Action of a Phospholipase C Preparation on the First Component of Complement of Guinea Pig and Human Serum: Lack of Correlation with Enzyme Activity

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Received for publication 5 May 1972

Interaction of a phospholipase C (from Clostridium welchii) preparation with the first component of human and guinea pig complement in serum is due to contaminants devoid of phospholipase C activity.

It has been reported that some enzymes have the ability to either activate or inactivate complement activity (3, 8). At least for two of these, streptokinase and L-asparaginase, anti- or pro-complementary effects were shown to be due to bacterial contaminants in the enzyme preparation (5, 6) and not to the enzymes themselves. We now present evidence that a phospholipase C preparation derived from Clostridium welchii was capable of interacting with C1 in serum and that this interaction was due to substances that are separable from enzyme activity. The interaction of the phospholipase C preparation with C1 in serum may result in activation or inactivation of C1 activity.

Reagents and buffers used in the following experiments were described by Rapp and Borsos (9). C1 and C1 activity in whole serum was determined by measuring the generation of EAC14 at the end of 10 min of incubation at 30 C of EAC4 with dilutions of whole serum. The dose-response curve of C1 under these conditions is linear (1), and a longer time of incubation at 30 C has no effect on the extent of lysis as a function of C1 concentration. On the other hand, the dose-response curve of precursor C1 is not linear (11), and further incubation leads to increased lysis for a given concentration of C1 without resulting in a linear dose-response curve (Fig. 1). Based on these observations, activated and non-activated C1 can be distinguished by comparing the shapes of their dose-response curves; furthermore, a shift in the angles of the dose-response curves may indicate activation of C1 to C1 or inactivation of C1 and C1. Serum samples from two individual humans (FS and MS) and one guinea pig served as the source of the non-activated first component of complement, C1. The activity of phospholipase C (C. welchii; 2.5 units/ mg; lot G1C-6890, Sigma Chemical Co., St. Louis, Mo.) was measured by its ability to lyse sheep erythrocytes as described in (10). Sephadex chromatography was performed as described (2).

To determine whether phospholipase C has an effect on C1 in guinea pig and human sera, equal volumes of undiluted guinea pig or un-
diluted human serum and dilutions of phospholipase C (diluted in Veronal-buffered saline, VBS, \( \mu = 0.065, \) pH 7.3) were mixed together and incubated for 30 min at 37 C. At this time, the mixtures were diluted in VBS, \( (\mu = 0.065) \), and the amount and nature of C1 were determined. The results in Fig. 2 show that the phospholipase C preparation activated C1 in guinea pig serum and human serum MS, and that phospholipase C did not convert C1 to C1. In contrast, phospholipase C was capable of activating C1 to C1 in human serum FS (Fig. 3); however, inactivation of C1 and C1 occurred when phospholipase C was incubated with human serum FS at 0 C (Fig. 3). Since phospholipase C was derived from bacteria, we considered the possibility that the effect of phospholipase C preparations on C1 may be due to a contaminant and not to the enzyme phospholipase C. To test this hypothesis, we subjected the phospholipase C preparation to molecular sieve chromatography on Sephadex G-100. The effluent was tested for phospholipase C activity and for interaction with serum C1. The interaction with serum C1 was tested by incubating equal volumes (0.1 ml) of undiluted serum and undiluted effluent for 30 min at 37 C. At this time, the mixtures were diluted (final serum dilution 1:100,000 in VBS, \( \mu = 0.065, \) pH 7.3), and the amount of C1 was determined as described above. The results of this experiment are shown in Fig. 4. We found two activities which inhibited serum C1; one appeared in the void volume and the other was retarded by the beads and its position with respect to serum proteins was between the 7S and albumin peaks. The phospholipase C activity was eluted after the second peak of anti-C1 activity. These results showed that the enzyme phospholipase C has no effect on C1 in human or in guinea pig serum. The inhibitory effect of the contaminant(s) on C1 of human as well as guinea pig serum may be due to proteinase(s) since it is known from work by Goldlust et al. that proteinases derived from Clostridium sp. inactivate whole serum complement activity (4, 7). The proteinase isolated by Goldlust inactivated C2 but not C1 (4); our experiments also showed that Clostridium preparations also contain an inactivator of C1. The activation of C1 noted in one of the sera tested after incubation with the phospholipase C preparation is explainable as a result of antibody-antigen-C1 interaction in which the source of the antigen is the phospholipase C preparation. The results of this study and of those on the interaction of complement with L-asparaginase and streptokinase indicate some of the difficulties that may be encountered in studying the effects of enzymes derived from bacteria on serum complement.

G. M. L. was a guest worker in the Biology Branch, National Cancer Institute, and was supported by the Deutsche Forschungsgemeinschaft, Lo. 188/1.
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