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Studies on the Interaction between the Rheumatoid Factor and Complement Components on Sensitized Sheep Erythrocytes

Recently, Laurell (1963) called attention to the relationship between the fourth component of complement (C'4) and the rheumatoid factor (RF) on the basis of the observation that the agglutinating activities of EAC'1,4, EAC'1,4,2, EAC'4 and EAC'4,2, prepared from sensitized sheep erythrocytes (EA) and human complement, by the sera of rheumatoid arthritic patients were lower than that of EA. Decreased agglutinability was found in all the intermediates containing C'4, regardless of whether C'1 or C'2 was present on the cells.

In this investigation, we re-examined Laurell's results by comparing the agglutinability of EA by RF with the agglutinabilities of the intermediates of immune hemolysis, i.e., EAC'1, EAC'1,4 and EAC'4 using guinea pig serum as complement instead of human sera. RF preparations were obtained by filtration of the sera of rheumatoid arthritic patients through Sephadex G-200 gel columns. The fractions corresponding to the first peak of protein showed the highest activity of EA agglutination and were therefore used as the RF preparation in the following experiments.

As shown in Table A, the titers of agglutination of EAC'1,4 (S)* and EAC'4, prepared from whole guinea pig complement (Nishioka and Linscott 1963), by RF were significantly lower than that of EA.

However, as shown in the control in Table A, when EA was washed and incubated by the same procedures as those used for preparing the intermediate complexes, the agglutination titer was also lowered to the same degree as those of EAC'-1,4 (S) or EAC'4, suggesting an important role of such mechanical procedures as incubation and washing in lowering the agglutinating activity of the cell.

Similar results were also obtained using EAC'1 and EAC'1,4(C)** prepared from EA and partially purified preparations of C'1 and C'4 (Inai, *et al.* 1964). This is shown in Table B. Thus, the agglutination titers of these intermediates against RF were similar to those of EA, when it had been treated by the same procedures used for preparing these intermediate complexes. In addition, it will be seen from these Tables that the decrease in the agglutination titer of EAC'1,4(C) is more remarkable than that of EAC'1,4(S). Although the exact reason for this is still uncertain, considering the procedures used for preparing EAC'1,4(S) and EAC'1,4 (C), the explanation of this phenomenon may be similar to that described

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* EAC'1,4(S): Cells were prepared from EA and guinea pig serum

** EAC'1,4(C) Cells were prepared from EA and partially purified preparations of guinea pig C'1 and C'4

Table. Agglutination of EA and of Intermediates of Immune Hemolysis by RF.

AGGLUTINATION OF EAC'1, 4(S), EAC'4 (PREPARED WITH WHOLE COMPLEMENT) AND EA BY RF (N.O.)

DILS OF RF	56	112	224	448	896	1792	3584	7168	14336	28672	CONTROL BUFFER
EA	###	###	###	###	###	###	+	+	+	—	—
EAC'1,4 (S)	###	###	###	###	###	+	+	—	—	—	—
Control cells	###	###	###	+	+	+	±	—	—	—	—
EAC'4	+	±	—	—	—	—	—	—	—	—	—
Control cells	+	±	±	—	—	—	—	—	—	—	—

B AGGLUTINATION OF EAC'1, EAC'1, 4 (C) (PREPARED WITH ISOLATED C'1 AND C'4) AND EA BY RF (N.O.)

DILS OF RF	56	112	224	448	896	1792	3584	7168	14336	28672	CONTROL BUFFER
EA	###	###	###	###	+	+	+	+	+	—	—
EAC'1	###	###	###	+	+	+	+	±	—	—	—
Control cells	###	###	+	+	+	+	+	±	—	—	—
EAC'1,4 (c)	+	±	—	—	—	—	—	—	—	—	—
Control cells	+	±	—	—	—	—	—	—	—	—	—

0.5 ml aliquots of the various cell suspensions were mixed with 0.5 ml of serial dilutions of RF in veronal buffer, ionic strength 0.09.

The degree of agglutination was noted after 1 hour's incubation at 37°C and over night storage in a refrigerator at 2-4°C.

— ~ ### : extent of agglutination
— : no agglutination
: strong agglutination
Control cells : EA was washed and incubated using the same procedures used for preparing each intermediate.

above.

The effect of RF on the hemolysis of EA and EAC'1 was also examined to see if there was any competition between RF and C'4 for the receptor on the cell. To a series of test tubes containing constant amounts of EA, serial dilutions of RF were added. After incubation at 30°C for 30 minutes the cells were centrifuged, washed twice and resuspended in the original volume of medium. The same amount of guinea pig complement was then added to all the tubes which were then incubated for 60 minutes at 37°C. The resultant hemolysis in all the tubes was practically the same. Similar treatment of EAC'1 with RF also failed to demonstrate any difference in reactivity with a constant amount of partially pur-

ified guinea pig C'4, when tested using C'2 and then C'-EDTA (Inai *et al.* 1963). This clearly indicates that EA and EAC'1 can exhibit the same degree of hemolytic reactivity with complement or C'4 regardless of the combination with RF.

The results obtained here suggest that the decreased agglutinabilities of the intermediates by RF are simply due to the mechanical procedures used in the preparations and are not due to the C'4 activity on the cells. Further, as far as guinea pig C'4 is concerned, our results are inconsistent with the hypothesis proposed by Laurell, that C'4 may compete with RF on the sensitized cell by steric hindrance or may destroy the structure of the γ-globulin which is to combine with RF.

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