave 88% lysis in presence of 108 ng of C2 and C-EDTA. When C2 was omitted from the system only 4% lysis was observed. The cells showed characteristic EACT\textsubscript{4} cell formation and decay profiles (Figure 1). These results show that the cells were sensitized up to the C1\textsubscript{4} stage. C2 as estimated by the proposed method and the method of Ngan et al.\textsuperscript{1} in 28 sera of healthy controls gave values ranging from 78 to 138% and 72 to 134%, respectively. Day-to-day precision was calculated from duplicates, of which the second sample was always analyzed on a different day than the first. In the range 80–120% the CV was about 9%. In the range 40–80% the CV was about 10%.

Comparison of results by the proposed method with those by the method of Ngan et al. (Figure 2), for normal as well as patients' sera (n = 37), gave the regression equation $y = 1.04x + 4.38\%$ ($r = 0.78$; standard error of the estimate 21.46%). A very good correlation between results by the method of Ngan et al. and the proposed method is not to be expected. The activators of the classical pathway such as immune complexes, if present in the serum, can cause a far greater decrease in C2 during incubation in the method of Ngan et al. than in the proposed method. In the former method, EA are incubated with a mixture of test and C2-deficient serum at 37°C for 60 min, whereas in the proposed method EACT\textsubscript{4} cells are incubated with the test serum at 32°C for 2 min. In this respect the proposed method appears to be superior to that of Ngan et al.

We conclude that EACT\textsubscript{4} cells suitable for use in the determination of C2 can be prepared by incubating EA cells

\[ 0.2 \text{ mL of EACT}_4 \text{ cells (2 \times 10^5)} \text{ for } 2 \text{ min at } 32 ^\circ \text{C}. \text{ Then } 0.4 \text{ mL of EDTA-GVB and } 0.2 \text{ mL of C-EDTA were added, and the mixture was incubated at } 37 ^\circ \text{C for } 60 \text{ min, with continuous shaking. The percent lysis was measured as described above and C2 CH50 units calculated. The results were presented in terms of percent of the normal serum pool.} \]

![Graph](image-url)

Fig. 1. EACT\textsubscript{4} formation and decay as a function of time

![Graph](image-url)

Fig. 2. Correlation between the proposed method and the method described by Ngan et al.\textsuperscript{1}

\[ n = 37; y = 1.04x - 4.38\%; r = 0.78; \text{ standard error of the estimate, 21.46}\% \]

with normal serum pool diluted enough in Ca\textsuperscript{2+}-GVB\textsuperscript{2} to decrease the magnesium concentration such that there is no further sensitization of C1\textsubscript{4} sites. Thus, for preparation of EACT\textsubscript{4} cells, there is no need of a serum rendered deficient in C2 as used by Joisel et al.\textsuperscript{(3)}, a serum rendered deficient in C3 as used by Thompson\textsuperscript{(4)}, or purified components as used by Nelson et al.\textsuperscript{(2)}. EACT\textsubscript{4} cells thus prepared can be used to detect gross abnormalities in C2 concentrations.

References


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