The Terminal Transformation of Immune Complement; Comparison of Human and Guinea-Pig C'3

In the sequence of reactions between sensitized sheep erythrocytes (i.e. sheep erythrocytes treated with hemolytic antiserum and referred to as EA) and complement (C'), an intermediate is formed (E*) which will undergo lysis in the absence of fluid phase complement; the lysis of E* is referred to as the terminal transformation[1]. HOFFMANN, in work referred to by MAYER[2], found that the rate of the terminal transformation of E* formed from guinea-pig complement (G.P. C') was greater than that formed from human complement (Hu C'). The present work provides evidence that the chief source of difference in the terminal transformation rates resides in the EAC'1,4,2 + C'3[3] reaction.

Materials and Methods. Sheep erythrocytes were collected as described by MAYER[2]. A commercial hemolysin was used to sensitize the cells. Veronal buffered saline (VBS) with Ca^{++} and Mg^{++} was prepared according to MAYER[2] and contained 0.05% gelatin. A 0.065 ionic strength buffer (0.065 buffer) containing sucrose isotonic with red cells was prepared according to RAPP and BORSOS[4]. EAC'4 cells were made (using guinea-pig complement) by the method of BORSOS and COOPER[5]. Partially purified guinea-pig C'1[6] and C'2[7] were prepared according to published procedures. Whole guinea-pig serum was the source of guinea-pig C'3. For this purpose it was diluted 1:200 or 1:300 with VBS free of Ca^{++} and Mg^{++} and containing 0.01*M* EDTA; this VBS with EDTA is referred to as EDTA buffer. A euglobulin fraction of human serum referred to as 'M' (for midpiece) was the source of human C'3 and was prepared according to MAYER[2]. A pool of 'M' was stored as a frozen precipitate; for use it was diluted 1:4 or 1:10 with regard to the original serum volume using EDTA buffer.

Results. In the first experiment EAC'1,4,2 cells made from guinea-pig complement were converted to E* using guinea-pig C'3 and human C'3, and the course of the terminal transformation was followed. Three samples of 6 ml each of EAC'4 at a concentration of $1.5 \cdot 10^8$ cells per ml were treated with 6 ml of partially purified C'1 (diluted 1:8000) and incubated at 30 °C for 20 min. 6 ml of C'2 was then added to yield a ratio of about 50 C'2*M* per cell; the mixtures were incubated 7 min at 30°C. All manipulations to this point were carried out in 0.065 ionic strength buffer. Then 36 ml of the appropriate C'3 diluted in cold EDTA buffer was added to the samples: 1:200 guinea-pig serum, 1:4 human 'M', and EDTA buffer (control). The samples were mixed and held at 15°C for 10 min. The tubes were chilled briefly in an ice slush then centrifuged at 0°C and drained. The cell buttons were then suspended in 20 ml of ice-cold 0.09*M* EDTA; FRANK, RAPP, and BORSOS[8] have shown that this operation strongly inhibits the EAC'1,4,2 + C'3 reaction and the terminal transformation. To initiate the terminal transformation the cells were centrifuged, drained and resuspended in 9.0 ml of VBS and held at 15 °C. The reaction mixtures were sampled at various times by removing 0.5 ml of the mixture to 4.0 ml ice-cold 0.09*M* EDTA. The suspension was centrifuged and the O.D. of the supernatant read at 412 nm on the Beckman DU spectrophotometer. The sample O.D.'s were corrected for the controls, and the % hemolysis calculated. Figure 1 shows that E* prepared from guinea-pig C'3 completes its terminal transformation in 5 h while E* prepared from human C'3 continues to react at 20 and 28 h.

In another experiment EAC'1,4,2 was prepared from human complement; this EAC'1,4,2 will be referred to as EAC'1,4,2(Hu). The terminal transformation of the corresponding E*'s was followed. EAC'1,4,2(Hu) was prepared in VBS according to a modified method of LEON[9]. 30 ml of EA at a concentration of $1.5 \cdot 10^8$ cells per ml were brought to 37 °C and treated with 15 ml of 1:100 human serum at 37 °C for 3.0 min. 90 ml of ice-cold VBS were then added and the mixture centrifuged at 1 °C, drained, and washed with 45 ml ice-cold VBS. The cells were suspended in 30 ml ice-cold VBS and stored in an ice slush.

E* was prepared from EAC'1,4,2(Hu) using G.P. C'3 and the terminal transformation initiated in the following

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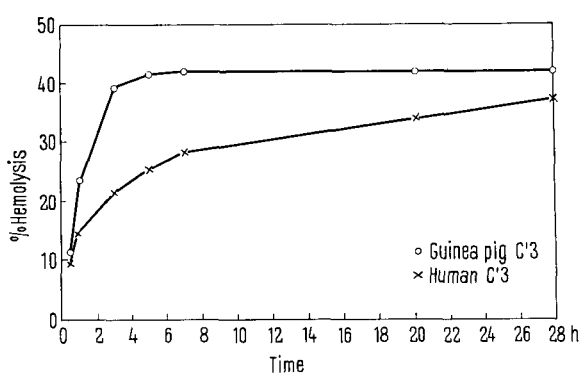

Fig. 1. Terminal transformation of EAC'1,4,2 cells prepared from guinea-pig complement and treated with guinea-pig and human C'3.

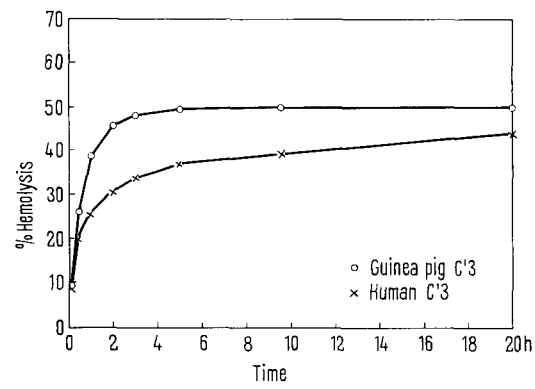

Fig. 2. Terminal transformation of EAC'1,4,2 cells prepared from human complement and treated with guinea-pig and human C'3.

way. To 12.5 ml of 1:100 G.P. serum diluted in EDTA buffer at 37 °C were added 4.0 ml ice-cold EAC'1,4,2(Hu). The mixture was held at 37 °C for 2 min then 13.3 ml ice-cold EDTA buffer added and the suspension was centrifuged and drained. The cells were washed once with 13.3 ml ice-cold EDTA buffer. The cells were suspended in 4.5 ml of cold VBS, a zero time sample taken, and the suspension transferred to the 15 °C bath. The reaction was sampled as before. E* made from EAC'1,4,2(Hu) and Hu C'3 was prepared by adding 4.0 ml ice-cold EAC'1,4,2(Hu) to 13.5 ml 1:10 'M' at 37 °C. This mixture was held at 37 °C for 2 min, then the E* preparation was completed and the terminal transformation followed as described above. In addition, a control was included which lacked a C'3 source but was otherwise manipulated as described. The data are represented in Figure 2 and show again that E* prepared from G.P. C'3 completes the terminal reaction more rapidly than does E* made from Hu C'3.

Discussion. From Figures 1 and 2 it seems reasonable to suggest that the lower rate of the terminal reaction of Hu C' compared to G.P. C' is due to the EAC'1,4,2 + C'3 step. That is, the nature of the E* with regard to its lytic rate seems to be determined by the reactants which convert EAC'1,4,2 to E*. When EAC'1,4,2 cells are prepared using Hu C', the terminal reaction will assume the higher rate if G.P. serum furnishes the C'3. While EAC'1,4,2 made from G.P. C' will produce a slow-reacting E* if Hu serum is the source of C'3. The reaction EAC'1,4,2 + C'3 → E* has been shown to proceed by more than one step in both G.P.[10] and Hu C'[11] due to the existence of several C'3 subcomponents. Further study of the difference of the Hu and G.P. terminal reaction rates will require the use of C'3 subcomponents to define precisely the intermediate at which the difference emerges[12].

Zusammenfassung. Mit Meerschweinchen-Komplement sensibilisierte Schaferythrocyten (EAC'1,4,2) wurden einerseits mit Meerschweinchen-, andererseits mit menschlichem C'3 in Intermediärform (E*) gebracht. Sie wurden im ersten Fall rascher lysiert, als im zweiten Fall. Es wird vermutet, dass hauptsächlich die EAC'1,4,2 + C'3-Reaktion für die unterschiedliche Wirkung von Meerschweinchen- und menschlichem Komplement in der Terminalphase der Hämolyse verantwortlich ist.

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Phospholipid Catabolism in Some Organs of Rabbit During Aseptic Autolysis

Almost nothing is known at present about phospholipid catabolism. In some tissues, phospholipase activities were shown in vitro[1-6]: phospholipase A activity, which was found in different tissues, was made evident only against rat liver endogenous phospholipids[7,8]. But the data available do not furnish conclusive information about the pathways that operate in vivo; they deal with a latent activity, which is in balance and is probably masked in tissues, in vivo, by other enzymatic activities.

Using, as method of research, aseptic autolysis, we tried to show changes in phospholipid content of different organs, due to hydrolysis by phospholipase activity. During aseptic autolysis[9], many enzyme activities, cathepsins, and other acid hydrolases become free – activities which are not seen in intact cells. Therefore we thought that possible phospholipase activity might become manifest under these conditions.

Material and methods. Portions of rabbit heart, kidney and liver, removed from animals immediately after beheading and bleeding in sterile conditions, were kept in aseptic autolysis at pH 4.5, according to the method used by DIANZANI[9]. After 6 days of incubation at 37 °C, the tissues were removed, weighed, and extracted for lipid analysis. In order to exclude the possibility that phospholipids in the same experimental conditions were hydrolysed, aliquots of phospholipids extracted from normal tissues and standard phospholipid were incubated in the same conditions of tissues in autolysis with and without phospholipase A (as source of phospholipase A snake venom *Naja naja* was used, supplied by Miami Serpentarium, Florida), in final solution of 2.5 mg% in the same buffer acetate pH 4.5; the medium in which the phospholipid was incubated contained also bovine serum albumin (supplied by the Instituto Sieroterapico Toscano Sclavo) in final concentration of 0.065%. The samples incubated with snake venom were used as standard for lysolecithin, previous to extraction with chloroform-methanol (2:1).

Lipid extraction was carried out either on some portion of different organs not in autolysis used as control, or on tissue in autolysis. Tissue was extracted 3 times with 20 volumes of chloroform-methanol 2:1 (v/v) and the combined extracts washed according to FOLCH[10]. Phospholipids were separated by silicic acid impregnated paper

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